Cadmium Immobilization in the Rice – Paddy Soil with Biochar Additive

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ABSTRACT
Cadmium (Cd) is toxic for humans, but its effects on the yield and quality of rice under contaminated irrigation conditions remain uncertain. In this study, paddy soils in the Red River Delta (Vietnam) were selected for experiments with the purpose of understanding the effects of Cd contaminated irrigation water on growth, yields, and grain Cd accumulation. In addition, biochar was produced from rice husk (BRH) and rice straw for preventing Cd infiltration into rice was also studied in this experiment. A field experiment was established with applied BRH and straw into polluted paddy soil, as a result the Cd content in grains reduced significantly. The Cd contaminated soil was added to the BRH and rice straw (RS) with six ratios, including: (1) RS 2.5\%, (2) BRH 2.5\%, (3) RS-BRH: 1.25–1.25\%, (4) RS 5.0\%, (5) BRH 5.0\%, (6) RS-BRH: 2.5–2.5\% (w:w). Besides, three content levels 0.01–0.05–0.5 mg/L of Cd in irrigation water were applied throughout crop season. The results showed that the Cd accumulation in rice was in the following order: roots > stems > seeds. With 3 contaminated irrigation levels which were applied, the Cd concentrations of 0.05 mg/L and 0.5 mg/L affected plant height and yield. However, the Cd content in grains under contaminated soil condition can be controlled from 82.47–83.94\% by applying a BRH ratio from 2.5–5\% (w:w).

Keywords: Cd contaminated soil, rice grain, Cd accumulation, biochar, straw.

INTRODUCTION
Cadmium (Cd) is a very toxic metal and listed as a top group carcinogen (Lu et al., 2019) by a serious extent of bioaccumulation (Banerjee et al., 2020). Due to the effect of pollution irrigation water, Cd can be found commonly in paddy soils and other agricultural soils. Irrigation water contaminated by wastewater can be main reason for Cd contamination in agricultural soils, in addition, the occurrence of Cd in soils may be due to the abuse of fertilizers employed to improve production (Di Pierro et al., 2017). In soils, Cd is an easily soluble heavy metal and is becoming mobile in soil solutions than other heavy metals (Li et al., 2015). This property leads to easy Cd intake by plants through roots, and translocation into different plant parts before the accumulation process (Adil et al., 2020).

Among plant species, rice (Oryza sativa L.) can absorb easily Cd through these plants’ roots system, as a result, Cd can translocate to stalks and continue its accumulation in grain (Rizwan et al., 2017). Compared with other metals, such as lead, copper, zinc and arsenic, the amount of Cd in the soils although much smaller, but is easily absorbed by plants such as wheat and rice. This is explained by the higher enrichment coefficient thus, Cd from soil solution moves to rice plants easier than other metals (Zhu et al., 2016). This is the reason that leads to Cd possibly becoming a more common toxic heavy metal found in rice (Rao et al., 2018).

Cd accumulation in rice seeds poses a major threat to the health and safety of humans. The Cd pollution on a large scale in agricultural land poses potential health risks for the people who eat the food which was produced from that region. Up to 20–40 μg Cd per day may be tolerated (Sebastian & Prasad, 2014). Once people begin to eat the agricultural products and drinking water which were contaminated with Cd on a daily basis, this leads to the accumulation of Cd and symptoms
of chronic Cd poisoning will appear after a certain period of time. Significant Cd accumulation in humans through food, can also make people suffer from diseases due to damaging the lungs, liver, kidneys, bones and reproductive organs. Cd is also toxic to the immune and the cardiovascular systems (Tian et al., 2012). Rice is the staple food for Vietnamese people and over 2 billion people in Asian populations (Honma, 2017), as well as for over 50% of the world population (Rizwan et al., 2016). However, in some soil rice products from certain agricultural regions were discovered to be polluted with Cd and other metals in recent years, especially in densely populated agricultural regions using wastewater as irrigation water (Xie et al., 2017).

