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Effects of Organic Fertilizer on Cd Bioavailability and Cd Accumulation in Rice Grown in Contaminated Paddy Soil

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Abstract

The effects of organic fertilizer on Cd phytoavailability and distribution in rice plants (Oryza sativa L.) were examined in pot and field experiments. The results of the pot experiment show that the addition of organic fertilizer increased the oxidizable Cd fraction (F3) and decreased the soluble and exchangeable Cd fraction (F1). There was also an increase in the dry matter yield when more organic fertilizer was applied. The Cd concentrations in the rice plant parts were observed in the following order: root > stem > grain. The accumulation index from the field experiment indicates that organic fertilizer application is likely to reduce the uptake of both Cd and Zn by rice.

Keywords: Cadmium; Organic fertilizer; BCR Bioavailability; Accumulation index

Introduction

Cadmium (Cd) can be normally found as an associated trace element around zinc (Zn) mining areas. Agricultural soils in Mae Sot, Tak, Thailand, have accordingly been found to be contaminated with Cd, which has been receiving attention as an environmental concern since 2003. The Cd contamination in paddy fields and agricultural areas is believed to have originated from Zn mining activities that have been in operation upstream for over three decades [1]. It has further been found that over 90% of the rice grain samples harvested from this area contained Cd at concentrations exceeding the Codex Committee on Food Additives and Contaminants (CCFAC) Maximum Permissible Level for rice grains of 0.4 mg Cd kg^{-1} [2].

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Organic matter (OM) generally affects the accumulation and transport (and subsequent leaching) of metal ions present in soil and water. Organic matter can absorb metal ions through its ion exchange mechanism, rendering them less mobile and therefore less bioavailable. Several studies in the literature have investigated the effects of organic matter such as manure, activated sludge, and biosolid compost on altering the metal uptake of plants [3,4,5]. These mentioned organic matters have commonly been used for the immobilization of Cd in soils [5].

Organic fertilizers or compost amendments were introduced, as an additional option, based on the theory that the organic matter in the fertilizer may bind with Cd to form organic complexes and hence decrease the mobility of Cd. Moreover, an organic fertilizer can simultaneously release macronutrients and micronutrients to the soils to be further absorbed by plants and improve soil qualities by improving its water holding capacity, buffering soil pH, and increasing of the soil CEC, for example [6]. The metal speciation in paddy soil is likely to undergo changes among different moisture regimes [7]. Paddy soil is traditionally submerged and the water level is maintained until the panicles are formed; then the paddy soil is allowed to become dry before the rice is harvested. These are the dynamic changes in forms of heavy metals related to bioavailability in rice plant. Therefore, in this study, the effects of an organic fertilizer (compost) added to soil were investigated on (1) Cd immobilization in the soil phase and (2) Cd accumulation in rice (Khowdawkmali 105) biomass, including the relation between the cadmium forms in each soil fraction.

Methodology

The pot experiment was conducted at the National Center for Genetic Engineering and Biotechnology, Kamphaengsan District, Nakhonpatom Province, in the central region of Thailand. Four field plots in the Mae Sot District, Tak Province, were selected to con-duct the field experiment and were denoted as LS1, LS2, HS1, and HS2 (Figure 1), where LS1& LS2 and HS1 & HS2 represented low and high Cd concentrations in soil, respectively. The Cd contamination levels of the four selected plots were in the range of 0.3-50 mg kg^{-1}.

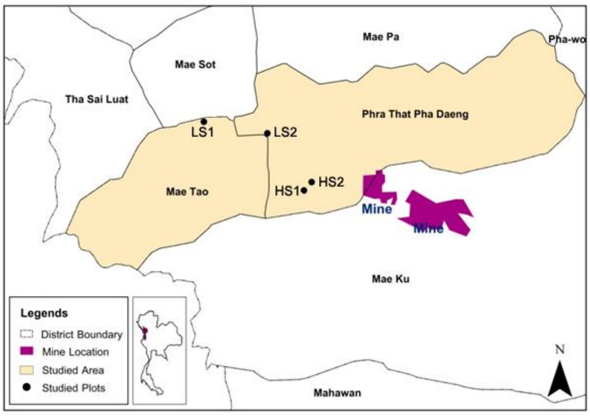

Figure 1 Locations of the field experimental sites, LS1, LS2, HS1 and HS4.

