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Six Years Monitoring of the Promising Low Grain-Cd Accumulating Rice, RD15 Cultivar Grown on Cd-contaminated Paddy Soils

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Abstract

The experiment was conducted on the Cd-contaminated paddy field, two of Thai rice cultivars, the prevailing KDML105 and RD15 were included together with determining grain-Cd accumulation. The results had revealed that the RD15 contained Cd in grains not only lower than KDML105 but also lower than the critical level as proposed by Codex (0.4 mg kg^{-1} polished rice). Thus, the RD15 was selected as the promising low grain-Cd accumulating rice cultivar, and farmers were encouraged to grow this cultivar. The grains of prevailing and promising rice cultivars were annually collected for Cd analysis. After six-years monitoring, the results revealed that the average concentration of Cd in KDML105 was 0.658 mg kg^{-1}, which exceeded the critical level. On the other hand, the average concentration observed in RD15 (0.127 mg kg^{-1}) was lower than the critical level. The human dietary intake of Cd through rice consumption was calculated, comparing to the provisional tolerable weekly intake (PTWI) as adopted by FAO/WHO (7 ug Cd kg^{-1} BW per week), the PTWI of KDML105 was far exceeded the critical level. Contrary, the RD15 had the PTWI lower than the critical level. Present results indicated that, by cultivating RD15 the promising low-grain Cd cultivar, Cd intake together with health risk of human could be reduced.

Keywords: Cadmium; Rice; RD15; Low grain-Cd accumulating rice cultivar; Mae Sot District, Thailand

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Introduction

Cadmium (Cd), the non-essential heavy metal that usually found in zinc (Zn) ores is now widespread into the environment by industrial and agricultural activities, resulting in harmful for human health [1]. According to its well-known renal toxicity, the Cd-polluted environment had raised growing concern by ordinary people. The "Itai-Itai disease" in Toyama Prefecture of Japan is said to be the most calamitous Cd pollution record of the world [2]. According to this disease, the wide range of symptoms were noticeable, as low grade of bone mineralization, high rate of fractures, increased rate of osteoporosis and intense bone associated pain [3].

Cadmium appears as a contaminant in most foodstuffs. For the average consumer, based on the typical contents of the pertinent foods, the Cd intakes of 30 μg d^{-1} has been estimated. More than 80% of this estimation was the result of cereals consumption, especially rice, legumes, pulses and wheat grains [4]. In Asia, because of its main staple crop, rice (*Oryza sativa* L.) may be the principal source of Cd intake. Rice grown in either slightly or moderately Cd-contaminated paddy soils was normally had high potential to uptake Cd and later accumulate in grain [5]. According to the joint of Food and Agriculture Organization/World Health Organization (FAO/WHO), the provisional tolerable weekly intake (PTWI) 7 μg kg^{-1} body weight (BW) per week has been established as a health-based value. For the polished rice grain, based on the PTWI value, the 0.4 mg Cd kg^{-1} was proposed by Codex Alimentarious Commission as the upper limit of acceptable concentration [6].

The Cd-contaminated soils which located downstream of the Mae Tao Creek in Mae Sot District of northern Thailand was firstly reported in 2002 by the International Water Management Institute (IWMI). In conclusion of the report, the Cd concentration in home-consumed rice would probably lead to Cd toxicity in exposed population [7–8]. A conclusion of the health impact survey of 7,697 residents in 12 villages of Phrathat Phadaeng, Mae Tao and Mae Ku Sub-districts of Mae Sot District by Swaddiwudhipong et al. [9] had revealed that 7.2% (527 residents) of the examined residents had urinary Cd levels higher than 5 μg g^{-1} creatinine, which among them, 19.9% had renal dysfunction. The highest proportion of persons who had urinary Cd levels higher than 5 μg g^{-1} creatinine was detected among those who consumed locally grown rice [10]. Since 2012, Swaddiwudhipong et al. [11] had revealed the 5-years follow-up of the 436 persons who had urinary Cd higher than 5 μg g^{-1} creatinine. The results showed significant increases in urinary β(2)-microglobulin (β(2)-MG), urinary total protein and serum creatinine, and a decrease of glomerular filtration rate (GFR). In 2012, Sriprachote et al. [12] had reported that the home-consumed rice including the preference Khao Dawk Mali 105 (KDML105) had Cd levels higher than 0.4 mg kg^{-1} - the upper limit of acceptable concentration of Cd in polish rice grains. Recently, Suwatvitayakorn et al. [13] revealed the current situation of Cd accumulating in rice grain grown on the Cd-polluted paddy fields of the Mae Tao Sub-district. The Cd concentration in households rice grains ranged from less than 0.1 – 1.941 mg kg^{-1} and less than 0.1 – 2.597 mg kg^{-1} for jasmine rice and sticky rice, respectively, and approximately 19% of the total samples each had Cd concentration higher than the Codex. Therefore, in order to minimize human intake of Cd, the reduction of Cd concentrations in rice grains is the main issue.

