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Combined effect of rice genotypes and soil characteristics on iron plaque formation related to Pb uptake by rice in paddy soils

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Abstract
Purpose The formation of iron plaques and their ability to sequester heavy metals are influenced by soil properties and rice genotype. Lead accumulation in paddy soil is increasing, but in order to assess soil suitability for rice cultivation, a better understanding of the effects of iron plaques on Pb uptake by paddy rice is needed.
Materials and methods Two rice cultivars, Taikang 16 (TK16) and Taichung Sen 10 (TCS10), were grown in pots to assess lead (Pb) uptake. Three paddy soils (C, D, and N) sampled from potentially contaminated sites in central Taiwan were used for the experiment, with additional Pb spiking (0, 150, and 300 mg kg^{-1}).
Results and discussion Pb uptake by rice plants was positively correlated with soil Pb availability. However, Pb uptake by rice plants in soils D and N, even with higher Pb availability, was much less than in soil C. We attribute this to the large amount of iron plaques coating the root surfaces of plants in soils D and N. Moreover, TCS10, which is an indica rice sensitive to metal stress, took up more Pb than TK16, which is a japonica rice. Iron plaque formation on the roots of TK16 was superior to that on the roots of TCS10.
Conclusions Iron plaque formation that inhibits Pb uptake appears to be driven primarily by the soil properties and secondarily by the rice genotype. Nevertheless, the capacity of iron plaques to sequester Pb is limited by a high level of Pb contamination in the soil.

Keywords DCB extractable · Metal sequestration · Metal stress · Paddy rice · Root oxidation

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1 Introduction

Paddy rice (*Oryza sativa* L.) is a major staple crop in Asia; however, heavy metal accumulation in paddy soil poses a risk to the sustainability of rice crops and is of growing concern (Liu et al. 2007; Williams et al. 2009; Ok et al. 2011). Due to a plethora of activities based around lead, including lead (Pb) additives in pigments and gasoline, mining, and smelting of Pb ores, effluents from storage battery industries, deposition of Pb shot and sinkers, and application of sewage sludge on soil (Eick et al. 1999; Lofts et al. 2007), Pb is increasingly accumulating in soils and in some cases is approaching 2 % of dry soil material (Kabata-Pendias and Pendias 1984). Although plant uptake of Pb is usually low, Pb concentration in plants has been found to be elevated as Pb contamination in soils increases (Rooney et al. 1999; Kibria et al. 2006).

Lead uptake in rice plants was generally assumed to be related to Pb availability (Ok et al. 2011), which in turn depends on the total Pb concentration in soil and soil properties (e.g., pH, organic carbon, and cation exchangeable capacity). The availability of Pb in paddy soil is of increasing concern due to Pb toxicity from human consumption of rice (Chamannejadian et al. 2013). The uptake and accumulation of trace elements by plants are governed by both soil and plant factors, and they differ significantly with plant species. Patra

et al. (2004) emphasized that the effects of soil Pb on plant growth depend on Pb concentrations, Pb species (i.e., types of Pb salt), soil properties, and plant species. A number of studies have focused on the differences among rice cultivars in the uptake, translocation, and grain enrichment of Pb in rice plants (Liu et al. 2003; Chen et al. 2008; Liu et al. 2011). However, limited information is available on the combined effects of soil properties and rice genotypes on Pb uptake by rice plants in contaminated paddy soils.

Earlier studies emphasized the oxidation states of the rhizosphere of wetland plants as a protection against the entry of phytotoxins, such as Fe^{2+}, Mn^{2+}, and S^{2-} (Armstrong 1967; Taylor and Crowder 1983). The formation of an iron oxide coating on the root surface, called an iron plaque, was typically observed during oxidation of the rhizosphere (Crowder and St.-Cyr 1991; Wang and Peverly 1999). In a paddy field, the presence of iron plaques has been suggested to be a barrier to heavy metals in soil and to prevent heavy metal uptake by and subsequent toxicity to rice plants. Greipsson and Crowder (1992) showed that iron plaque formation on the rice root surface resulted in the amelioration of Cu and Ni toxicities to rice plants. Liu et al. (2004) reported that the As sequestrated by iron plaques (i.e., dithionite–citrate–bicarbonate [DCB] extractable As) on root surfaces was about 75–89 % of the total rice plant As and suggested that iron plaques may act as a barrier to prevent As absorption and translocation by rice plants. More recently, Lei et al. (2011) showed that As, Cd, and Pb accumulations in iron plaques on the root surface relative to rice plant total accumulation reached about 88, 44, and 34 %, respectively. It has been suggested that iron plaques on rice roots could provide a barrier to soil Pb stress and increase sequestration of Pb on the rice root surface (Liu et al. 2011), and this has been further supported by Zheng et al. (2012) who found that iron plaques sequestered much more Pb than Cd, Zn, and As. Iron plaque coatings on root surfaces appear to be a prohibitive factor for Pb uptake in paddy field rice plants. Lead accumulation in paddy soil is increasing, but in order to assess the soil suitability for rice cultivation, a better understanding of the effects of iron plaques on Pb uptake by paddy rice is needed. The objective of the present study is to examine the effects of both rice genotypes and soil characteristics on iron plaque formation in order to assess Pb uptake by rice plants from paddy soil.

