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Factors affecting cadmium toxicity to rice germinated in soils collected from downstream areas of abandoned zinc mines

P. Chanpiwat^{1,2}, A. Numprasanthai^{2,3,*}

¹ Environmental Research Institute, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

² Center of Excellence in Environmental Innovation and Management of Metals, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

³ Department of Mining and Petroleum Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

| ARTICLE INFO | ABSTRACT | |
|---|--|--|
| Article History: Received 24 March 2023 Revised 01 July 2023 Accepted 11 August 2023 | BACKGROUND AND OBJECTIVES: Cadmium conta concentration 19 times the national food standard at s been reported since 2005. These cultivated rice grain increased the risk of renal dysfunction in residents. D cadmium accumulation in rice should be considered toxicity and accumulation of cadmium in rice cultivate heen reported this study was conducted to investig | miniation in rice grains with a maximum ites downstream of zinc mines in Thailand has ns are consumed by local residents and have ecreasing negative health effects by reducing d. Since the soil characteristics affecting the ed in cadmium-contaminated soils have never the the soil characteristics affecting the plant |
| Keywords: Fractionation Mae Tao Mobility Phytotoxicity Plant availability Seed germination Thailand | Availability and mobility of cadmium in paddy soils ar rice seed germination and accumulation in rice. METHODS: The study area is the Mae Tao Subdist downstream of abandoned zinc mines in northwest were reported to produce rice grain with cadmium of cadmium in rice (0.4 milligrams per kilogram) were is collection. The physicochemical characteristics of the cation exchange capacity, potential of hydrogen, orga chemical speciation and concentration of plant-avai of cadmium to rice and the cadmium accumulatio germination of Khao Dok Mali 105, a popular rice va study area. FINDINGS: Total cadmium concentrations of 0.20 to soils, with 64 percent of all samples containing value in agricultural soils. Up to 74.2 percent and 99.5 perc of mobile- and plant-available cadmium, respectively reductions in the number of seeds germinated and | It is a solution of the solution of the plant is the impacts of these soli characteristics on rict, Mae Sot District, Tak Province, located ern Thailand. A total of 36 paddy fields that contents exceeding the national standard for randomly selected for composite soil sample soils, including soil texture, redox potential, nic matter, total cadmium concentration, and lable cadmium, were analyzed. The toxicity n ability in rice were assessed through the ariety for cultivation and consumption in the 89.87 milligrams/kilogram were found in the s greater than the national background value sent of total cadmium was found in the forms y. Plant-available cadmium toxicity to rice was |
| DOI: 10.22034/gjesm.2024.01.10 | positively affected by the concentrations of exchange concentrations of plant-available, exchangeable, car affected the accumulation of cadmium in germinate available cadmium was the main factor responsible for CONCLUSION : Based on the overall analyses of sc plant availability of cadmium in soils and its toxicit immobilization of plant-available cadmium in soils to soils is recommended. In addition, oxidizing soil cultivation to reduce the phytoavailability of Cd in soil | able, plant-available and total cadmium. The bonate-bound, and total cadmium strongly d roots. Cluster analysis showed that plant- r high cadmium accumulation in rice. il characteristics affecting the mobility and y and accumulation in germinated rice, the by adding organic matter-rich amendments conditions should be maintained during rice ls. |
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| *Corresponding Author: | | |
| Email: <i>Apisit.Nu@chula.ac.th</i> | | |
| Phone: +66 2218 8114 | | |
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INTRODUCTION

Cadmium (Cd) is principally considered a naturally occurring metal in most environmental samples, including igneous rocks, soils, sediments, freshwater, seawater and crops. Cd is ranked as the 64th most abundant element in the Earth's crust, with an average concentration of 0.15 to 0.20 milligrams per kilogram (mg/kg) (Adriano, 2001; Nurhasanah et al., 2023). Although Cd is used in various manufacturing industries, such as the automotive, pigment, coating and plating, plastics (Sabilillah et al., 2023), and battery industries, its usage has drastically decreased since the first discovery of Itai-itai disease in Japan, in which an epidemiological study showed a positive correlation between the disease prevalence rate and the levels of Cd contamination in paddy soils (KMU, 2023; WHO, 2019; Adriano, 2001). In general, the main source of Cd contamination in the environment is anthropogenic activities, including industrial production processes (e.g., mining and smelting of ferrous and nonferrous metals, cement production, application of Cd-containing fertilizer and municipal sewage sludge, disposal and recycling of electronic waste and municipal waste incineration) (WHO, 2019; Nordberg et al., 2018). Of all environmental media, soil is expected to be the major sink of Cd (Adriano, 2001). Once released into the soil, Cd can be absorbed and accumulate in plants at toxic levels, affecting their growth and eventually causing detrimental health effects to humans via the ingestion of contaminated edible plants. In principle, Cd uptake by plants is generally controlled by various soil characteristics as well as plant species. Plant uptake of Cd normally decreases as the soil potential of hydrogen (pH), cation exchange capacity (CEC), oxidation-reduction potential (ORP), and organic matter (OM) increase. On the other hand, a greater amount of Cd uptake by plants is observed in response to higher total and available concentrations of Cd in soil. After uptake, Cd is translocated and accumulates in several plant parts. Since Cd is a nonessential plant nutrient, its accumulation in plants can cause phytotoxic effects, including general growth reduction, leaf curling, chlorosis, necrosis, leaf yellowing, and lowered productivity and yield (Khanna et al., 2022; Haider et al., 2021; Zhao and Wang, 2020). The phytotoxicity of Cd mainly depends on Cd concentration and bioavailability, exposure time, plant species, and plant stage (Khanna et al., 2022; McLaughlin et al., 2021; Samimi, 2024). Rice can accumulate higher amounts of Cd than other staple food crops (Zhao and Wang, 2020). In addition, as Cd is highly soluble in soil, which enhances its transfer to the food chain, its accumulation in grains to levels that can cause potential human health impacts has been reported worldwide (Hussain et al., 2021; Zhao and Wang, 2020). Therefore, reducing Cd bioavailability, uptake, and translocation to grains is expected to be a principal strategy to lower Cd accumulation in grains to a safe level for consumption as well as to protect public health. Since 2005, studies have reported Cd contamination in rice grains in Thailand, especially in the areas downstream of the country's most abundant zinc (Zn) deposit areas in Tak Province, as a result of high Cd background concentrations in the soil and the irrigation of rice using Cd-contaminated water (Srisawat et al., 2021; Chanpiwat et al., 2019; Simmons et al., 2005). The highest Cd concentrations determined in agricultural soils and rice grains were 300.9 mg/kg and 7.7 mg/ kg, respectively (Chanpiwat et al., 2019; Simmons et al., 2005). Due to the extremely high levels of Cd contamination in agricultural soils and in rice grains, which are consumed by local residents, extensive research, particularly monitoring studies of Cd contamination in environmental media and human health impact assessments of Cd exposure through rice consumption, has been conducted in the area (Srisawat et al., 2021; Sriprachote et al., 2020, 2012; Suwatvitayakorn et al., 2020; Chanpiwat et al., 2019; Somprasong, 2019; Kosolsaksakul et al., 2018, 2014; Nishijo et al., 2014; Simmons et al., 2005). Briefly, total Cd concentrations in agricultural soils and rice grains exceeding the background concentration in agricultural soil (1.7 mg/kg) and the standard for Cd in polished rice (0.4 mg/kg) have been reported. In addition, more than 40% of local residents were found to have increased risks of renal dysfunction due to Cd-contaminated rice consumption (Nishijo et al., 2014). As a consequence, reduced rice cultivation and a shift toward nonfood crop production, especially sugarcane, have been proposed to local farmers. However, these approaches were not successfully adopted in the long term due to the lower market price of sugarcane than rice. In addition, local residents mainly rely on their homegrown rice for daily consumption (BOD, 2012). Thus, rice production is still the main crop produced in the



