IJERD – International Journal of Environmental and Rural Development (2020) 11-1

Research article

The Influence of Percolation Patterns on Copper and Cadmium Uptake, Growth and Yield of Rice Plants in Copper- and Cadmium-polluted Stratified Paddy Fields

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Received 20 Jan 2020 Accepted 5 March 2020 (*Corresponding Author)

Abstract Combined pollution in farmland soil is a recognized issue in Japan. The measurement of combined pollution has been discussed because the interaction between heavy metals is complex. To date, soil dressing has been the primary method employed to tackle this problem. The objective of this study is to clarify how the percolation patterns of polluted subsoil affect Cu and Cd uptake, and the growth and yield of paddy rice plants. We prepared six stratified paddy-field models to test growth with plowsole and subsoil in open and closed percolation patterns. Each model consisted of a 12.5-cm-thick plowed layer and an upper plowsole made of a non-polluted soil dressing and underlying polluted plowsole and subsoil. Three soils with three levels of Cu contaminations (100, 250, and 500 mg Cu kg^{-1}) were prepared by adding a Cu solution to Cd-contaminated paddy-field soils (1.7 mg Cd kg^{-1}). Three of the six models were open systems, and the other three were closed systems. During the tests, a constant water-ponding system was adopted. As a result, the polluted plowsole and subsoil became oxidized in the open system and reduced in the closed system. The Cu and Cd concentrations in the rice grains were 5% higher in the open models than in the closed models, regardless of the original Cu concentrations in the polluted soils. Interestingly, the Cd concentrations in the grains had an inverse relationship with the Cu concentrations. No significant difference was observed in the growth and yield of the rice plants among the models. We concluded that the Cu and Cd concentrations in rice plants were affected by the percolation patterns of polluted plowsole and subsoil, even though they were covered with non-polluted soil-dressing layers.

Keywords copper, cadmium, soil dressing, percolation patterns, rice

INTRODUCTION

Heavy metal contamination became an apparent problem when severe damage was caused by Cu contamination to paddy fields in the lower reaches of Watarase River near Ashio Copper Mine and Cd contamination caused Itai-itai disease among people living near Jintsu River, Toyama prefecture (Asami, 2010). In the 1970s, the *Agricultural Land Soil Pollution Prevention Act* came into effect, identifying Cu, Cd, and As as harmful substances.

Arao et al. (2010) reviewed the history of heavy metal contamination in agricultural soil in Japan and the countermeasures against contamination such as soil dressing, water management, and chemical washing. They pointed out that while soil dressing was very effective in reducing Cd absorption by paddy rice, the biggest problem was its high cost.

When it comes to combined heavy metal contamination in agricultural lands, soil dressing has mainly been applied for remediation (e.g., Ogoya Copper Mine, Ishikawa prefecture, Japan; Asami, 2010). However, recently low-cost countermeasures have gradually been introduced such as multiple heavy metal insolubilization treatments and heavy metal absorption materials (Kanamori et al., 2019). Clarifying the complex interaction among multiple heavy metals and its effect on the growth and yield of crops is a lengthy process.

It has been pointed out that the contaminated lower soil layers of stratified paddy fields with soil dressing are under oxidizing and reducing conditions in open and closed percolation patterns, respectively (Fan et al., 2018a). Paul et al. (2011a, 2011b) and Sasaki et al. (2016a, 2016b) focused on these phenomena and studied the effects of percolation patterns on root uptake of Cd and the growth and yields of crops using models of contaminated stratified paddy fields with a soil dressing. They found that Cd concentration in brown rice was significantly higher when the contaminated layer was in the open system percolation pattern than its closed system percolation pattern counterpart. Paul et al. (2011a), who used Andosol in their experiments, found a significant difference in the growth and yield of paddy rice, between open and closed system percolation patterns. On the contrary, Sasaki et al. (2016a), who used alluvial soil, did not find any such difference. Fan et al. (2018a, 2018b) conducted a growth experiment by using stratified paddy field models with a soil dressing on a Cu contaminated soil layer under open and closed system percolation patterns. They reported that Cu concentrations in brown rice were higher in the open system percolation pattern than those in the closed system percolation pattern. They also found no significant difference in growth and yield between the two percolation patterns.

