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ORIGINAL PAPER

Cadmium (Cd) and zinc (Zn) accumulation by Thai rice varieties and health risk assessment in a Cd–Zn co-contaminated paddy field: Effect of soil amendments

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Abstract Zinc mining and smelting activities result in cadmium (Cd) and zinc (Zn) contamination in rice grains, causing deleterious impacts on human health and local economies. Here, we investigated the effects of soil amendments, including mixtures of dicalcium phosphate with cattle manure (T1) and leonardite (T2), on soil physicochemical properties as well as growth performance and accumulation of Cd and Zn among three commercial Thai rice varieties: Khao Dok Mali 105 (KDML105), Phitsanulok2 (PSL2) and RD3, grown in a Cd–Zn co-contaminated paddy field. Human health risk was assessed using the health risk index (HRI) and Daily Intake of Metal (DIM). Application of the amendments, particularly T1, decreased Cd and Zn bioavailability by 60% and 39%, respectively, increased biomass production in PSL2 and RD3 varieties, and substantially reduced Cd uptake in the KDML105 variety by 47%. While levels of Zn in whole plant tissues of all treatments did not exceed maximum levels of undesirable substances in fodder, Cd contents in grain of PSL2 and RD3 exceeded the maximum allowable concentration of 0.2 mg kg^{-1}. The HRI values for Cd of PSL2 and RD3 varieties were relatively high and are considered to pose a potential risk to human health. KDML105 in the T1 treatment had the lowest HRI value (0.05 ± 0.03), which was within acceptable limits. Our results suggest that Cd and Zn accumulation in rice and associated human health risks could be reduced by application of amendments to paddy soils, but the effectiveness depends on amendment types, rice varieties and soil physicochemical properties.

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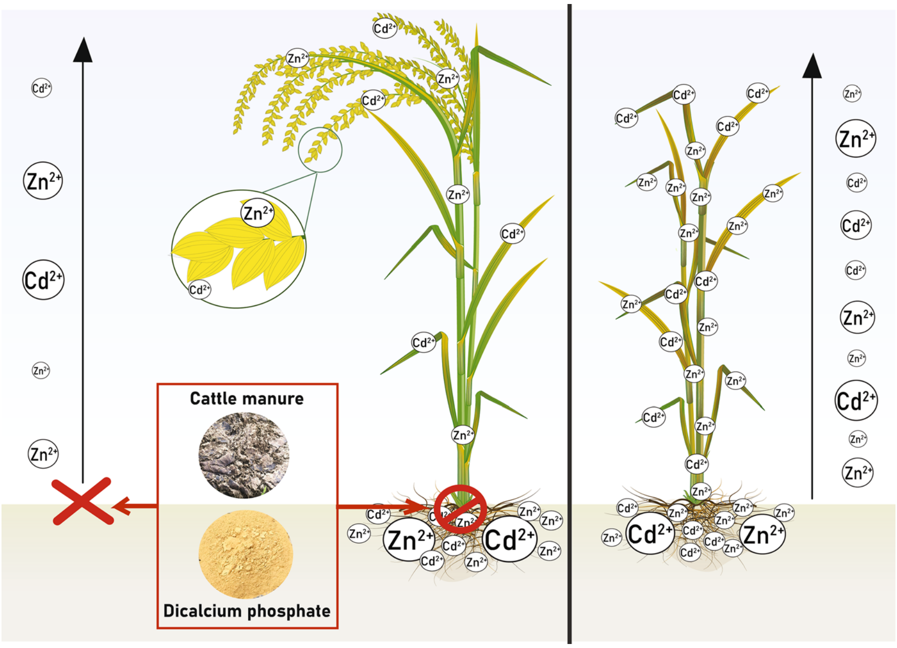
Graphic abstract



Keywords Cadmium · Zinc · Amendment application · Field experiment · Human risk index · Health

Introduction

Cadmium (Cd) and zinc (Zn) are widely distributed in strata and soil. While Zn is an essential micronutrient required for all organisms (Gupta et al. 2016; Swamy et al. 2016), Cd is one of the most toxic heavy metals with a slow elimination rate according to ATSDR rankings (Tophuria et al. 2017). Co-contamination of Cd and Zn in the environment as a consequence of mining and smelting activities has been reported in many rice-farming countries including Japan, China and Thailand (Chaney 2015; Zhang et al. 2012). The presence of high Cd and Zn concentrations in agricultural soils and irrigation water can markedly reduce growth and yield of rice plants by interfering with numerous plant physiological processes, and biochemical and genetic responses (Szopiński et al. 2019). In humans, daily intake of Cd via rice consumption has been shown to be a major cause of diseases such as itai-itai, nephrosis/nephritis, cardiovascular disease, cerebrovascular disease, osteoporosis and cancer. Chronic diseases associated with Cd exposure among those living in and around in rice fields have been reported with increasing frequency (Songprasert et al. 2015).

In Thailand, for example, Cd and Zn have been detected locally as a consequence of Zn mining operations in the northwest region. However, the trend of Cd and Zn accumulation in agricultural soils has declined approximately tenfold following the closure of Zn mining operation almost ten years ago (Nak-ai et al. 2018). As it stands, substantial Cd and Zn concentrations in such areas remain and can be transferred in the food chain, resulting possible health risks to consumers. To reduce risks to local communities, various campaigns and policies have been initiated by the Thai government. These include: (1) ceasing rice farming and other crop cultivation in affected locations; (2) have the government purchase contaminated edible products, particularly rice grains, and remove them from the market and consumption cycle, while farmers are encouraged to grow non-edible or energy crops such as sugarcane, rubber and teak trees and *Jatropha curcas*, to produce ethanol;

and (3) solving health and environmental problems among farmers and local villagers (Nak-ai et al. 2018). However, many households still rely on consuming and selling rice and vegetables grown in the Cd and Zn co-contaminated fields. In this case, the application of organic and inorganic amendments could immobilize heavy metals and enhance the geochemical stability of Cd in contaminated soil, thereby reducing contamination in agricultural products (Bashir et al. 2019).

