



Full Length Article

Effects of Soil Cadmium Contamination on Grain Yield and Cadmium Accumulation in Different Plant Parts of Three Rice Genotypes

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Abstract

The zinc (Zn) - rich tailing from Zn mines often causes cadmium (Cd) poisoning in people living downstream. This study evaluated the effect of soil Cd contamination on grain yield and accumulation of Cd and Zn in different plant parts among three rice genotypes. The experiment was conducted to study the interaction effect of the three rice varieties namely KH CMU, KDML 105 and Sang Yod and the soil mixtures with different level of Zn tailings mine from Mae Sot District, Tak Province, Thailand, which designated M₀, M₁, M₂₀ and M₄₀. Increasing doses of the tailings increased the brown rice Cd and Zn in all three genotypes, while increasing the grain yield in Sang Yod, depressing it in KDML105 and having only a mild effect in KH CMU. The genotype KDML105 had the highest concentration of Cd and Zn in its brown rice. Higher application rate of the mine tailings increased both grain and straw yield in Sang Yod, while increased straw yield but decreased grain yield in KDML105, and decreased grain yield at the highest rate of tailings in KH CMU. Different response of rice genotypes to soil Cd with respect to yield and Cd accumulation in rice grain should be exploited by rice breeders to tailor more efficient rice genotypes for the areas of soil Cd contamination. © 2019 Friends Science Publishers

Keywords: Rice; Zinc mining industry; Ore tailings; Cadmium contamination; Grain zinc concentration

Introduction

Rice (*Oryza sativa* L.) grown on land down-stream from Zn mines has been reported to accumulate large amounts of Cd from water-soluble Cd contained in the mine waste; which leads to serious Cd toxicity in people who depend on this rice as their staple (Simmons *et al.*, 2005). Cadmium toxicity in people has focused attention on Cd contamination in rice soils and its accumulation in the grain. In Toyama prefecture of Japan, 1,500 ha of rice fields were declared contaminated with Cd from the stream and irrigation water (Yanagisawa, 1984). Records of excessive levels of Cd in the rice soils and grain in villages downstream from a Zn mine in Thailand (Simmons *et al.*, 2003, 2005) were followed by reports of prevalence of renal problems associated with Cd toxicity (Limpatanachote *et al.*, 2009). The older leaves of rice accumulated higher Cd than the younger, but the effect of Cd accumulation in plant parts was depended on absorption and translocation among different genotypes (McKenna *et al.*, 1993; Das *et al.*, 1997). On the other hand, Zn is rich in the Zn mine tailing which could lead to the phytotoxicity when plants accumulate in

excess of 300–1,000 mg Zn kg⁻¹ dry weight in their leaves (Chaney, 1993). The association between Cd and Zn accumulation in plant tissues was found that the Cd/Zn ratio of 1:100 in the contaminated soil showed a natural limit to Cd uptake due to Zn phytotoxicity (Chaney, 2015).

In addition, rice genotypes have also been reported to differ widely in their grain Cd (Sriprachote *et al.*, 2012a) and Zn (Saenchai *et al.*, 2012) concentration. The rice genotypes used in this experiment were Kam Hom CMU (KH CMU), Khao Dawk Mali 105 (KDML105) and Sang Yod Phattalung (Sang Yod). The KDML105 (12–17% amylose) is one of Thailand's non-glutinous mega-varieties that produces high quality and priced Thai jasmine rice, KH CMU (2–5% amylose), is a purple glutinous upland rice variety from the highlands and Sang Yod (16–18% amylose), is a special quality non-glutinous variety. Some variation in the concentration of Cd has been noted in Song Yod and KDML 105 which reported its accumulation only a fraction of the grain Cd as similar as in KDML105 (Sriprachote *et al.*, 2012b), while no information has been made for KH CMU in both Cd and Zn accumulation in the contamination soil.

This study was designed to evaluate the effect of Zn mine tailing on yield and accumulation of Cd and Zn in different plant parts including grain of 3 selected traditional rice genotypes with different morpho-physiological traits and exceptional grain quality *e.g.*, amylose content, aroma flavor and gelatinization temperature, originated from different regions of Thailand.

Materials and Methods

Experiment Design and Soil Preparation

The experiment was conducted in pots during the rainy season (June–September) of 2014 at the Faculty of Agriculture, Chiang Mai University. The average temperature during cropping season was 26.3°C with 75.0% relative humidity (NMC, 2015). The treatments were factorial combinations of 3 rice genotypes and 4 rates of mine tailings, with three independent replications arranged in a completely randomized design. The soil used in the experiment was Sansai series with sandy loam texture, pH 6.35, with background concentration of 0.1 mg Cd kg⁻¹ and 2.2 mg Zn kg⁻¹. The soil was sun dried for 3 days before grinding and sieved to make the uniform particles of < 0.2 mm. The ore tailings were derived from a Zn mine in Mae Sot District, Tak Province, Thailand. The DTPA extractable analysis indicating that the ore tailing containing of 487 ± 6 mg Zn kg⁻¹, 199 ± 2 mg Cd kg⁻¹, 18.5 ± 1.5 mg Pb kg⁻¹, 1.63 ± 0.02 mg Cu kg⁻¹, 1.1 ± 0.03 mg Fe kg⁻¹ and 2.7 ± 0.03 mg Mn kg⁻¹.