In Japan, paddy fields with Cu and Cd combined contamination have been recognized downstream of some mines for decades. There has been an introduction of countermeasures against such contamination, soil dressing being the most popular. However, few studies have clarified the characteristics of heavy metal absorption by rice plants, the degree to which contamination varies based on the percolation pattern used, nor the impact of the percolation pattern on the growth and yield of rice crops. The purpose of this study is to investigate the effects of percolation patterns on heavy metal absorption (for Cd and Cu) and the growth and yield of rice plants in paddy fields with combined heavy metal contamination.

The experimental devices and the stratified paddy field models adopted in this study were the same as those in Sasaki et al. (2016a). Three soils with three levels of Cu contamination (100, 250, and 500 mg Cu kg^{-1}) were prepared by adding a Cu solution to Cd contaminated paddy field soils (approximately 1.72 mg Cd kg^{-1}; Sasaki et al., 2016a). We found that Cd and Cu concentrations in brown rice were higher in open system percolation patterns than those in closed system percolation patterns. No significant difference was found in the growth and yield of the rice crop. As a result, we concluded that the type of percolation patterns had a significant impact on Cd and Cu absorption but little on the growth and yield of the paddy rice plants.

METHODOLOGY

Materials and Methods

In this study, we used stratified paddy field models and conducted a growth experiment in open and closed percolation systems following Sasaki et al. (2016a). Table 1 details the physical and chemical properties of the soils used in this study. Non-contaminated soil (light clay; International Union of Soil Science) was sampled from the plow layer of the paddy field at the Kanagi Farm of Hirosaki University, Aomori prefecture (hereafter referred to as “Kanagi soil”). Cd and Cu combined contaminated soil was prepared by adding $CuCl_2 \cdot 2H_2O$ to Cd contaminated soil (clay loam), collected from the plow layer of Cd contaminated paddy fields in Japan (hereafter referred to as “contaminated soil”). The 0.1 M HCl extracted Cu concentrations in the original contaminated soil were 12.2 mg Cu kg^{-1}, and we made three concentrations of Cu contaminated soil; 100, 250 and 500 mg Cu kg^{-1}. This meant that one was below the Japanese standard of 125 mg Cu kg^{-1}, and the other two were above that. The Kanagi soil and gravel were 3.7 and 0.8 mg Cu kg^{-1}, respectively.

Table 1 Physical and chemical properties of soil samples and gravels

	Density	Soil Texture	MgO	Na_2O	CaO	K_2O	Cd	Cu	T-C	T-N	C/N	OM
	(g/cm^3)				(mg/kg)				(%)			(%)
Kanagi Soil	2.62	LiC	120	64	400	120	0.14	3.70	2.07	0.16	13.3	3.6
Contaminated Soil	2.44	CL	640	128	2280	288	1.81	12.2	2.96	0.21	14.1	5.1
Gravel	2.68	-	147	18	539	600	0.13	0.80	-	0.00	-	0.1

The Cu concentrations in the combined contaminated soils were 20 to 100 times higher than those in average non-contaminated soils of paddy fields in Japan (which are approximately 5 mg Cu kg^{-1}) (Asami, 2010). In this study, we prepared such highly Cu contaminated soils because of a regionally unique situation in the Aomori prefecture. In this prefecture, apple has long been the center of the economy and culture, and apple orchards often use a Bordeaux mixture (i.e., a mixture of copper sulfide and calcium carbonate), as a pesticide. As a result, Cu concentrations in surface soil have at times been found to be higher than 500 mg Cu kg^{-1} (Aoyama, 2009). Nowadays the number of apple producers has been decreasing due to the lack of successors and parts of apple orchards have been converted into other land uses, including paddy fields. This is especially the case in lowlands, where some of them were once used as paddy fields.

Notably, no significant change was found in soil Cu concentrations before and after the experiment.