2 Materials and methods

2.1 Soil characterization and Pb treatments

Paddy soils were drawn from three potentially contaminated sites (C, D, and N) in central Taiwan. The three soil sites are typical rural soils in major rice production areas where most paddy soils are classified as non-calcareous slate alluvial soils. About 50 kg of topsoil (0–15 cm) was randomly sampled by using augers from each site. Soil samples were air dried and passed through a 2-mm sieve for Pb-spiking treatments. Sand and clay contents were determined using the hydrometer method (Gee and Bauder 1986). Soil pH was measured using a 1:1 soil-to-water ratio (weight/volume) (McLean 1982). Organic matter (OM) of the soil was determined using the Walkley–Black method (Nelson and Sommers 1996). The calcium carbonate equivalent (CCE) was determined for the soil samples by acid neutralization (Loeppert and Suarez 1996). Amorphous iron oxide (Fe_{ox}) was extracted with Tamm's reagent (i.e., ammonium oxalate at pH 3.0) and measured according to McKeague and Day (1966). Free iron oxide (Fe_{DCB}) was determined with dithionite–citrate–bicarbonate extraction (Mehra and Jackson 1960). Total iron (Fe_{total}) was determined by using the aqua regia digestion method (Chen and Ma 2001).

In Taiwan, a regulation threshold of 300 mg kg^{-1} for soil Pb digested by aqua regia is used to state soil suspected of having Pb contamination. And in some routine surveys, Pb concentrations in rice grain exceeded the hazardous limit (0.2 mg kg^{-1}), while rice plants were grown in paddy soils with Pb concentration less 300 mg kg^{-1}. In order to mimic soils contaminated with a level of Pb near the regulation threshold, one subsample of each soil used was spiked with one of three levels of Pb (0, 150, and 300 mg kg^{-1}) in the form of $Pb(NO_3)_2$ for a total of three subsamples per soil. For stabilization, the Pb-spiked soils underwent a wetting–drying cycle that comprised a wetting period with soil moisture at field capacity for 2 weeks and an air-drying period for a further 2 weeks, both at room temperature (25 °C). After approximately 3 months of three wetting–drying cycles, Pb-spiked soil samples were ground, passed through a 2-mm sieve, and thoroughly mixed in preparation for measuring the soil Pb content and use in the pot experiment.

2.2 Total Pb and Pb availability in Pb-spiked soils

The total Pb content in each soil sample was determined using the aqua regia digestion method (Chen and Ma 2001). We used 0.1 N HCl extractable Pb as an index of Pb availability in the soils (Chen 1991; Yu et al. 2004). This method was recommended by the Environmental Protection Administration (EPA), Taiwan (EPA-TW 1991, 1994), for estimating the availability of soil heavy metals and is commonly used in Taiwan. The Pb concentrations of aqua regia digested and 0.1 N HCl extracted solutions were determined by flame atomic absorption spectrophotometry (FAAS) (iCE 3000; Thermo Scientific).

2.3 Plant materials and the pot experiment

We used two rice cultivars, Taikang 16 (TK16; a japonica rice) and Taichung Sen 10 (TCS10; an indica rice), which were both

bred in Taiwan. Lee et al. (2013) compared iron plaque formation of 28 rice cultivars; japonica rice TK16 and indica rice TCS10, representing high and low iron plaque formation capability, respectively, were selected from these 28 cultivars. Japonica rice has a genetic ability to form iron plaques that is superior to that of indica rice, and compared to indica rice, japonica rice appears to have lower accumulations of Pb (Liu et al. 2011). Thus, these two rice types represented different iron plaque formation capacities and ability to absorb Pb for this study.