The pots were 28 cm in diameter and 30 cm deep, each containing 10 kg of soil mixture with the ore tailings powder at the rate of 0, 2.5, 50 and 100 g kg⁻¹ soil to achieve the variation of Cd concentration at 0, 5, 100 and 200 mg Cd pot⁻¹. The soil mixture was incubated for 2 weeks before planting and analysis for DTPA-extractable Cd and Zn with Atomic Absorption Spectrophotometer (Perkin Elmer 3110, Germany) following the dry-ashing with the method of Lindsey and Norvell (1978). The treatments, designated soil mixture with Cd and Zn containing at 0.1 mg Cd kg⁻¹ soil and 2.2 mg Zn kg⁻¹ soil (M₀), 1.3 mg Cd kg⁻¹ soil and 17.0 mg Zn kg⁻¹ soil (M₁), 21.1 mg Cd kg⁻¹ soil and 279 mg Zn kg⁻¹ soil (M₂₀) and 37.5 mg Cd kg⁻¹ soil and 436 mg Zn kg⁻¹ soil (M₄₀) (Table 1).

Rice Genotypes and Sample Collection

Three improved rice genotypes used in this experiment were Kam Hom CMU (KH CMU), Khao Dawk Mali 105 (KDML105) and Sang Yod Phattalung (Sang Yod) which had the initial seed Cd and Zn concentration in brown rice at 0, 0.1 and 0.1 mg Cd kg⁻¹ dry weight and 40.0, 38.0 and 33.0 mg Zn kg⁻¹ dry weight, respectively. The rice seed was soaked in distilled water for 48 h at room temperature (25–30°C). Two week old seedlings were transplanted into the

prepared pots, at 5 plants per pot. The pots were maintained in flooded condition with 3–5 cm of water above the soil surface during growing period of 90–140 days (depending on genotypes) until maturity. Fertilizer applied was 15-15-15 (N-P₂O₅-K₂O) at 0.9 g per pot, split into 4 equal applications at 7 days after transplanting, tillering, booting and flowering stages.

Plant development was recorded in days from planting to flowering and days from flowering to maturity (Table 2). At maturity, all plants were manually harvested, and separated into roots, stems, flag leaf, remaining leaf blades, and grain. All samples were carefully washed with filtered water then deionized water to avoid contamination. The grain was sun-dried for 2–3 days to reach at 14% moisture content before separating into brown rice and husk. All samples were oven-dried at 75°C for 72 h and ground for chemical analysis.

Chemical Analysis

The grain samples were analyzed for amylose content by iodine reaction method (Juliano, 1971). The analysis of Cd, Zn, Pb, Cu, Fe and Mn in the Zn mine tailing and Cd and Zn concentration in different plant parts was conducted by Atomic Absorption Spectrophotometer (Perkin Elmer 3110, Germany) following dry-ashing of test solutions with method of Neggers and Lane (1995). Feeding stuffs (PTCH-FAO2-1401 Ca, Cu, Fe, Mg, Mn, K, Na, Zn and P) were used as certified reference material in each batch during all analyses.

Data Analysis

Data of Cd and Zn concentration and content in different plant parts were transformed to log₁₀ and tested for uniformity and homoscedasticity before analysis. Statistical analysis of all the data was performed by using the Statistic 9 (analytical software SX). Analysis of variance (ANOVA) was used to detect difference among treatments and least significant difference (LSD) at *P* < 0.05 was used to compare means.

Results

Plant Growth, Yield and Yield Components

The soil treatments at the higher rates of ore tailings, at M₂₀ and M₄₀, delayed development from flowering to maturity in Sang Yod by 6 days, but no effect on its development time from planting to flowering in KDML105 and KH CMU for their entire growing period (Table 2).

Grain and straw dry weight were affected by the soil treatments differently among the rice genotypes (Fig. 1). Increasing rate of ore tailings depressed grain yield in KDML105, but increasing in Sang Yod, while having only

Table 1: The DTPA-extractable Cd and Zn in the soil samples after applying the ore tailings in the mixture of M_0 , M_1 , M_{20} and M_{40} and incubated for two weeks

Treatments	Concentration (mg kg ⁻¹)	
	Cd	Zn
M_0	0.1 ± 0.0	2.2 ± 0.1
M_1	1.3 ± 0.0	17.0 ± 0.1
M_{20}	21.1 ± 0.7	279.0 ± 3.3
M_{40}	37.5 ± 1.2	436.0 ± 4.5

The values are mean ± SD (n = 3)

Table 2: Effect of soil Zn tailing on days taken from planting to flowering and days taken from flowering to maturity of three rice genotypes