Experimental Design

We adopted the design of Sasaki et al. (2016a), where we used two types of stratified paddy field models in our experiment; an open system percolation model and a closed system percolation model. Each stratified field model consisted of a 12.5 cm soil dressing (12.5 cm Kanagi paddy soil), a 15 cm soil polluted by Cd and Cu, and a 35 cm gravel layer. We defined O-100 as an open system percolation model in which Cu concentration in the plowsole and subsoil was 100 mg Cu kg^{-1}. C-100 was the closed system percolation model in which the Cu concentration in the sub-layer (plowsole and subsoil) was 100 mg Cu kg^{-1}. Similarly, O-250 and C-250 were defined as models with 250 mg Cu kg^{-1} in the sub-layer whilst O-500 and C-500 were defined as models with 500 mg Cu kg^{-1} in the sub-layer. Note that ‘O’ and ‘C’ stand for the open system percolation pattern and the closed system percolation pattern, respectively. The two percolation patterns have been used by Sasaki (1992). Each stratified paddy field model was constructed in an iron box (30 × 50 × 70 cm), filled with three layers of soil. The box has several holes on its side walls. The plow layer was 0 to 10 cm deep, with dry density in puddling condition of 1.04 g cm^{-3}. The plowsole was 10 to 20 cm deep with dry bulk densities of 1.23 and 0.89 g cm^{-3} at depths of 10 to 12.5 cm and 12.5 to 20 cm, respectively. The subsoil was 20 to 62.5 cm deep. It had dry bulk densities of 0.89 and 1.40 g cm^{-3} at depths of 20 to 27.5 cm and 27.5 to 62.5 cm, respectively. These layers were formed by compaction. The ground water levels of the open and closed system percolation models were controlled at 57.5 cm and 12.5-20 cm depth, respectively. In the closed system percolation models, the holes on the side walls of iron boxes were blocked to prevent the penetration of air. In the open system percolation models, these holes remained open at the lower part of the plowsole and the upper part of the subsoil to enable aeration of these layers. After those two types of models were prepared, fifteen paddy seedlings (plant length and leaf stage were about 18 cm and about 5 leaves, respectively), named ‘*Oryza sativa* L., Tsugaru Roman’ were transplanted as per Sasaki et al., (2016a) and Fan et al., (2018b). The paddy seedlings were transplanted in 10 cm intervals. The fertilizer used consisted of 2 g of N, 2 g of P_2O_5 and 2 g of K_2O for each model. This fertilizer was mixed with the entire plow layer before transplanting. During the cultivation period, a water ponding condition was adopted and there was no mid-summer drainage. Paddy seedling transplants and harvesting was conducted at the end of May and the end of September, respectively. Our experiment was conducted in a greenhouse on the university campus.

Measuring Method

Examination of rice plants such as plant length, leaf stage, the number of stems and panicles, the weight of straw, the number of brown rice and its weight was done using the Iwate Agricultural Experimental Station standard methods (1981). The quantitative analysis of Cu concentrations in rice grains, stems and leaves, roots and soils extracted by HCl solution was measured using atomic absorption spectroscopy (AAS) (MAFF, 1979). Other measurements were conducted with the standard methods used in Japan. The Oxidation-Reduction Potential (ORP) meter (Central Kagaku Co., Ltd., model UC-203), was used for measuring oxidation-reduction potential (Eh). An ORP sensor was set in each soil layer.

RESULTS AND DISCUSSION

Oxidation-reduction Potential (Eh)