Chemical remediation, phytoremediation and application of soil amendments are some methods for restoring Cd-contaminated paddy soils [14–17]. In addition, screening of rice cultivars with low Cd concentrations in grain is a promising measure to decrease Cd obtained through the food web in human. For a wide range of the soil with slightly to moderately contamination, growing the crop cultivars with low accumulation of heavy metal is a wisely alternative choice.

It is well documented that there is great difference among crop species and genotypes in Cd uptake and accumulation. Genetic variations in Cd accumulations in rice grains are reported [18–21]. Sriprachote et al. [22] have studied varietal difference in Cd uptake among prevailing *Indica* rice cultivars in the contaminated paddy fields of the Pha Te Village of Mae Sot District, and concluded that RD15 cultivar had lowest Cd concentration in grain (0.197 mg kg^{-1}) compared with the prevailing cultivar KDML105 (0.58 mg kg^{-1}), and lower than the maximum concentration of 0.4 mg kg^{-1} polished rice. The results indicated that by selecting rice genotype with low-grain Cd cultivars, human Cd intake from rice consumption and the resulting health risk could be reduced. Since 2012, the authors had encouraged farmers' replacement of the prevailing cultivar KDML105 with the promising low-grain Cd cultivar RD15 in their paddy fields.

Therefore, the objective of this study was to present the long-term observation of Cd concentration in the prevailing cultivar KDML105 and the promising low grain-Cd rice cultivar RD15 grown in the contaminated paddy fields at the Pha Te Village. The result would probably be valuable for the administrations to substantiate relevant environmental policies against soil-rice Cd pollution to ensure food safety and human health.

Materials and methods

1) Study area

Six-years monitoring studies (2012 to 2017) were carried out in the same farmer's paddy field in the Pha Te Village, Phratat Phadaeng Sub-district, Mae Sot District, Tak Province, Thailand (Latitude 93° 37' 59.7", Longitude 16° 40' 34.4'') (Figure 1). In 2012, the physical and chemical properties of soil were analyzed followed the method of Page et al. [23], the mineral concentrations and soil properties are presented in Table 1. For the first years of encouragement, seeds of two rice cultivars, KDML105 and RD15 were brought from Ubonrachathani Rice Seed Center and provided to the farmer. Thirty days after sowing, seedlings of each cultivars were transplanted into the area of approximately 8,093.71 m^2 (approximately 2 acres) with the planting space of 30 cm x 30 cm during the rainy season (July to November) under the same field management. An average rainfall over Mae Sot District during July to November which was obtained from the Department of Meteorology [24], were 2.79.9, 244.9, 157.9, 218.5, 155.3 and 146.5 mm for the years of 2012, 2013, 2014, 2015, 2016 and 2017, respectively.