Genotypes	Treatments	DPF (Day)	DFM (Day)
KH CMU	M_0	66	27
	M_1	66	27
	M_{20}	66	27
	M_{40}	66	27
KDML105	M_0	80	33
	M_1	80	33
	M_{20}	80	34
	M_{40}	80	34
Sang Yod	M_0	105	28
	M_1	105	28
	M_{20}	105	34
	M_{40}	105	34

Here DPF =days from planting to flowering; DFM =days from flowering to maturity; M_0 = 0.1 mg Cd and 2.2 mg Zn kg⁻¹ soil; M_1 = 1.3 mg Cd and 17.0 mg Zn kg⁻¹ soil; M_{20} = 21.1 mg Cd and 279 mg Zn kg⁻¹ soil; M_{40} = 37.5 mg Cd and 436 mg Zn kg⁻¹ soil

minor effect in KH CMU. Straw dry weight in both KDML105 and Sang Yod both increased with increasing rate of ore tailings, but not affected in KH CMU.

Increasing rate of ore tailings depressed the number of spikelet panicle⁻¹ and grain filling percentage in KDML105 but only the slight effects on these was found in yield components in the other two genotypes (Fig. 2). The number of tillers plant⁻¹, panicles plant⁻¹ and 1,000 grain weight were different among the rice genotypes, while showing little effect of the soil treatments, except that tillering was generally promoted by the addition of the ore tailings.

Plant Cd and Zn Content and Partitioning in Straw, Brown Rice and Husk

All three rice genotypes had higher Cd and Zn contents with increasing doses of tailings, but with different rates of increasing (Table 3). KH CMU accumulated the least amount of both elements at all levels of tailings, followed by KDML105 and Sang Yod. Most of the Cd and Zn in the plant were in the straw, while most contents of Cd and Zn was allocated to the husk only a fraction to brown rice.

Cd and Zn Concentration in Brown Rice and Other Plant Parts

Increasing doses of the ore tailings increased Cd and Zn concentration in brown rice and other plant parts, but with

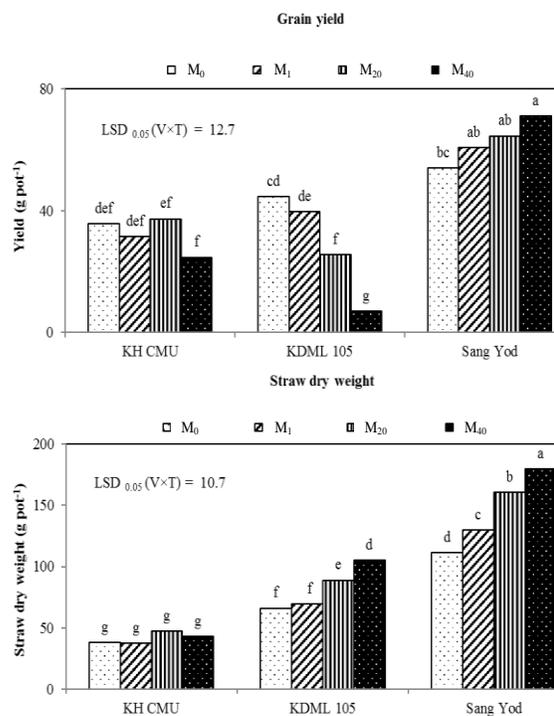


Fig. 1: Effect of soil Zn tailing on grain yield and straw dry weight of three rice genotypes. M_0 = 0.1 mg Cd and 2.2 mg Zn kg⁻¹ soil; M_1 = 1.3 mg Cd and 17.0 mg Zn kg⁻¹ soil; M_{20} = 21.1 mg Cd and 279 mg Zn kg⁻¹ soil; M_{40} = 37.5 mg Cd and 436 mg Zn kg⁻¹ soil. Different letters indicate significant differences at $P < 0.05$ (n = 3)

different extents in different genotypes and the plant parts (Table 4). In general, both Cd and Zn were much more concentrated in the stem and leaves than in brown rice and husk. Adding the ore tailings had smallest effect on Cd and Zn in brown rice, which rose from almost undetectable Cd and approximately 40 mg Zn kg⁻¹ in M_0 to 10–15 mg Cd kg⁻¹ and 50–70 mg Zn kg⁻¹ in M_{40} , compared with up to hundred-folds increases in Cd and up to 30-folds increases in Zn in the stem and leaves. A notable exception was the Cd concentration in the stem of KDML105 that remained relatively low even with the highest rate of tailings. Among the rice genotypes, the brown rice Cd was increased the most by the ore tailings in KDML105, followed by Sang Yod and the least in KH CMU. Brown rice Zn of Sang Yod, which was the lowest among the genotypes in M_0 , was also increased the least with increasing rates of tailings. The genotype KDML105 on the other hand had the highest Zn concentration in its brown rice with the higher rates of tailings. The husk Cd concentration was affected by addition of ore tailings in the same direction as the brown rice Cd, but largely to a less extent. The husk Zn concentration relative to brown rice Zn, however, was different among the genotypes at higher doses of tailings, being about the same in Sang Yod, significantly higher in the husk in KDML105 but higher in brown rice in KH CMU. Differential effects of the ore tailings on Cd and Zn