The combined use of inorganic and organic amendments leads to increased metal complexation, precipitation, redox reactions and/or sorption, resulting in reduced metal mobility and bioavailability of metal(loid)s in contaminated soil and water, and increased soil fertility. Additionally, amendments promote microbial and enzyme activity and enhance plant nutrition (Lwin et al. 2018). Metals commonly form complexes directly in the solid phase with humic substances having hydroxyl-, phenoxyl- and carboxyl-reactive groups. Furthermore, the mobility, bioavailability and potential toxicity of polyvalent cations such as Cd^{2+} and Zn^{2+} in plants are influenced by their strong interactions with organic matter, variable charge minerals and microorganisms in soil. To some extent, these metallic elements may be strongly adsorbed to edges of phyllosilicate minerals due to the presence of –SiOH or –AlOH groups. Such chemisorption of ions reduces metal retention and mobility through soil and irrigation water (Caporale and Violante 2016). While the reduction of toxic metal concentrations in soil is essential for growing edible crops in contaminated areas, a decrease in concentrations of beneficial metallic micronutrients can lead to plant stress, resulting in decreased crop production which eventually affects human nutrition (Kihara et al. 2020). Excessive fertilizer application, on the other hand, may cause deleterious effects to terrestrial and aquatic ecosystems (i.e., high uptake of metals in biota, eutrophication, etc.) and decrease antioxidant activities in crop plants (Arshad et al. 2016). Thus, the type and amount of fertilizer used in heavy metal-contaminated soil must be carefully considered in support of environmental sustainability and plant productivity.

In the present study, three commercial Thai rice varieties, Khao Dok Mali 105 (KDML105), RD53 and Phitsanulok2 (PSL2), were grown in a Cd and Zn co-contaminated paddy field in Thailand. Selected amendments were added to the contaminated soils. Plant growth performance, Cd and Zn uptake and accumulation and the potential health risks associated with Cd were determined.

Materials and methods

Study site and soil profiles

The study site occurs in a Cd-contaminated area of Mae Sot district, Tak province, Thailand (E 16° 67′ 38.6″ N 98° 62′ 63.1″), located downgradient of an inactive Zn mine, the putative point source of Cd and Zn at the site. The site is a paddy field with a history of rotation between maize, rice and beans. The field was plowed by tractor and flooded before being planted with KDML105 cultivar, a common rice strain typically grown during the rainy season (August–December) in this area. The field was irrigated with local Cd-contaminated surface water. Each plot was flooded and the water level was maintained at 5 cm above the soil surface, following which it was gradually drained at 21–25 days after flowering until harvest.

The study was conducted during the growing season of 2017. Total rainfall and mean temperature were approximately 1421.6 mm and 27.9 °C, respectively. Prior to the experiment, rhizosphere soil samples were collected from 5 locations (the corners and the middle) of the study site and oven-dried at 70 °C for 5 days. The dried soil samples were ground finely with an agate mortar and pestle and sieved through a 2-mm mesh sieve. The sieved soil was then mixed thoroughly and stored in plastic bags prior to analysis.

Soil pH and electrical conductivity (EC) were determined using a glass electrode pH meter (Accumet® AP115, USA); organic matter (OM) content was determined by the Walkley–Black titration method (Walkley and Black 1934) and soil texture by the hydrometer method (Allen et al. 1974). Cation exchange capacity (CEC) was determined by leaching with 1 N ammonia acetate (Sumner and Miller 1996). Total N was determined by the Kjeldahl method (Black 1965), extractable P via the Bray II method (Bray and Kurtz 1945) and extractable K via atomic absorption spectrophotometry after extraction with NH_4OAC (Warncke and Brown 1998). Total and extractable Cd and Zn concentrations were determined by flame atomic absorption spectrophotometry (FAAS; AAnalyst 200, PerkinElmer®) or graphite furnace atomic absorption spectrophotometry (GF-AAS; AAnalyst 600, PerkinElmer®), depending on metal concentration.

Plant materials

Three varieties of commercial Thai rice (*Oryza sativa* L.), KDML105, RD53 and PSL2, were assessed. These varieties were selected based on reports by Saengwilai et al. (2017) and Sriprachote et al. (2012) describing their contrasting abilities in Cd accumulation in plant parts and seeds. KDML105 has been shown to be a high Cd accumulating variety, whereas PSL2 is a low Cd accumulator as demonstrated in both mesocosm and field studies (Saengwilai et al. 2017). Seeds were obtained from the Bureau of Research and Development (Bangkok, Thailand).

Seeds were surface-sterilized with 5% sodium hypochlorite for one night and then soaked for two days in deionized (DI) water in an incubator at 40 °C and a 16 h light period day^{-1}. Sterilized seeds were placed on a 3-mm-thick bed of moistened paper towels and placed in Petri dishes (30 seeds per dish) at room temperature. The tops of the Petri dishes were covered with plastic wrap to prevent evaporation. Germinated seeds that showed normal roots and small embryo buds were considered for transfer to a rice seedling plastic tray (434 holes). Only one germinated seed was placed in commercial soil (i.e., without heavy metal contamination). Deionized water was sprayed evenly over the plastic tray twice daily in order to maintain consistent moisture content. The one-month-old seedlings were then transferred to the field site.

Field experiment

Field plots measured 2 × 1 m^2 with five replicates in each treatment. Plot size and arrangement were established according to a previous study (Saengwilai et al. 2020). Each replicate (one plot) consisted of 135 rice seedlings planted in 5 × 9 rows (45 holes). Three rice seedlings were grown in one hole with a spacing of 23 cm in one direction and 20 cm in the other. The experimental design was a completely randomized block nested with different rice varieties within each treatment. Soil without amendment (Cont treatment) served as the control treatment. Dicalcium phosphate and cattle manure (T1), and dicalcium phosphate and leonardite (T2), mixed in a 1:1 (w:w) ratio, were the amendment treatments. Dicalcium phosphate served as an inorganic amendment, whereas cattle manure and leonardite served as organic amendments. The amendments were added to the paddy soil prior to planting at a rate of 500 Mg ha^{-1}. This rate is typical for local farming practices in Thailand (Miadnok and Promatar 2019). The amendments were broadcast over the surface and mixed with soil manually prior to planting. Following fertilizer application, the three varieties of rice seedlings were planted and covered by shading nets to prevent exposure to direct sunlight and bird attacks. Inorganic fertilizer (16-20-0) and urea were added at one and three months after transplanting to maintain plant health.

Three plants representative of each plot were collected at the flowering and physiological maturity stage. Phitsanulok2 and RD53 were collected in month 4, while KDML105 was collected in month 6. Three rice samples were collected randomly in the middle row (row 3). Plants at the edge of a plot were not chosen in order to minimize errors arising from border effects (Gomez and De Datta 1971). Plants were separated into shoots, roots and rice grains for drying, weighing and analysis of Cd and Zn via FAAS. Roots were excavated using a steel spade. Soil attached to roots was removed carefully by shaking and then soaking in tap water, followed by low-pressure rinsing and then a DI water rinse.

Metal analysis in plant and soil

Dried plant materials were ground with a mortar and pestle (IKA; A11 basic), sieved through a 2-mm mesh sieve and weighed. A total of 0.5 g of plant sample was prepared in a vessel tube for acid digestion [conc. 70% nitric acid (HNO_3) and 37% hydrochloric acid (HCl)], using microwave digestion (ETHOS One; Milestone Inc.), and analyzed with either FAAS or GFAAS, depending on metal concentration.

