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Accumulation of cadmium in the grain of *Oryza sativa* varieties in the Philippines

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Abstract

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Cadmium contamination of paddy soil is a widespread problem in rice-growing areas. For this study, soil containing 1.60 mg/kg of cadmium, the maximum allowable concentration, was used. The cadmium resistance levels (extra vigour) of seventeen rice varieties were tested using a seedling vigour test, fourteen days after seeding. The cadmium-resistant varieties were then transplanted into severely cadmium-contaminated soil under controlled conditions. The accumulation of cadmium in the grain and the biological accumulation coefficient of the cadmium-resistant rice varieties were also determined in both unpolished and polished grains, using the maximum allowable level of cadmium in rice grains (0.15 mg/kg). The soil used in the study contained 10.23 mg/kg of cadmium and was categorised as severely contaminated. Seven rice varieties were identified as cadmium-resistant even in this soil. However, three varieties were found to have low cadmium accumulation in unpolished grains (0.1 mg/kg), a low biological accumulation coefficient value (0.01), zero cadmium content (0.00 mg/kg) and no biological accumulation coefficient value (0.00) in their polished grains. Therefore, it is recommended that cadmium-resistant rice varieties which accumulate low levels of cadmium in their grains (i.e. cadmium-excluder rice) be planted in severely cadmium-contaminated paddy soil.

Keywords: bioaccumulation, cadmium excluder, grain, heavy metals, phytostabilisation.

INTRODUCTION

Some metals and metalloids are poisonous even at low concentrations (parts per billion) and have a comparatively high density. Heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), and chromium (Cr), as well as the metalloid arsenic (As) are identified as toxic even at low concentrations (Duruibe et al., 2007; WHO, 2017). One of the significant ecological problems facing the globe today is the contamination of the environment with heavy metals resulting from human activity. Heavy metals are present in the soil, but human activity and geological processes can increase their concentration, which can be harmful to the environment, animals, plants, and people (Alloway, 2009; Masindi & Muedi, 2018).

Among the heavy metals, cadmium (Cd) is one of the most toxic to humans, animals, plants and the environment. Contamination of soils with Cd is the result of previous and present manufacturing and farming activities (Aoshima & Horiguchi, 2019). Aside from inhalation of Cd fumes and dust, eating or smoking with contaminated hands is also a serious way of taking Cd (Piscator et al., 1976; Satarug & Phelps, 2020). Eating cadmium-contaminated food from crops can lead to cadmium poisoning or accumulation in the body, which can result in various diseases. Crops with hyperaccumulating plants are plants that significantly absorb heavy metals in high concentrations. Hyperaccumulating crops, including rice, can expose humans to heavy metals, including cadmium (Martin & Griswold, 2009). Fortunately, several techniques have been developed to reduce the absorption of heavy metals from the soil, as they are known to have low accumulation of heavy metals and are known as heavy metal excluder plants. This method is modest, inexpensive, and has no adverse environmental impact in terms of stabilising and reducing pollutant bioavailability (Jadia & Fulekar, 2009).

Rice (*Oryza sativa* L.) is an essential food for more than 50% of the Earth's population, and 90% of the total global production originates from Asian countries with more than 110 000 cultivated varieties (Fukagawa & Ziska, 2019). In 2024, the top rice-producing countries were China (27%), India (27%), Bangladesh (7%), Indonesia (6%), Vietnam (5%), Thailand (4%), and 8% production from the Philippines, Myanmar, Pakistan, and Brazil (USDA, 2025). In the Philippines, 19.3 million metric tons of rice were produced in 2024, making it the staple food of Filipinos (DA-Philippines, 2025). However, one of the problems that arises in rice farming is the contamination of heavy metals, specifically cadmium. Cadmium is one of the abundant contaminants in rice grains in rice-consuming countries. Understanding the levels of these metals can help guide agricultural practices and food safety regulations (Shimbo et al., 2001).