Soil sampling, preparation and analysis

Pot experiment

Soil for the pot experiment was collected from Mae Sot District and then ground, air dried, and mixed to be homogenized. Each pot containing 5 kg of soil was treated with organic fertilizer; four levels of fertilizer were used: 0 (control, C), 2 (O-1), 4 (O-2), and 20 (O-3) mg kg^{-1} soil. Soil samples were collected four times, according to the growth stage of the rice: at the initial stage (IS); 30 days after the IS, the so-called vegetative (VG) stage; when the rice formed panicles or the panicle formation (PF) stage; and at the maturation (MT) stage.

Field experiment

For the field study, two separated paddy soils represented by different Cd contamination levels were designed: one with a low Cd concentration (LS) and the other with a high Cd concentration (HS), as illustrated in Figure 1. Each field was divided into two plots: one for the control (C) and the other for organic fertilizer addition (OF) at a ratio of 3,125 kg ha^{-1}. While the pot experiment had four soil sample collection periods, in the field study, there were five.

The first and second sampling periods differed slightly. The first soil sampling was done before seedlings started, known as the background (BG) period. The second soil sampling was done once the plot had been prepared, about 2 weeks after the organic fertilizer was applied to the soil (OA). Since rice is grown by the transplanting technique in this area, during the first two soil sampling periods, rice sprouts were not transplanted into the paddy field. The third sampling period was about 30 days after transplanting, known as the vegetative growth (VG) period, which includes the seedling period; plant was age at 60 days. Then the fourth and fifth samplings were conducted at the panicle formation (PF) stage, and the maturation (MT) stage, which occurred at around 90 and 120 days, respectively.

Soil samples were collected from the upper layer (0-30 cm), air-dried, ground and homogenized by sieving through a 0.2 mm screen. Three fractions of Cd in the soil were determined through a three-stage BCR sequential extraction procedure (proposed by the Standards, Measurements and Testing Programme of the European Union, SM&T). The contaminated soils were digested by the microwave-assisted acid digestion procedure, in accordance with USEPA Method 3052 [8]. The concentrations of Cd were analyzed by a flame atomic absorption spectrophotometer.

Plant sampling, preparation and analysis

Total plant samples were collected at the MT stage from both the pots and field sites for five replications. The rice plants were washed and air dried to measure their fresh weight; then, the plant samples were separated into 3 parts: the roots, stems with leaves, and grains. Each part of the plant samples was dried prior to being grinded in a stainless steel grinder. Five gram of each above ground plant samples (roots, stems, and grains) were digested by concentrated nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) by AOAC Method 999.10 [9]. The 0.5 g of root parts were digested by 12 mL of aqua regia reagent (3:1 v/v HCl: HNO_3). The concentration of Cd in the digested solution was measured by a graphite furnace atomic absorption spectrophotometer.

Quality control and data analysis

To validate the method, the accuracy of the total digestion procedure for determining metals in the extracts was compared to the results of the CRM 025-050 (RTC) Lot No: JG025, Product of Resource Technology Corporation (RTC), USA. Five replicates of all samples and reference materials were performed. The LOD and LOQ for the GFAAS were 1.64 and 4.18 μg L^{-1}, respectively. An analysis of variance was performed using the Statistical Package for the Social Sciences (SPSS) program. Means were compared using the analysis of variance (one way ANOVA) with a significance level of $P < 0.05$. The Duncan’s New Multiple Range Test (DMRT) was performed to determine the significant difference between the treatments of organic fertilizer addition on the concentrations of Cd in plant parts and Cd fractionation in the soils in the pot experiment.

Results and discussion

Soils and organic fertilizer properties

The organic fertilizers used for this study were produced by leaves, branches and biomass residues mixed with cow manure and a mixed culture inoculum of Por Dor 1, which was produced by the Thai Land Development Department. The Cd and Zn concentrations and the percentage of OM contained in the organic fertilizer (O) are shown in Table 1. The properties of the studied soil for the pot experiment and from experimental field sites are shown in Table 2. All of these properties are considered factors that influence heavy metal mobilization. Clay, high SOM and high CEC were introduced to immobilize the toxic heavy metals.