In the world of agriculture soil, there are about $2.35 \times 10^2$ m$^2$ in a pollution situation by heavy metals that include the occurrence of Cd (Bermudez et al., 2012). In Asia, the situation of Cd contamination is occurring widely in some rice-growing areas, such as in China, the Cd content of grains has been observed to increase in recent years (Zhu et al., 2016). In China alone, about $2.786 \times 10^8$ m$^2$ of agricultural soils are seriously polluted by Cd (Liu et al., 2017), accounting for one fifth of the total arable land, with Cd content to exceeding the standard by about 7% (Rao et al., 2018).

The occurrence of Cd in agricultural land is due to polluted irrigation water resources, fertilizers, pesticides, activity of mineral mining and usage of fossil fuels. The climate change leads to irrigation water scarcity in the dry season in many cultivated regions became, forcing them to make use of wastewater as a source of main irrigation. This leads to the accumulation of Cd metal in cultivated soil, which is the cause of Cd entering the crops and threatens human health (Zhu et al., 2016). In Vietnam, most of the irrigation systems for rice were faced water scarcity during the dry season, so many regions must use wastewater as irrigation sources. In the Red River delta, irrigation systems of Nhue river, Cau Bay river, Bac Hung Hai... are the main irrigation sources for rice systems in this region. In addition to the role of providing irrigation water, these irrigation systems are the places that will receive a large amount of wastewater from domestic and production activities, potentially risking heavy metal pollution, including Cd. A survey at 61 locations scattered throughout the North showed great differences in the Cd content in rice grains between low-lying and upland areas (Bui et al., 2020).

While Cd was not observed in most rice grain samples from upland fields, it was found in rice in lowland areas with an average concentration of 0.033 ppm. The main cause of the difference is Cd pollution from irrigation water sources. In upland fields, the type of irrigation water that was used was primarily rain water which was distributed from reservoir systems. In contrast, the irrigation system in the delta includes surface water, rain water and wastewater, so the water quality can be polluted. Once Cd is present in irrigation water, it will accumulate in agricultural lands and enter the rice plant (Peng et al., 2019).

Si is considered as being useful improving the Cd stress phenomenon for crops (Rizwan et al., 2016, Yu et al., 2016). Many plant species have high Si accumulation capacity, for example rice can accumulate Si to more than 10% of its dry weight (Majumdar et al., 2019). To produce 1 ton of rice, rice plants absorbed NPK nutrients and about 80–103 kg of SiO$_2$ (FAO). As a result, the Si content accounts for 27 kg per ton of rice husk and about 40 kg of Si per ton of rice straw. Si is stored in the cell wall in the form of phytoliths, if the straw returns to the agricultural soil it will re-add the amount of Si absorbed from the soil. Si is an essential element for healthy growth and development of plants; however, the available Si content in soil was very low compared to total Si. When the Si accumulation increases in soil and plants, the physical and biochemical protection can improve in Cd contaminated soil (Rizwan et al., 2016). The reason is because Si can absorb Cd ions which contributed to reducing Cd toxicity in paddy soil (Guerriero et al., 2016). Furthermore, Si can be used to decrease soil heavy metal toxicity and reduce the transportation of Cd into rice plants (Li et al., 2015) with role of alteration of plant cellular mechanisms and biochemical interactions with the external growth condition (Wang et al., 2004). The available Si in the soil can reduce the flexibility of heavy metals by the focusing on silicate complexes, Si polyphenol complexes, and insoluble metal silicates in soil (Shim et al., 2014). The additions of Si into soil can decrease the content of available heavy metal in metal-contaminated soil by their joining in the silicates precipitate, also bound with organic matter in soil (Etesami & Jeong, 2018). In addition, the application of biochar rice husk and rice straw could effectively increase the pH of the soil which will reduce the available Cd form in the soil solution. Besides, the application of crop
straw and biochar can improve the Cd uptake by plants, because of ligands in organic matter connects with Cd in soils solution (Huang et al., 2017; Xu et al., 2016) which is contributed for reducing the available Cd.

Therefore, evaluating Cd accumulation and limiting Cd content in rice grains in contaminated soil is the main purpose of this research to reduce its negative effects on humans. Biochar is increasingly often applied for soil improvement to increase the soil fertility and reduce heavy metal toxicity in plants. Thus, the biochar from rice husk and straw manure was used in the experiment.