Table 3: Effect of soil Zn tailing on Cd and Zn content and allocation to the straw, brown rice and husk of three rice genotypes

Genotypes	Tailing treatments	Cd content				Zn content			
		mg pot ⁻¹	Percentage in			mg pot ⁻¹	Percentage in		
			Straw	Brown rice	Husk		Straw	Brown rice	Husk
KH CMU	M ₀	0.02	95.2	0	4.8	2.8	52.0	1.8	46.2
	M ₁	0.23	87.0	0.4	12.6	6.4	76.8	1.2	21.9
	M ₂₀	1.43	87.0	0.8	12.3	29.1	93.3	0.4	6.3
	M ₄₀	1.67	89.2	1.0	9.8	29.2	95.4	0.3	4.3
KDML105	M ₀	0.04	85.7	2.9	11.4	5.8	73.5	2.1	24.4
	M ₁	0.53	78.8	2.4	18.8	17.9	89.4	2.3	8.3
	M ₂₀	3.33	91.5	1.1	7.4	84.3	97.8	0.7	1.5
	M ₄₀	5.02	97.9	0.5	1.6	109.5	99.5	0.2	0.3
Sang Yod	M ₀	0.03	90.9	3.0	6.1	9.0	82.2	0.8	17
	M ₁	0.69	88.2	0.3	11.6	27.3	92.8	0.3	6.9
	M ₂₀	4.89	89.3	0.9	9.8	143.6	97.9	0.4	1.6
	M ₄₀	7.86	91.1	1.3	7.6	184.4	98.1	0.4	1.5
F-test (<i>p</i> < 0.05)		LSD _(0.05)				F-test (<i>p</i> < 0.05)		LSD _(0.05)	
Variety (V)	**	1.02				Variety (V)	**	1.05	
Treatment (T)	**	1.02				Treatment (T)	**	1.05	
Plant part (P)	**	1.02				Plant part (P)	**	1.05	
V × T	**	1.05				V × T	**	1.10	
V × P	**	1.05				V × P	**	1.10	
T × P	**	1.05				T × P	**	1.10	
V × T × P	**	1.10				V × T × P	**	1.17	

Data were log₁₀ transformed before subjected to analysis of variance, ** = significant at *P* < 0.01

M₀ = 0.1 mg Cd and 2.2 mg Zn kg⁻¹ soil; M₁ = 1.3 mg Cd and 17.0 mg Zn kg⁻¹ soil; M₂₀ = 21.1 mg Cd and 279 mg Zn kg⁻¹ soil; M₄₀ = 37.5 mg Cd and 436 mg Zn kg⁻¹ soil

Table 4: Effect of soil Zn tailing on Cd and Zn concentration in different plant parts; stem, leaf sheath, leaf blade, flag leaf, husk and brown rice among three rice genotypes

Genotypes	Tailings treatments	Cd concentration (mg kg ⁻¹)						Zn concentration (mg kg ⁻¹)					
		Stem	Leaf sheath	Leaf blade	Flag leaf	Brown rice	Husk	Stem	Leaf sheath	Leaf blade	Flag leaf	Brown rice	Husk
KHCMU	M ₀	0.6	0.2	0.7	0.3	0	0	75	28	26	21	43	8
	M ₁	4.6	6.0	5.7	7.0	1.1	0.2	264	118	72	42	55	15
	M ₂₀	22.0	18.8	40.1	47.3	5.6	1.8	589	448	783	637	59	17
	M ₄₀	29.4	30	45.9	53.8	8.1	3.5	676	827	472	470	63	19
KDML105	M ₀	0.6	0.3	0.4	0.6	0.1	0.1	148	43	28	25	39	14
	M ₁	8.5	6.8	3.9	5.6	3.3	1.4	543	160	95	87	49	44
	M ₂₀	7.4	50.4	52.6	34.7	12.3	7.1	1305	970	716	483	63	113
	M ₄₀	7.1	65.4	70.1	54.7	14.6	16	1397	1018	872	622	69	133
Sang yod	M ₀	0.4	0.1	0.4	0.3	0.1	0.1	144	35	33	22	35	6
	M ₁	10.1	4.7	1.0	0.6	1.6	0.2	491	138	34	29	38	8
	M ₂₀	29.6	35.8	19.4	12.6	9.1	3.9	1354	763	707	338	44	52
	M ₄₀	58.1	38.7	29.1	18.6	10.3	7.9	1763	984	463	212	49	55
F-test (<i>p</i> < 0.05)		LSD _(0.05)					F-test (<i>p</i> < 0.05)		LSD _(0.05)				
Variety (V)	**	1.05					Variety (V)	**	1.05				
Treatment (T)	**	1.05					Treatment (T)	**	1.05				
Plant part (P)	**	1.07					Plant part (P)	**	1.07				
V × T	**	1.10					V × T	**	1.10				
V × P	**	1.12					V × P	**	1.12				
T × P	**	1.15					T × P	**	1.15				
V × T × P	**	1.26					V × T × P	**	1.26				