Soil materials were collected at the rhizosphere. Soil samples were dried, ground with a mortar and pestle and subsequently sieved through a 2-mm sieve. A 0.5 g portion of air-dried soil was digested with conc. 70% HNO_3 and 30% hydrogen peroxide (H_2O_2) using microwave digestion. Cadmium and Zn concentrations were determined by FAAS. NIST 1515 apple leaves and 2711a Montana soil were used to calculate percentage recovery for plant and soil samples, respectively. The mean percentage recovery ranged from 94.3 to 108.5% and 97.5 to 110.3%, respectively, and the percentage relative standard deviation (%RSD) ranged from 3.5 to 5.2% and 1.8 to 4.7%, respectively.

Data analyses

Growth index

Growth rate in biomass Growth rate in biomass (GRB) was used to compare total dry matter production between plant samples (Meeinkuirt et al. 2016).

$$\mathrm{GRB} = \mathrm{TDBP}_{\mathrm{AH}} - \mathrm{TDBP}_{\mathrm{BP}}/\mathrm{DT}$$

where $\mathrm{TDBP}_{\mathrm{AH}}$ (g) is the total dry biomass production after harvesting, $\mathrm{TDBP}_{\mathrm{BP}}$ (g) is the total dry biomass production before planting and ΔT is the interval between planting periods, measured over four months for the PSL2 and RD53 varieties and six months for the KDML105 variety, respectively.

Heavy metal and translocation indices

Metal uptake Metal uptake from contaminated soil indicates the metal concentrations in whole plant tissue (Meeinkuirt et al. 2013).

$$\text{Metal uptake} = C_{\mathrm{PT}}x \text{ dry plant biomass production } (g)$$

where C_{PT} is the metal concentration in plant tissue (mg kg^{-1}).

Bioconcentration coefficient (BCF) The bioconcentration coefficient indicates the accumulation efficiency of metal in whole plant tissue. It can also indicate hyperaccumulation potential, where metal accumulates primarily in the aboveground part of the plant (Sricoth et al. 2018b).

$$\mathrm{BCF} = C_{\mathrm{PT}}/S_{\mathrm{Ext.metal}}$$

where $S_{\mathrm{Ext.metal}}$ is the extractable metal concentration in soil (mg kg^{-1}).

Translocation factor (TF) The translocation factor of a metal verifies the degree of metal movement from media (soil) to plant tissue or from plant root to aerial parts (e.g., shoot and grain). A TF value of > 1 indicates a high translocation potential of the metal to the aerial part of the plant (Marchiol et al. 2004).

$$\mathrm{TF}_{\mathrm{soiltoroot}} = C_{\mathrm{root}}/C_{\mathrm{soil}}$$

$$\mathrm{TF}_{\mathrm{roottoshoot}} = C_{\mathrm{shoot}}/C_{\mathrm{root}}$$

$$\mathrm{TF}_{\mathrm{shoottograin}} = C_{\mathrm{grain}}/C_{\mathrm{shoot}}$$

Translocation index (TI) The translocation index indicates possible metal movement in the plant part (shoot or grain) (Rahman et al. 2013; Rehman et al. 2018a).

$$\text{Translocation index } (\%) = \frac{\text{Metal concentration in shoot (or grain)}}{\text{Metal concentration in whole plant [or shoot (grain + straw)]}}$$

where the unit of Cd concentration in the plant part is mg kg^{-1}.

Cadmium harvest index (%) Cadmium harvest index indicates the Cd uptake ability of plant tissue, which can imply a risk to the health of humans due to Cd-related diseases from long-term exposure (Rehman et al. 2018b).

$$\text{Cadmium harvest index}(\%) = \frac{\mathrm{CdG} + \mathrm{CdS}}{(\mathrm{CdG} + \mathrm{CdS} + \mathrm{CdSo})} \times 100$$

where CdG is Cd concentration in grain, CdS is Cd concentration in straw and CdSo is Cd concentration in soil. The unit of Cd concentration in the plant part is mg kg^{-1}.

Health risk assessment for Cd

Health risk index (HRI) The HRI for Cd through intake of contaminated rice grains is used to evaluate chronic health risk. A health risk index of ≥ 1 is considered a health risk to humans. Cadmium HRI depends upon the daily intake of metal (DIM) and the oral reference dose (Rf_{D}) (Rehman et al. 2018a; Rizwan et al. 2017).

$$\text{Health risk index} = \mathrm{DIM}/\mathrm{Rf}_{\mathrm{D}}$$

Daily intake of metal (DIM) was calculated as given below.

$$\text{Daily intake of metal} = C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{foodintake}} / B_{\text{averageweight}}$$

where C_{metal} indicates Cd concentration in grain (mg kg^{-1}), C_{factor} is the factor (0.085) for conversion of fresh to dry weight grains, $D_{\text{food intake}}$ is daily intake of grains taken as 0.4 kg per person per day and $B_{\text{average weight}}$ is the average body weight, which was considered to be 70 kg for an adult.

The oral reference dose is an estimate of a daily exposure of metal to the human body that has no hazardous effect during a lifetime (Barnes and Dourson 1988). In this study, the Rf_D value for Cd is 0.001 mg kg^{-1} day^{-1} (Rehman et al. 2018a; Rizwan et al. 2017).

Statistical analysis

R program version 2.15.1 (R Development Core Team 2012) was used to statistically analyze the data. All data were expressed as mean ± standard deviation (SD). Differences among treatments were determined using a one-way analysis of variance (ANOVA), while least significant difference (LSD) post hoc comparisons ($p < 0.05$) were used to compare means for significant differences. ANOVA outputs for each parameter are provided as supplementary information (Table S1–S26).

Results and discussion

Soil and amendment properties

Physicochemical properties of soil and the amendments are presented in Tables 1 and 2. Generally, leonardite had the lowest pH value (pH 2.6), compared to dicalcium phosphate (pH 8.2) and cattle manure (pH 8.5) (Table 1). Both leonardite and cattle manure possessed high electrical conductivity (EC), organic matter content (OM) and extractable K, Ca and Mg contents (Table 1), whereas dicalcium phosphate had high available P, K, Ca and Mg contents. Application of amendments did not affect soil pH values (Table 2). The substantial EC, OM, total N, extractable P, K, Ca and Mg contents were measured in the amended treatments. The application of amendments increased soil EC by approximately 1.6–4.7 times. While the EC values of the amendments were relatively high, they did not cause salt damage to plants when incorporated into the contaminated soils. This was consistent with Saengwilai et al. (2017) who tested similar rice varieties and amendments in a mesocosm system.