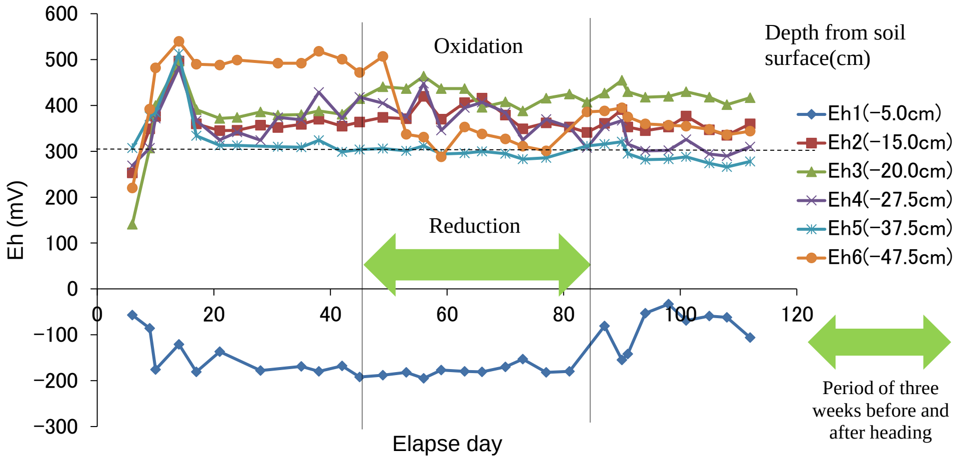

Fig. 1 The Eh of the open system percolation model (O-250)

The trend of Eh in each soil layer showed similar changes from transplanting to harvest among O-100, O-250 and O-500, and among C-100, C-250 and C-500. Figure 1 presents the Eh of the open system percolation model (O-250) and Figure 2 presents the Eh of the closed system percolation model (C-250). The oxidized layer in a paddy field is defined as the layer in which the Eh value is greater than +300 mV (Yamane, 1982). In the open system percolation model (O-250), the Eh of the plow layer became a reduced layer (less than 0 mV) during cultivation of paddy rice. The Eh of the plowsole and the subsoil became oxidized layers (> +300 mV). On the other hand, in the closed system percolation model (C-250), the Eh of all layers gradually declined and became reduced layers after transplanting. In all open system percolation models (O-100, O-250 and O-500), the plowsoles became oxidized layers during the cultivation period. In contrast, all layers of the closed system percolation models (C-100, C-250 and C-500) became reduced layers during the cultivation period. The absorption of Cu and Cd by rice plants was influenced by the redox potential of the soil

(Matsunaka, 2014). Based on these results, we anticipated adverse effects of soluble Cu and Cd on rice in the open system percolation models. The variation of Eh values in each layer was attributed to non-uniform contact between the Eh sensor and soil particles.

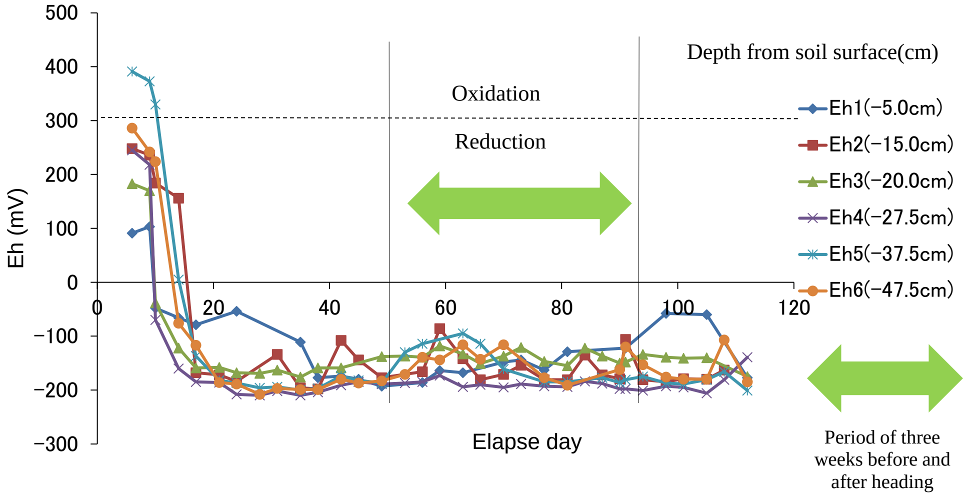

Fig. 2 The Eh of the closed system percolation model (C-250)

Copper Concentrations in Rice Plants

The results of Cu concentrations in rice plants are listed in Table 2.