Fig. 1: The research framework of the current study

area. Although several studies, as mentioned earlier, have been conducted in the area, studies on the soil characteristics affecting the plant availability of Cd in paddy soils and the effects of plant-available Cd on rice growth have never been conducted. It was hypothesized that i) soil pH, redox potential (Eh), CEC, and OM would be the soil factors controlling the mobility and availability of Cd in soils, and ii) the higher the total and mobile Cd concentrations in the soil were, the higher the plant-available Cd concentrations and their negative effects on rice growth would be. The results obtained from this study are expected to lead to appropriate mitigation strategies for local farmers and people in other Cdcontaminated areas to reduce the phytotoxicity and accumulation of Cd in rice cultivated in soils with elevated Cd concentrations. Therefore, the aims of the current study are to i) investigate the physical and chemical characteristics of Cd-contaminated paddy soils, ii) determine the soil characteristics affecting the plant availability of Cd in soil, and iii) investigate the effects of plant-available Cd in soil on rice growth. This study was carried out in the Mae Tao Subdistrict, Mae Sot District, Tak Province, and at Chulalongkorn University in 2021. A schematic diagram showing the scope of the study is shown in Fig. 1.

MATERIALS AND METHODS

Information on the study area

The study area is the Mae Tao Subdistrict, Mae Sot District, Tak Province, located in northwestern Thailand (Fig. 2). Since this area is rich in primary and secondary Zn deposits, Zn mines yielding 110,000 metric tons/year were operated in the area until 2017 (Chanpiwat et al., 2019; Sriprachote et al., 2012). Following the first report by the International Water Management Institute (IWMI) on Cd contamination in agricultural soils and rice grains in 2005, the Department of Primary Industries and Mines (DPIM) conducted a comprehensive survey on Cd contamination in the area. Particular attention was given to the agricultural areas along Mae Tao Creek, a main source of irrigation water. Since Mae Tao Creek flows through the mine areas, elevated sediment Cd concentrations of up to 7.86 mg/kg have been found (Weeraprapan et al., 2015). In addition, the runoff carrying Cd-laden sediment from the mine areas to the creek during rainfall was reported to increase the contamination level in the creek (Somprasong, 2019). As a result, elevated soil Cd concentrations greater than the background Cd concentration in agricultural soils of 1.7 mg/kg were found. According to a survey conducted by the DPIM, agricultural soils in the area



Fig. 2: Geographic location of the study area in the Mae Tao Subdistrict in Thailand, background Cd concentrations in agricultural soils and sampling locations

were categorized into areas with elevated (3 to 30 mg/kg) and high (>30 mg/kg) total Cd contamination (Chanpiwat *et al.*, 2019), as shown in Fig. 2. Rice cultivation is the most popular agricultural practice in the wet season. Cultivation of other crops, such as corn, soybean, and Chinese parsley, is normally adopted in the dry season. The survey also reported a total of 58 paddy fields producing rice grains with total Cd concentrations exceeding the national and Codex standards for total Cd in polished rice of 0.4 mg/kg (Chanpiwat *et al.*, 2019).

Sample collection

Considering the number of paddy fields (58 sites) that produced rice grains containing total Cd concentrations higher than the food standard (0.4 mg/kg) (Chanpiwat *et al.*, 2019), a total of 36 paddy fields were randomly selected as the sampling sites (Fig. 2) with a 90% confidence level that these areas could represent all paddy fields that produced Cd-contaminated rice grains. A composite soil sampling technique was applied in this study. At each sampling site, surface soil samples within a 15 centimeter (cm) depth were randomly collected from 5 sampling

points using a shovel. An area of 10'10 square centimeters (cm²) was marked as a subsampling point. Thus, approximately 2 kg of composite soil sample was thoroughly mixed and collected in a clean plastic bag before delivery to the laboratory.

Soil preparation and analyses

Samples were air-dried and turned over for 3 days in a laboratory. Afterward, the samples were dried in a hot air oven (105 degrees Celsius (°C)) for 16 hours to completely remove soil moisture. Afterward, the samples were ground using an agate mortar and a pestle before sieving to obtain 2-millimeter (mm) soil particles. Finally, the homogenized samples were placed in a clean plastic bag and stored in a desiccator until further analysis.