Three fractions of Cd concentrations in the soil

Soil samples both from the pot and field experiments were collected at different periods as described earlier. Three fractions of soil were analyzed in order to evaluate the effect of the organic fertilizer on the Cd fraction in the soil. It should be noted here that in this study, it was assumed that fraction 4 (BCR4), which is considered as residue, was relatively constant (not transferable into other fractions and vice versa); therefore the following results will describe Cd fractions based on the first 3 fractions: BCR1 (F1), BCR2 (F2) and BCR3 (F3).

Pot experiment

The proportions of Cd from different fractions in the soil exhibited similar trends throughout the collection stages (Figure 2). Cd in the oxidized form (F3) fraction was expected to increase due to the addition of the organic fertilizer; however, only a tiny change was observed. During the experiment, the Cd proportions in the different fractions followed a similar trend in all the stages: F1 > F2 > F3. More than 70% of the Cd was found in a leachable and exchangeable form (F1), which leads to a higher risk of bioavailability and Cd contamination in the ecosystem. The highest level of organic fertilizer addition (O-3) provided the greatest Cd reduction of F1 in all the stages. The reducible forms of metals (F2) were normally found in the flooded condition or bound to hydrous oxides of Fe and Mn [10]. They are thermodynamically unstable under anoxic conditions [11]. The Cd in the F2 fraction significantly increased as the amount of organic fertilizer increased. The Cd in its oxidizable form (F3), which occurs when it is generally bound to organic matter, was found as the lowest fraction. However, this fraction was found to slightly increase in the VG stage, especially when the highest amount of organic fertilizer was added (O-3), showing a significant difference in F3 between the control pot and O-3 pot. Therefore, to evaluate an effect on transferring of fraction, the concentration of the organic fertilizer is an important factor. However, organic matter can degrade naturally under oxidizing conditions in water. Consequently, the Cd in F3 can be released and transform into its soluble form or reducible form, depending on the surrounding geological environment and the types of anthropogenic and natural activities that occur.

Table 1 Organic matter, Cd and Zn concentrations in the organic fertilizer amended in the study soils.

	Total Cd (mg kg^{-1})	Total Zn (mg kg^{-1})	Organic matter (%)
Organic fertilizer (O)	0.302	83.953	32.584

Table 2 Soil properties and characteristics of the pot and field experiments.

Soil characteristics	Pot experiment	Field experiment			
		LS1	LS2	HS1	HS2
pH 1:1H_2O	7.51	6.42	6.69	7.50	7.62
SOM [a] (%)	5.42	4.05	5.21	4.62	5.32
CEC [b] (meq/100g)	18.66	15.99	21.64	13.02	14.11
Soil texture [c]	Loam	Loam	Silty clay loam	Loam	Loam
- % Clay	19.77	27.44	36.99	24.84	13.63
- % Silt	44.80	47.63	47.87	42.13	47.90
- % Sand	35.43	24.93	15.14	33.03	38.47
Total Cd (mg kg^{-1})	81.89	0.32	3.97	9.35	47.03
Total Zn (mg kg^{-1})	3307.27	107.02	125.32	264.41	1936.43

[a] SOM: Soil organic matter (Walkley and Black method)

[b] CEC: Cation exchange capacity (NH4OAc, pH 7.0)

[c] Soil texture (Pipette method)