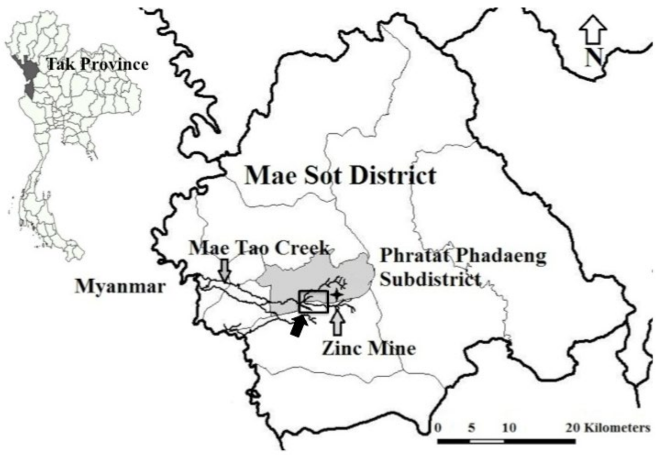

Figure 1 The grey shading and the black arrow and box denotes the study area at Pha Te Village, Phratat Phadaeng Sub-district, Mae Sot District, Tak Province, Thailand.

Table 1 Physicochemical properties of soil of the Pha Te Village paddy field, Mae Sot District

Soil properties	Value
Textural class	Loam
Sand (g kg^{-1})	370
Silt (g kg^{-1})	450
Clay (g kg^{-1})	180
pH 1:5 H_2O	6.41
EC 1:5 H_2O (µS cm^{-1})	160
Organic matter (g kg^{-1})	24.2
Available phosphorus (mg kg^{-1})	7.17
Cation exchange capacity (cmol kg^{-1})	12.4
Extractable bases (mg kg^{-1})	
Potassium (K)	67.8
Calcium (Ca)	1228
Magnesium (Mg)	119
DTPA-extractable (mg kg^{-1})	
Iron (Fe)	240
Manganese (Mn)	36.7
Copper (Cu)	3.12
Zinc (Zn)	11.0
Cadmium (Cd)	0.38
Total Cd (mg kg^{-1})	1.05

2) Rice grain sampling and preparation

Thirty sampling points were randomly collected throughout the whole 2 acres area of each cultivar. Each point, husk rice grains (grain with hull) were randomized sampling from five hills surrounding the sampling point. To minimize the variation of analytical data, especially Cd concentration, 10 sampling points were grouped together to become one sample. Finally, each cultivar had three composite samples. Samples were kept in separate plastic bags and then bring back to the laboratory. Husk rice samples were oven-dried at 70°C for 72 h to kept constant weights, then, manually milled before getting ground through a 0.40 mm (40 mesh) screen of a Thomas scientific model 3384-L40. The rice powder was stored in a desiccator until analysis.

3) Laboratory analysis

Cadmium was determined with inductive coupled plasma optical emission spectrometer (ICP-OES; PerkinElmer model Avio500) after digestion. The sensibility of ICP-OES for Cd was 1 µg L^{-1}, three measurements were performed for each sample. Briefly, about 0.2000 g of rice powder sample was weighed into the digestion tube with three replications for each sample, and 4 mL of 65% HNO_3 and 1 mL of 60% $HClO_4$ were added and digested with a block digester. Initially, the digest procedure at 80°C was undertaken for 30 min then, the temperature was slowly raised until white fumes of $HClO_4$ appear. Afterward, the temperature was increased to 205°C and digest for 30 min or until clearer solution was observed [25]. After cooling, the digested sample was filled up to 10 mL with distilled water. The SPSS version 19.0 was applied in statistical analysis of the monitoring data in each year. Mean of triplicate samples in each year were compared by *t*-test ($p<0.05$).

4) Provisional tolerable weekly intake (PTWI)

The PTWI of toxic metals is dependent on both the metal concentration in rice or meals and the amount of consumption of the respective food. The PTWI of Cd was determined using the following Eq. 1.

$$\text{PTWI} = \frac{\text{Weekly Cd intake}}{\text{Body weight (BW)}} \qquad \text{(Eq. 1)}$$

Where the weekly Cd intake (µg) is a multiple of rice daily intake (kg d^{-1}) within a week (7 d) and rice grains Cd concentration (µg kg^{-1}).