Table 1 Physicochemical properties of the amendments used

Parameter	Unit	DCL	Leo	CM
pH		8.2	2.6	5.5–8.5
EC	dS m^{-1}	0.12	3.92	> 10
OM	%	0.1	20.1	15.31
Total N	%	0.01	0.6	0.77
Ext. P	%	6.6	BDL	0.65
Ext. K	%	0.23	0.17	1.63
Ext. Ca	%	24.1	1.7	0.86
Ext. Mg	%	0.33	0.28	0.17
Total Cd	Mg kg^{-1}	4.57	0.69	5.7
Total Zn	Mg kg^{-1}	302.16	39.98	71.1

EC electrical conductivity; *C* carbon; *N* nitrogen; *OM* organic matter; *P* phosphorus; *K* potassium; *Ca* calcium; *Mg* magnesium; *Cd* cadmium; *Zn* zinc; *Ext.* extractable; *DCP* dicalcium phosphate; *Leo* leonardite; *CM* cattle manure

Cation exchange capacity (CEC) did not differ between Cont and T1 treatments; however, the T2 treatment exhibited the highest CEC (21.7 cmol kg^{-1}), which was 1.4 times higher than the control (Table 2). The carboxyl, phenol, hydroxyl and ketone functional groups in the humic acid (HA) of leonardite could be a key factor in increasing soil CEC (Jomhataikool et al. 2019; Zhou et al. 2019). After amendment application, the sandy clay loam in the unamended soil changed to loam in the amended treatments (Table 2). Loam was the typical soil type in Cd-contaminated paddies in the Mae Tao river basin (Meeinkuirt et al. 2016), and this soil type exhibited the highest grain Cd pollution risk (Han et al. 2012).

Soil organic matter and phosphate content in the amended treatments increased by 1.5–1.7 times and 5.7–6.4 times, respectively, compared to the controls. Elevated organic matter content in soils treated with organic amendments is considered the principal factor reducing soluble metal concentration in contaminated soil, thereby reducing Cd and Zn phytoavailability and decreasing the transfer of the metals through the food chain (Liu et al. 2015; Ullah et al. 2020). Stable soil organic matter (‘humus’) plays an important role in the retention, release and bioavailability of heavy metal

Table 2 Physicochemical properties of the soil samples and metal concentrations in the soils after plant harvest

	Parameter	Unit	Soil treatment		
			Cont	T1	T2
	Texture		Sandy clay loam	Loam	Loam
	Sand	%	55	46	37
	Silt	%	20	37	45
	Clay	%	25	17	25
	pH		7.7	7.6	7.7
	EC	dS m^{-1}	0.46	0.73	2.17
	OM	%	2.38	4.10	3.64
	CEC	Cmol kg^{-1}	15.6	13.3	21.7
	Total N	%	0.12	0.21	0.18
	Ext. P	mg kg^{-1}	15.7	101.0	88.8
	Ext. K	mg kg^{-1}	54.6	749.6	181.6
	Ext. Ca	mg kg^{-1}	2903	4073	7510
	Ext. Mg	mg kg^{-1}	282	355	497
Metal concentrations in the soils before planting					
	Total Cd	mg kg^{-1}	3.6	2.3	3.7
	Ext. Cd	mg kg^{-1}	2.0	0.8	1.5
	Total Zn	mg kg^{-1}	92.8	57.4	89.3
	Ext. Zn	mg kg^{-1}	13.6	8.3	10.6
Metal concentrations in the soils after plant harvest					
Genotype					
KDML105	Total Cd	mg kg^{-1}	41.8 ± 14.9aA	33.5 ± 9.1aA	31.6 ± 9.6aA
	Ext. Cd	mg kg^{-1}	6.6 ± 4.0aB	5.5 ± 2.3aA	3.9 ± 1.4aB
	Total Zn	mg kg^{-1}	1458.4 ± 700.0aA	1017.1 ± 342.0aA	954.3 ± 337.4aA
	Ext. Zn	mg kg^{-1}	71.6 ± 44.4aA	71.6 ± 44.4aA	42.3 ± 16.2aA
PSL2	Total Cd	mg kg^{-1}	53.7 ± 9.3aA	41.3 ± 12.7abA	37.2 ± 7.6bA
	Ext. Cd	mg kg^{-1}	10.7 ± 2.3aA	6.7 ± 2.1bA	6.7 ± 2.5bA
	Total Zn	mg kg^{-1}	1923.1 ± 281.3aA	1264.9 ± 434.6bA	961.5 ± 192.7bA
	Ext. Zn	mg kg^{-1}	106.9 ± 12.7aA	84.0 ± 23.5abA	60.6 ± 18.2bA
RD53	Total Cd	mg kg^{-1}	46.0 ± 9.8aA	34.7 ± 3.6aA	35.1 ± 11.5aA
	Ext. Cd	mg kg^{-1}	8.7 ± 1.4aAB	5.9 ± 1.6bA	5.5 ± 2.0bAB
	Total Zn	mg kg^{-1}	1332.3 ± 294.7aA	1012.7 ± 95.4aA	1009.7 ± 496.6aA
	Ext. Zn	mg kg^{-1}	85.6 ± 11.2aA	67.4 ± 11.4bA	57.7 ± 14.7bA

EC electrical conductivity; *OM* organic matter; *CEC* cation exchange capacity; *N* nitrogen; *P* phosphorus; *K* potassium; *Ca* calcium; *Mg* magnesium; *Cd* cadmium; *Zn* zinc; *Ext.* extractable; *Cont* control; *T* treatment

Values followed by the same letter are not significantly different; small letters show the difference of treatments of the same soil treatment (LSD, $p < 0.05$); capital letters indicate the difference of soil metal concentrations in the same treatment (LSD, $p < 0.05$)

ions and cation exchange capacity in soils, since it can combine electrostatically with metal ions. Humus compounds can form both simple and chelated complex compounds (Koncewicz-Baran and Gondek 2012; Lasota et al. 2020). Organic matter in cattle manure is documented to form strong complexes with Cd resulting in low mobility of Cd in soil (Palansooriya et al. 2020).

Long-term continuous application of certain amendments and fertilizers can result in metal contamination in soil and plants (Gonçalves Jr et al. 2014). In this study, trace amounts of Cd and Zn were detected in all amendment materials. Highest Cd content was in the cattle manure (5.7 mg kg^{-1}), and the highest Zn content was in the dicalcium phosphate amendment (302.2 mg kg^{-1}; Table 1). The lowest Cd and Zn contents were found in leonardite (0.7 and 40 mg kg^{-1}, respectively). The contamination levels did not, however, exceed the Thai agricultural standard for the composite amendment (NBACFS 2005). Following amendment application, soil dilution may further decrease Cd and Zn concentrations, as seen in the total and extractable Cd and Zn contents in the study soils (Table 2). This phenomenon is consistent with previous reports (Paul and Rufus 2017; Satachon et al. 2019). It is important to note that both Cd and Zn contents in soil after plant harvest were higher than those at the beginning of the season (Table 2). Since paddy fields were continuously irrigated by local surface water, the dramatic increase in soil metal concentrations throughout the growing season was likely due to irrigation water originating from a contaminated local water reservoir located near the zinc mine in Mae Sot district (Charoenpanyanet and Huttagosol 2020).