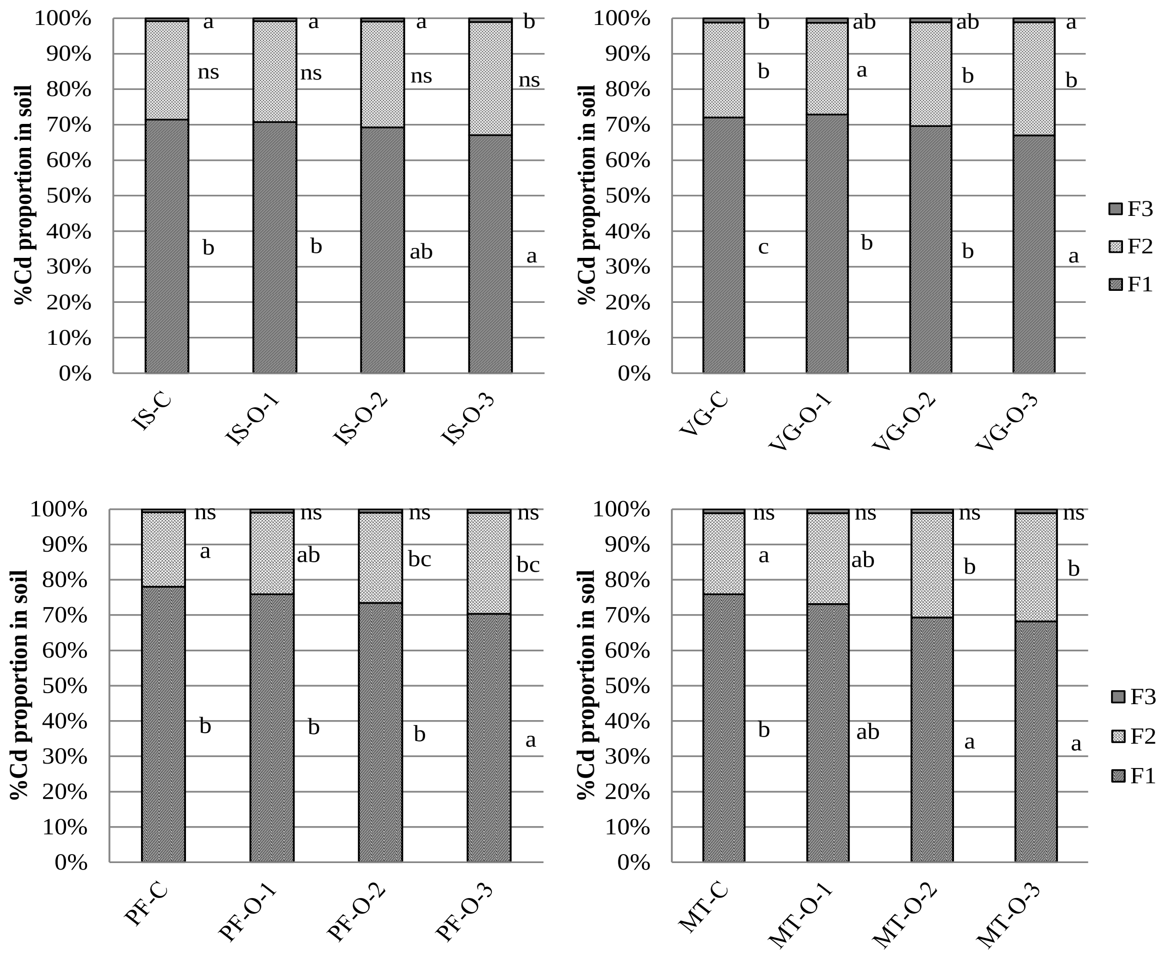

Figure 2 Cd fractionations F1, F2 and F3 in the soils with organic fertilizer at all growth stages (IS = initial stage, VG = vegetative stage, PF = panicle formation stage, MT = maturation stage); C, O-1, O-2 and O-3 represent the control (0), 2, 4 and 20 mg kg^{-1} soil, respectively.

Field experiment

Unlike in the pot experiment, in the field experiment, only one concentration of organic fertilizer was added into the four plot sites, which contained different Cd and Zn concentrations (Table 2). Each field experiment site (LS1, LS2, HS1 and HS2) was divided into two plots: one as the control, which received the same treatment but without the addition of organic fertilizer, and the other one with the organic fertilizer.

The Cd fractions from the five soil sampling periods were plotted to compare the control (C) and organic fertilizer addition (O) samples from each of the field experiment sites (LS1, LS2, HS1 and HS2) (Figure 3). The field experiment yielded different results from the pot experiment. F1 was not the dominant fraction in the field experiment that it was in the pot experiment. However, F1 and F2 were shown to be present in the same proportions, approximately 30-40%, and the combination of F1 and F2 produced the main proportion of Cd fractions, which was greater than 80%. The increase of F2 might be related to the natural existing Mn and Fe, which are rich in the studied soil. The Mn and Fe concentrations found in the soil were respectively about 50 and 120 times higher than the Cd concentration (data not shown here). These results agreed with the work of Akkajit and Tongcumpou [13], who reported that Cd, Mn, Zn and Pb have a strong affinity for Fe/Mn oxides, to which more than 30% of the total was adsorbed in the reducible fraction.