In this survey, the PTWI value (µg Cd kg^{-1} BW per week) was focusing on the adults aged during 35 – 65 years old, the most populations who have consumed rice grown in their own land. It was assumed that the average rice consumption per day was 0.268 kg which was 53.06 kg in BW for female and 58.54 kg in BW for male (National Bureau of Agricultural Commodity and Food Standards; ACFS) [26]. The PTWI were compared with the tolerable daily intake of Cd (7 µg kg^{-1} BW per week), as recommended by Codex [6].

Results

1) Concentrations of Cd in rice grain

Six-years monitoring from 2012 – 2017, the results revealed that under the same paddy field, similar farming practices and environmental condition, the Cd concentrations in grains were different between the prevailing cultivar KDML 105 and the promising low grain-Cd rice cultivar RD15 (Figure 2). In general, grain Cd concentration of KDML105 was significantly higher than the RD15 ($p<0.01$). The average Cd concentration in KDML105 grain was 0.658 mg kg^{-1}, ranging from 0.033 to 1.168 mg kg^{-1} (Table 2). The results showed that the Cd level of KDML 105 grain was higher than the criteria of CODEX, 0.4 mg Cd kg^{-1} polished grain [6]. On the other hand, grain of RD15 contained Cd of 0.127 mg kg^{-1}, ranging from 0.004 to 0.285 mg kg^{-1}, lower than the critical level. The Cd concentration of RD15 was only 20% of KDML105.

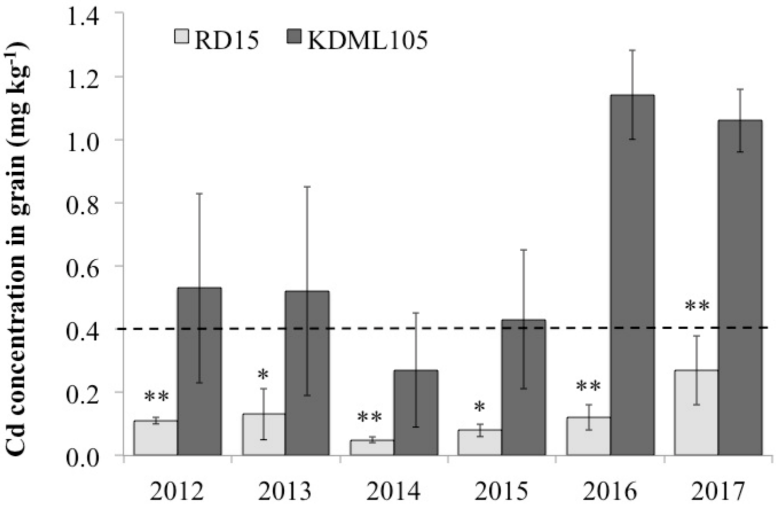

Figure 2 Grain Cd concentrations of the prevailing cultivar KDML105 and the promising low-grain Cd cultivar RD15 grown in a Cd-contaminated paddy field for the year of 2012 to 2017. The error bar indicated the standard deviation of 3 replications. The * and ** symbols indicate the significantly different to the t-test at the $p<0.05$ and $p<0.01$, respectively. The dashed line indicates the critical level of Cd, 0.4 mg kg^{-1} polished rice.

Table 2 Grain Cd concentrations of the prevailing cultivar KDML105 and the promising low grain-Cd RD15 grown in the Cd-polluted paddy fields in the Pha Te Village, Phratat Pha Daeng Sub-district, Mae Sot District, Tak Province, Thailand (n = 3)

Year	**Cadmium concentration (mg kg^{-1})**	
	KDML105	**RD15**
2012	0.53 ± 0.30[a]	0.11 ± 0.01
2013	0.52 ± 0.33	0.13 ± 0.08
2014	0.27 ± 0.18	0.053 ±0.01
2015	0.43 ± 0.22	0.081 ± 0.02
2016	1.14 ± 0.14	0.12 ± 0.04
2017	1.06 ± 0.10	0.27 ± 0.11
Mean	0.658 ± 0.360	0.127 ± 0.079
T-test	**	

Remark: [a] Data represent the mean and standard deviation of three replicates composite sample collected from thirty sampling points.