Plant growth

The effects of soil amendments on growth and yield production depended on rice variety and amendment type. While KDML105 had the highest biomass production compared to the other rice varieties, it was not responsive to T1 and T2 treatments (Fig. 1a and b). PSL2 and RD53 varieties had significant responses via increased growth rate and yield production in the amended treatments, particularly T1 (Fig. 1). Among rice varieties, KDML105 has the lowest yield production in all treatments, while highest yield production was in the PSL2 variety treated with the cattle manure and dicalcium phosphate mixture (T1) (Fig. 1c). Differences in growth response and yield production to soil amendments among rice varieties grown in Cd and Zn co-contaminated soil are well documented (Saengwilai et al. 2020). The present results emphasize that plant response to soil amendments is complex and influenced by genetics, environmental factors and their interaction. In many cases, an increase in soil organic matter level from amendment application has been shown to reduce Cd toxicity to rice grown in Cd-contaminated environments (Rehman et al. 2017). However, the mixture of inorganic and organic amendments has been proposed to offer the greatest benefit for crop production, as use of organic fertilizers alone might not provide sufficient nutrients to meet plant requirements (Mahmud et al. 2016).

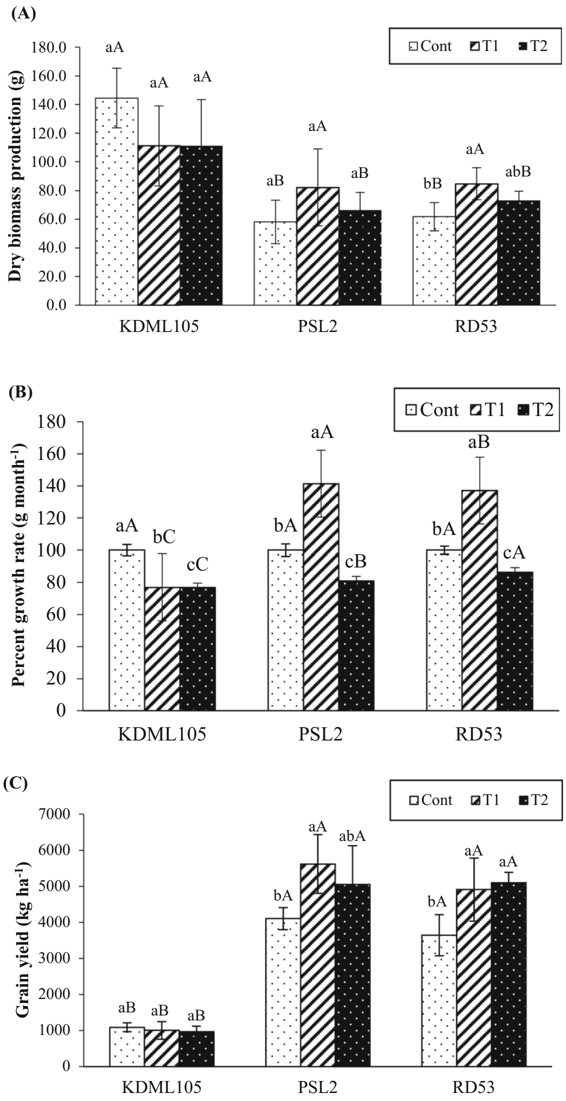
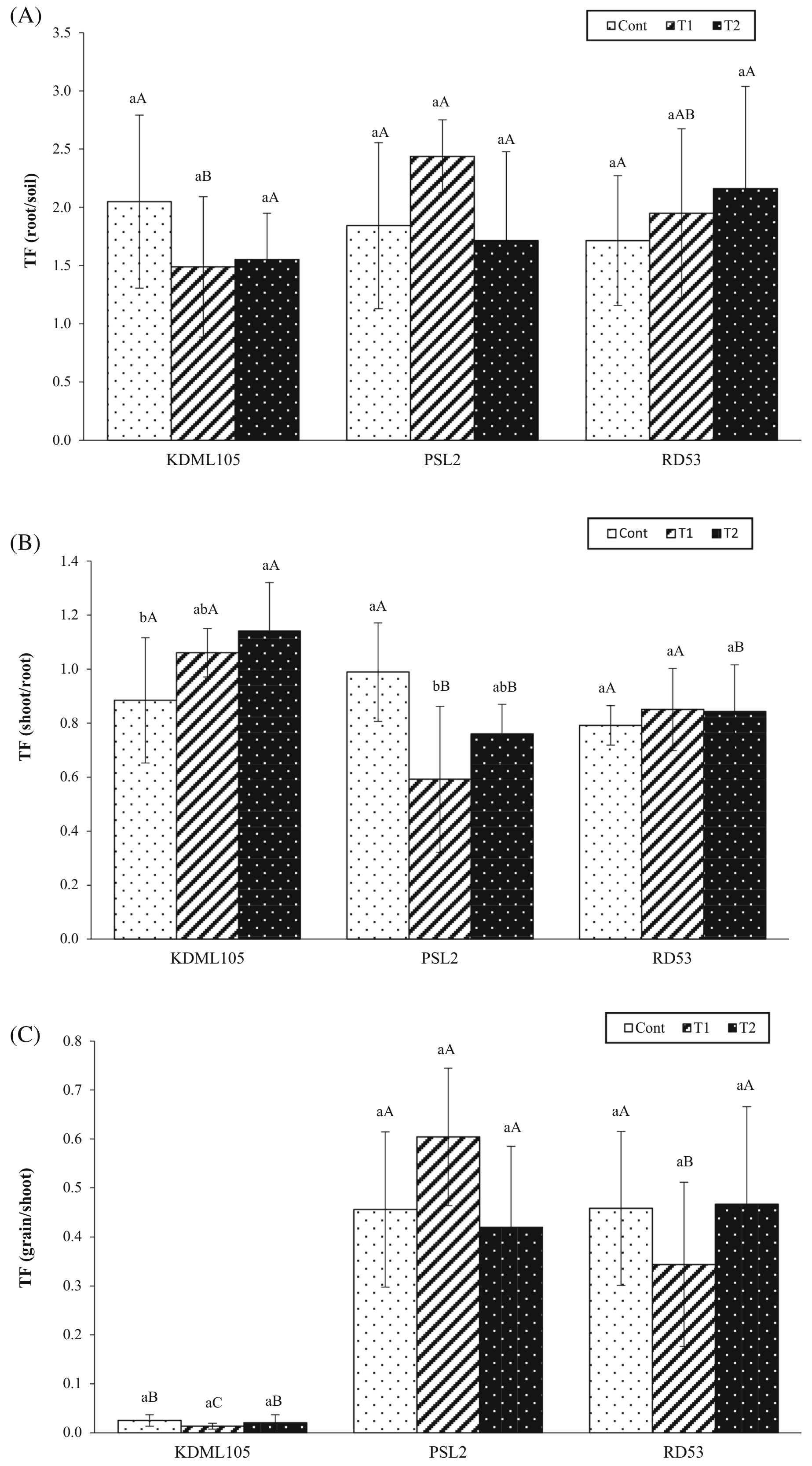