In 2001, due to heavy rainfall brought about by a typhoon, the mine tailings ponds leaked from the upper part of the Agno River, and heavy metal-contaminated sediments, including cadmium, were buried one metre below the rice paddies in the Municipalities of San Manuel and Binalonan, the Province of Pangasinan, the Philippines (Cordillera Peoples Alliance, 2007).

Aside from cadmium-resistant (extra vigorous) rice varieties, low grain-cadmium accumulating rice (cadmium-excluder rice varieties) is the best option to ensure that the rice grains, exceptionally unpolished and polished grains, are low in cadmium content or cadmium-free. At present, several rice varieties can be potentially cultivated in severely cadmium-contaminated rice paddy soils. However, cadmium-resistant and low-grain-cadmium-accumulating rice are not yet known. Hence, this study was conducted to identify cadmium-resistant, low-grain-cadmium-accumulating rice varieties (cadmium excluder rice varieties) in severely cadmium-contaminated rice paddy soil. The following questions were addressed in severely cadmium-contaminated rice paddy soil: (1) How to identify the cadmium-resistant rice varieties? (2) How to determine the low cadmium accumulation in unpolished and polished grains of the cadmium-resistant rice varieties? (3) How many rice varieties are identified as cadmium-excluding rice varieties? (4) What is more advisable to eat, the unpolished grain or the polished grain of the cadmium-resistant rice varieties?

MATERIALS AND METHODS

Selected rice varieties

The study aimed to identify *Oryza sativa* L. varieties that are resistant to cadmium and accumulate low levels of cadmium in their grain in severely cadmium-contaminated soil under controlled environmental conditions.

In the Philippines, rice varieties or rice cultivars (Rc) are certified by the National Seed Industry Council and are named NSIC Rc. Seventeen rice varieties were acquired from the Philippine Rice Research Institute. The rice varieties were grouped into Japonica rice cultivars, characterised by short grains (NSIC Rc 304, 414, 482, 484, and 584) and Indica rice cultivars, characterised by long grains (NSIC Rc 160, 222, 338, 402, 462, 506, 508, 510, 512, 528, 556, and 604).

Identification of cadmium resistance using the seedling vigour test

Seventeen rice varieties were screened using a seedling vigour test for up to 14 days after seeding. The facility consisted of plastic boxes measuring 60 cm in length, 40 cm in width, and 20 cm in depth. The soil used in the study was severely cadmium-contaminated, containing 10.23 mg/kg of cadmium. The seeds were sown 10 cm apart along the width of the plastic box. The pre-germinated seeds for each rice variety were sown 5 cm apart along the row. The Standard Evaluation System for Rice (IRRI, 2013) was used as the parameter for the seedling vigour test, using the following scale: Extra vigorous (1-EV): very fast-growing, 5–6 leaf stage and two or more tillers; vigorous (3-V): fast-growing, 4–5 leaf stage and 1–2 tillers; normal (5-N): 4 leaf stage; weak (7-W): stunted growth, 3–4 leaf stage, thin and no-tillers; and very weak (9-VW): stunted growth and yellowing of leaves. The extra vigorous (1-EV) rice varieties were identified as cadmium-resistant. They qualified for the cadmium accumulation grain test.

Test for cadmium accumulation in grain

Cadmium-resistant rice varieties were identified and used in the experiment. Cadmium-contaminated soil weighing 500 kg was collected, placed in a wooden box measuring 5.0 m in length, 1.0 m in width, and 0.25 m in depth, and soaked with water. The box was then covered with a plastic sheet to prevent leakage. After soaking the soil for a week, it was thoroughly mixed until saturated. Healthy 10-day-old seedlings of the cadmium-resistant rice varieties were planted in the box and tended for three days after transplanting. A water level of 3 cm was maintained in a controlled environment (Fig. 1). The sample plants were fertilised with 120 kg of nitrogen, 60 kg of phosphorus and 90 kg of potassium during the dry season. Upon reaching maturity, the grains of the entries (i.e. the cadmium-resistant rice varieties) were separated and processed into unpolished rice (containing bran) and polished rice (without bran).