2) Dietary intake of Cd

The estimation of PTWI values of Cd for adults (35 – 65 years old) in the Cd-polluted area through consumption of rice is presented in Table 3. Based on an average grain Cd concentration of KDML105 (0.658 mg kg^{-1}) and RD15 (0.127 mg kg^{-1}), KDML105 resulted in the higher Cd through rice consumption (23.26 and 21.09 μg Cd kg^{-1} BW per week for female and male, respectively). Meanwhile, the consumption of RD15 had not only shown the lower the PTWI of Cd (4.49 and 4.07 μg Cd kg^{-1} BW per week for female and male, respectively) than those observed from KDML105 consumption, but also far lower than the FAO/WHO tolerable daily intake of 7 μg Cd kg^{-1} BW per week (Table 4).

Table 3 Estimated PTWI Cd values (μg Cd kg^{-1} BW per week) based on rice grain Cd concentrations

Rice cultivar	Gender[a]	
	Female	Male
KDML105	23.26	21.09
RD15	4.49	4.07

Remark: [a] An average rice consumption of 35 – 65 years old population at 0.268 kg per day, which was 53.06 kg in BW for female and 58.54 kg in BW for male [26].

Table 4 Estimated PTWI of Cd (μg Cd kg^{-1} BW per week) based on rice grain Cd concentrations (n = 3)

Rice cultivar	Year	Gender[a]	
		Female	Male
KDML105	2012	18.73	16.98
	2013	18.39	16.66
	2014	9.546	8.652
	2015	15.20	13.78
	2016	40.31	36.53
	2017	37.48	33.97
RD15	2012	3.889	3.525
	2013	4.596	4.166
	2014	1.768	1.602
	2015	2.828	2.564
	2016	4.243	3.846
	2017	9.546	8.653

Remark: [a] Average rice consumption of 35 – 65 years old population at 0.268 kg per day, which was 53.06 kg in BW for female and 58.54 kg in BW for male.

Discussion

According to the results, Cd levels in soils are not a directly indicator the level of Cd in rice grain. With exception of extreme contamination, as other factors such as Cd species and Cd mobility in soil, the variety of crop, farming practice and environmental conditions are an important. DTPA-extractable Cd in a study plot is 0.38 mg kg^{-1} (Table 1) this might be due to soil pH, OM and other soil conditions. Although absolute DTPA-Cd concentration found here was lower than the phytotoxically excessive level 3.0 mg kg^{-1} [27], our previous research has been revealed that more than 70% of total Cd in paddy-soils occurred primary in the carbonate and exchangeable fraction. These fractions would be released and becomes bioavailable to rice when the soil pH changes into acidic condition [28]. As presented in Figure 2, the grain Cd concentrations of KDML105 and RD15 even grown in the same field, revealed fluctuation year to year. This might be due partially to fluctuation of water level in the paddy field caused by erratic rainfalls. Flood condition reduced the concentration of Cd in rice grain, because flooding decreases the redox potential of the soil and decreases the Cd concentration in soil solution together with the pH becomes nearly neutral. Therefore, Cd ions were transformed to an insoluble form by the production of Cd sulfide (CdS), which is difficultly taken up by paddy rice. However, due to the irregular rains during the midseason, the soil becomes an aerobic and again, Cd returns to soluble ionic form which more easily taken up by rice [29]. Yanagisawa et al. [2], Huang et al. [30] and Sriprachote [31] had been reported that water management of paddy fields at the maturing, grain filling period, is important to control the grain Cd contamination. In the Mae Sot area, October is the rice ripening period and at the same time, was the end of the rainy season, thus low rainfall makes it difficult to control the water levels in the paddy fields. Although, the water management of paddy fields is effective to reduced Cd uptake by rice plants and Cd accumulation in the grain, water management is hardly to be applied to the Mae Sot paddy fields.