Analyses of physical and chemical characteristics of paddy soils

The physicochemical characteristics of the soil samples, including soil texture, Eh, CEC, pH, OM, total Cd concentration, chemical speciation and plantavailable Cd concentration, were analyzed following the methods summarized in Table 1. According to

| Soil characteristic | Soil sample amount used for analysis (g) | Method of analysis | Reference |
|------------------------|---|--|-------------------------------|
| Soil texture | 5 | Bouyoucos hydrometer method | DOA, 2010 |
| (sand, silt, and clay) | | | |
| Eh | 10 | Mixing with deionized water (1:2 weight per weight (w:w)) and Eh measurement using a glass electrode (ORP meter) | LDD, 2010 |
| CEC | 5 | Ammonium saturation method | DOA, 2010 |
| рН | 10 | Mixing with deionized water (1:2.5 w:w) and pH measurement using a glass electrode (pH meter) | LDD, 2010 |
| OM | 1 | Walkley Black modified acid-dichromate digestion | DOA, 2010 |
| Total Cd | 0.5 | Aqua regia | Chanpiwat <i>et al.,</i> 2010 |
| Cd speciation | 0.5 | Tessier sequential extraction | Tessier <i>et al.,</i> 1979; |
| | | | Chanpiwat <i>et al.,</i> 2010 |
| Plant-available Cd | 10 | Diethylenetriaminepentaacetic acid (DTPA) soil extraction | Lindsay and Norvell, 1978 |

Table 1: Methods of soil sample analyses

the different extraction methods, three different categories of Cd, as shown in Table 1, were categorized in this study. Each category of Cd indicates different aspect of Cd contamination in soils. Total Cd was used to indicate gross contamination. The chemical speciation of Cd was used to indicate the mobility of Cd under different environmental conditions. Finally, plant-available Cd was used to indicate the concentration of Cd in soil that is readily taken up through the roots of plants. The concentrations of Cd were quantitatively analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Plasma Quant 9000 Elite, Analytik Jena). The limit of quantitation was 5 micrograms per liter (μ g/L). The accuracies of soil sample digestion for total Cd determination and ICP-OES analysis were validated with standard reference material (SRM) 1944 (New York/New Jersey waterway sediment) and SRM 1643e (trace elements in water) from the National Institute of Standards and Technology (NIST). The results of digestion method validation (86.3%) and guality assurance and quality control of the ICP-OES analysis (95.0%) were within ±15% of the certified values. All tests were carried out in triplicate. All glassware and plasticware used for the experiments were previously rinsed with 10% volume per volume (v/v) nitric acid (HNO₂) and deionized water prior to use. Analyticalgrade chemicals were used in all experiments.

Seed germination test

The seed germination test is a simple toxicity

assessment that is initially used to indicate the potential toxic effects of metals on both plants and humans (Pokorska-Niewiada et al., 2018; Bae et al., 2014). The toxicity of plant-available Cd extracted from the soil samples (Table 1) to the germination of Khao Dok Mali 105 (KDML 105) rice, a popular rice variety for cultivation and consumption in the study area, was assessed in this study. Prior to the test, seed disinfection (Tornuk et al., 2011), comprising soaking in 400 parts per million (ppm) sodium hypochlorite (NaOCl) for 30 minutes and rinsing with deionized water to remove the residual disinfectant, was performed. The germination test was conducted according to Pokorska-Niewiada et al. (2018); Bae et al. (2014) and Walter et al. (2006) with some modifications for 96 hours. In brief, 5 milliliter (mL) of plant-available Cd solution that was extracted from each soil sample was poured into a plastic petri dish (10 cm in diameter and 1.5 cm in depth) containing No. 1 Whatman filter paper. Afterward, 15 disinfected grains were uniformly placed onto the filter paper without touching each other. The dish was then incubated at 25°C in the dark for 96 hours. In addition, deionized water was used as a control in the germination test. Germination tests of each soil sample were conducted with five replicates. After 96 hours of incubation, the number of seeds with visible breakage of the seed coat was counted as the germinated seeds, and the root length was measured as the key parameter of rice growth. Finally, the percentage of relative seed germination (%RSG),

percentage of relative root growth (%RRG), and germination index (%GI) were calculated using Eqs. 1 to 3 (Walter *et al.*, 2006).

$$%RSG = \frac{\text{Number of seeds germinated in test group}}{\text{Number of seeds germinated in control group}} \times 100$$
 (1)

$$%RRG = \frac{\text{Mean root length of test group}}{\text{Mean root length of control group}} \times 100$$
 (2)

$$%GI = \frac{RSG \times RRG}{100}$$
(3)

Assessment of the bioconcentration of Cd in germinated rice

After the completion of the seed germination test, all germinated seeds from each test were collected and washed with deionized water three times. Afterward, the germinated seeds were dried in a hot air oven at 80°C for 4 hours before grinding with an agate mortar and pestle. Then, the samples were sieved through a 425 micrometer (µm) sieve and dried at 80°C until a constant weight was obtained. A mixture (3:1 v/v) of concentrated, extra-pure nitric acid (67-69% HNO₃, Val de Reuil, France) and hydrogen peroxide (H₂O₂) (35% Chemsupply, Australia) was added to a clean plastic tube containing approximately 0.5 grams (g) dry weight of germinated seeds and predigested overnight before digestion at 80°C in a dry heating block for 2 hours. Finally, the extracted solution was adjusted to 25 mL with deionized water, filtered through a 0.45-µm filtration unit into a new plastic tube and kept at 4°C for total Cd determination by inductively coupled plasma–mass spectrometry (ICP–MS, Agilent 7500c). The limit of quantitation was $1 \mu g/L$. The accuracies of the germinated seed digestion and ICP-MS analyses were validated with NIST SRM 1568a (rice flour) and NIST SRM 1643e (trace elements in water). The results of digestion method validation (92.3%) and quality assurance and quality control of the ICP-MS analysis (88.2%) were within ±15% of the certified values. Rice sample digestion was carried out in triplicate. Finally, the bioconcentration factor (BCF), as shown in Eq. 4 (Islam et al., 2020; Wang et al., 2020), was calculated to assess the capability of rice plants to adsorb and accumulate Cd. The higher the BCF value is, the greater the capacity of rice to accumulate Cd. According to Wang et al. (2020), plants showing BCFs larger than 1 can be considered hyperaccumulator plants for that particular soil pollutant.