Fig. 1 Dry biomass production (**a**), percent growth rate in biomass per month (**b**) and grain yield (**c**) of the study rice varieties. Values are mean of 5 replicates. Bars with different lowercase letters indicate that values are significantly different among experimental treatments in the same rice variety (LSD, $p < 0.05$), while bars followed by different capital letters are significantly different among rice varieties within the same treatment (LSD, $p < 0.05$)

Table 3 Cadmium and Zn accumulation and uptake, and bioconcentration coefficient (BCF) of Cd and Zn among rice varieties in field study over 4 (RD53 and PSL2) and 6 (KDML105) months (n = 5)

Genotype	Treatment	Cd accumulation in plant (mg kg^{-1})					Cd uptake (mg plant^{-1})	BCF
		Shoot	Root	Grain	Grain + Shoot	Whole Plant		
KDML105	Cont	8.0 ± 1.4aAB	9.2 ± 1.1aA	0.19 ± 0.08aB	4.1 ± 0.7aA	6.3 ± 1.6aB	917.7 ± 298.0aB	1.4 ± 0.5aB
	T1	8.2 ± 0.6aA	7.8 ± 0.7bA	0.11 ± 0.05aC	4.2 ± 0.3aA	4.6 ± 1.3abB	490.9 ± 122.2bB	0.9 ± 0.4bB
	T2	9.0 ± 0.7aA	8.0 ± 0.9abA	0.19 ± 0.17aB	4.6 ± 0.4aA	3.7 ± 0.6bB	415.3 ± 150.6bAB	0.7 ± 0.2bB
PSL2	Cont	8.9 ± 1.5aA	9.1 ± 1.3aA	3.95 ± 1.06aA	6.4 ± 0.9aA	3.7 ± 0.9aB	223.7 ± 105.1aB	0.7 ± 0.2aB
	T1	6.8 ± 1.2bB	16.1 ± 13.1aA	4.06 ± 1.05aA	5.4 ± 0.9abA	2.8 ± 2.5aB	218.6 ± 163.2aB	0.9 ± 1.2aB
	T2	7.0 ± 0.8bB	9.3 ± 0.9aA	2.88 ± 0.89aA	5.0 ± 0.5bA	2.5 ± 0.6aB	163.1 ± 27.7aB	0.5 ± 0.3aB
RD53	Cont	7.0 ± 0.5aB	8.9 ± 0.4aA	3.23 ± 1.16aA	5.1 ± 0.7aA	14.1 ± 8.2aA	1823.2 ± 1125.9aA	7.6 ± 5.2aA
	T1	7.6 ± 0.5aAB	9.1 ± 1.3aA	2.57 ± 1.09aB	5.1 ± 0.4aA	13.9 ± 7.1bA	1791.9 ± 1373.0aA	2.8 ± 1.8aA
	T2	7.3 ± 0.5aB	9.1 ± 2.3aA	3.41 ± 1.50aA	5.4 ± 0.8aA	11.7 ± 7.6bA	890.5 ± 627.4aA	3.9 ± 3.5aA
Genotype	Treatment	Zn accumulation in plant (mg kg^{-1})					Zn uptake (mg plant^{-1})	BCF
		Shoot	Root	Grain	Grain + Shoot	Whole Plant		
KDML105	Cont	22.7 ± 2.4aA	77.3 ± 17.9aA	22.1 ± 1.9aB	22.4 ± 1.3aA	51.3 ± 19.3aB	7459.5 ± 3240.6aB	0.4 ± 0.1aB
	T1	20.6 ± 2.6aA	63.3 ± 4.8abA	18.1 ± 4.0bB	19.4 ± 2.5aA	40.8 ± 8.6aB	4483.8 ± 1084.3bB	0.3 ± 0.1abB
	T2	21.8 ± 3.4aA	50.9 ± 8.8bB	13.9 ± 1.1cB	17.8 ± 1.9aA	39.0 ± 3.6aB	4256.5 ± 858.1bB	0.3 ± 0.1bB
PSL2	Cont	20.1 ± 2.1aA	90.2 ± 11.5aA	21.2 ± 4.0aB	20.6 ± 2.1aA	55.3 ± 7.8aB	3278.7 ± 1125.6aB	0.4 ± 0.1aB
	T1	19.7 ± 1.6aA	145.4 ± 115.8aA	24.5 ± 2.1aA	22.1 ± 1.4aA	58.1 ± 15.2aB	4648.2 ± 1299.9aB	0.5 ± 0.2aB
	T2	27.4 ± 10.9aA	93.0 ± 16.6aA	23.5 ± 1.6aA	25.5 ± 5.6aA	58.9 ± 10.3aB	3950.0 ± 1145.6aB	0.4 ± 0.2aB
RD53	Cont	19.9 ± 1.9aA	90.1 ± 14.1aA	26.1 ± 1.5aA	23.0 ± 1.1aA	71.3 ± 34.2aA	5632.3 ± 2315.1aA	3.5 ± 2.8aA
	T1	23.0 ± 4.9aA	96.1 ± 32.4aA	25.4 ± 1.5abA	24.2 ± 3.2aA	73.5 ± 24.1aA	5782.2 ± 3123.2aA	2.1 ± 1.8aA
	T2	23.4 ± 5.8aA	81.8 ± 13.9aA	23.5 ± 1.3bA	23.5 ± 3.4aA	63.5 ± 31.4aA	4983.5 ± 2580.7aA	1.9 ± 1.5aA

Values followed by the same letter are not significantly different; small letters show the difference of treatments of the same rice cultivar (LSD, $p < 0.05$); capital letters indicate the difference of metal uptake and accumulation among rice varieties in the same treatment (LSD, $p < 0.05$)