We conducted a pot experiment to test the uptake and distribution of Pb in the TK16 and TCS10 rice plants, which were grown in Pb-spiked soils. Plastic pots (9-cm diameter×9 cm high) were each filled with 1 kg of Pb-spiked soils. Three rice seedlings, in the morphological development stage with three leaves completely emerged, were transplanted into each pot. Three pots were used as replicates for each Pb-spiked level. Basal fertilizers were added to each pot as 110 mg N (as $CO(NH_2)_2$), 22 mg P (as KH_2PO_4), and 75 mg K (as KH_2PO_4 and KCl) at the start of the pot experiment to ensure adequate nutrition for the growth of the rice seedlings. In order to mimic soil water conditions in a paddy field, each pot was submerged with 1 cm of water above the soil surface to keep the soil water content at saturation. Plants were then grown in a greenhouse under natural sunlight. The mean temperature throughout the experimental period was about 36 °C and within the range of 28–43 °C. The mean relative humidity was 67 % with a range of 50–75 %. Thirty-three days after transplanting, the rice plants in each pot were harvested and washed thoroughly with deionized water, and the root length and plant height were measured.

2.4 DCB extraction of iron plaque and determination of plant Pb concentrations

The harvested rice plants were separated into roots and shoots. The roots were dipped into a DCB solution (0.03 M sodium citrate+0.125 M sodium bicarbonate+0.015 g ml^{-1} sodium dithionite) to remove iron plaques from the root surfaces (Liu et al. 2004). The washed root samples and the shoot samples were then oven dried at 65 °C for 3 days, and their dry weights were measured. Next, the concentrations of Fe in the DCB extracts were measured by FAAS. The concentrations of Pb in the DCB extracts were measured by an inductively coupled plasma-mass spectrometer (ICP-MS) (7700×; Agilent). Following $HNO_3/HClO_4$ digestion, as adopted by Allen (1989), the Pb concentrations in the digests of the root and shoot samples were determined by FAAS and ICP-MS, respectively.

2.5 Statistical analysis

For the assessment of Pb availability influenced by soil characteristics, analysis of variance (ANOVA) was used to determine the significance of the effects of soil variety and Pb spiking on total (i.e., aqua regia digested) and available (i.e., 0.1 N HCl extractable) Pb. The effects of rice cultivar, soil variety, and Pb spiking on rice plant growth characteristics (i.e., root length, plant height, and root and shoot dry weights) and Pb concentrations in shoot, root, and plaque were also assessed with ANOVA by using the generalized linear model (GLM) (SAS Institute Inc. 1985). Linear relationships between plant Pb concentrations and 0.1 N HCl extractable Pb, given the soil variety and different rice cultivars, were determined by using a regression procedure of the GLM (SAS Institute Inc. 1985).

3 Results and discussion

3.1 Soil characteristics and Pb availability

The three soils (C, D, and N) were found to be acidic (Table 1). Soil C had a lower lime factor of CCE than did soils D and N; however, the pH of soil C was higher than those of soils D and N. In addition, soil N had a much higher clay content compared to soils C and D. The OM content and amorphous iron oxides (i.e., oxalate extractable Fe) in soils D and N were notably higher than in soil C. The content of free iron oxides (i.e., DCB extractable Fe) in soil C was similar to that in soil N but higher than that in soil D. Nevertheless, the content of total Fe (i.e., *aqua regia* digested Fe) in soil

Table 1 Selected properties of the soils (C, D, and N) used in this study

Soil	Texture class %	Sand	Clay	OM[a]	pH	CCE[b] g kg^{-1}	Fe_{ox}[c]	Fe_{DCB}[d]	Fe_{total}[e]	Fe_{ox}/Fe_{total} %
C	Sandy loam	61	10	1.18	6.27	13.05	4.52	11.25	22.82	19.81
D	Loam	46	16	2.68	5.56	13.08	6.61	7.25	22.39	29.52
N	Silty clayey loam	23	35	2.55	6.18	14.05	8.64	14.49	29.84	28.95