$$BCF = \frac{\text{Total Cd concentration in germinated rice root}}{\text{Plant} - \text{available Cd concentration in soil}}$$
(4)

Data analysis

All descriptive and inferential statistical analyses in this study were performed using the Statistical Package for Social Science (SPSS) (version 23). The normality of the data distribution was analyzed by the Shapiro-Wilk test (n<50) before conducting further analyses. As the data were not normally distributed, significant differences in all soil characteristics, the concentrations of all Cd species, the concentrations of Cd in germinated roots, and all phytotoxicity indices (%RSG, %RRG, and %GI) among different soil types were analyzed by the Mann–Whitney U test and Kruskal–Wallis H test. Spearman correlations were performed to determine the soil characteristics affecting the plant availability of Cd in soils and the toxic effects of plant-available Cd on the early stage of rice growth. Cluster analysis was used to group soil samples based on their major common characteristics causing different levels of Cd accumulation in the germinated rice roots. A p value of 0.05 was used to determine significance.

RESULTS AND DISCUSSION

Physicochemical characteristics of paddy soils

The basic physicochemical characteristics of the soils (n=36) are summarized in Table 2. According to their different percentages of sand, silt and clay particles, the soil samples can be categorized into 5 types, including sandy loam, loam, clay loam, silty clay and clay. Approximately 42% and 39% of all samples were loam and clay loam, respectively. Regarding the classification of soil pH (LDD, 2010), one sample was very acidic (pH of 5.1). The remaining samples had pH values ranging from 6.1 (slightly acidic) to 7.8 (slightly basic), with 75% of the samples having a neutral pH (6.6 to 7.3). Based on a review of fieldscale soil Eh values (Husson, 2013), all samples were moderately reduced soils with Eh values between +100 and +400 millivolt (mV). According to the DOA (2010), various classifications of soil CEC and OM were found in this study. Approximately 19%, 56%, and 25% of all samples had low (3 to 10 centimole per kilogram (cmol/kg)), moderate (10 to 15 cmol/ kg), and high CEC values (>15 cmol/kg), respectively. The OM concentrations in the soils ranged from very low (0.5% to 1.0%) to high (3% to 5%). Most of the soils (67% of all samples) had low OM contents (1%

| Statistical value | Sand | Cil+ (%) | Clay(%) | Eh(m)/l | nH | CEC | OM (%) | Total Cd |
|-------------------|------|-----------|-----------|-----------------|-----|-----------|--------|----------|
| | (%) | Siit (70) | Clay (76) | LII (IIIV) | pn | (cmol/kg) | | (mg/kg) |
| | | | S | andy loam (n= | =2) | | | |
| Minimum | 53.4 | 38.0 | 8.6 | 276.2 | 6.2 | 3.3 | 0.7 | 0.20 |
| Maximum | 58.4 | 25.0 | 16.6 | 278.8 | 6.5 | 8.4 | 1.4 | 3.95 |
| Mean | 55.9 | 31.5 | 12.6 | 277.5 | 6.3 | 5.9 | 1.0 | 2.08 |
| Median | 55.9 | 31.5 | 12.6 | 277.5 | 6.3 | 5.9 | 1.0 | 2.08 |
| SE | 2.5 | 6.5 | 4.0 | 1.3 | 0.1 | 2.6 | 0.3 | 1.88 |
| | | | | Loam (n=15) | | | | |
| Minimum | 30.2 | 28.2 | 14.6 | 172.4 | 6.1 | 6.3 | 1.1 | 0.40 |
| Maximum | 51.2 | 46.0 | 26.6 | 262.1 | 7.3 | 12.6 | 1.9 | 27.53 |
| Mean | 40.8 | 36.0 | 23.1 | 224.1 | 6.8 | 10.4 | 1.5 | 5.82 |
| Median | 40.2 | 34.2 | 24.6 | 231.6 | 6.9 | 10.5 | 1.5 | 2.34 |
| SE | 1.4 | 1.3 | 0.9 | 6.9 | 0.1 | 0.4 | 0.1 | 1.96 |
| | | | (| Clay loam (n=1 | .4) | | | |
| Minimum | 25.2 | 28.0 | 28.6 | 171.4 | 5.1 | 10.1 | 1.6 | 0.99 |
| Maximum | 41.4 | 40.2 | 37.6 | 278.0 | 7.8 | 17.2 | 2.8 | 33.56 |
| Mean | 33.4 | 33.7 | 32.9 | 222.9 | 6.8 | 14.5 | 2.1 | 8.92 |
| Median | 33.3 | 32.6 | 34.1 | 214.5 | 6.8 | 14.9 | 2.0 | 6.25 |
| SE | 1.3 | 1.1 | 0.8 | 11.5 | 0.2 | 0.5 | 0.1 | 2.68 |
| | | | | Silty clay (n=2 | 2) | | | |
| Minimum | 19.4 | 40.0 | 40.6 | 208.0 | 6.5 | 17.6 | 3.2 | 2.00 |
| Maximum | 19.4 | 40.0 | 40.6 | 258.0 | 7.2 | 21.4 | 3.4 | 89.87 |
| Mean | 19.4 | 40.0 | 40.6 | 233.3 | 6.9 | 19.5 | 3.3 | 45.93 |
| Median | 19.4 | 40.0 | 40.6 | 233.3 | 6.9 | 19.5 | 3.3 | 45.93 |
| SE | 0.0 | 0.0 | 0.0 | 25.0 | 0.4 | 1.9 | 0.1 | 43.94 |
| Clay (n=3) | | | | | | | | |
| Minimum | 18.2 | 35.0 | 40.6 | 223.7 | 7.0 | 12.8 | 1.6 | 0.40 |
| Maximum | 24.4 | 36.2 | 45.6 | 257.1 | 7.2 | 21.4 | 2.3 | 10.72 |
| Mean | 22.0 | 35.7 | 42.3 | 238.6 | 7.1 | 16.8 | 1.9 | 5.38 |
| Median | 23.4 | 36.0 | 40.6 | 235.1 | 7.1 | 16.2 | 1.8 | 5.01 |
| SE | 1.9 | 0.4 | 1.7 | 9.8 | 0.1 | 2.5 | 0.2 | 2.99 |
| | | | A | ll samples (n= | 36) | | | |
| Minimum | 18.2 | 25.0 | 8.6 | 171.4 | 5.1 | 3.3 | 0.7 | 0.20 |
| Maximum | 58.4 | 46.0 | 45.6 | 278.8 | 7.8 | 21.4 | 3.4 | 89.87 |
| Mean | 36.0 | 35.1 | 28.9 | 228.3 | 6.8 | 12.8 | 1.8 | 9.01 |
| Median | 37.2 | 34.6 | 28.6 | 232.4 | 6.8 | 12.6 | 1.8 | 3.98 |
| SE | 1.6 | 0.8 | 1.4 | 5.7 | 0.1 | 0.6 | 0.1 | 2.68 |