Concerning over safety agricultural products, selection of rice with low grain-Cd contents that can be cultivated in slightly and moderately contaminated soils had been reported. Sriprachote et al. [22] had revealed that growing on the same contaminated paddy fields in Mae Sot District, specific Thai-rice cultivars RD5 (0.108 mg kg^{-1}), RD15, KNU1 (0.125 mg kg^{-1}) and KNU2 (0.179 mg kg^{-1}) had significantly lower Cd concentrations in their grain than the prevailing cultivars KDML105 and RD6 (0.740 mg kg^{-1}). The current survey for 6-years monitoring which the authors had encouraged the farmer to grow the promising low grain-Cd rice cultivar RD15 instead of the prevailing cultivar KDML105, since 2012, in harmony with the previous research [22], under the same conditions, the RD15 had lower Cd concentration than the one determined from the KDML105. Comparison between both cultivars, the RD15 had 80% of Cd lower than the KDML105. The result indicates the stability of the genetic response to the level of Cd contamination in soil in term of the accumulation of Cd in grains. The same pattern has been previously observed in paddy rice [18–21].

Rice is the greatest source of the dietary Cd intake for the world population and especially for the Asia peoples. Therefore, Cd contamination in rice grain is a serious threat to people, because the Cd intake may cause Itai-Itai disease. The national average daily rice intake of rice is 0.268 kg for adults aged during 35 – 65 years old. According to Cd concentration in rice grains, the dietary intake of Cd through the prevailing cultivar KDML105 consumption was higher than the PTWI 7 µg Cd kg^{-1} BW per week recommended by FAO/WHO [6]. On the other hand, the estimated PTWI showed that the promising low-grain Cd RD15 could reduce the values of Cd intake to be lower than the FAO/WHO limit. Current data shows that, if Cd in rice grain is higher than 0.2 mg kg^{-1}, using the average daily rice intake of 0.268 kg and mean body weight for women and men (35 – 65 years old) of 53.06 kg and 58.54 kg, respectively, PTWI values would exceed 7 µg Cd kg^{-1} BW per week (Table 4). Therefore, based on the amount of rice consumption and average body weight in Thailand, critical level in rice grain should not be higher than 0.2 mg kg^{-1}.

A low grain-Cd accumulating cultivar, RD15, was bred through gamma-ray mutation of KDML105, which suggest that mutation may occur on some genes that control Cd accumulation in the grains. Recently, Uraguchi et al. [32] have identified a rice Cd transporter, *OsLCT1*, involved in Cd transports to grains of Japonica rice. Although the grain Cd contents of these two cultivars were different, the quality of cooked rice was similar. After cooking, both of them resulted in soft texture and natural fragrant smell. In addition, yield of both cultivars were approximately 400 kg per rai (1,600 m^2). Moreover, Sriprachote et al. [33] has reported that there was no significant effect of Cd on Fe and Zn concentration in RD15 grains, even though the RD15 had low grain-Cd accumulation, the levels of Fe and Zn still meet the human requirement. From this monitoring, the results confirm the possibility of controlling and reducing Cd concentrations in rice grains through selecting and developing the cultivar with low grain-Cd accumulation.

Conclusions

This study presents a long-term monitoring result of Cd concentration in the prevailing cultivar KDML105 and the promising low grain-Cd cultivar RD15 in the Pha Te Village, Mae Sot District. Comparing to KDML105, grain Cd concentration of RD15 was lower than the permissible limit of 0.4 mg kg^{-1} of Cd in polished rice. Moreover, the estimated PTWI of Cd through rice consumption showed that RD15 could reduce the values of Cd intake to be lower than the FAO/WHO limit (7 µg Cd kg^{-1} BW per week). From these results, it is noticeable that health risk through rice containing high Cd consumption could be reduced substantially by cultivating the low grain-Cd rice cultivar. Promising rice cultivar with low grain-Cd accumulations RD15 provide an important material in breeding programs to reduce health risk for human consumption. Breeding low grain-Cd rice could contribute an option for farmers to get through with the risk and reduce the influx of pollutants to the human food web, especially in the cases that the soil remediation and water control are hardly to be applied. Moreover, identifying a rice Cd transporter gene involves in Cd transports to grains of Indica rice is an interesting task for the future study.

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