Research article

Studies on Reducing Cadmium Uptake of Paddy Rice (*Oryza sativa* L.) by both Soil Dressing and Mixing Tillage

KIICHI SASAKI*
The United Graduate School of Agricultural Sciences, Iwate University, Morioka, Iwate, Japan
Email: watersapphire0919@gmail.com

CHOICHI SASAKI
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

CHIHIRO KATO
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

AKIRA ENDO
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

TAKEYUKI ANNAKA
Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata, Japan

SHIGEOKI MORITANI
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

NOBUHIKO MATSUYAMA
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

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Abstract Though soil dressing is one of the most effective methods for reducing cadmium (Cd) uptake in rice plants of Cd contaminated paddy fields, it needs large amount of soil and thus often requires a high cost and heavy environmental loads. In this study, we investigated any possibility of minimizing the thickness of soil dressing by utilizing mixing tillage before the soil dressing. 15 cm-thick mixed contaminated soil and overlying 12.5 cm-thick soil dressing (the conventional thickness of soil dressing being 20-30 cm in Japan) were used to form the usual stratified paddy field of the three layers (plow layer, plowsole, and subsoil). Cd concentration of the contaminated soil was adjusted to approximately 2.0, 1.0 and 0.5 mg/kg by mixing tillage. Then rice plants were grown under ponding condition during the cultivation and the experiments of each treatment were conducted under two different water flow systems, open and closed systems. As a result, Cd concentration in brown rice with water flow in an open system was 0.07 mg/kg, 0.05 mg/kg, and 0.17 mg/kg when Cd concentration of soil was 0.5, 1.0 and 2.0 mg/kg, respectively. Cd concentration in brown rice of 2.0 mg/kg soil was 5% significantly higher by 5% than those of other treatments. These results indicated that it was effective to dilute soil Cd concentration to 1.0 mg/kg for reducing Cd uptake of rice plants. There was no significant difference in growth and yield a way these treatments. However, we conclude that mixing tillage may have potential to minimize the thickness of soil dressing to half of the conventional thickness, 20 -30 cm, under ponding condition during the cultivation.

Keywords Cadmium, rice, water flow system, mixing tillage, soil dressing

INTRODUCTION

Heavy metal contamination of agricultural lands resulting from human activities has been causing serious health damages all over the world. Alarmed by this situation, Codex Alimentarius Commission has decided upon the limits of the cadmium (Cd) contents in food, e.g. 0.4 mg/kg in rice (MAFF, 2015a). Separately from the international standard, some countries have employed stricter standards and thus with a view to international trading, it is necessary for any country to reduce Cd contamination level of agricultural products as much as possible.

Cd health damages in Japan have mainly resulted from the intake of rice, Japanese staple food. Cd from mine waste water had accumulated in the paddy field soil, and the intake of the high-Cd rice caused the famous Itai-itai Disease (Kobayashi, 1978). In Japan, soil dressing of 20-30 cm-thick has been employed as the usual countermeasures for Cd contaminated agricultural fields. Though soil dressing is effective to reduce the Cd uptake of rice, it needs such a large amount of soil and therefore it costs much and causes heavy environmental loads.

The Ministry of Agriculture, Forestry and Fishery in Japan recommends that rice should be cultivated under ponding condition as a treatment for reducing the Cd uptake of rice (MAFF, 2015b) since solubility of Cd is low under reduction condition. However, Pongpattanasiri et al. (2005), Sasaki et al. (2010, 2012) and Paul et al. (2011) pointed out that even under ponding condition, the plowsole and subsoil layers showed oxidation condition when water flow in these layers was the open-system percolation and thus Cd might be dissolved in the soil water and absorbed by roots. Sasaki et al. (2010) and Paul et al. (2011) conducted rice growing experiments using paddy field models with 12.5 cm-thick soil dressing and reported that the percolation pattern in the plowsole and subsoil affected the degree of the Cd uptake of rice. In these cases, however, the Cd concentration of the subsoil was 3.39 mg/kg, which was twice as high as that of the average contaminated soils in Japan (Shibuya et al., 1980). Other than those mentioned above, there are few studies which considered the percolation patterns (Adachi and Sasaki, 1999; Sasaki et al., 2001).