(A)
TF (root/soil)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
□Cont
▨T1
■T2
aA
aB
aA
aA
aA
aA
aA
aAB
aA
KDML105
PSL2
RD53
(B)
TF (shoot/root)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
□Cont
▨T1
■T2
bA
abA
aA
aA
bB
abB
aA
aA
aB
KDML105
PSL2
RD53
(C)
TF (grain/shoot)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
□Cont
▨T1
■T2
aB
aC
aB
aA
aA
aA
aA
aB
aA
KDML105
PSL2
RD53

◀**Fig. 2** Translocation factor (TF): **a** soil to root; **b** root to shoot; **c** shoot to grain; translocation index (TI): **d** shoot; **e** grain; **f** Cd harvest index. Values are mean of 5 replicates. Bars with different lowercase letters indicate that values are significantly different among experimental treatments in the same rice variety (LSD, $p < 0.05$), while bars followed by different capital letters are significantly different among rice varieties within the same treatment (LSD, $p < 0.05$)

Cadmium and zinc concentration in rice tissues

The mean levels and ranges of Cd and Zn concentrations in plant tissue are shown in Table 3. The descending order of Cd accumulation in different plant parts was as follows: root $\approx$ shoot $>$ whole plant $\approx$ shoot $+$ grain $>$ grain. Slightly elevated Cd concentrations were measured in shoots of the KDML105 variety in the amended soil treatments. Both the PSL2 and RD53 varieties exhibited slightly higher Cd concentrations in roots than in shoots. Previous studies using different commercial rice varieties such as KDML105, PSL2, RD53, Shendao5, Tianfu1, Fuhe90 and Yanfeng47 reported that rice is an excluder for Cd and accumulates Cd primarily in roots (Chi et al. 2018; Saengwilai et al. 2017; Zhan et al. 2013). In the current study, the RD 53 variety had the highest potential to accumulate Cd in tissue, with a Cd uptake value of 1823.2 mg kg^{-1} in the Cont treatment, followed by 1791.9 mg kg^{-1} and 890.5 mg kg^{-1} in the T1 and T2 treatments, respectively. These results were consistent with Meeinkuirt et al. (2019) who reported that RD53 accumulated the highest amount of Cd in roots and shoots compared to the KDML105 and PSL2 varieties. The T1 and T2 treatments tended to decrease Cd uptake in whole plants, but the significance of the effects depended on plant part and rice variety. Among the varieties, KDML 105 showed significant decreases in overall Cd uptake, while PSL2 exhibited both significant reduction in the Cd accumulation in shoots and grain yield under amendment treatments (Table 3).

The descending order of Zn accumulation for rice varieties differed from that of Cd as follows: root $>$ whole plant $>$ shoot $\approx$ shoot $+$ grain $\approx$ grain. In the amended soil treatments, Zn values in roots of the PSL2 variety were 7.4 times higher than that of shoots in the T1 treatment, followed by the RD53 variety in the T2 treatment (4.2 times greater than shoots). A higher concentration of heavy metals in roots is a typical characteristic of monocot excluder species. These results are consistent with those reported by Saengwilai et al. (2020). Rice root has been shown to act as a barrier to metal transport to shoots, acting as a tolerance mechanism to elevated Zn concentrations in soil. Zinc accumulation in roots could reach as high as 3400 mg kg^{-1}. However, shoots are more sensitive to high levels of Zn than are roots (Souza et al. 2020). It has been shown that Zn toxicity and alterations in certain physiological processes (i.e., transpiration and photosynthesis) can occur in plant tissue at concentrations greater than 200 mg kg^{-1} (Richardson et al. 1993). However, Zn concentrations in the whole plant tissue in this study were below 100 mg kg^{-1}, implying that the plants might not be adversely affected by Zn. Furthermore, Zn concentrations in rice grains (13.9–26.1 mg kg^{-1}) were within maximum acceptable limits for undesirable substances in fodder ($<$ 100 mg kg^{-1}) (Borowczak and Hołtra 2017); the Zn-contaminated rice biomass can be used for compost or as a biofuel (Malinowska et al. 2015).

A bioconcentration coefficient factor (BCF) $>$ 1 indicates the accumulation potential of heavy metals in plant tissue (Baker and Brooks 1989). In our study, only the RD53 variety had BCF values $>$ 1 in all treatments; thus, the RD53 variety had the highest accumulation potential of the tested metals in its tissues. A translocation factor (TF) $<$ 1 indicates a low translocation efficiency of metals in plant tissue (Kandziora-Ciupa et al. 2017). In this study, TF values for Cd in all study plants were generally $<$ 1 (Fig. 2a, b, c) except for those of the KDML105 variety in the amended treatments (T1 = 1 and T2 = 1.1). Nevertheless, it is not considered a hyperaccumulator for Cd and Zn. The PSL2 and RD53 varieties appeared to be excluders for Cd and Zn as they accumulated the metals primarily in roots with a TF value $<$ 1. These results are consistent with those reported by others (Radovanovic et al. 2020; Sebastian and Prasad 2014). The translocation index (TI) is used for determining the degree of Cd translocation in plant parts. The TI values for shoots of plants in all treatments were relatively high ($>$ 60) (Fig. 2d). Compared to other monocot species such as *Triticum aestivum*, the rice in our study had relatively high TI for Cd (Rehman et al. 2018a). The TI values for grains (Fig. 2e) showed a similar trend with TF values. Low TI values for grains in the KDML105 variety (1.3–2.4) indicated low Cd uptake, which emphasize that Cd was restricted to the