Data were log₁₀ transformed before subjected to analysis of variance, ** = significant at *P* < 0.01

M₀ = 0.1 mg Cd and 2.2 mg Zn kg⁻¹ soil; M₁ = 1.3 mg Cd and 17.0 mg Zn kg⁻¹ soil; M₂₀ = 21.1 mg Cd and 279 mg Zn kg⁻¹ soil; M₄₀ = 37.5 mg Cd and 436 mg Zn kg⁻¹ soil

status of three rice genotypes were indicated by concentration of the elements in the flag leaf, which were indistinguishable among the genotypes in M₀ and M₁, but significantly lower in Sang Yod than KDML105 and KH CMU in M₂₀ and M₄₀.

Correlation between Cd and Zn Concentration among Different Plant Parts and Grain Yield

Positive correlation was found between Cd and Zn

concentration in brown rice and other plant parts in all genotypes (*P* < 0.01), except between Cd concentration in brown rice and the stem of KDML105 (Table 5). On the other hand, the correlation between grain yield and the concentration of Zn and Cd was different among the genotypes (Table 6). In KDML105, there was negative correlation between grain yield and Zn and Cd concentrations in all plant parts except between grain yield and the stem Cd. In Sang Yod, grain yield was positively correlated with Zn concentration in the stem, leaf sheath and

Table 5: Correlation between Cd and Zn concentration in brown rice and plant parts of three rice genotypes; stem, leaf sheath, leaf blade and flag leaf

Plant parts	Brown rice Cd concentration (mg kg ⁻¹)		
	KHCMU	KDML105	Sang Yod
Cd Stem	0.99**	0.56 ^{ns}	0.93**
Leaf Sheath	0.99**	0.99**	0.98**
Leaf blade	0.95**	0.97**	0.97**
Flag leaf	0.96**	0.95**	0.96**
	Brown rice Zn concentration (mg kg ⁻¹)		
Zn Stem	0.94**	0.98**	0.97**
Leaf Sheath	0.83**	0.96**	0.94**
Leaf blade	0.71**	0.96**	0.74**
Flag leaf	0.76**	0.97**	0.71**

ns = not significant, ** = significant at $P < 0.01$

Table 6: Correlation between Cd and Zn concentration in different plant parts and grain yield of three rice genotypes; stem, leaf sheath, leaf blade and flag leaf

Plant parts	Yield (g pot ⁻¹)		
	KHCMU	KDML105	Sang Yod
Cd Stem	-0.42 ^{ns}	-0.42 ^{ns}	0.58*
Leaf sheath	-0.49 ^{ns}	-0.88**	0.44 ^{ns}
Leaf blade	-0.31 ^{ns}	-0.90**	0.54 ^{ns}
Flag leaf	-0.32 ^{ns}	-0.88**	0.57 ^{ns}
Brown rice	-0.44 ^{ns}	-0.88**	0.55 ^{ns}
	Yield (g pot ⁻¹)		
Zn Stem	-0.38 ^{ns}	-0.80**	0.61*
Leaf sheath	-0.49 ^{ns}	-0.83**	0.60*
Leaf blade	0.05 ^{ns}	-0.87**	0.43 ^{ns}
Flag leaf	-0.03 ^{ns}	-0.86**	0.42 ^{ns}
Brown rice	-0.43 ^{ns}	-0.89**	0.64*

ns = not significant, * = significant at $P < 0.05$, ** = significant at $P < 0.01$

brown rice, but not in the leaf blade and flag leaf, while positive correlation with grain yields was found only with Cd concentration in the stem. No correlation was observed between grain yield and Cd and Zn concentration in all plant parts of KH CMU.

Discussion

This study has shown that all rice genotypes were affected differently by soil contamination with tailings from Zn mining on grain yield and the concentration of Cd and Zn in the rice grain. Genotypic variation in the rice grain Cd concentration previously reported (Sriprachote *et al.*, 2012b), has been confirmed here but with much smaller difference between KDML105 and Sang Yod, suggesting a strong interaction between genotype and environment effects on Cd accumulation in the rice grain. This study has also shown how rice genotypes may differ in Cd accumulation and exclusion in their various tissues which may or may not be associated with plant growth and yield. Increasing rates of tailings also increased brown rice Zn concentration in all three rice genotypes, but only to levels that are considered nutritionally beneficial (*e.g.*, see Saenchai *et al.*, 2012), and confirming previous reports of insensitivity of rice grain Zn to the level of Zn present in the soil (Wissuwa *et al.*, 2008;