[a] Organic matter

[b] Calcium carbonate equivalent

[c] Oxalate extraction

[d] Dithionite–citrate–bicarbonate extraction

[e] *Aqua regia* digestion

N was higher than total Fe in soils C and D. The ratios of amorphous iron oxide to total iron content for soils D and N were similar to each other and higher than that for soil C. In Table 2, total Pb and available Pb appeared to increase with an increase in Pb-spiked levels. The recoveries of Pb spiked (i.e., the ratios of the difference in the measured total Pb concentrations between 0 and 150 or 0 and 300 mg kg^{-1} Pb-spiked samples to the spike level 150 or 300 mg kg^{-1}, respectively) were also used to investigate the contribution of Pb spiked on the measured total Pb concentrations by *aqua regia* digestion. In soil N, the recoveries of Pb spiked were higher than 90 %; but in soil D, those were about 80 %. For soil C, the recovery of Pb spiked was less than 80 % under Pb-spiked level of 150 mg kg^{-1}, but that was higher than 90 % under Pb-spiked level of 300 mg kg^{-1}. The variation of the recovery of Pb spiked for the soils would be referred to the influence of soil properties on *aqua regia* digestion (Chen and Ma 2001). According to the soil properties shown in, clay content of soil N was much higher than those of soil C and D, and organic matters of soils D and N were higher than twofold those of soil C. Thus, Pb-spiked levels of 150 and 300 mg kg^{-1} were reflected differently for the soils on the measured total Pb concentrations by *aqua regia* digestion. In addition, for soils D and N, the ratios of available Pb to total Pb at Pb-spiked levels of 150 and 300 mg kg^{-1} were higher than that at 0 mg kg^{-1}. For soil C, the ratios of available Pb to total Pb at Pb-spiked levels of 150 and 300 mg kg^{-1} were lower than that at 0 mg kg^{-1}. Thus, Pb spiking contributed a greater amount of available Pb in soils D and N than in soil C. These results support previous findings that the availability of Pb in soil is not only dependent on the total Pb content but is also governed by the soil properties (Brown et al. 2003; Si et al. 2006; Hashimoto et al. 2011). As shown in Table 1, the lower pH of soils D and N favors the spiked Pb in high availability. Moreover, the lower DCB extractable Fe in soil D may be the reason why Pb spikes resulted in relatively high Pb availability in soil D compared with soils C and N. Consequently, for each soil, the availability of Pb is positively related to the total Pb content; however, there may be significant differences in available Pb among different soils with the same levels of total Pb.

3.2 Rice plant growth as influenced by Pb treatments

Following 1-month growth in Pb-spiked soils, root length, plant height, and root and shoot dry weights of rice cultivars TK16 and TCS10 were measured for assessment of the influence of the Pb treatments. Significant differences in root length and dry weights of root and shoot were observed between the two cultivars (Table 3). The indica rice TCS10 presented more vigorous growth in terms of root length and dry weights of roots and shoots than did the japonica rice TK16. In addition, root length, plant height, and dry weight of shoots differed significantly among the three soils. Rice plants grown in soil N were more vigorous than those grown in soils C and D, but there were no significant differences in root length, plant height, and dry weights of roots and shoots among the Pb-spiked soils tested. This suggested that soil N influenced rice plant growth more than did soils C and D. In addition, the Pb-spiked levels of 150 and 300 mg kg^{-1} did not approach the level indicating Pb toxicity. These results are in agreement with those of Liu et al. (2011) who found no significant influence from soil treated with Pb at levels less than 500 mg kg^{-1} on rice growth.

Table 2 Total and available Pb concentrations (i.e., soil Pb measurements by *aqua regia* digestion and 0.1 N HCl extraction) in the soils (C, D, and N) with Pb spikes (0, 150, and 300 mg kg^{-1})

Soil	Pb spiked	Total Pb (*aqua regia* digested)	Available Pb (0.1 N HCl extracted)	Recovery of Pb spiked	Ratio of available Pb to total Pb
	mg kg^{-1}			%	
C	0	143.87 (0.49)[a]	80.13 (1.98)		55.69
	150	261.14 (6.36)	109.16 (1.31)	78.18	41.80
	300	436.28 (4.71)	191.57 (3.33)	97.47	43.91
D	0	229.18 (3.43)	137.80 (5.24)		60.12
	150	349.24 (1.76)	228.21 (20.5)	80.04	65.35
	300	476.76 (2.76)	420.29 (11.3)	82.53	88.16
N	0	297.08 (2.10)	127.63 (19.2)		42.96
	150	444.92 (0.89)	250.73 (6.72)	98.56	56.35
	300	574.09 (8.56)	313.87 (12.6)	92.34	54.67
ANOVA for significance of					
Soil		(N > D > C)***	(D > N > C)***		
Pb spiked		(300>150>0)***	(300>150>0)***		

[a] Values in the parentheses are standard errors (n=3)

***p<0.001

Table 3 Effects of Pb (0, 150, and 300 mg kg^{-1}) spiked in the soils (C, D, and N) on plant growth characteristics (i.e., root length, plant height, and root and shoot dry weights) of the rice cultivars (TK16 and TCS10)