In this study, aiming at the reduction of Cd uptake in rice plants, we investigated any possibility to minimizing the thickness of soil dressing by both utilizing soil dressing and mixing tillage and cultivation under ponding condition regulating the percolation systems in the subsoil.

METHODOLOGY

Soil Properties

In this study, the soil from plow layer of the Cd contaminated paddy field, where the mine waste water had been used as the irrigation water, was sampled for the experiments. Non-contaminated soil for soil dressing and gravel were obtained from the plow layer of a paddy field and a mountainous district in Tohoku region, or the north-eastern part of Japan.

Table 1 Physical and chemical properties of soil samples and gravel

	Density	Soil Texture	MgO	Na_2O	CaO	K_2O	Cd	T-C	T-N	C/N	OM
	(g/cm^3)		*	*	*	*	*	(%)	(%)		(%)
Non Polluted Soil	2.62	LiC	120	64	400	120	0.14	2.07	0.16	13.27	3.6
Polluted Soil	2.44	CL	640	128	2280	288	1.81	5.30	0.39	13.57	9.1
Gravel	2.68	-	147	18	539	600	0.13	-	0.00	-	0.1

*Note: * mg/kg*

Table 1 shows the physical and chemical properties of the soil samples and gravel. The soil textures of contaminated and non-contaminated soils were CL and LiC, respectively, and their contents of soil organic matter were 9.2% and 3.6%, respectively. The Cd concentration of the contaminated, non-contaminated and gravel were, 1.81, 0.14, and 0.13 mg/kg, respectively. By mixing these two kinds of soils, the Cd concentration of the experimented soils was adjusted to the aforementioned three levels. The experimental apparatus was designed after the fashion of paddy fields near a river and thus gravel was also used for the lower layer.

Experimental Design

Two kinds of stratified paddy field models were used for the experiments (Fig.1). The air entry values were determined by the method of Sasaki (1992). These models consisted of an iron box (30 cm × 50 cm × 70 cm) filled with soils and gravel in three layers. Plow layer (1st layer) was from 0 cm to 10 cm deep (dry density was 1.02 Mg/m^3): plowsole (2nd layer) was from 10.0 cm to 20.0 cm deep (dry density at the depth from 10.0 cm to 12.5 cm and from 12.5 cm to 20.0 cm were 1.23 Mg/m^3 and 0.75 Mg/m^3, respectively) : subsoil (3rd layer) was from 20.0 cm to 62.5 cm deep (dry density from 20.0 cm to 27.5 cm was 0.75 Mg/m^3 and that from 27.5 cm to 62.5 cm was 1.4 Mg/m^3, those layers were formed by compaction). The thickness of non-polluted soil dressing was 12.5 cm deep, and beneath the soil dressing there lay the polluted soil of 15.0 cm depth, the gravel layer was placed beneath the polluted soil layer.

In order to make three different levels of contaminated concentration of the plowsole and subsoil in the stratified paddy field models, polluted and non-polluted soils were mixed. The authors defined O-1 and C-1 as the setting value of 0.5 mg/kg of the stratified paddy field model (the actual Cd concentration after mixture became 0.57 mg/kg). Similarly, O-2 and C-2 were defined as the setting value of 1 mg/kg of Cd concentration (the actual Cd concentration after mixture became 1.21 mg/kg). Moreover, we defined O-3 and C-3 (the actual Cd concentration 1.81 mg/kg) as the polluted soil without dilution. Note that 'O' and 'C' mean the open-system and the closed-system percolation, respectively. The ground water levels of the open-system and the closed-system percolation models were controlled at 57.5 cm and 20.0 cm depths, respectively. In the closed-system percolation models, the holes in the side walls of iron box were blocked in order to prevent the penetration of the atmosphere. On the other hand, in the open-system percolation models the holes in the side walls of the iron box were open in the lower part of the 2nd layer and the upper part of the 3rd layer in order to aerate those layers.