Soil organic matter (SOM) is a factor that represents the level of soil fertility, which affects Cd adsorption [14]. The organic fraction released in the oxidizable step (F3) is not considered very mobile or available since it is thought to be associated with stable high molecular weight humic substances that can possibly release small amounts of metals in a rather slow manner [15]; however, metals in the BCR3 fraction may be mobilized by decomposition processes and transferred into a more mobile fractions.

Cd concentration in plant parts

Pot experiment

The roots exhibited the highest Cd concentration as compared to the stems and grains in all treatments, including the control (Figure 4). The Cd accumulation (mg kg^{-1}) in different parts of the rice was observed as follows: roots > stems > grains. This is because the root is the part in direct contact with the soil, and all nutrients, including available forms of metals, are uptaken via the root before translocating to other parts of the plant. Consequently, heavy metals (including toxic ones) are found in higher concentrations in the roots [12]. The addition of organic fertilizer insignificantly influenced Cd accumulation in the rice plant; however, at the organic fertilizer addition rate of 20 mg kg^{-1} soil, O-3 showed a relatively lower Cd concentration in the whole plant as compared to that of the control. The average Cd concentrations of the grains obtained in pots O-1 and O-2 were slightly lower than those of the control. However, the Cd concentration in the rice grains obtained from both the treatment and control were not significantly different and all were below the Codex standard for polished rice of 0.4 mg kg^{-1}.

Field experiment

Cd was mainly uptaken and accumulated in the underground part of the plant. The maturation results show that the Cd concentrations were as follows: root > stem > grain (Figure 5). The addition of organic fertilizer tended to decrease the Cd uptake to the rice plant, especially in the low Cd contaminated soils (LS1 and LS2). The rice grain is the part of greatest concern, and it was found that only the rice grain obtained from HS2 contain a