Cultivar	Soil	Pb spiked	Root length	Plant height	Dry weight	
					Root	Shoot
		mg kg^{-1}	cm		g pot^{-1}	
TK16	C	0	15.17 (1.48)[a]	70.16 (1.61)	0.0843 (0.0129)	0.7203 (0.0435)
		150	14.67 (1.76)	68.00 (2.36)	0.0607 (0.0084)	0.5932 (0.0256)
		300	15.90 (0.38)	72.17 (1.28)	0.0858 (0.0178)	0.7763 (0.0556)
	D	0	10.00 (0.58)	67.60 (2.20)	0.0888 (0.0035)	0.7091 (0.0547)
		150	11.10 (0.70)	70.37 (0.72)	0.1085 (0.0165)	0.8656 (0.0688)
		300	9.30 (0.78)	62.70 (0.79)	0.0851 (0.0175)	0.6291 (0.0064)
	N	0	13.00 (2.65)	78.83 (4.52)	0.0798 (0.0315)	1.2521 (0.1082)
		150	12.20 (1.47)	70.17 (3.72)	0.0467 (0.0086)	0.6946 (0.1136)
		300	15.67 (0.33)	83.17 (1.38)	0.1074 (0.0171)	1.0771 (0.0729)
TCS10	C	0	18.00 (0.58)	72.11 (1.80)	0.1127 (0.0137)	1.3513 (0.0506)
		150	14.67 (2.19)	72.43 (1.94)	0.1173 (0.0284)	1.2058 (0.2710)
		300	17.67 (1.67)	72.34 (2.14)	0.1451 (0.0339)	1.2275 (0.1878)
	D	0	16.63 (0.98)	68.84 (0.79)	0.0909 (0.0153)	1.4071 (0.0467)
		150	14.23 (2.15)	68.81 (0.61)	0.1028 (0.0009)	1.2698 (0.0763)
		300	12.07 (0.20)	65.31 (0.95)	0.1442 (0.0075)	1.4009 (0.0882)
	N	0	20.00 (0.76)	76.88 (1.18)	0.1835 (0.0225)	2.0043 (0.2670)
		150	20.00 (1.73)	78.88 (1.07)	0.1890 (0.0295)	2.0732 (0.2297)
		300	15.83 (2.62)	76.13 (1.28)	0.1213 (0.0154)	2.0100 (0.0632)
ANOVA for significance of						
Cultivar			(TCS10 > TK16)***	*ns*	(TCS10 > TK16)***	(TCS10 > TK16)***
Soil			(N > D and C > D)***	(N > C > D)***	*ns*	(N > C and N > D)***
Pb spiked			*ns*	*ns*	*ns*	*ns*

[a] Values in the parentheses are standard errors (n=3)

ns not significant

***p<0.001

3.3 Pb concentrations in rice plants and iron plaque formation for different rice genotypes

Lead concentrations in the root and shoot tissues of the TCS10 plants were significantly higher than in the TK16 plants (Table 4). Liu et al. (2011) also found that Pb concentrations in the root tissue and shoots of indica rice (e.g., TCS10) were much higher than those of japonica rice, such as TK16. Compared with japonica rice, an indica rice has been found to be a more sensitive genotype to metal stress and also showed a greater ability to accumulate metal (Hsu and Kao 2003; Kuo and Kao 2004). In addition, we found that the DCB extractable Fe concentrations from the TK16 roots were significantly higher than those from the TCS10 roots. These findings indicate that TK16 has a greater ability to form iron plaques on root surfaces compared to TCS10. On the other hand, the difference between TK16 and TCS10 in the pooled Pb concentrations of the root tissue and DCB extract was not significant. However, the ratios of the Pb concentration in root tissue to the pooled Pb concentration of root tissue and DCB extract were lower for TK16 than for TCS10, suggesting that the amount of Pb sequestered by the iron plaques of TK16 roots may be higher than the iron plaques of TCS10 roots. In previous studies (Liu et al. 2004; Lei et al. 2011; Zheng et al. 2012), iron plaque formation on rice roots was suggested to be a barrier to metal stress and to enhance sequestration of metal on root surfaces. In our study, TK16 produced more iron plaque formation than TCS10, which resulted in more Pb accumulating on root surfaces. In summary, Pb uptake in rice plants varies by genotype and is related to iron plaque formation.

There were significant differences in the Pb concentrations in the roots and shoots among the three soils (C, D, and N) (Table 4). The pooled Pb concentrations of root tissue and DCB extract for soil C were significantly higher than those for soils D and N. Lead concentrations in the root tissue were in the following order: soil N > soil C > soil D. However, Pb concentrations in the shoots of plants grown in soil N were

Table 4 Effects of Pb (0, 150, and 300 mg kg^{-1}) spiked in the soils (C, D, and N) on Pb concentrations in root and in shoot and plaque Fe of the rice cultivars (TK16 and TCS10)