Analysis of cadmium concentration

Dried and pulverised grain samples (200 g) were baked at 300°C for three hours, after which the temperature was increased to 500°C for a further two hours until the ash turned white. The plant ash samples were mixed with 5N HNO_3 (3 mL). The plant samples were dried on a heat-regulated hot plate. Concentrated HCl (3 mL) was then added to the samples until they were dry, after which they were baked for one hour to dehydrate the silica. The plant ash samples were then removed from the hot plate and allowed to cool. Then, 5 mL of 2N HNO_3 was

Fig. 1. *Oryza sativa* cultivated in wooden boxes in a controlled environment with severely cadmium-contaminated soil.

Fig. 2. Site of collection of soil samples in the Municipality of San Manuel, in the Province of Pangasinan on the Island of Luzon in the Philippines (Google Maps, 2024).

added and stirred with a rubber stirrer to dissolve the salt residue. The plant tissue extract was filtered and stored in a polypropylene container. The plant tissue extract was analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Nutrients, physicochemical properties, and cadmium concentration of the soil samples

Collection of the soil samples

The soil samples used in this study were collected at a depth of 20 cm from sampling sites in San Manuel Municipality, Pangasinan Province, the Philippines. The precise geographic coordinates of the sampling locations are 16.074947° N latitude and 120.668934° E longitude (Fig. 2). The samples were analysed and categorised using the Chiroma et al. (2014) contamination and pollution index for cadmium. This scale categorises soil as slightly contaminated if the concentration of cadmium is between 0.16 mg/kg and 0.40 mg/kg, moderately contaminated if the concentration is between 0.40 mg/kg and 0.80 mg/kg, severely contaminated if the concentration is between 0.81 mg/kg and 1.20 mg/kg, and very severely contaminated if the concentration is between 1.21 mg/kg and 1.60 mg/kg. The maximum allowable cadmium concentration in soil is 1.6 mg/kg (Crommentuijn et al., 2000).

Soil analysis

Various standard analytical methods were employed to assess the nutrient status of the soil samples (Miura et al., 1995). Total nitrogen (N) content was determined using the Kjeldahl method, a widely accepted procedure involving digestion with sulfuric acid and subsequent distillation to measure organic nitrogen and ammonium (Kirk, 1950). The amount of available phosphorus (P), which indicates the amount of phosphorus readily accessible to the soil, was assessed using the Olsen method (Elbasiouny et al., 2020). Available potassium (K) was measured using a flame photometry method that quantifies potassium based on its emission of light at a specific wavelength (Pratt, 1965). Additionally, soil pH and electrical conductivity, which are indicators of soil reaction and salinity, were measured in a 1:1 soil-to-water suspension. The organic carbon content, reflecting the amount of organic matter and the potential for microbial activity in the soil, was determined using the Walkey-Black method (Sikora & Moore, 2014).

The soil sample contains 1.34% organic matter, which suggests a moderate level of organic content. The total nitrogen content is 0.069%, indicating relatively low nitrogen reserves. Available phosphorus is at a level of 5.0 mg/kg, which limits plant growth. Finally, the available potassium content is 0.40 cmol(+)/kg, which is a moderate range for plant availability. In terms of texture, the soil is classified as silty clay and is composed of 48.3% clay, 40.7% silt and 11.0% sand. The soil sample's cation exchange capacity is 32.51 cmol(+)/kg, indicating a high capacity to hold essential nutrients (Miura et al., 1995). The cadmium content of the soil sample was recorded as 10.23 mg/kg, based on the compilation by Crommentuijn et al. (2000).

To extract cadmium from the soil sample, the aqua regia digestion method was employed. This method involves a mixture of concentrated HNO_3 and HCl in a 1:3 ratio. The concentration of cadmium in the digested samples was analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES), a highly sensitive technique for detecting and quantifying cadmium and other heavy metals in soil samples.

Data assessment

The accumulation of cadmium in the grains of cadmium-resistant rice varieties was compared using the maximum allowable level of cadmium in rice grains, which is 0.15 mg/kg. Rice varieties with a cadmium content lower than or equal to the maximum permissible level are low-grain cadmium accumulators or cadmium excluders (Fu et al., 2015).