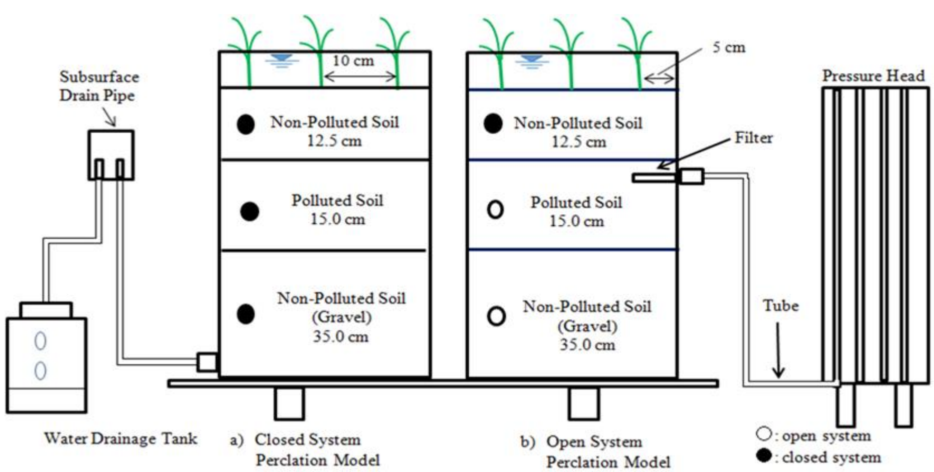

Fig. 1 Layout of the experimental design

After the two types of models were prepared, fifteen paddy seedlings (plant length and leaf stage were from 14.0 to 18.0 cm and from 5.4 to 6.8 leaves, respectively) named '*Oryza sativa* L. Tsugaru Roman' were transplanted. The paddy seedlings were transplanted by 10.0 cm intervals. As for fertilizer, 2.0 g of N, 2.0 g of P_2O_5 and 2.0 g of K_2O was put per model and mixed with the whole plowed layer before transplanting. While the cultivation period, the water ponding condition was constantly adopted, and the mid-summer drainage was not done. Transplanting of the paddy seedlings and harvesting were conducted at the end of May and at the end of September, respectively. This experiment was conducted without repetition, and Tukey-Kramer methods were used to test the statistical significance of the data of growth and yields of rice plants.

Measuring Method

The ORP meter (Central Kagaku co Ltd, model UC-203) was used for measuring oxidation -reduction potential (Eh), and it was set at each soil layer. We measured plant length, leaf number, the number of stems and panicles, the weight of straws, the number and weight of brown rice. Before the quantitative analyses of Cd concentrations in the stems and leaves, roots, brown rice and soils, the samples were treated with HNO_3 and HCl to extract Cd as sample preparation, and then absorbance of extracted solution was measured with atomic absorption spectroscopy (MAFF, 1979). Other measurements i.e., (density of soil particle, soil texture, grain size analysis, C/N ratio) were also conducted in standard methods in Japan.

RESULTS AND DISCUSSION

Oxidation-reduction Potential (Eh)

The oxidation-reduction state in the soil is an important factor for Cd mobility. In the oxidized layer, the solubility of Cd increases in soil solution (the main form of Cd component is $CdSO_4$) and so the uptake of Cd from plant roots is promoted. On the other hand, in the reduced layer, the solubility of Cd decreases (the main form of Cd component is CdS) and so the uptake of Cd from plant roots is limited (Iimura, 1981). From these, the measurement of oxidation-reduction potential (Eh) is very important for judging the mobility of Cd. The oxidized layer in paddy fields is defined as the layer whose Eh value is higher than +300 mV (Yamane, 1982).