Cultivar	Soil	Pb in root				Pb in shoot	Fe in DCB ext.
		Pb spiked	Tissue + DCB ext.	Tissue	Tissue/(tissue+ DCB ext.)		
		mg kg^{-1}			%	mg kg^{-1}	g kg^{-1}
TK16	C	0	208.6 (34.5)[a]	57.7 (21.2)	27.66	1.13 (0.32)	74.2 (54.4)
		150	560.0 (52.7)	173.6 (10.3)	31.00	10.13 (0.72)	131.2 (20.1)
		300	718.4 (53.0)	481.9 (43.0)	67.08	19.29 (0.97)	93.3 (18.3)
	D	0	117.3 (7.6)	76.6 (8.6)	65.30	3.41 (0.98)	395.8 (34.7)
		150	147.8 (16.6)	114.4 (11.4)	77.40	5.55 (0.86)	293.4 (23.6)
		300	239.2 (8.8)	183.7 (7.5)	76.80	15.15 (3.37)	341.0 (24.8)
	N	0	98.7 (9.6)	56.8 (14.0)	57.55	2.42 (0.99)	235.7 (31.0)
		150	141.4 (26.4)	77.7 (5.6)	54.95	5.36 (0.98)	369.8 (3.7)
		300	261.2 (21.7)	230.0 (11.4)	88.06	8.72 (0.86)	265.9 (24.0)
TCS10	C	0	231.3 (36.6)	116.7 (17.1)	50.45	1.86 (0.04)	133.3 (19.1)
		150	628.6 (44.0)	316.7 (16.1)	50.38	7.78 (1.06)	106.2 (6.0)
		300	935.0 (78.2)	617.8 (62.2)	66.08	32.04 (2.60)	75.9 (3.8)
	D	0	129.2 (0.03)	74.6 (6.7)	57.74	5.91 (1.78)	282.4 (93.2)
		150	111.2 (17.7)	90.1 (8.0)	81.03	9.16 (1.27)	333.9 (32.0)
		300	316.3 (36.2)	220.1 (13.4)	69.59	21.91 (2.99)	279.0 (28.6)
	N	0	116.4 (19.3)	95.9 (9.0)	82.39	2.92 (1.43)	178.4 (69.9)
		150	200.7 (10.0)	168.9 (7.2)	84.16	6.84 (2.67)	148.3 (0.1)
		300	188.7 (4.8)	180.3 (4.6)	95.99	7.26 (1.58)	251.6 (1.3)
ANOVA for significance of							
Cultivar			*ns*	(TCS10 > TK16)*		(TK10 > TCS16)**	(TK16 > TCS10)**
Soil			(C > N and C > D)***	(N > C > D)***		(C > N and D > N)***	(D > N > C)***
Pb spiked			(300>150>0)***	(300>150 and 300>0)***		(300>150>0)***	*ns*

[a] Values in the parentheses are standard errors (n=3)

ns not significant

***p<0.001; ** p<0.01; * p<0.05

significantly lower than those in soils C and D. A significant difference in DCB extractable Fe among soils C, D, and N was also observed in the following order: soil D > soil N > soil C, indicating that characteristics of soils D and N were more favorable to the formation of iron plaque than those in soil C.

Spiking the soil with Pb had significant effects on the uptake and distribution of Pb in the rice plants (Table 4). The pooled Pb concentrations of root tissue and DCB extract increased with increasing Pb-spiked levels. Lead concentrations in root tissue at a Pb spike of 300 mg kg^{-1} were higher than at spiked levels of 0 and 150 mg kg^{-1}. Lead concentrations in shoots also significantly increased in correlation with an increase in Pb spiking. Thus, Pb concentrations in root and shoots increase by increasing soil Pb levels, depending on rice genotype and soil characteristics. Nevertheless, no significant difference in DCB extractable Fe was found among the three Pb-spiked levels, indicating that Pb spiking to 300 mg kg^{-1} did not inhibit the oxidation ability of rice roots and thus did not change the potential for iron plaque formation.

3.4 Relationship between rice Pb uptake and soil Pb availability

According to the results in Tables 2 and 4, one could find that Pb concentrations in plant were positively correlated to soil available P (i.e., 0.1 N HCl extracted Pb). And, rice cultivar TCS10 presented significantly more vigorous growth into dry mass of root and shoot than did rice cultivar TK16 (Table 3). The difference in biomass yields between TK16 and TCS10 thus was taken into account for evaluation of Pb uptake by rice plant. The scatter plots of Pb uptakes (i.e., multiplying Pb concentration with dry weight) in root and shoot versus 0.1 N HCl extractable Pb, which were specified for rice cultivars TK16 and TCS10 grown in soils C, D, and N, were shown in Figs. 1 and 2. The Pb uptakes in root and shoot all were increased with increasing 0.1 N HCl extractable Pb concentrations. This revealed that for each soil, Pb uptake by rice plants was positively correlated with Pb availability in the soil. However, the positive correlations between plant Pb