The biological accumulation coefficient is the relationship between the cadmium concentration in rice grains (both unpolished and polished) and the total cadmium concentration in soil. The biological accumulation coefficient of unpolished and polished grains of cadmium-resistant rice varieties was determined by dividing the cadmium concentration in the grain (mg/kg) by the cadmium concentration in the soil (mg/kg).

Unpolished and polished grains from cadmium-resistant rice varieties with a biological accumulation coefficient value lower than 1.0 are considered low-grain cadmium accumulators or cadmium excluders. In comparison, a value higher than 1.0 is regarded as a high-grain cadmium accumulator or hyperaccumulator (Hakeem et al., 2015).

Statistical analysis

The cadmium accumulation in the grain and the biological accumulation coefficient of unpolished and polished grains of cadmium-resistant rice varieties were determined with three replications. Microsoft Office Excel 2017 was used to determine the means and standard deviation. The Kruskal-Wallis test in *RStudio* software was used to determine significant differences ($p < 0.05$) and effect size estimates among groups with different medians of cadmium content in unpolished and polished grains of the different cadmium-resistant rice groups.

RESULTS

Cadmium-resistant rice varieties

The cadmium resistance of the rice varieties was determined using the seedling vigour test in severely cadmium-contaminated soil for up to 14 days after seeding. Seven of the 17 rice varieties were identified as extra vigorous or resistant in severely cadmium-contaminated soil (NSIC Rc 414 and Rc 482, Japonica rice cultivars; NSIC Rc 528, Rc 604, Rc 222, Rc 508 and Rc 512, Indica rice cultivars). This was in an irrigated lowland environment. These varieties produced two tillers and five leaves and were categorised as extra vigorous (1-EV) or cadmium-resistant. Conversely, ten rice varieties (NSIC Rc 304, Rc 484, Rc 584, Japonica rice cultivars; NSIC Rc 338, Rc 462, Rc 556, Rc 160, Rc 402, Rc 506 and Rc 510, Indica rice cultivars) were categorised as weak (7-W) or cadmium non-resistant (Fig. 3, Table 1).

Table 1. Assessment of *Oryza sativa* varieties according to the seedling vigour scale 14 days after seeding. Values are the mean ± SD (n = 5). Rice variety numbers (Rc) are provided according to the National Seed Industry Council (NSIC). Abbreviations of the seedling vigour scale: 1-EV – extra vigorous, 7-W – weak

Variety	Rice cultivar	Tiller number	Leaf number	Seedling resistance and vigour scale value
Rc 160	Indica	0 ± 0.00	2 ± 0.45	Non-resistant, 7-W
Rc 222	Indica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 304	Japonica	0 ± 0.00	2 ± 0.71	Non-resistant, 7-W
Rc 338	Indica	0 ± 0.00	2 ± 0.55	Non-resistant, 7-W
Rc 402	Indica	0 ± 0.00	2 ± 0.00	Non-resistant, 7-W
Rc 414	Japonica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 462	Indica	0 ± 0.00	2 ± 0.71	Non-resistant, 7-W
Rc 482	Japonica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 484	Japonica	0 ± 0.00	2 ± 0.71	Non-resistant, 7-W
Rc 506	Indica	0 ± 0.00	2 ± 0.45	Non-resistant, 7-W
Rc 508	Indica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 510	Indica	0 ± 0.00	2 ± 0.00	Non-resistant, 7-W
Rc 512	Indica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 528	Indica	2 ± 0.00	5 ± 0.00	**Resistant, 1-EV**
Rc 556	Indica	0 ± 0.00	2 ± 0.71	Non-resistant, 7-W
Rc 584	Japonica	0 ± 0.00	2 ± 0.45	Non-resistant, 7-W
Rc 604	Indica	2 ± 0.00	5 ± 0.00	Resistant, 1-EV