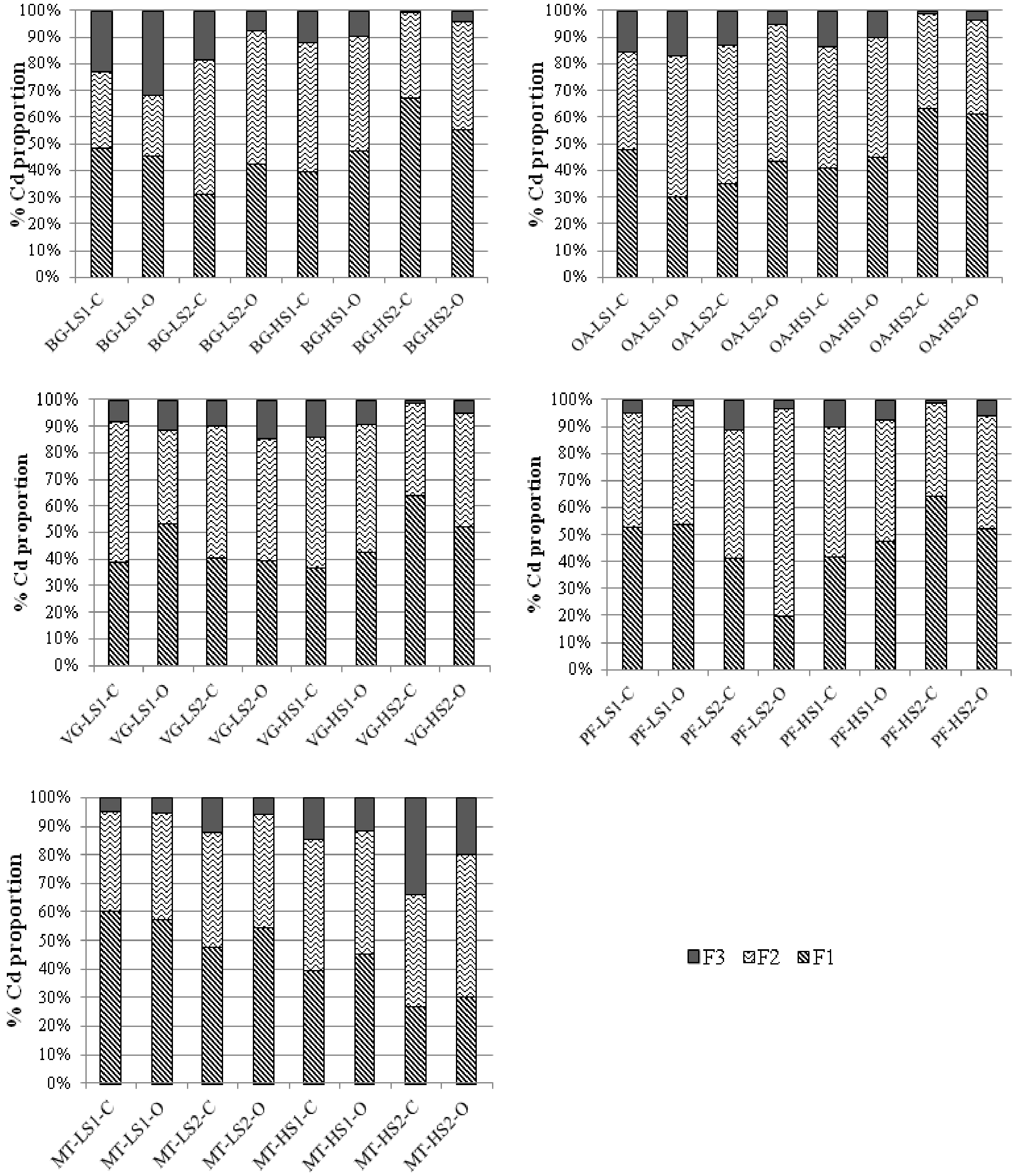

Figure 3 The percentages of Cd in the 3 fractions (F1, F2, and F3) at all five growth stages, the background (BG), organic fertilizer addition (OA), vegetation growth (VG), panicle formation (PF) and maturation (MT) stages, in comparison to the control (C) and organic fertilizer addition (O) in the four field experimental plots: LS1, LS2, HS1 and HS2

Cd concentration that exceeded the Codex standard for polished rice (0.4 mg Cd kg^{-1}). When focusing only on the rice grains, the use of organic fertilizer seems to reduce Cd accumulation.

In a study by Kashem and Singh [4], Cd and Zn concentrations significantly decreased in rice plant parts when a biosolid compost was added. This result was similar to that of Maftoun and Moshiri [16], who reported that organic waste (municipal waste compost and poultry manure) applied to soil was able to reduce Cd and Pb concentrations in rice shoots. On the contrary, the results of Bolan et al. [5] indicate that the effect of biosolid compost on Cd^{2+} adsorption was inconsistent, yet they conceded that it could greatly inhibit the phytoavailability of Cd. The addition of a biosolid increases the surface charge.

Effect of organic fertilizer application on Cd and Zn accumulation in rice plants

In order to evaluate the effect of organic fertilizer application on the Cd contaminated soil, the accumulation index introduced by Kashem and Singh [4] was used in this study to compare the control and organic fertilizer application plots. They mention that the accumulation index is a likely indicator of the relative availability of metals to a plant. It can be calculated by following equation:

Acculumation Index (AI)
= Mean metal concentration in plant / Total metal concentration in soil

In this study, the AI (%) was determined for Cd and Zn for both the control and organic fertilizer applications of the field experimental plots, as shown in Table 3. The AI values for both metals, Cd and Zn, for the low and high Cd contaminated soil plots, show that the accumulation of both Cd and Zn in the organic fertilizer application plots having AI values lower than those of the control plot (no organic fertilizer application) except HS1 for Cd accumulation. This is presumably because that organic fertilizer application was able to reduce the bioavailability of the Cd and Zn in the soil.