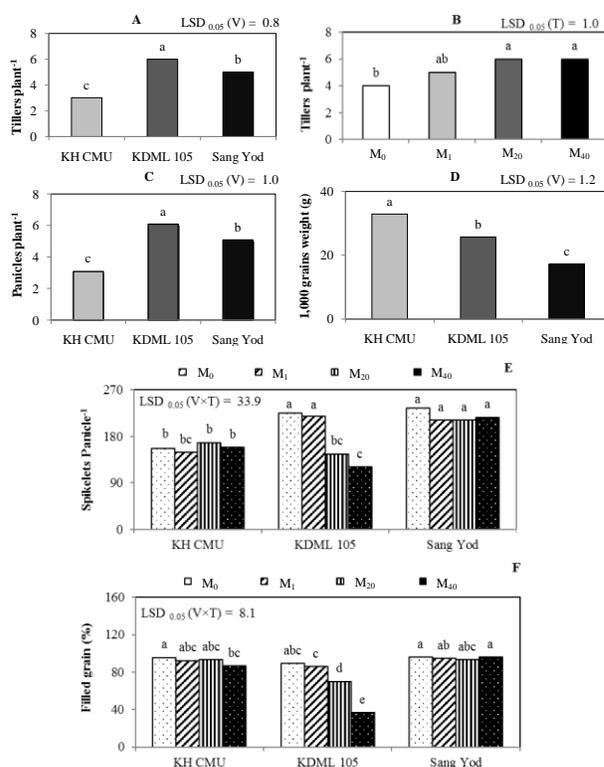


Fig. 2: Effect of soil Zn tailing on number of tiller plant⁻¹ (A, B), panicle plant⁻¹ (C), 1,000 grains weight (D), spikelet panicle⁻¹ (E) and percent filled grain (F) among three rice genotypes. M₀ = 0.1 mg Cd and 2.2 mg Zn kg⁻¹ soil; M₁ = 1.3 mg Cd and 17.0 mg Zn kg⁻¹ soil; M₂₀ = 21.1 mg Cd and 279 mg Zn kg⁻¹ soil; M₄₀ = 37.5 mg Cd and 436 mg Zn kg⁻¹ soil. Different letters indicate significant differences at $P < 0.05$

Phattarakul *et al.*, 2012). Differential yield responses to the tailings were illustrated by grain yield depression in the genotype KDML105, mediated through the number of spikelets/panicle and percentage of filled grain which positively affected on grain and straw dry weight in Sang Yod and relatively mild affected on KHCMU. Phytotoxic effects from both Cd and Zn are well established (Chaney, 1993; Das *et al.*, 1997), with a report of rice growth and yield depression by high Cd from Bangladesh (Kibria *et al.*, 2006). Phytotoxic effects of Cd ranged from reduction of chlorophyll levels, inhibition of photosynthesis (Kabata-Pendias and Pendias, 2001; Liphadzi and Kirkham, 2006), to interruption of respiration, cell division and cell elongation, and inhibition of RNA transcription and activity of a ribonuclease (Shah and Dubey, 1995; Toppi and Gabbrielli, 1999). Phytotoxicity of Zn was associated with depressed vegetative growth, including inhibition of root elongation (Wong and Bradshaw, 1982) and depression of photosynthesis as the result of interference by Zn with the uptake of iron and magnesium (Woolhouse, 1993; Sagardoy *et al.*, 2009). Thus, phytotoxicity from high concentration of Cd and Zn in soil mixture inhibited biochemical and

physiological mechanisms resulted in depression of grain yield, especially in KDML105, while the yield increase in the case of Sang Yod cannot not be explained which could be about the higher requirement of Cd and/or Zn in Sang Yod compared with the other varieties and/or the mechanism of internal tolerance.

No critical concentration for Cd toxicity in plants is available in the literature, in the case of Zn the range of 300–1,000 mg Zn kg⁻¹ dry weight in the leaves has been suggested (Chaney, 1993). In the present study, a possibility of genotypic variation in internal tolerance was suggested by similar levels of flag leaf Cd that were inversely associated with grain yield in KDML105 but showing no significant relationship in KH CMU. The higher Zn concentration in the stem of the genotype Sang Yod, on the other hand, could be indicative of either its greater tolerance or ability to keep excessive Zn in the stem and preventing it to concentrate in the leaves and grain. In barley, the genotype Tokak was reported as a Cd tolerant by its ability of heavy metal entering the cells (Ha *et al.*, 1999; Tiryakioğlu *et al.*, 2006). It was reported that the presence of Zn can lower Cd toxicity in wheat and maize by minimizing membrane damage (Wu *et al.*, 2003; Koleli *et al.*, 2004; Fahad *et al.*, 2015) offers a possible explanation to the apparent tolerance to very high Cd levels in the tissues of rice genotypes like Sang Yod and KHCMU. In view of widespread occurrence of potentially harmful levels of Cd and Zn in agricultural soil polluted by manufacturing industry as well as mining (Chaney, 1993) these also deserves further investigation.