Cadmium accumulation in grains of the resistant varieties

Lower cadmium accumulation was observed in polished rice grains compared to unpolished rice grains with bran (Table 2). Among unpolished (with bran) grains, low cadmium accumulation was observed in three varieties (NSIC Rc 222, Rc 414 and Rc 508), containing 0.1 mg/kg of cadmium. The highest accumulation was observed in one variety (NSIC Rc 604), with a concentration of 0.6 mg/kg. Interestingly, no cadmium was detected in three varieties of polished rice grains (NSIC Rc 222, Rc 414 and Rc 508). In comparison, a cadmium accumulation of 0.1 mg/kg was recorded in four cadmium-resistant rice varieties (NSIC Rc 482, Rc 512, Rc 528 and Rc 604). A significant difference was observed in at least one group median of the unpolished (H = 18.94, p = 0.004) and polished (H = 17.00, p = 0.009) grains of the different cadmium-resistant rice varieties using the Kruskal-Wallis test.

The unpolished grains of the three cadmium-resistant rice varieties (NSIC Rc 222, Rc 414 and Rc 508) recorded the lowest biological accumulation coefficient value of 0.01 (Table 3). In contrast, the highest value of 0.06 was recorded in one variety (NSIC Rc 604). No biological accumulation coefficient value was recorded in the polished grains of the three varieties (NSIC Rc 222, Rc 414 and Rc 508). Four cadmium-resistant varieties (NSIC Rc 482, Rc 512, Rc 528 and Rc 604) exhibited a biological accumulation coefficient value of 0.01 in their polished grains.

Table 2. Cadmium accumulation in unpolished and polished grains of cadmium-resistant rice varieties compared to the maximum allowable level of cadmium in rice grains. Values are mean ± SD (n = 3). The rice variety numbers (Rc) are provided according to the National Seed Industry Council (NSIC)

Variety	Cadmium accumulation in grain (mg/kg)	
	Unpolished	Polished
Rc 222	0.1 ± 0.00	0.0 ± 0.00
Rc 414	0.1 ± 0.00	0.0 ± 0.00
Rc 482	0.4 ± 0.05	0.1 ± 0.01
Rc 508	0.1 ± 0.00	0.0 ± 0.00
Rc 512	0.5 ± 0.05	0.1 ± 0.00
Rc 528	0.4 ± 0.09	0.1 ± 0.01
Rc 604	0.6 ± 0.05	0.1 ± 0.00

DISCUSSION

The present study identified cadmium-resistant rice varieties with low grain cadmium accumulation, also known as ‘cadmium-excluding rice’. The seedling vigour test, cadmium accumulation in the grain and the biological accumulation coefficient were used to identify these varieties. The seed vigour test measures the ability of seeds to produce normal seedlings in

Fig. 3. Morphological appearance of seedlings of rice varieties with different levels of resistance: (A) non-resistant rice seedlings with stunted growth, no-tillers and yellowing leaves, and (B) resistant rice seedlings with two tillers and five leaves.

severely cadmium-contaminated soil. Some rice varieties (seven out of seventeen) exhibited characteristics of cadmium resistance (extra vigour) without affecting the growth and development of tillers and leaves. However, most of the rice varieties evaluated (ten out of seventeen) are non-resistant, as indicated by weak characteristics such as stunted growth, no-tillers development and single-leaf formation (Foolad et al., 2007). Cadmium-resistant rice varieties have extremophyte characteristics that enable them to survive and evolve in adverse environments, such as leaf hydration, freezing stress, temperate deserts, highly acidic volcanic soils, high levels of salt or desiccation, and high altitudes, including contaminated soil containing heavy metals. Non-resistant rice varieties are categorised as

Table 3. The biological accumulation coefficient values of cadmium accumulation in unpolished and polished grains of cadmium-resistant rice varieties. The rice variety numbers (Rc) are provided according to the National Seed Industry Council (NSIC)

Variety	Biological accumulation coefficient value	
	Unpolished	Polished
Rc 222	0.01	0.00
Rc 414	0.01	0.00
Rc 482	0.04	0.01
Rc 508	0.01	0.00
Rc 512	0.05	0.01
Rc 528	0.04	0.01
Rc 604	0.06	0.01

having a genetic makeup that cannot cope with harsh environments. Most of the rice varieties used in the study were non-resistant (Kumar et al., 2021; Barak & Farrant, 2016; Fan et al., 2018; Akshitha et al., 2020; Nagasawa et al., 2023; Gajardo et al., 2024).