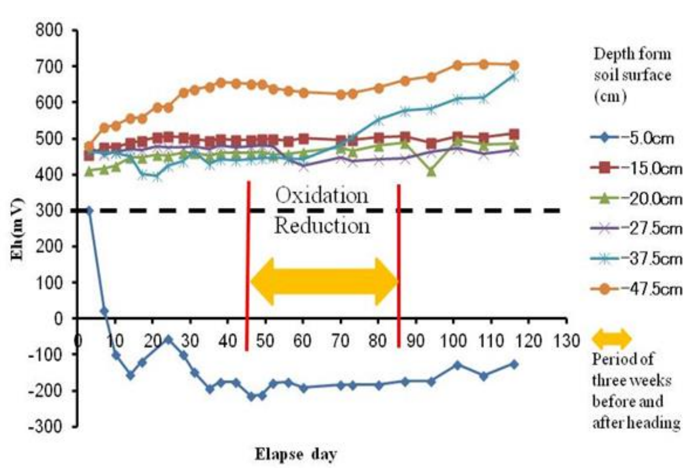

Fig. 2 Oxidation-reduction values in soil layers of the open system percolation model

The trends of Eh in each soil layer of O-1, O-2 and O-3, and of C-1, C-2 and C-3 were similar change from the transplanting to the harvest. Therefore, we have used below the Eh of open-system percolation model (O-3) in Fig. 2 and the Eh of closed-system percolation model (C-3) in Fig. 3. In the open-system percolation model, the Eh of the first layer (5.0 cm depth) had been about -100 mV during cultivation of paddy rice and so the first layer was a reduced layer. The Eh of the second layer (15 cm and 20 cm depth) and the third layer (27.5 cm, 37.5 cm and 47.5 cm depth) had been higher than +400 mV and those layers were oxidized layers. On the other hand, in the closed system percolation model, the Eh of all layers had been lower than -100 mV since 20 days after transplanting to harvest and so those layers were reduced layers.

It is said that Cd uptake of paddy rice is relatively high during three weeks before and after heading (MAFF, 2015b). In the open-system percolation model (Fig. 2), the second layer and the third layer were oxidized layers during three weeks before and after heading. On the other hand, all layers of the closed-system percolation model (Fig. 3) were reduced layers during the period. From these results, it is estimated that soluble Cd affects to paddy rice in the open-system percolation model.

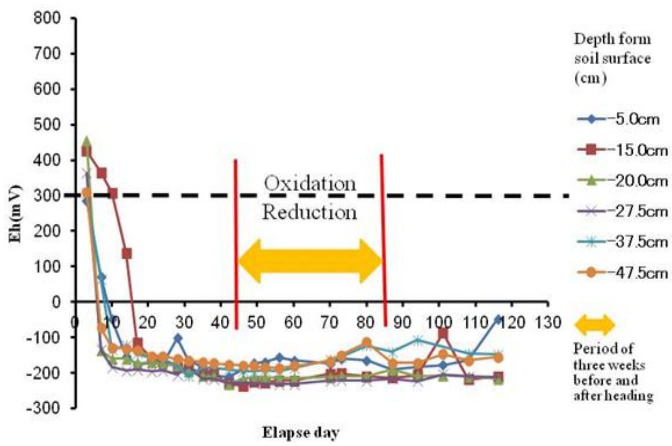

Fig. 3 Oxidation-reduction values in soil layers of the closed system percolation model

Table 2 Parameters of rice plant growth

Model	Plant length (cm)	No. of stems (Stems/hill)	Dry weight of straw (g)
O-1	94.8±4.0[ab]	15.2±2.3[a]	16.1±3.9[a]
O-2	97.5±3.2[b]	14.8±3.2[a]	16.4±3.9[a]
O-3	98.1±3.6[b]	17.1±2.5[a]	17.3±3.8[a]
C-1	92.5±2.5[a]	17.5±2.5[a]	18.1±4.6[a]
C-2	94.9±2.9[ab]	13.8±3.9[a]	20.9±4.7[a]
C-3	98.3±3.5[b]	17.1±3.2[a]	20.7±6.3[a]

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference (n=10). The numerical value of ± shows standard deviation.