Rice grains: Cu concentrations in rice grains (n = 7) ranged from 2.5 to 5.4 mg kg^{-1}. The Cu concentrations based on different percolation patterns were O-100 > C-100, O-250 > C-250 and O-500 > C-500. Any increase in Cu concentrations in the rice grains was not recognized despite increased Cu concentrations in soil. The range of Cu concentrations in our brown rice approximated to values indicated by Fan et al. (2018a, b) and Asami (2010). However, our figures were slightly higher than the 2.7 mg kg^{-1} in the Japanese rice ingredient table (MEXT, 2019). Unfortunately, there is an absence of studies applying the 'percolation pattern' concept other than Fan et al. (2018a, b) and Paul et al. (2011b). In our study, Cu concentrations in brown rice were significantly higher (at the 5% level) in the open system percolation models, than those in the closed system percolation models. A significant difference was also observed in brown rice Cu concentrations in Paul et al. (2011b) and Fan et al. (2018a, b). Moreover, Paul et al. (2011b) found that Cu concentrations in brown rice ranged from 2.5 to 4.2 mg kg^{-1} despite low Cu concentrations in the soils (12 mg Cu kg^{-1}). This result suggests that the range of Cu concentrations in brown rice with the difference in Cu concentrations in the lower layer, which ranged from 12 to 500 mg kg^{-1}, was 2.5 to 5 mg kg^{-1}, evidence of the impact of the percolation pattern. One reason for this may be that the immobilization effect is lower than that of Cd as pointed out by Kanamori et al. (2019). Based on these results, it was inferred that the variation of Cu concentrations in brown rice was due to the difference between percolation systems of the stratified paddy fields under combined pollution conditions.

Stems and leaves: The Cu concentrations in the stems and leaves ranged from 1.9 to 4.4 mg kg^{-1}, and there was a significant difference (at the 5% level) between the concentrations in the open system percolation models and the closed system percolation models, where the values of the former were higher than the latter. Fan et al. (2018a, b) and Paul et al. (2011b) also observed a similar concentration difference due to percolation systems. However, Fan et al. (2018a, b), did not observe significant differences in Cu concentrations of 70 and 100 mg kg^{-1} based on the type of percolation system. This was presumably because of the difference in soil type. This indicates that there may be no significant difference in Cu concentrations in the stems and leaves when soil Cu concentrations in the lower layer are less than 250 mg kg^{-1}. However, when soil Cu concentrations are greater than 250 mg kg^{-1}, the difference in the percolation system may affect the Cu concentrations in the stems and leaves.

Roots: The Cu concentrations in roots (n = 5) ranged from 12.7 to 18.6 mg kg^{-1}. The difference in the soil Cu concentrations in this study was 400 mg kg^{-1}. However, the difference in root Cu concentrations was approximately 6 mg kg^{-1}. The Cu concentrations in the roots observed by Fan et al. (2018a, b) also ranged from 13.7 to 20.3 mg kg^{-1}, and they could not identify whether this difference was due to the type of percolation system. However, there was no significant difference in Cu concentrations in the roots, providing clarity on the extent to which the type of percolation pattern influences brown rice, and the stems and leaves. Based on these results, it is presumed that there is a difference in Cu concentrations due to the type of percolation patterns in the transport mechanism of Cu in the above-ground biomass of rice plants.

The Cu concentrations in rice plants among the six models were in the order of roots > rice grains > stems and leaves on average, and the ratio was 5.7: 1.3: 1. This ranking was similar to that of the paddy rice indicated by Shibuya (1979) and Paul et al. (2011b), and that of the soybeans indicated by Li et al. (2017). This result is considered to be a result of the Cu transport mechanism in the rice plant. It is surmised that Cu concentrations in rice plants tend to be maintained within a certain range, similar to Zn concentrations (Shibuya et al., 1979), despite an increase in soil Cu concentrations.

The results indicated that there was a significant difference in Cu concentrations in rice grains, stems and leaves due to the type of percolation patterns. In contrast, such difference was not discernible for Cu concentrations in the roots even if the percolation pattern was different.