to 2%). There were significant differences in CEC and OM concentrations in different soil types (*p*<0.05), in which higher values of both characteristics were found in the silty clay soil than in the remaining soil types. The highest CEC (21.4 cmol/kg) and OM (3.4%) were observed in the silty clay soil (Table 2). On the other hand, compared to the other soil types, significantly lower CEC (3.3 cmol/kg) and OM (0.7%) values were determined in the sandy loam soil. This is because sand particles do not have any electrical charge on their surface; as a result, the higher the percentage of sand particles in the soil is, the lower the CEC value and the lower the capability of the soil to hold and exchange cations on its surface. When comparing the overall physical and chemical characteristics of

all collected soils to the suitable soil characteristics for rice production in Thailand as recommended by the Ministry of Agriculture and Cooperative (LDD, 2019), in which loamy or clayey soils with pH values ranging from slightly acidic to slightly basic as well as OM contents ranging from low to high are considered appropriate for rice production, it was found that two soil samples should not be used as a medium to grow rice, as one soil sample (G24) had a very low OM content (0.7%) and another sample (G34) was very acidic (pH=5.1), which are not appropriate conditions for rice growth. Therefore, soil improvement should be adopted to increase the OM content as well as the pH level of both soils before they can be used as a medium to sustain rice production.

Total Cd concentrations in paddy soils

A wide variation in total Cd concentrations (0.20 to 89.87 mg/kg; n=36) in soil samples was observed, as summarized in Table 2. Compared to the national soil quality standard for commercial and agricultural activities to protect against chronic exposure to contaminants (PCD, 2021), in which Cd is limited to not more than 762 mg/kg, all samples had lower total Cd concentrations. However, when comparing the total Cd concentrations in all samples to the national background Cd concentration in agricultural soils (1.7 mg/kg; n=3,186) according to a survey conducted by the LDD (Chanpiwat et al., 2019), approximately 64% of the samples (23 out of 36 samples) contained total Cd concentrations 1.2 to 52.9 times greater than the background value. Although a significant difference in mean total Cd concentration among soil types was not observed (p>0.05), the silty clay soils contained approximately 5.1 to 22.1 times the total Cd concentration of the other soil types and exhibited the greatest total Cd concentration in this study (89.87 mg/kg). The highest total Cd concentration was determined in the soils collected from the uppermost stream sampling site, located the closest to the abandoned Zn mines. It is also interesting that 91% of all soils (21 out of 23 samples) containing total Cd concentrations higher than the background Cd concentration were collected from paddy fields that received water for rice cultivation from Mae Tao Creek. When comparing the total Cd concentrations in soils in this study to the total Cd concentrations in agricultural soils collected from areas downstream of the same Zn mines within the past decade (2014 to 2023), as reported by Taeprayoon *et al.* (2023); Srisawat et al. (2021); Sriprachote et al. (2020); Waleeittikul et al. (2019); Kosolsaksakul et al. (2018, 2014), similar magnitudes were found in previous studies (<0.04 to 106.43 mg/kg) and this study (0.20 to 89.87 mg/kg). In particular, the highest soil total Cd concentrations in this study and in studies by Kosolsaksakul et al. (2018, 2014) were found in paddy fields in the upstream section of Mae Tao Creek. However, the sampling site with the highest total soil Cd concentration (106.43 mg/kg) reported by Srisawat et al. (2021) was not assessed in the current study.