The toxic effect of either Cd or Zn or both on the yield formation process in KDML 105 was indicated by significant negative correlation between its grain yield and Cd and Zn concentration in various tissues. In contrast, the other two genotypes exhibited no such inverse relationship between grain yield and tissue Cd and Zn. It remains to be clarified whether the limiting element was Cd or Zn or both. By its much lower Cd and Zn content, lower Cd concentration in its leaf sheath and leaf blades, and lower Zn concentration in the stem and leaves as well as its brown rice, the genotype KHCMU was the most effective in excluding these heavy metals. The sensitive KDML105, with its significantly higher content of Cd and Zn, suffered from excessive concentration of these elements and its dry weight was greatly depressed at higher doses of tailings. In contrast, the Cd and Zn concentration in the leaves of Sang Yod was kept lower in spite of the much higher contents of these elements, by dilution effect as its grain and straw dry weight that were increased with increasing doses of tailings.

This study has shown how rice genotypes may differ in their ability to exclude Cd and Zn from the plant and grain. KH CMU had the lowest plant Cd content and brown rice Cd concentration. Sang Yod, on the other hand, maintained lower brown rice Cd concentration than KDML105 because of Cd in the panicle was allocated to the

husk. The Cd and Zn concentration in brown rice in all genotypes increased with increasing doses of the ore tailings in concordance with the concentrations the leaves and stem, except for Cd in the stem of KDML105 which remained low regardless of the level of tailings. The higher Cd concentration in brown rice in KDML105 than Sang Yod found here is in agreement with a report of an evaluation of grain Cd in 42 Thai rice cultivars in farmers' fields contaminated by the tailings from a Zn mine in Thailand that found Sang Yod to be among the low Cd varieties and KDML105 among the high Cd varieties (Sriprachote *et al.*, 2012b). The present study found even lower Cd in the grain than Sang Yod in the genotype KHCMU. High Cd concentration in the rice grain is a cause for concern, with the maximum contamination limit for rice set at 0.4 mg kg⁻¹ (CAC, 2011). To understand the difference in homeostatic mechanisms for Cd accumulation in brown rice among genotypes and the genetic control, further studies should focus on mechanisms controlling brown rice Cd concentration through the partitioning into brown rice as in Sang Yod as well as minimizing its uptake as in KH CMU, in contrast to a mega-variety prone to accumulate more Cd in the grain like KDML105.

Conclusion

Results disclosed that rice genotypes behaved differently to the applied ore tailings from Zn mining, in both their grain yield and nutritional quality. The genotype KH CMU could be suggested for the further in depth physiological study on the effectiveness of Cd exclusion from the grain and other plant parts. However, further studies are needed to explore the mechanisms behind the different uptake and translocation of Cd to the different plant parts among rice genotypes.

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References

- Chaney, R.L., 2015. How does contamination of rice soils with Cd and Zn cause high incidence of human Cd disease in subsistence rice farmers. *Curr. Pollut. Rep.*, 1: 13–22
- Chaney, R.L., 1993. Zinc phytotoxicity. In: *Zinc in soils and plants*, pp: 135–150. Robson, A.D. (ed.). Kluwer Academic Publishers, Dordrecht, The Netherlands
- Codex Alimentarius Commission (CAC), 2011. Joint FAO/WHO food standards programme codex committee on contaminations in foods, fifth session. Available at: http://www.fao.org/tempref/codex/Meetings/CCCCF/CCCCF5/cf05_IN_F.pdf (Accessed: 5 March 2016)
- Das, P., S. Samantaray and G.R. Rout, 1997. Studies on cadmium toxicity in plants: A review. *Environ. Pollut.*, 98: 29–36