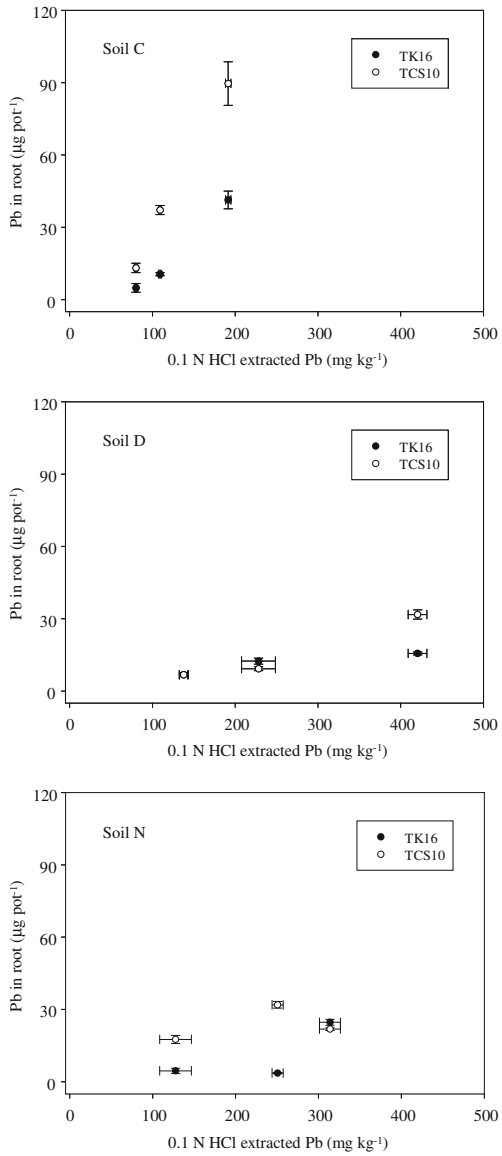

Fig. 1 Pb uptakes in root correlated with 0.1 N HCl extracted Pb for rice cultivars TK16 and TCS10 which were grown in soils C, D, and N, respectively. *Vertical* and *horizontal bars* denote standard error (n=3)

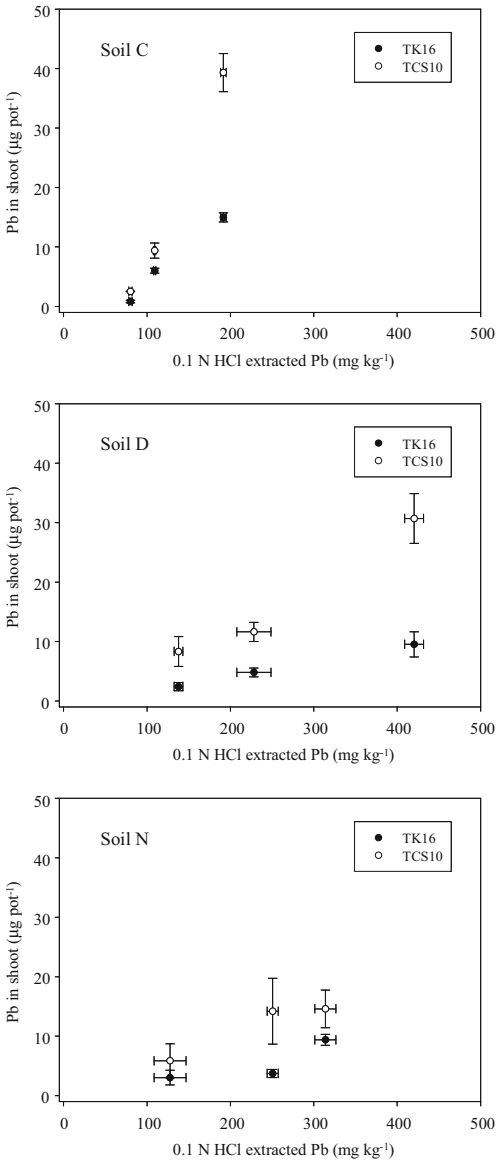

Fig. 2 Pb uptakes in shoot correlated with 0.1 N HCl extracted Pb for rice cultivars TK16 and TCS10 which were grown in soils C, D, and N, respectively. *Vertical* and *horizontal bars* denote standard error (n=3)

concentrations and 0.1 N HCl extractable Pb for soil C were much higher than those for soils D and N; even with higher Pb availability, Pb uptake by rice plants in soils D and N was much less than in soil C. The less Pb uptake by rice plants in soils D and N than in soil C could be attributed to the larger amounts of iron plaque deposition on root surfaces when rice plants were grown in soils D and N (Table 4). In a paddy field, higher OM and amorphous iron would promote iron plaque formation on root surfaces (Syu et al. 2013), and this was noted on the root surfaces of plants in soils D and N. Higher organic matter content will enhance more reductive dissolution of Fe oxides under flooding conditions to increase ferrous ion (Fe^{2+}) concentrations in soil solution. The amount of iron plaque formed on rice roots is primarily dependent on dissolved Fe^{2+} concentrations in soil pore water (Chen et al. 1980). The dissolved Fe as the form of Fe^{2+} can move into rhizosphere and then be oxidized on rice root surfaces to form plaques (Xu et al. 2008). Compared with soil C, soils D and N had relatively high OM and amorphous iron contents (Table 1). This revealed that there would be more dissolved Fe moving into rhizospheres in soils D and N. Therefore, the significantly more iron plaques on root surface by DCB extraction were found in soils D and N than in soil C.