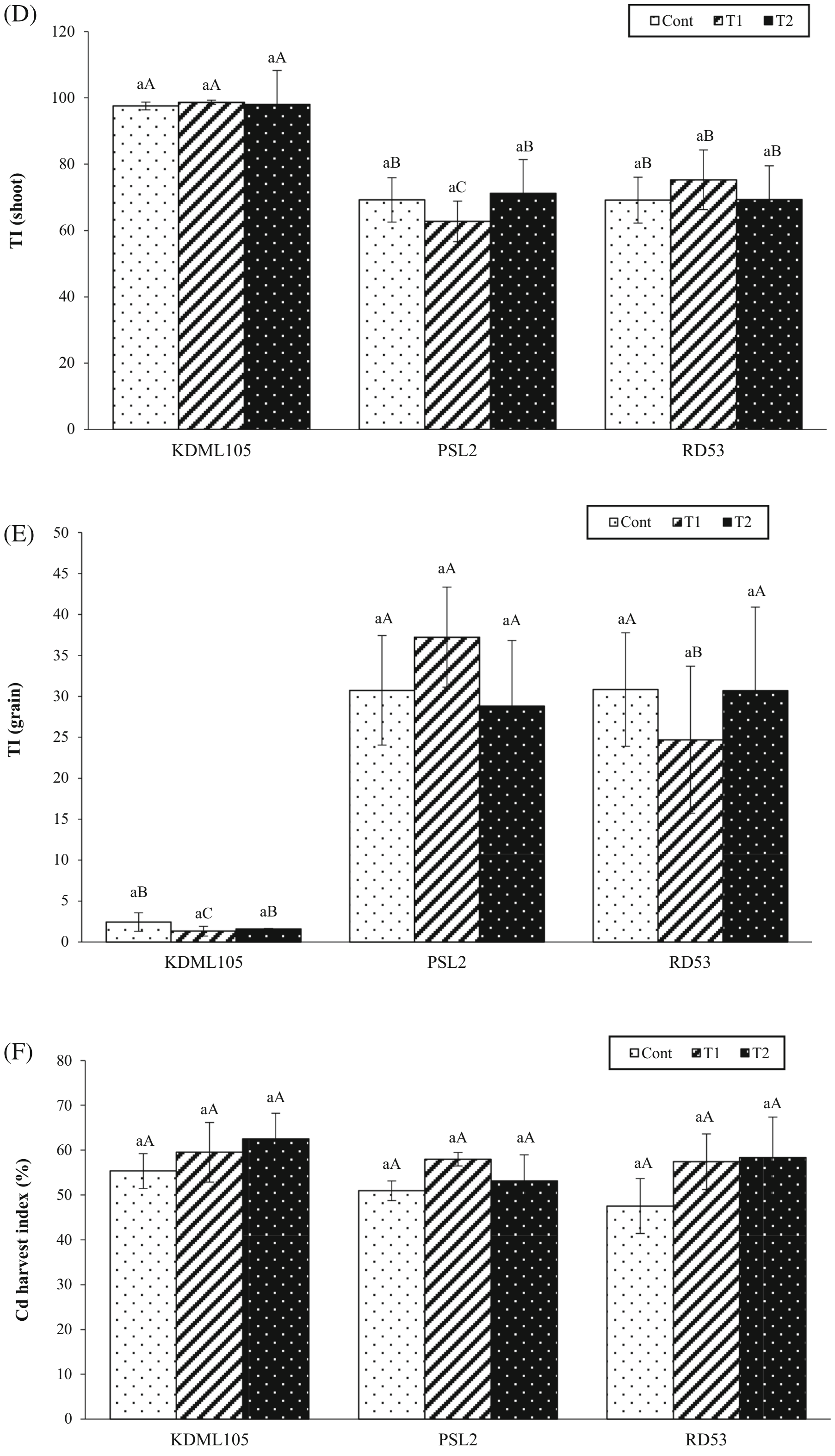


Fig. 2 continued

roots and moved minimally to edible parts. The high retention of Cd in roots could be associated with a number of metal-binding molecules such as phytochelatins (PCs). Phytochelatins chelate Cd and the low molecular weight Cd–PC complex is translocated across the tonoplast and then sequestered into the central vacuoles of root cells by means of the ATP-binding cassette (ABC) transporter *HMT1*, thereby decreasing the Cd content in the cytosol of plant cells (Rea 2012; Yamazaki et al. 2018).

Human health risk assessment

No significant differences were found in Cd harvest index values among different rice varieties (Fig. 2f). This result suggests that concentrations of potentially bioavailable Cd among all varieties were similar in all experimental treatments. Although the level of Cd contamination in the paddy soil was not excessively high (total concentration < 5 mg kg^{-1}), most grain samples collected from rice in this study (except those of KDML105 in the T1 treatment) exhibited 1.3–27.1 times greater Cd accumulation levels than the average level of Thai rice (0.15 mg kg^{-1}) (Zarcinas et al. 2004). The level of Cd accumulation in grains of PSL2 and RD53 exceeded the current Chinese maximum allowable concentrations (MAC) of 0.2 mg kg^{-1}, which is considered to pose a potential health risk to humans (Batista et al. 2012; Ke et al. 2015).

Daily intake of metal (DIM) and health risk index (HRI) were used to evaluate the potential human health risk due to ingestion of rice grains. DIM values were much lower than 1 (Table 4). The DIM of the KDML105 was lower than that of PSL2 and RD53 by up to 19 times and 17 times, respectively. HRI values of Cd ranged from 0.05 to 2 (Table 4). The lowest HRI value was observed in the T1 treatment for the KDML105 variety, which was approximately 80% lower than the control treatment. However, HRI values in all treatments for the KDML105 variety were within acceptable limits (HRI < 1) for edible plants, indicating no risk to human health (U.S. EPA 2005). In the case of the RD53 and PSL2 varieties, the HRI values (1.3–2) were relatively high and considered to pose a potential risk to human health.

Table 4 Daily intake of metal (DIM) and health risk index (HRI) for Cd

Genotype	Treatment	DIM	HRI
KDML105	Control	0.0001 ± 0.0000aB	0.09 ± 0.04aB
	T1	0.0001 ± 0.0000aB	0.05 ± 0.03aC
	T2	0.0001 ± 0.0001aB	0.09 ± 0.08aB
PSL2	Control	0.0019 ± 0.0005aA	1.92 ± 0.52aA
	T1	0.0020 ± 0.0005aA	1.97 ± 0.51aA
	T2	0.0014 ± 0.0004aA	1.40 ± 0.43aA
RD53	Control	0.0016 ± 0.0006aA	1.57 ± 0.56aA
	T1	0.0012 ± 0.0005aA	1.25 ± 0.53aB
	T2	0.0017 ± 0.0007aA	1.66 ± 0.73aA

Values are mean of 5 replicates. Different lowercase letters indicate that values are significantly different among experimental treatments in the same rice variety (LSD, $p < 0.05$), while different capital letters are significantly different among rice varieties within the same treatment (LSD, $p < 0.05$)

Conclusions

Contamination of Cd and Zn in rice has long been a serious issue for the economy, environment and human health. Our study demonstrated that the application of combined organic and inorganic amendments enhanced rice growth and yield while reducing both Cd accumulation in grains and the health risk to humans from rice consumption. A combination of dicalcium phosphate and cattle manure demonstrated the most efficient immobilization of Cd in soil and plant parts, rendering the widely grown KDML105 rice strain ‘safe’ for human consumption. Our findings also indicate that Cd uptake and accumulation differ among rice varieties. Thus, plant selection and breeding programs for low Cd accumulating rice varieties should be implemented and applied for cultivation of contaminated agricultural soils. We conclude that the use of organic–inorganic soil amendment mixtures suitable for heavy metal-contaminated soils and crop nutritional requirements, and heavy metals is a practical, sustainable and effective approach to reduce heavy metal contamination in rice.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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