In this experiment, the biochar from rice husk was burned under a low oxygen condition. The experiment of planting rice in pots system was conducted under greenhouse conditions with paddy soil which was contaminated by Cd. Biochar was added to planted soil with different rates, including 2.5 and 5% (w/w), to soil improvement. Besides, another experiment was conducted with irrigation water contaminated with Cd with three content levels including 0.01, 0.05, and 0.5 mg/L of Cd. The results showed that plant growth and yield reduced while the Cd concentrations were increased in irrigation water. The Cd accumulation in rice parts was in the following order: roots > stems > grains. The Cd accumulation in grains occurs due to Cd pollution in irrigation water; however, it can be controlled by biochar from rice husk and straw which contributed to the Cd concentration reduction in plants and grains.

MATERIALS AND METHODS

Experimental design

The experimental soil samples were collected from the upper 0–20 cm layer of a paddy field in Gia Lam, Ha Noi (21°14′33″ B, 105°48′13″ E), Vietnam. The experimental soil properties were fluvial with the pH\textsubscript{KCl} from 6.6–6.8, soil organic carbon (SOC) content 35.3 g kg\textsuperscript{-1}, total N of 3.42 g kg\textsuperscript{-1}, total organic N of 29.36 mg kg\textsuperscript{-1}, and cation-exchange capacity (CEC) of 21.23–22.41 mmol/kg, total Cd of 0.001 mg/kg, clay of 37.4%, silt of 42.2% and sand of 20.4%.

The experiments were designed in a field experimental area at the Vietnam National University of Agriculture, Gia Lam, Ha Noi, Vietnam (21°0′35″ B, 105°49′29″ E), from May 2019 to May 2021 in field with an area of 0.03 hectares in size with 4 rice crops including (two spring and two summer seasons). The soil samples were air dried and ground to pass through a 2-mm sieve. Afterwards, about 10 kg of soil was transferred into a pot with 30 cm diameter and higher of 40 cm. The total of pots were 50, including 15 pots for first experiment treatment, 30 pots for second experiment treatment, the control formula (CF) was 5 pots. Each experiment formula was repeated 3 times.

In the first experimental treatment: Cd accumulation in parts of rice affected by contaminated soil. The experiment was designed for contaminated water irrigation with three levels of Cd content (0.01–0.05–0.5 mg/L) in irrigation water throughout the crop season. Using drip irrigation system with the amount of water irrigation of 1000 mL every 3 days.

The second experiment treatment: Biochar rice husk and rice straw in limiting the Cd uptake of rice plant. In this experiment, the biochar from rice husks was used. The biochar was produced under anaerobic heating condition at 400–450°C for 4 hours. Rice straw after harvest was dried before being cut into 1 cm long pieces.

Cadmium was added to experimental soil by chemical Cd(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O. An exact amount 0.14 grams of Cd(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O was dissolved in 5 liters of distilled water before mixing well with 10 kg of soil sample in each pot. The total Cd content in the experiment soil after mixing was determined at 5.125 mg/kg. The experiment started immediately after the addition of Cd metal into the soil. The soil contaminated Cd was added biochar rice husk (BRH) and rice straw (RS) materials with ratios including: (1) RS 2.5%, (2) BRH2.5%, (3) RS-BRH 1.25–1.25%, (4) RS 5.0%, (5) BRH 5%, (6) RS-BRH 2.5–2.5% (w:w). Each experimental treatment was repeated three times, and a total of 30 pots were used.

The control formula (CF) without biochar and irrigation water without Cd was used.

Variety

A Bac Thom No 7 (BT) rice variety, a pure Chinese rice variety, selected and purified by Thai Binh Seed Corporation (Vietnam), which widely grown in the northern provinces of Vietnam, was chosen for the crop experiments. The BT variety is a healthy growing variety with the growing time in Spring crop 125–135 days, Winter season 105–110 days.
Reagents

In order to extract Cd from the soils and plants, the solutions of 30% HClO₄, 98% HNO₃, and 37% HCl (Xichlong, China) were used. Cadmium was added into soil as Cd(NO₃)₂·4H₂O (Merck).