Table 3 Accumulation index (%) of Cd and Zn in the rice grown in the control plots and organic fertilizer application plots (OF)

Field Plot	Accumulation Index (%)			
	Cd		Zn	
	Control	OF	Control	OF
LS1	24.42	18.55	11.52	11.06
LS2	3.46	3.29	11.74	9.03
HS1	1.17	1.44	5.98	5.29
HS2	1.81	1.40	1.53	1.02

Conclusion

With regard to the pot experiment, the addition of organic fertilizer increased the SOM content. During the experiment, the proportions of Cd were observed in the following order: F1 > F2 > F3. Cd fractionation shows that the F3 fraction increased, while the F1 fraction decreased. The results from most of the experimental field plots (except for HS1) show that F1 tended to decrease after organic fertilizer application or during the background stage. However, in the following stages (i.e., the vegetation, panicle formation and maturation stages), the F1 fraction of the OF application plots tended to increase and be relatively stable. A comparison of the soil treated with organic fertilizer with the control demonstrates the positive effect the organic fertilizer had on reducing the Cd concentration in the grains, as observed in LS1 and HS2, which is a promising result that may lead to its use in practical applications. The AI results of both Cd and Zn show reductions of metal accumulation in the rice plants due to the addition of the organic fertilizer.

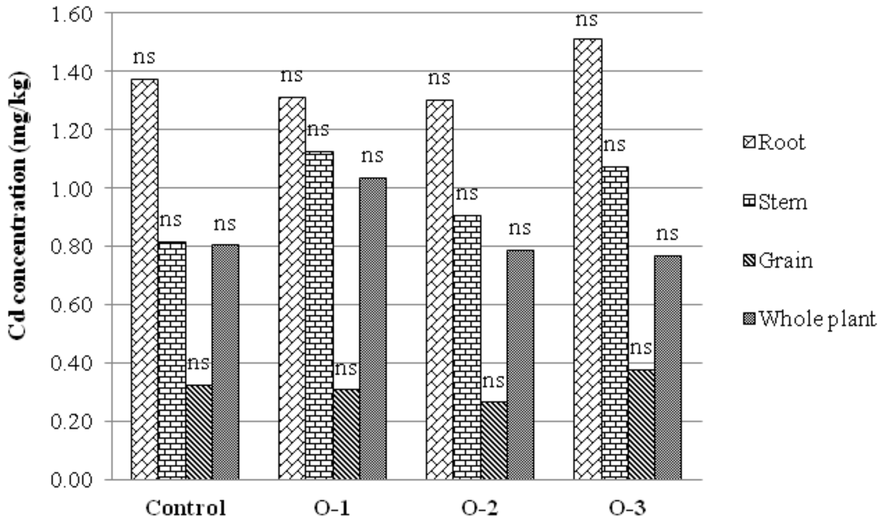

Figure 4 Cd concentrations in rice plant parts of the control and organic fertilizer addition soils.

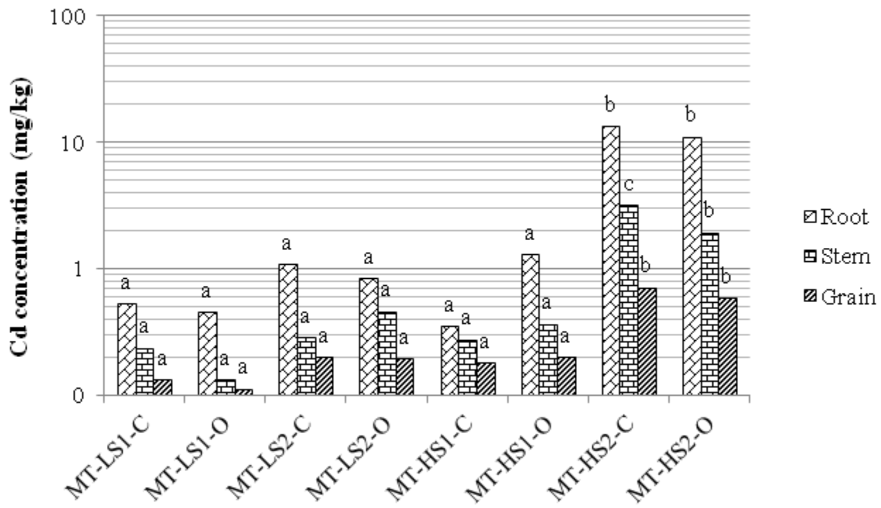

Figure 5 Cd concentrations of the rice plant parts in the maturation stage.

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