Table 2 Cu concentrations in rice grains, stems, leaves and roots in the plow layer

Model	Rice grains n = 7	Roots of plow layer n = 5	Stems and leaves n = 5
O-100	4.77 ± 0.50^{a}	16.19 ± 1.19^{ab}	3.38 ± 0.31^{b}
C-100	2.99 ± 0.34^{c}	17.82 ± 3.12^{a}	1.90 ± 0.10^{cd}
O-250	5.40 ± 0.64^{a}	18.56 ± 1.28^{a}	4.35 ± 0.66^{a}
C-250	2.94 ± 0.20^{c}	15.06 ± 2.89^{ab}	1.89 ± 0.18^{cd}
O-500	3.72 ± 0.30^{b}	15.58 ± 1.42^{ab}	2.42 ± 0.52^{c}
C-500	2.53 ± 0.20^{c}	12.72 ± 0.85^{b}	1.53 ± 0.41^{d}

Note: Tukey-Kramer test was performed at the 5% level; letter indicates significant difference. The numerical value of ± shows standard deviation. Unit: mg kg^{-1}

Cadmium Concentrations in Rice Plants

The results of Cd concentrations in rice plants are listed in Table 3.

Rice grains: Cd concentrations in rice grains (n = 7) ranged from 0.01 to 0.16 mg kg^{-1}. The Cd concentrations associated with the different percolation systems were O-100 > C-100, O-250 > C-250 and O-500 > C-500. This concentration range approximated the range from 0.03 to 0.17 mg kg^{-1} found by Sasaki et al. (2016a, b). In addition, the figures were higher in the open system percolation model (O-100 and O-250) than the 0.06 mg kg^{-1} in rice grains from Japan (MAFF, 2019). The increase in Cd concentrations in rice grains due to the increase in Cu concentrations was not observed in C-100, C-250 and C-500. However, these Cd concentrations in the open system percolation models were higher in the order of O-100> O-250> O-500. Until now there research on heavy metal concentrations caused by different percolation systems is lacking, with the exception of Sasaki et al. (2016a, b) and Paul et al. (2011b). In this study, Cd concentrations in brown rice were significantly higher (P value < 5%) in the open system percolation pattern than in closed system percolation pattern, except for 500 mg Cu kg^{-1}. Similarly, the Cd concentrations in rice grains indicated by Paul et al. (2011b) and Sasaki et al. (2016a, b) showed a significant difference due to the type of percolation patterns. In addition, models with an open system percolation pattern in the lower layer, were found to have Cd concentrations in rice grains that decreased in inverse proportion to the increase of Cu concentrations in the lower layer ranging from 100 to 500 mg kg^{-1}. One reason for this may be that an increase in the Cu concentrations suppresses the uptake of Cd.

Based on these results, the type of percolation pattern of stratified paddy field models under a combined pollution condition would create differences in Cd concentrations in brown rice. Additionally, when contaminated soil was in the closed system percolation pattern, the uptake of Cd was suppressed.

Stems and leaves: The Cd concentrations in the stems and leaves were range from 0.06 to 1.04 mg kg^{-1}, and a significant difference (at the 5% level) was confirmed in Cd concentrations between the open system percolation models and the closed system percolation models, with concentrations in the former being higher than the latter. The exception to this was for 500 mg kg^{-1}. Sasaki et al. (2016b) and Paul et al. (2011b) also observed similar results, where concentration differences were due to the type of percolation pattern. In these studies the soils of the models were only contaminated by Cd. This was presumably because the contaminated soils were in the open system percolation models, which were oxidized, so the solubility of Cd increased compared to those in the closed system percolation models (Arao et al., 2010).

These results indicate that the type of percolation patterns affects the Cd concentrations in the stems and leaves under combined contamination conditions when the Cu concentrations in the contaminated soils are lower than 500 mg kg^{-1}.

Roots: The Cd concentrations in the roots (n = 5) ranged from 1.00 to 1.97 mg kg^{-1}. The Cd concentrations in the contaminated soils in this study were approximately 1.72 mg kg^{-1}. However, the biggest difference in the Cd concentrations among the roots was 0.97 mg kg^{-1}. Regardless of the type of percolation model, which was under the same condition (1.81 mg kg^{-1}) as in Sasaki et al. (2016), no significant difference was observed.