In addition to cadmium resistance, low cadmium accumulation in grain is one of the most effective ways to reduce cadmium mobilisation in the environment. The current study found that three rice varieties (NSIC Rc 222, Rc 414 and Rc 508) had low levels of cadmium accumulation in both unpolished and polished grains, compared to the maximum allowable level of cadmium in rice grain (0.15 mg/kg). Low cadmium accumulation in both types of rice grain is an indicator that the grain is safe for human consumption (Fu et al., 2015). By contrast, the unpolished rice grain of the cadmium-resistant varieties contained higher levels of cadmium than the polished grain, as cadmium accumulates in the bran (outer layer). In contrast, the bran and germ layers are removed by milling in the production of polished grain. This could also be due to the ability of these varieties to retain cadmium in the root zone of the rice plant, thereby preventing it from accumulating in the upper part of the plant, particularly in the grain portion (Pignattelli et al., 2012; Jadia & Fulekar, 2009). Possible biological mechanisms include precipitation, sorption, metal valence reduction and complexation, which are present in the three rice varieties (Ghosh & Singh, 2005). At the cellular level, low-grain cadmium-accumulating rice is capable of phytostabilisation, such as the sequestration of metal ions into vacuoles and root exudates and their binding to the cell wall and chelation by binding molecules (Ginn et al., 2008; Kumpienė et al., 2012; Gerhardt et al., 2009). Xu & Wang (2005) and Chaignon et al. (2002) have reported that Poaceae family plants, such as rice, secrete phytosiderophores – stable complexes of amino acids that stabilise cadmium uptake and play a crucial role in phytostabilisation. Furthermore, certain rice plants express genes that limit cadmium uptake (Chen et al., 2023). The exclusion of cadmium in the upper part of rice plants may be due to cadmium-resistant genes (Hussain et al., 2021).

According to Liu et al. (2003) and He et al. (2006), rice cultivars or varieties exhibiting notable genetic variation in terms of cadmium uptake limitation are an essential source of functional alleles and genetic advancement. The effectiveness of phytostabilisation, or the accumulation of low cadmium levels in grain, depends on organic matter, redox potential, pH and temperature (Chaignon et al., 2002).

The biological accumulation coefficient is used to evaluate how effectively a plant accumulates and translocates metals. Rice varieties with a BAC value greater than 1.00 are considered cadmium hyperaccumulating plants and are, therefore, unable to phytostabilise. Conversely, rice varieties with a BAC value below 1.00 are low-grain cadmium accumulators and are recommended for use in severely cadmium-contaminated rice paddy soil (Yanqun et al., 2005; Sun et al., 2009). In the present study, the BAC was used to evaluate high and low cadmium accumulation in the unpolished and polished grains of seven cadmium-resistant rice varieties (NSIC Rc 414, Rc 482, Rc 528, Rc 604, Rc 222, Rc 508 and Rc 512) (Yoon et al., 2006; Li et al., 2007; Xu et al., 2022). These results suggest that the capacity of various rice varieties to absorb and translocate cadmium from the soil varies. The cadmium-resistant rice varieties identified in the present study exhibited cadmium accumulation in grains with biological accumulation coefficient values lower than 1.00 in both unpolished and polished grains. The cadmium content of polished and unpolished grains is crucial information for determining the safety of rice for human consumption.

CONCLUSION

Seven rice varieties (NSIC Rc 414, Rc 482, Rc 528, Rc 604, Rc 222, Rc 508 and Rc 512) were identified as being resistant to cadmium in soil with severe cadmium contamination. However, only three of these varieties (NSIC Rc 414, Rc 222 and Rc 508) exhibited low cadmium accumulation in both unpolished and polished grains. Therefore, they can be planted in rice fields with severely cadmium-contaminated paddy soil. Polished grains are recommended over unpolished grains.

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