Chemical species of Cd in paddy soils

Since the total concentration can be used to indicate

gross contamination without consideration of the potential effects of different forms of the element bound to soil particles, sequential extraction is normally applied to soils to determine the concentration of the chemical form that may be released into the surroundings once the environmental conditions change (Tessier et al., 1979). Five chemical fractions of Cd, including exchangeable (F1), carbonate-bound (F2), Fe- and Mn-bound (F3), organic-bound (F4) and residual Cd (F5), are the chemical fractions of interest in this study. The percentage distributions of each Cd species in the soils are depicted in Fig. 3. As clearly shown in Fig. 3, significant differences in the distributions of each Cd fraction in different soil types were not observed (p>0.05). On average, the order of the percentage distribution was F1 (38.6%) > F5 (20.6%) ≈ F2 (20.0%) > F3 (13.7%) > F4 (7.6%). The paddy soil with the highest concentration of total Cd (89.87 mg/kg) contained the highest concentrations of all Cd fractions except for F5. In contrast, the lowest concentrations of all Cd fractions were determined in the soil sample with the lowest concentration of total Cd. Regarding the environmental conditions that could leach Cd from solid particles, F1 and F2 are generally considered mobile fractions since they can be released from particles when there is a change in the ionic composition and pH of water, respectively (Tessier et al., 1979). The percentage of mobile Cd (F1+F2) in this study ranged from 28.1% to 74.2% (mean±SE: 58.6% ± 1.8%). However, Feand Mn-bound Cd (F3), which is normally unstable under anoxic conditions, accounted for 3.1% to 37.1% (mean±SE: 13.7% ± 1.1%). This fraction of Cd is moderately mobile. Moreover, the less mobile fractions had contents of approximately 7.6% ± 1.1% (F4) and 20.6% ± 2.4% (F5). The major distribution of mobile Cd in the soils in this study agrees well with the fact that Cd is one of the most mobile elements in the environment. At environmental pH values of 5.1 to 7.8 and Eh values of 171 to 279 mV, which were found in the soils in this study, the majority of Cd is soluble Cd²⁺, according to the pH-Eh diagram of Cd species (Adriano, 2001). Moreover, Kubier et al. (2019) found that Cd is the only metal showing an affinity for being easily solubilized and released in the form of the water-soluble, exchangeable, and acid-soluble fractions, which are considered bioavailable Cd. The results obtained in this study were in accordance with the percentages of mobile Cd found in soil collected

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| Statistical value | Co | oncentration (mg/kg) | | (d plant availability (%) |
|-------------------|-----------------|----------------------|----------|---------------------------|
| | Exchangeable Cd | Plant-available Cd | Total Cd | Cu plant availability (%) |
| | | Sandy loam (n=2) | | |
| Minimum | 0.06 | 0.07 | 0.20 | 33.4 |
| Maximum | 2.20 | 3.49 | 3.95 | 88.4 |
| Mean | 1.13 | 1.78 | 2.08 | 60.9 |
| Median | 1.13 | 1.78 | 2.08 | 60.9 |
| SE | 1.07 | 1.71 | 1.88 | 27.5 |
| | | Loam (n=15) | | |
| Minimum | 0.14 | 0.55 | 0.40 | 9.6 |
| Maximum | 6.29 | 9.35 | 27.53 | 99.5 |
| Mean | 1.88 | 2.94 | 5.82 | 40.0 |
| Median | 1.09 | 1.25 | 2.34 | 37.9 |
| SE | 0.53 | 0.83 | 1.96 | 6.1 |
| | | Clay loam (n=14) | | |
| Minimum | 0.22 | 0.13 | 0.99 | 13.4 |
| Maximum | 8.74 | 8.31 | 33.56 | 67.1 |
| Mean | 2.91 | 2.82 | 8.92 | 32.7 |
| Median | 2.16 | 1.71 | 6.25 | 27.2 |
| SE | 0.75 | 0.78 | 2.68 | 4.5 |
| | | Silty clay (n=2) | | |
| Minimum | 1.06 | 0.55 | 2.00 | 27.4 |
| Maximum | 23.36 | 27.48 | 89.87 | 30.6 |
| Mean | 12.21 | 14.01 | 45.93 | 29.0 |
| Median | 12.21 | 14.01 | 45.93 | 29.0 |
| SE | 11.15 | 13.47 | 43.94 | 2.3 |
| | | Clay (n=3) | | |
| Minimum | 0.13 | 0.07 | 0.40 | 18.5 |
| Maximum | 5.57 | 3.35 | 10.72 | 31.3 |
| Mean | 2.67 | 1.58 | 5.38 | 25.4 |
| Median | 2.30 | 1.33 | 5.01 | 26.5 |
| SE | 1.58 | 0.96 | 2.99 | 3.7 |
| | | All samples (n=36) | | |
| Minimum | 0.06 | 0.05 | 0.20 | 9.6 |
| Maximum | 23.36 | 27.48 | 89.87 | 99.5 |
| Mean | 2.88 | 3.33 | 9.01 | 36.4 |
| Median | 1.68 | 1.50 | 3.98 | 31.3 |
| SE | 0.70 | 0.83 | 2.68 | 3.4 |

Table 3: Concentrations of exchangeable Cd, plant-available Cd and total Cd in paddy soils

from the same study area as reported by Kosolsaksakul *et al.* (2018, 2014). The mean percentages of mobile Cd in the soils containing low (<10 mg/kg), medium (10–50 mg/kg), and high total Cd contents (>50 mg/kg) in this study, according to the contamination categorization scheme in the previous study, were 60.0%, 58.9%, and 59.1%, respectively. These results were lower than the mobile Cd contents of 82.6%, 94.7% and 95.0% found in soils with low, medium, and high Cd contamination, respectively, as reported by Kosolsaksakul *et al.* (2018, 2014). The differences in findings between the two studies can be explained by the highly heterogeneous nature of soil. The high Cd mobility in this study (mean±SE: 58.6% ± 1.8%;

maximum: 74.2%) clearly indicates the potential release of Cd into the environment, which may cause environmental impacts to the surroundings due to its high toxicity.

Plant-available Cd concentrations in paddy soils

The concentration of plant-available Cd and percentage of plant availability, which is calculated as the ratio of the plant-available Cd concentration to the total Cd concentration, are summarized in Table 3. This fraction of Cd represents Cd species that are readily available for uptake by plants. To obtain results comparable to previous studies conducted in the same study area, the DTPA soil extraction method was