In addition, some notable differences in Pb uptake between rice cultivars TK16 and TCS10 were found in Figs. 1 and 2. The Pb uptakes of TCS10 were much higher than those of TK16, which were corresponding to relatively high 0.1 N HCl extracted Pb concentrations (Figs. 1 and 2). This suggested that the differences in Pb uptake between TK16 and

TCS10 were not only related to their iron plaque formation abilities (Table 4) but also promoted by their biomass yields (Table 3). Compared with Pb uptakes in root (Fig. 1), Pb uptakes in shoot being higher by TCS10 than by TK16 (Fig. 2) were showed more significantly. And, the differences in Pb uptake between TK16 and TCS10 were more pronounced in soils C and D than in soil N (Figs. 1 and 2). Therefore, Pb uptake by rice plant would be a conditional function of available Pb in soil simultaneously given rice genotypes and soil characteristics.

3.5 Pb uptake by rice plants in relation to iron plaque formation

As discussed above, Pb concentrations in rice plant in relation to iron plaque formation are influenced by rice genotype and soil properties. A rice cultivar that has a higher capacity of root oxidation to produce more iron plaque (Lee et al. 2013), such as TK16, will sequester more Pb on root surfaces rather than absorbing it into root tissue. As such, iron plaque formation could be used as an index for assessing the root oxidation intensity of paddy rice; however, iron plaque formation is also dependent on soil properties (e.g., pH, OM, and Fe content) (Syu et al. 2014). As shown in Table 4, the deviations in DCB extractable Fe among the three soils were greater ($p<0.001$) than those between the two rice cultivars ($p<0.05$). Thus, the contribution of iron plaque formation to the reduction of Pb uptake by rice plants is dominated principally by soil properties and secondarily by rice genotypes.

However, the sequestration of Pb by iron plaques is limited when high levels of Pb contamination are present. In our study, the ratios of Pb concentration in root tissue to the pooled concentration of Pb in root tissue and DCB extract were relatively high for soil samples with high Pb spiking (Table 4). Our results are in agreement with those of Liu et al. (2011) that iron plaques would gradually become saturated due to Pb sequestration. Moreover, iron plaques in soils D and N did not show higher Pb sequestration; nevertheless, the ratios of the Pb concentration in root tissue to the pooled concentration of Pb in root tissue and DCB extract were higher in soils D and N than in soil C (Table 4). Iron plaque deposition occurs not only on the root surfaces but also on soil solid surfaces near the root surface by root oxidation (Mendelssohn et al. 1995; Wang and Peverly 1999). In soils D and N, the OM and the ratios of amorphous iron oxide to total iron content for soils D and N were much higher than those for soil C (Table 1). In the case where characteristics of soils D and N promote a large amount of iron plaques deposited on soil solids in the rhizosphere, the soil available Pb would be prevented from reaching the rice root surfaces and from absorption by the plant. As a consequence, we found significantly low Pb concentrations in the DCB extract and in plant tissues (root and shoot) grown in soils D and N despite the Pb availability in these soils being higher than in soil C (Table 2).

4 Conclusions

We showed that indica rice (e.g., TCS10), which is considered to be a genotype that is more sensitive to metal stress, accumulated more Pb than japonica rice (e.g., TK 16). For the three soils studied, Pb uptake by rice plants was positively correlated with Pb availability in the soil. In soils D and N, due to the relatively high OM and amorphous iron contents, a large amount of iron plaques formed on the rice roots, which significantly reduced Pb absorption by the rice plants. In addition, iron plaque formation on the roots of TK16 was superior to formation on the roots of TCS10. Thus, Pb uptake by rice plants in relation to iron plaque formation is also influenced by the soil properties and rice genotypes. However, the sequestration of Pb by iron plaques on root surfaces will be limited by saturation levels of Pb contamination. If the oxidation intensity of the rice roots can influence a large area of the rhizosphere, iron plaques may be deposited more on soil solids than on root surfaces. In this case, Pb will be mainly sequestered by the plaques on soil solids in the rhizosphere.

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