Fertiliser

Fertiliser was used in the experiment with nitrogen/phosphorus/potassium (NPK) ratio of 125 g of compost + 1.25g N + 0.75 g P₂O₅ + 0.75 g K₂O per pot. A pesticide, namely Nouvo 3.6EC was used for the prevention of disease. For the field experiment, the amount of fertiliser was applied as 10 tons compost + 60 kg N + 60 kg P₂O₅ + 60 kg K₂O per ha.

Analysis

After harvesting, rice plants were collected, including plants, seeds, and roots. The plants and roots were washed with tap water and were dried at temperatures under 70 °C for 72 h.

Plants sampling: the Cd contents in plants, grains and roots were determined by digesting wet method by a concentrated solution of HNO₃-HClO₄ (3:1, v:v) and analysis on atomic absorption spectrophotometer (Ryan et al., 2001).

Soil sampling

Soil was taken after harvesting, oven dried (40°C), and sieved (2 mm). The physical and chemical characteristics of soil samples including CEC, organic carbon content (OC), phosphorus (TP) and nitrogen (TN) content were used by the methods of ammonium acetate, Walkley-Black, and Kjeldahl for analysing.

Growth indicators

Including plant height and yield were determined after harvest in the greenhouse experiment.

Statistical analysis

All statistical analyses for the result data from experiment were compiled by Microsoft Excel version 5.5 (Microsoft, USA). Each value represented the average of three replications. The data was subjected to analysis of variance (ANOVA), and significant differences in mean values were determined using Duncan’s multiple range test (P < 0.05).

RESULTS AND DISCUSSION

Cd accumulation in rice from contaminated irrigation water

The Cd accumulation in rice plant including Cd contents in roots, rice stalks and grains in three stages including 5th week, 9th week and after harvest. In addition, the Cd accumulation in paddy soil was determined (Figure 1).

The Cd content in paddy soil under pollution irrigation is observed after the harvest. With three Cd content levels in irrigation water applied, the results indicated that the Cd content in soil of irrigation treatment of Cd 0.5 is higher than 150–170 times, compared to treatments of Cd 0.01 and Cd 0.05, respectively. The Cd content in soil of Cd 0.05 treatment is higher than 2 times compared to the Cd 0.01 treatment. Thus, the irrigation water with Cd 0.5 level causes significant Cd accumulation in soil.

Besides, the experiment results of the four crop seasons indicated that the amount of Cd accumulation in the stalks and roots increases from 5th week to 9th week to the harvest. At the harvest, the Cd content in soil and roots can be seen the highest. Infiltration of Cd into rice plant at the moment in the order of roots > stems > seeds can be observed in all of treatments. With the treatment of Cd 0.01, the Cd content in grain with 0.001 ppm corresponds to low accumulation level. However, the Cd content in stalks and roots is much higher compared to Cd in grain, which were greater 27 and 334 times, respectively.

The Cd accumulation was observed to increase significantly in the treatments with higher Cd concentration in irrigation water. The Cd absorption rate in grain of Cd 0.05 treatment increased 64 times compared to Cd 0.01 treatment with Cd content in rice grain of Cd 0.05 treatment is 0.064 ppm. In addition, the Cd content in stalk and roots in 0.05 treatments shows an increase of 3.15 times and 10.18 times, respectively, compared to the Cd 0.01 treatment. Compared with the 0.01 Cd treatment, the Cd 0.5 treatment dramatically increases the Cd content in some rice parts, such as root and grain with 0.173 ppm Cd in roots, and 0.012 ppm Cd in grains. However, in this experiment, the total Cd concentration in the roots region was the highest in grain, followed by stalks.
The Cd accumulation in paddy soils was observed after the harvest. The Cd accumulation rate of the paddy soils in the Cd 0.5 and Cd 0.05 treatments is 244.81 and 14.0 times higher compared to the 0.05 treatment, respectively. The reason for these results, is that the uptake of Cd from soil and irrigation water by rice plants depends on the Cd concentration and rice bioavailability property (Roth et al., 2006). In the transportation mechanic, the entry of Cd metals into plant cells occurs through trans-membrane carriers to take up micronutrients such as Mg, Ca, Fe, Zn and Cu (Roth et al., 2006). From polluted paddy soil, Cd can easily be taken up by roots system, after that, Cd was transported to other rice parts (Uraguchi et al., 2009). Other plant species have different uptake level of Cd but at certain accumulation concentrations, it can induce phytotoxicity (Verbruggen et al., 2009).