Fig. 3: The distribution of Cd fractions in different soil types

applied in this study. Significant differences in both plant-available concentrations and the percentages of plant availability among different soil types could not be determined (p>0.05). The highest plant-available Cd concentration (27.48 mg/kg) was determined in the soil containing the highest total Cd concentration (89.87 mg/kg) as well as the highest concentration of mobile Cd (F1: 23.36 mg/kg and F2: 29.61 mg/kg). The order of the mean percentage of plant availability was sandy loam (60.9%) > loam (40.0%) > clay loam (32.7%) > silty clay (29.0%) > clay (25.40%). Variations in the concentration of plant-available Cd were found not only in this study (0.05 to 27.48 mg/kg) but also in previous studies (0.38 to 11.8) conducted by Sriprachote et al. (2020) and Phaenark et al. (2009). It is also worth noting that plant availability of Cd among soils can vary by a factor of 10 (Smolders and Mertens, 2010). A comparison of the concentrations of plant-available Cd found in all soil samples in this study to the soil guideline values set to protect human health and ecosystems, including the predicted noeffect concentration (PNEC) of 0.9 mg/kg regulated by the European Chemical Agency (ECHA, 2022), the ecological soil guideline value (Eco-SGV) to protect soil quality, particularly for New Zealand productive agricultural land, of 1.5 mg/kg (Gray and Cavanagh, 2022) and the proposed ecological protective concentration for Korean agricultural soil of 1.58 mg/kg (Lee et al., 2013), indicated that 36% of all soil samples in this study were expected to be safe to all receptors and for all purposes of use. However, approximately 47% of all samples may cause negative effects on terrestrial life and lead to the accumulation of Cd to a level that might not be safe for human consumption. Among various organisms, plants and soil microorganisms are more sensitive to Cd toxicity (Australian Government, 2019, 2017; Adriano, 2001). In addition, root crops, leafy vegetables and cereal grains normally show higher Cd accumulation in their plant parts (Adriano, 2001). The finding that the average percentages of plant-available Cd in all soil samples ranged between 25.4% and 60.9% (Table 3) clearly confirms that not all of the Cd present in the soils can be released and is available for plant uptake. Since Cd is a nonessential element for plant growth and cereal grains exhibit high Cd accumulation, the toxicity and accumulation of plant-available Cd in rice in this study were investigated.

Factors affecting the mobility and phytoavailability of Cd in paddy soils

Table 4 summarizes the soil characteristics that have significant effects on the mobility and phytoavailability of Cd in soils. The levels of association between soil characteristics of concern were interpreted according to Schober *et al.* (2018). The statistical analyses indicated that the speciation of Cd in the soils was significantly positively and strongly affected by the

| Characteristic | % Sand | % Silt | % Clay | Eh | Ηd | CEC | MO | Total Cd | Plant- available Cd | F1ª | F2 ^b | F3° | F4 ^d | F5 ^e |
|-----------------------|--------------------------|--------------|----------------------------|-----------------------|-------------------------|------------|---------------------------|-------------------|------------------------|-------|-----------------|------------|-----------------|-----------------|
| % Sand | 1.00 | | | | | | | | | | | | | |
| % Silt | -0.46 | 1.00 | | | | | | | | | | | | |
| % Clay | -0.85 | -0.03 | 1.00 | | | | | | | | | | | |
| Eh | 0.08 | 0.05 | -0.10 | 1.00 | | | | | | | | | | |
| Нд | -0.10 | -0.04 | 0.16 | -0.49* | 1.00 | | | | | | | | | |
| CEC | -0.70 | -0.13 | *06:0 | -0.12 | 0.04 | 1.00 | | | | | | | | |
| MO | -0.58* | -0.07 | 0.71* | -0.26 | -0.03 | 0.80* | 1.00 | | | | | | | |
| Total Cd | -0.04 | -0.20 | 0.21 | -0.53* | 0.45* | 0.20 | 0.38* | 1.00 | | | | | | |
| Plant available Cd | 0.16 | -0.24 | 0.01 | -0.44 | 0.38* | 0.04 | 0.22 | 0.91* | 1.00 | | | | | |
| F1 ^a | -0.03 | -0.20 | 0.17 | -0:40 | 0.32 | 0.18 | 0.38* | . 96*0 | °060 | 1.00 | | | | |
| F2 ^b | -0.05 | -0.22 | 0.22 | -0.55* | 0.51^{*} | 0.24 | 0.42* | 0.94 [*] | 0.85* | 0.86* | 1.00 | | | |
| F3 ^c | -0.03 | -0.19 | 0.19 | -0.57* | 0.51^{*} | 0.22 | 0.41* | 0.95* | 0.85* | 0.89* | 0.97* | 1.00 | | |
| F4 ^d | -0.09 | -0.19 | 0.24 | -0.58* | 0.46* | 0.21 | 0.40* | 0.93* | 0.79* | 0.88* | 0.88* | 0.91^{*} | 1.00 | |
| F5 ^e | -0.08 | -0.09 | 0.16 | -0.54* | 0.31 | 0.17 | 0.30 | 0.91* | 0.82* | *06:0 | 0.79* | 0.82* | 0.83* | 1.00 |
| *significa | nt correlatior | n at the 95% | confidence le | vel (<i>p</i> <0.05) | | | | | | | | | | |
| ^a exchangt | able Cd; ^b ca | Irbonate-bou | und Cd; ^c Fe- a | nd Mn-bour | nd Cd; ^d org | anic-bound | l Cd; ^e residu | ual Cd7 | | | | | | |

Table 4: Correlations between soil characteristics and different Cd species in paddy soils

total Cd concentration in the soil (p < 0.05). In addition, significant strong positive correlations were found between Cd fractions (p<0.05). The phytoavailability of Cd was found to be significantly strongly controlled by the concentrations of total Cd and of all Cd species, with correlation coefficients of more than 0.9 (p<0.05). Moreover, significant positive effects of pH and OM on the mobility and availability of Cd in soils were found at moderate levels (p < 0.05). Negative moderate correlations were obtained between Eh and all Cd species (F1 to F5) as well as between Eh and plant-available Cd. Srisawat et al. (2021) also reported positive correlations of pH and OM with the concentrations of Cd in agricultural soils collected from the same study area. Previous studies also reported significant effects of total Cd contamination, soil pH, OM, and chemical species on the mobility and availability of Cd (Li et al., 2021a; McLaughlin et al., 2021; Kubier et al., 2019; Smolders and Mertens, 2010). OM that accumulates at the soil surface has the ability to retain metals, particularly cations, on soil particles (Adriano, 2001). The different directions of the relationships between pH and Cd mobility and availability in this study and those in previous studies (Kubier et al., 2019; Smolders and Mertens, 2010; Adriano, 2001) require further investigation.