Cd concentrations in rice plants were in the order of roots > stems and leaves > rice grains on average, and the ratio was 28:7:1. This ratio was slightly lower than 100:10:1, as found in Ito and Iimura (1976).

Table 3 Cd concentrations in rice grains, stems, leaves and roots in the plow layer

Model	Rice grains n = 7	Roots of plow layer n = 5	Stems and leaves n = 5
O-100	0.16 ± 0.05^{a}	1.60 ± 0.15^{ab}	1.04 ± 0.35^{a}
C-100	0.01 ± 0.00^{c}	1.06 ± 0.13^{c}	0.09 ± 0.02^{b}
O-250	0.08 ± 0.03^{b}	1.34 ± 0.23^{bc}	0.71 ± 0.24^{a}
C-250	0.01 ± 0.00^{c}	1.00 ± 0.24^{c}	0.10 ± 0.02^{b}
O-500	0.04 ± 0.01^{c}	1.97 ± 0.31^{a}	0.18 ± 0.05^{b}
C-500	0.01 ± 0.01^{c}	1.65 ± 0.18^{ab}	0.06 ± 0.02^{b}

Note: Tukey-Kramer test was performed at the 5% level; letter indicates significant difference. The numerical value of ± shows standard deviation. Unit: mg kg^{-1}

Growth and Yield of Rice Plants

The results of this experiment for the growth and yield of rice plants are shown in Tables 4 and 5, respectively.

Growth of rice plants: The average plant height (n=8) of each model was almost equal, and was between 99.6 to 101.7 cm (Table 4). The leafage of each model ranged from 14.3 to 15.0 leaves, showing very little difference among them. The total straw weight was 12.1 to 15.6 g $hill^{-1}$. No significant difference was observed in plant height, leafage and total straw weight regardless of the percolation system. Paul et al. (2011a) conducted stratified paddy field model experiments using Andosol and reported that the growth items above showed significantly higher figures in the closed system percolation models than the open system percolation models. Sasaki et al. (2016a, 2016b) and Fan et al. (2018a, b), used percolation systems that were clearly conditioned as per our experiments, but did not observe any significant difference (at the 5% level) in the growth of rice plants between the closed and open system percolation models. Shibuya et al. (1979) reported that Cu concentrations in the Cu polluted soil layer had an influence on the growth of rice plants. However, in our study, any influence of the Cu and Cd concentrations on the growth of rice plants was not noticeable, and may be a result of the soil dressing application.

Yield of rice plants: The weight of one panicle and the number of grains of brown rice per unit hill were between 2.3 and 2.8 g panicle^{-1} and between 597 and 724 grains hill^{-1}, respectively (Table 5). In addition, the percentage of ripening and the 1000 grain weight of brown rice were between 85.5 and 97.6% and between 20.2 and 22.2 g, respectively. No significant differences (at the 5% level) were found in any of the items of the models for the different percolation systems. Paul et al. (2011a) reported that yield components of the closed system percolation model were significantly higher than those of the open system percolation model although their experiment was conducted using a different soil type for Cd polluted soil layers. Sasaki et al. (2016a, b) and Fan et al. (2018a, b), used percolation systems that were clearly conditioned as per our experiments, and did not observe any significant difference (at the 5% level) in the yield components between closed and open system percolation models. Shibuya et al. (1979) reported that Cu concentrations had an influence on the number of panicles and the percentage of ripening. However, in our study, the mixed effect of the Cu and Cd concentrations on the growth of rice plants was unremarkable, which may be attributed to the application of soil dressing.

Transfer prevention of nutrients from the stems and leaves to grains (100 × weight of brown rice / weight of total straws) ranged from 82 to 120%. In the models of O-500 and C-500, rather low values of 83 and 82% were obtained, respectively, suggesting influence from the higher Cu concentration of 500 mg kg^{-1}. Sasaki et al. (2016b) did not recognize any transfer prevention in their experiments using Cd contaminated soils and controlling percolation patterns in the plow sole and subsoil. This suggests that Cu has much more influence on paddy rice growth and yield than Cd (Yamane et al., 1997).