**Effect of Cd on rice growth**

**Plant height**

To clarify the effect of Cd on the plant growth rates for each experiment, growth parameter measurements were performed after every week. Measurements were based on plant height with the number of rice plants observed per season being 60, of which 45 plants were showed normal growth, 15 had a slow-growth rate at the end of the observed period (Fig. 2).

Overall, in the first 3 weeks, the plant height of rice was in the order of Cd 0.01 > CF > Cd 0.05 > Cd 0.5. In the next 5 weeks (4th to 8th week), the height of the rice plant that had the Cd 0.05 treatment was superior, with an average increase from 8.1–15.1% compared to the CF, while the plant height of rice in the Cd 0.01 treatment was only over the CF 1.8%. There was a variation in plant height of the Cd 0.01 treatment, compared...
to CF. The mean height of plant in Cd 0.5 was lowest in this stage. From the 9th to the harvest, the plant height in Cd 0.01 and the CF were the same (P > 0.05). The plant height of CF was higher than the Cd 0.05 treatment by 11.2–14.2%, and higher than the Cd 0.5 treatment by 15.2–17.3%.

The results from experiment show that the Cd concentrations 0.05 mg/L and 0.5 mg/L in irrigation water cause disparate plant height development compared to CF.

Critical leaf concentrations of Cd from 5 to 10 μg Cd g⁻¹ dry matter can be toxic to most plants (White & Brown, 2010). Cd can inhibit the leaf photosynthesis process through affecting the chlorophyll biosynthesis and metabolic mechanism (Dong et al., 2005). For rice plants, Cd dramatically reduced the growth of roots and shoots (Zhang et al., 2002), therefore reducing shoot growth and decreasing nutrient uptake (Khan et al., 2016).

**Grain yield**

Cd is not a nutrient element for plants, so Cd accumulation in large concentrations will be harmful to plant growth and have an effect on yield (Bari et al., 2019). Accumulation of Cd does not only have a negative influence on nutrient uptake but also decreases the growth and yield of rice (Bari et al., 2019). The results indicated that the yield of the CF is higher than others with 83.04 g/pot, while the yield of the Cd 0.05 and Cd 0.5 treatments reduced by 5.9% and 7.6%, respectively. These results are consistent with the studies of
evolutionary, nutritional, and environmental reasons (Wang et al., 2016; Tran & Popova, 2013) of all non-essential heavy metals, cadmium with the effect of Cd accumulation on the process of photosynthesis and absorption of nutrient elements leading to a decrease in grain productivity (Fig. 3).

Cd inhibits the plant nutrient uptake, as well as influences growth development and contributes to nutrient deficient in plant (Khan et al., 2015). Cd can cause nutrition deficiency in roots region by competing absorption with minerals which have similar properties to Ca and Mg (Catalan et al., 2006). In addition, bioaccumulation of Cd in plants can cause the change of N, P metabolism mechanic which affects physiological functions and the growth of plants (Chaffei et al., 2004). The decrease in N and P content of rice also other crop plant were also reported under the condition of Cd contamination of soil (Dražić et al., 2004). Moreover, Cd was proven to limit the uptake of micronutrients such as Cu, Fe, Zn, and Mo in vegetable and crop plants (Street et al., 2010).

Biochar from rice husk and straw on limiting Cd uptake of rice plant

Rice husk and straw are more popular materials in agricultural countries, including Vietnam. It was used to produce the compost manure for crop cultivations to help enhance soil properties, including nutrition, moisture and remarkably increased soil pH (Catalan et al., 2006). Straw and the biochar which was produced from rice husk have role as amendments which may increase the pH value in soil and reduce the mobility of Cd metal via absorption Cd in its components. In addition, the silicon content was noted in straw and biochar. The spectroscopic results show that the biochar from rice husk ash has a higher Si content than the biochar from rice straw (Fig. 4).

In this experiment, 6 treatments were applied for biochar with a purpose to limit Cd uptake into rice plants (Table 1).