Growth of Paddy Rice

Table 2 shows the growth of paddy rice in the open-system percolation model and the closed-system percolation model. The average plant length ranged from 92.5c m to 98.3 cm in the two types of models. The average number of stem ranged from 13.8 to the 17.5 in the two types. The average dry weight of straw ranged from 16.1 g to 20.9 g in two types. There were no significant differences in the growth parameters between the open-system percolation model and the closed-system percolation model. Paul et al. (2011) reported that the growth parameters of the closed-system percolation model were significantly higher than those of the open-system percolation model in the case of 3.39 mg/kg in plowsole. Furthermore, Ito and Iimura (1976) and Asami et al. (1995) reported that the increase of Cd concentration in the soil caused the decrease of plant length, number of stems and dry weight of straw. Those previous researches had different results from ours and that in probably because in our experiments the root system mainly existed in the non-polluted soil dressing and the Cd concentration in the soil was relatively low (less than 2 mg/kg).

Yield Components of Paddy Rice

Table 3 shows the yield components of paddy rice both in the open-system percolation model and the closed-system percolation model. The average number of panicles ranged from 9.9 to 13.2 in the two types of models. The average weight of brown rice ranged from 15.0 g to 21.7 g in the two types. The average number of brown rice grain ranged from 634 to 938 in the two types. The average 1000 grain weight of brown rice ranged from 18.7 g to 19.6 g in the two types. There were no significant differences in the yield components between the open-system percolation model and the closed-system percolation model. However, Paul et al. (2011) reported that the yield components of the closed-system percolation model were significantly higher than those of the open-system percolation model. Sasaki et al. (2010) and Pongpattanasiri et al. (2005) also reported that the average number of panicles in the closed-system percolation model was significantly higher than that of the open-system percolation model. Furthermore, Ito and Iimura (1976) and Asami et al. (1995) reported that the increase of Cd concentration in the soil caused the decrease of the weight of brown rice. The reasons why there were no significant differences in our experiments in the yield components between the two types of models was thought to be the effects of soil dressing and the relatively low Cd concentration of less than 2 mg/kg in the plowsole and subsoil.

Table 3 Parameters of rice plant yield

Model	No. of Panicles (Panicles/ hill)	Weight of brown rice per unit hill (g)	Number of brown rice grain per unit hill	1000 grain weight of brown rice (g)
O-1	$10.1±2.2^{a}$	$15.0±4.2^{a}$	$634±173^{b}$	$19.6±1.4^{a}$
O-2	$10.0±1.9^{a}$	$16.6±4.4^{ab}$	$720±189^{ab}$	$19.3±0.4^{a}$
O-3	$12.9±2.5^{ab}$	$17.4±4.2^{ab}$	$790±189^{ab}$	$18.9±0.4^{a}$
C-1	$10.1±2.2^{a}$	$17.5±3.7^{ab}$	$740±173^{ab}$	$18.7±2.1^{a}$
C-2	$9.9±2.9^{ab}$	$15.9±2.9^{ab}$	$701±110^{ab}$	$18.7±0.9^{a}$
C-3	$13.2±2.7^{b}$	$21.7±6.3^{b}$	$938±260^{a}$	$19.4±1.1^{a}$

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference (n=10). The numerical value of ± shows standard deviation.

Cadmium Concentration in Rice Plants

Root: Table 4 shows the Cd concentration in roots in each soil layer: a range of 1.8-3.7 mg/kg was observed in the plow layer, though these values were not appreciably different among the experimental models; in the plowsole, a range of 1.70-17.00 mg/kg was observed. The result of larger amount of Cd concentration in O-3 than other could be explained by the experimental condition of open-system percolation. In the subsoil, a range of 1.2-4.5 mg/kg was observed, which was 10 times higher than that of 0.13 mg/kg in the gravel layer. This result proved the occurrence of Cd movement from contaminated soil layer in the direction of downward or upward. The similar tendency was reported by Sasaki (2010, 2012) and Paul et al. (2011). This fact may provide a new perspective regarding the phytoremediation. The concentration factor, which is defined as the ratio of Cd concentration in roots in each soil layer, showed the ranges of 12.9-26.4, 2.0-9.4, and 9.2-34.6 from the plow layer to subsoil, respectively. The concentration factor in the plowsole was lower than both the plow layer and subsoil.