- Fahad, S., S. Hussain, S. Saud, S. Hassan, D. Shan, Y. Chen, N. Deng, F. Khan, C. Wu, W. Wu, F. Shah, B. Ullah, M. Yousaf, S. Ali and J. Huang, 2015. Grain cadmium and zinc concentrations in maize influenced by genotypic variations and zinc fertilization. *Clean-Soil Air Water*, 43: 1433–1440
- Ha, S.B., A.P. Smith, R. Howden, W.M. Dietrich, B. Bugg, M.J. O'Connell, P.B. Goldsbrough and C.S. Cobbett, 1999. Phytochelatin synthase genes from *Arabidopsis* and they east *Schizosaccharomyces pombe*. *Plant Cell*, 11: 1153–1163
- Juliano, B.O., 1971. A simplified assay for milled rice amylose. *Cer. Sci. Today*, 16: 334–340
- Kabata-Pendias, A. and H. Pendias, 2001. *Trace Elements in Soils and Plants*, 3rd edition, pp: 114–131. CRC Press LLC, New York, USA
- Kibria, M.G., K.T. Osman and M.J. Ahmed, 2006. Cadmium and lead uptake by rice (*Oryza sativa* L.) grown in three different textured soils. *Soil Environ.*, 25: 70–77
- Koleli, N., S. Eker and I. Cakmak, 2004. Effect of zinc fertilization on cadmium toxicity in durum and bread wheat grown in zinc-deficient soil. *Environ. Pollut.*, 131: 453–459
- Limpatanachote, P., W. Swaddiwudhipong, P. Mahasakpan and S. Kirintratun, 2009. Cadmium exposed population in Mae Sot District, Tak Province: 2. Prevalence of renal dysfunction in the adults. *Med. J. Med. Assoc. Thail.*, 92: 1345–1353
- Lindsey, W.L. and W.A. Norvell, 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Amer. J.*, 42: 421–428
- Liphadzi, M.S. and M.B. Kirkham, 2006. Physiological effects of heavy metals on plant growth and function. In: *Plant-Environment Interaction*, pp: 243–270. Huang, B. (ed.). CRC, Taylor & Francis, Boca Raton, Florida, USA
- McKenna, I.M., R.L. Chaney and F.M. Williams, 1993. The effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. *Environ. Pollut.*, 79: 113–120
- Negggers, Y.H. and R.H. Lane, 1995. Minerals, ch. 8. In: *Analyzing Food for Nutrition Labeling and Hazardous Contaminants*, p: 185. Jeon, I.J. and W.G. Ikins (Eds.). Marcel Dekker, New York, USA
- Northern Meteorological Center (NMC), 2015. Climate in Chiang Mai. Available at: <http://www.cmmet.tmd.go.th/index1.php> (Accessed: 10 January 2015)
- Phattarakul, N., B. Rerkasem, L.J. Li, L.H. Wu, C.Q. Zou, H. Ram, V.S. Sohu, B.S. Kang, H. Surek, M. Kalayci, A. Yazici, F.S. Zhang and I. Cakmak, 2012. Bio-fortification of rice grain with zinc through zinc fertilization in different countries. *Plant Soil*, 361: 131–141
- Saenchai, C., C. Prom-u-thai, S. Jamjod, B. Dell and B. Rerkasem, 2012. Genotypic variation in milling depression of iron and zinc concentration in rice grain. *Plant Soil*, 361: 271–278
- Sagardoy, R., F. Morales, A.F. López-Millán, A. Abadía and J. Abadía, 2009. Effects of zinc toxicity on sugar beet (*Beta vulgaris* L.) plants grown in hydroponics. *Plant Biol.*, 11: 339–350
- Shah, K. and R.S. Dubey, 1995. Effect of cadmium on RNA level as well as activity and molecular forms of ribonuclease in growing rice seedlings. *Plant Physiol. Biochem.*, 33: 577–584
- Simmons, R.W., P. Pongsakul, D. Saiyasitpanich and S. Klinphoklap, 2005. Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: Implications for public health. *Environ. Geochem. Health*, 27: 501–511
- Simmons, R.W., P. Pongsakul, R.L. Chaney, D. Saiyasitpanich, S. Klinphoklap and W. Nobuntou, 2003. The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean: Implications for human health. *Plant Soil*, 257: 163–170
- Sripachote, A., P. Kanyawongha, K. Ochiai and T. Matoh, 2012a. Current situation of cadmium-polluted paddy soil, rice and soybean in the Mae Sot District, Tak Province, Thailand. *J. Soil Sci. Plant Nutr.*, 58: 349–359
- Sripachote, A., P. Kanyawongha, G. Pantuwan, K. Ochiai and T. Matoh, 2012b. Evaluation of Thai rice cultivars with low-grain cadmium. *J. Soil Sci. Plant Nutr.*, 58: 568–572
- Tiryakioglu, M., M. Eker, F. Ozkutlu, S. Husted and I. Cakmak, 2006. Antioxidant defense stem and cadmium uptake in barley genotypes differing in cadmium tolerance. *J. Trace. Elem. Med. Biol.*, 20: 181–189
- Toppi, L.S.D. and R. Gabbrielli, 1999. Response to cadmium in higher plants. *Environ. Exp. Bot.*, 41: 105–130
- Wissuwa, M., A.M. Ismail and R.D. Graham, 2008. Rice grain zinc concentrations as affected by genotype, native soil–zinc availability, and zinc fertilization. *Plant Soil*, 306: 37–48
- Wong, M.H. and A.D. Bradshaw, 1982. A comparison of the toxicity of heavy metals, using root elongation of ryegrass (*Lolium perenne*). *New Phytol.*, 91: 255–261
- Woolhouse, H.W., 1993. Toxicity and tolerance in the responses of plants to metals. In: *Encyclopedia of Plant Physiology New Series*, pp: 245–300. Lange, O.L., P.S. Nobel and C.B. Osmond (Eds.). Springer-Verlag, Berlin, New York, USA
- Wu, F.B., G.P. Zhang and D. Peter, 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environ. Exp. Bot.*, 50: 67–78
- Yanagisawa, M., 1984. Heavy metal pollution and methods of restoration of polluted soil in the Jinzu River basin. *Bull. Toyam. Agric. Exp. Stn.*, 15: 1–110

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