Soil pH

Both of BRH and RS had strong effects on the soil pH. Relative to CF, both BRH and RS application could effectively increase the pH of soil. The pH of soil was 16.64% higher compared to CF. Meanwhile, RS increased pH by 8.54% (2.5% treatment) and 13.34% (5% treatment), respectively (P < 0.05). On average, the pH of all treatments increased by 12.46%, compared to CF (Fig. 5).

The condition of increasing the pH of the soil resulted in the availability of Cd to form hydroxide precipitations Cd(OH)$_2$ as a result decreased the exchangeable Cd content in soil (Yuan et al., 2011). Besides, the increase of pH promotes the formation of CdCO$_3$, which may be the reason for the increase of the content of carbonate-bound Cd in soil (Lu et al., 2019).

Si weight in soil

Silicon-rich amendments in soil increased the plant Si concentrations under added straw and biochar. Silicon concentrations in the BRH 2.5% was

| Table 1. Some properties of biochar |
|-------------------------------------|
| Properties       | Biochar from rice husk (BRH) | Rice straw (RS) |
| pH               | 8.7–8.9                      | 8.6–8.8         |
| CEC              | 49.8–55.2 mmol/kg            | 45.6–47.8 mmol/kg |
| Porosity         | 57.24–60.14%                 | 55.32–56.13%    |

Figure 4. Infrared spectra of DOM in biochar from rice husk (left) and straw (right)
11.05–16.28% higher the CF under conditions of adding biochar, 2.5% and 5% respectively (p < 0.05). Independent of mixing ratio of biochar, the treatment of husk amendment with the BRH 5% increased the Si content by 4.7% relative to the BRH 2.5% (p < 0.05). Straw Si contributed to Si-rich amendments in soil (p < 0.05) being 1.12 – 1.2 times higher compared to the CF. In addition, the RS5% treatment resulted in significantly higher soil Si than CF 20.48% (Fig. 6).

**Cd content in grain**

Total Cd concentration is 5.125 ppm, available Cd content is 0.048 ppm in experiment soils. There is competition of Si with Cd for plant uptake and translocation to grain (Seyfferth et al., 2016) leads to high grain Si, soil Cd is also high. The Si accumulation in rice can help to reduce the Cd content in rice plant and limit the transfer Cd from straw to rice grain (Liang et al., 2007) (Fig. 7).

Biochar supplementation experiments indicated quite positive results in limiting Cd accumulation in rice plants. Specifically, the mixing ratio of RS 2.5% treatment reduced the Cd content in rice by 47.92% compared to the CF. With the same mixing ratio (2.5%, w:w), the biochar from rice husk reduced 67.89% of Cd in rice, compared with CF. Thus, the results of mixing ratio of 2.5% by weight showed that biochar from rice husk strongly reduces the Cd accumulation in rice. This result is demonstrated in the figure.

**Figure 5.** The soil pH of treatments of the BRH and the RS

**Figure 6.** Si (%wt) in soils of treatments of the BRH and the RS
organic materials for soil, which affects the absorption and desorption mechanism of Cd (Wu et al., 2007). Biochar and crop straw put into soil a solution at a large quantity of dissolved organic matter. This organic matter is easy to chelate and create a complex with ligands, including Cd and then reduce the availability of Cd content in soil (Chen & Chen, 2002).

The results of this experiment indicated that the adding biochar and crop straw, effectively reduces the uptake of Cd in rice plant and rice grains. Previous studies showed that the addition of biochar can inhibit the Cd absorption into the rice plant. Biochar and rice straw could limit the Cd content in different parts of rice, might be based on the reason that there is an increase of Cd content joined and bound with organic matter (Bian et al., 2013). For this complex, the reaction contributes to reduced absorption metabolism of Cd by rice plants.

CONCLUSIONS

The accumulation of Cd in rice plant under the condition of polluted Cd irrigation in this study indicated the following order: roots > stems > grain. The application of biochar and straw manure into paddy soils could decrease the toxic Cd accumulation in rice grains. The results of this study showed that Si from biochar rice husk and straw can successfully limit the Cd content in rice grain on the paddy soils contaminated by Cd.
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