Table 4 Cd concentration in roots of different soil layers in the open and closed system percolation models

Model	Plow layer Non-polluted soil (mg/kg)	Plowsole polluted soil (mg/kg)	Subsoil Gravel (mg/kg)
O-1	2.90	4.20	1.20
O-2	3.20	2.70	1.60
O-3	3.00	17.00	4.50
C-1	3.00	1.70	1.80
C-2	1.80	6.00	1.70
C-3	3.70	3.60	2.20

Stem and Leaves: Table 5 shows the Cd concentration of stem and leaves. Except for O-3 (0.86 mg/kg), the range of Cd concentration was 0.18-0.33 mg/kg in the rest of five kinds of models. Thus, there was a significant difference between O-3 and the other models. It was inferred that the obvious Cd increment in stems and leaves became remarkable at a Cd concentration above 2 mg/kg in the contaminated soil layer. The similar tendency of a Cd increment had been recognized in open-system percolation (Sasaki et al., 2010; Paul et al., 2011). They reported similar values of Cd concentrations of stem and leaves under almost the same formation of soil layers except for the Cd concentration of 3.39 mg/kg in the contaminated soil layer. As Shibuya et al. (1980) pointed out that, the Cd concentration in contaminated soils higher than 1.5-2.0 mg/kg provides an appropriate lower limit and may be valid for our results in the stratified paddy field models.

Table 5 Cd concentration in the stem and rice grain

Model	Rice grains (mg/kg)	Stem and leaves (mg/kg)
O-1	0.07 ± 0.04^{b}	0.33 ± 0.13^{b}
O-2	0.05 ± 0.01^{ab}	0.28 ± 0.14^{b}
O-3	0.17 ± 0.05^{c}	0.86 ± 0.45^{a}
C-1	0.05 ± 0.01^{ab}	0.21 ± 0.10^{b}
C-2	0.04 ± 0.01^{ab}	0.18 ± 0.03^{b}
C-3	0.03 ± 0.01^{a}	0.24 ± 0.13^{b}

Note: Tukey-Kramer test was performed at 5% level;letter indicates significant difference (n=10). The numerical value of ± shows standard deviation.

Rice Grains: Table 5 shows the Cd concentration of rice grain. Except for O-3 (0.17 mg/kg), the Cd concentrations of rice grains were less than 0.07 mg/kg in the rest of five models. However, Cd concentrations in rice grains harvested from the contaminated soil with Cd concentration of below 1.0 mg/kg were similar regardless of percolation types. These values were consistent with the value of 0.06

mg/kg produced in non-contaminant paddy fields in Japan (MAFF, 2015b). There was a statistically significant difference between O-3 and the other five models.

Itou and Iimura (1976) reported that the value dividing Cd concentration of rice grains by that of straw was ranged between 0.1 and 0.2. Our experimental data brought about the same result, 0.1-0.2. This suggested that the ratio range of 0.1-0.2 may be common under widely variable cultivation conditions, such as pot experiments, field experiments (Tokunaga et al., 1977) and model experiments for stratified paddy fields (Sasaki et al., 2012).

CONCLUSION

As the countermeasures against Cd-polluted paddy fields, we experimented with six models of stratified paddy fields that had ponding during cultivation and soil dressing (12.5 cm), thickness being half of the conventional soil dressing in Japan, and also two different percolation patterns, closed-system percolation and open-system percolation of the plowsole and the subsoil. Those models had Cd concentration of 0.57 mg/kg, 1.21 mg/kg and 1.81 mg/kg in the plowsole and the subsoil.

As a result, the open-system percolation layers became an oxidation condition and the closed-system percolation layers became a reduction condition. But we could not recognize statistically significant difference in growth and yield in rice plants with those models. Cd concentration in rice grains, stems and leaves in the open-system percolation models with 2.0 mg/kg Cd-polluted soil was statistically significant and higher than those models of the others. Consequently, we found that with Cd-polluted soil models of less than 1.0 mg/kg made by mixing tillage we just needed almost half of the usual amount of soil dressing to significantly reduce the Cd concentration in the rice grains, stems and leaves. Thus, we found that the countermeasures which combined soil dressing and mixing tillage were an effective method with economic and safety merits.

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