Table 4 Parameters of rice plant growth

Model	Plant length (cm)	Leaf age (leaf)	Weight of dry straw (g hill^{-1})
O-100	101.66 ± 5.52^{ab}	14.88 ± 0.35^{ab}	12.61 ± 2.58^{a}
C-100	99.60 ± 1.97^{b}	14.25 ± 0.46^{c}	12.13 ± 3.59^{a}
O-250	100.48 ± 4.22^{ab}	14.63 ± 0.52^{abc}	13.15 ± 3.45^{a}
C-250	99.90 ± 6.03^{b}	14.38 ± 0.52^{bc}	13.51 ± 3.29^{a}
O-500	101.40 ± 1.54^{ab}	15.00 ± 0.00^{a}	14.45 ± 3.38^{a}
C-500	106.15 ± 3.50^{b}	15.00 ± 0.00^{a}	15.61 ± 4.41^{a}

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

Table 5 Parameters of rice plant yield

Model	Weight of one panicle （g）	Percentage of ripening （%）	Number of brown rice grains per unit hill （grains hill^{-1}）	Weight of 1000 rice grains （g）
O-100	2.27 ± 0.50^{b}	93.17 ± 1.30^{ab}	700.13 ± 143.73^{a}	20.23 ± 0.59^{b}
C-100	2.59 ± 0.18^{ab}	89.69 ± 4.93^{bc}	597.38 ± 134.56^{a}	20.24 ± 0.68^{b}
O-250	2.51 ± 0.36^{ab}	91.60 ± 2.74^{abc}	761.38 ± 217.21^{a}	21.01 ± 0.31^{ab}
C-250	2.71 ± 0.38^{ab}	85.50 ± 7.50^{c}	628.13 ± 127.81^{a}	20.44 ± 1.50^{b}
O-500	2.63 ± 0.27^{ab}	95.97 ± 4.73^{ab}	685.63 ± 133.67^{a}	22.21 ± 0.77^{a}
C-500	2.79 ± 0.27^{b}	97.57 ± 0.82^{a}	723.88 ± 136.85^{a}	22.18 ± 0.79^{a}

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

CONCLUSION

Using six types of Cu and Cd combined in polluted stratified paddy field models, we conducted an experiment to clarify the effects of percolation patterns in the sub-layer (plowsole and subsoil) on Cu and Cd concentrations in rice plants and growth and yield. The models had a 15 cm thick Cu and Cd polluted soil layer and a 12.5 cm thick non-polluted soil dressing with a Cu and Cd concentration of 3.7 mg kg^{-1} and 0.14 mg kg^{-1}, respectively. The six polluted soils were prepared by mixing each with a different concentration of Cu (100, 250 and 500 mg kg^{-1}) and a Cd concentration of 1.72 mg kg^{-1}.

The results of our experiment show that in open system percolation models the sub-layers became oxidized and in the closed system percolation models the sub-layers became reduced. Cu and Cd concentrations in the rice grains of the open system percolation models were significantly higher (at the 5% level) than those observed in the closed system percolation models. Cu and Cd concentrations in the stems and leaves also showed a significant difference between the models for different percolation systems. It was found that in the open system percolation models a higher Cu concentration reduced the Cd concentration in the brown rice, suggesting a coexistent effect of Cu and Cd. However, there was no significant difference in the growth and yield of rice plants between the two percolation systems.

Under the above conditions, the difference in percolation systems of the stratified paddy field models did little to affect the growth and yield of rice plants. However, it had an influence on Cu and Cd concentrations in the rice plants.

ACKNOWLEDGEMENTS

The authors appreciate the cooperation of Li Sontao, Fu Uzyou, Takahashi Hideki, Nakamura Mikako and Nara Ikumi who cooperated in carrying out this research. In addition, we would like to thank everyone concerned, such as farmers who most willingly provided us with the soil sample.

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