Toxicity of Cd to rice growth and Cd accumulation in germinated roots

Since Cd has chemical and charge characteristics similar to those of several macro- and micronutrients for plant growth, including Fe(II), Zn, Mn, and Ca, it competes with these essential nutrients for uptake and translocation by plants (McLaughlin et al., 2021; Samimi et al., 2023). However, Cd is a nonessential element and is highly toxic to plants (Khanna et al., 2022; Haider et al., 2021; McLaughlin et al., 2021). The toxicity of plant-available Cd extracted from the soils to rice growth in this study was assessed by using the seed germination test. Table 5 summarizes the toxicity of plant-available Cd in the soil solution to the germination and growth of rice seeds. Significantly lower percentages of RSG, RRG and GI were obtained in all test groups than in the control group (p<0.05). Values of 100% for all indices were obtained for the control group. However, differences in the percentages of RSG, RRG and GI among soil types could not be identified (p>0.05). The number of germinated rice seeds and root length for the control group were 14 seeds and 1.87 cm, respectively. However, the corresponding values for the test groups varied from 1 to 8 seeds (mean±SE: 4.3±0.3 seeds) and 0.02 to 1.03 cm (mean±SE: 0.42±0.04 cm), respectively. Fig. 4 shows the Cd toxicity to rice germination and root development at the end of the 96-hour incubation. The results shown in Table 5 and Fig. 4 clearly confirmed the toxic effects of Cd on the germination of KDML 105 rice. These results are in line with the findings from previous studies that general visible growth abnormalities induced by Cd include a reduction in plant root elongation after germination (Khanna et al., 2022; Haider et al., 2021). This is mainly caused by the retardation effects of toxic metals on water penetration into seeds (Pokorska-Niewiada et al., 2018). Thus, a lower degree of germination was found compared to the control group that used deionized water for germination. The plant-available Cd concentrations in the test groups that caused significant reductions in rice seed germination and root length ranged from 0.05 to 27.48 mg/kg (mean±SE: 3.33 ± 0.83 mg/kg). Compared to the concentration of Cd causing reductions in the rice root elongation and germination of seedlings (5.40 mg Cd) (Haider et al., 2021), the toxicity of Cd in this study showed similar negative effects at a lower Cd concentration (mean±SE: 3.33 ± 0.83 mg/kg; minimum: 0.05 mg/kg). The differences in the toxic concentrations of Cd in this study and the previous study might be attributed to the differences in the Cd species, growth media, incubation environment and tested rice variety. As the germination in this study was conducted for only 96 hours, it is expected that a longer germination period in Cd-contaminated soil would cause more negative effects on growth and greater accumulation of Cd in rice roots. In previous studies, cultivation time and soil Cd concentration showed positive relationships with Cd accumulation in the roots, shoots, and grains of rice (Uraguchi et al., 2009). Cd in soil is transported into grains via xylem-phloem transfer, with a reported range of Cd grain-to-soil ratios of 0.058 to 5.96 (Zhao and Wang, 2020; Uraguchi et al., 2009). As the mean Cd concentration in germinated roots (1.04 mg/kg) in this study exceeded both the national and Codex standards for Cd in rice (0.4 mg/kg), it is highly likely that the accumulated Cd concentrations in the grains would exceed the standards.

Table 6 summarizes the relationships of the Cd

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| Statistical value | RSG (%) | RRG (%) | GI (%) | Cd concentration in germinated roots (mg/kg) | BCF |
|-------------------|---------|---------|------------------|--|------|
| | | Sa | andy loam (n=2) | | |
| Minimum | 12.4 | 11.6 | 1.4 | 0.04 | 0.63 |
| Maximum | 55.0 | 44.4 | 24.4 | 4.02 | 1.15 |
| Mean | 33.6 | 28.0 | 12.9 | 2.03 | 0.89 |
| Median | 33.6 | 28.0 | 12.9 | 2.03 | 0.89 |
| SE | 21.4 | 16.4 | 11.5 | 1.99 | 0.26 |
| | | | Loam (n=15) | | |
| Minimum | 10.0 | 1.3 | 0.1 | 0.03 | 0.17 |
| Maximum | 54.3 | 41.0 | 14.1 | 2.09 | 4.06 |
| Mean | 30.6 | 21.5 | 7.0 | 0.75 | 0.59 |
| Median | 30.7 | 21.1 | 5.5 | 0.36 | 0.29 |
| SE | 3.5 | 2.8 | 1.2 | 0.20 | 0.25 |
| | | C | lay loam (n=14) | | |
| Minimum | 15.0 | 7.9 | 1.2 | 0.07 | 0.21 |
| Maximum | 57.9 | 54.0 | 25.1 | 5.38 | 0.69 |
| Mean | 29.9 | 19.3 | 6.6 | 1.09 | 0.38 |
| Median | 24.6 | 15.1 | 3.5 | 0.45 | 0.32 |
| SE | 3.6 | 3.3 | 1.7 | 0.40 | 0.04 |
| | | | Silty clay (n=2) | | |
| Minimum | 22.9 | 8.0 | 1.8 | 0.19 | 0.21 |
| Maximum | 45.0 | 32.8 | 14.8 | 5.88 | 0.35 |
| Mean | 33.9 | 20.4 | 8.3 | 3.03 | 0.28 |
| Median | 33.9 | 20.4 | 8.3 | 3.03 | 0.28 |
| SE | 15.7 | 17.5 | 9.2 | 4.02 | 0.09 |
| Clay (n=3) | | | | | |
| Minimum | 20.7 | 15.5 | 3.2 | 0.04 | 0.02 |
| Maximum | 45.7 | 55.0 | 25.1 | 0.48 | 0.57 |
| Mean | 36.2 | 35.9 | 14.7 | 0.19 | 0.32 |
| Median | 42.1 | 37.2 | 15.7 | 0.05 | 0.28 |
| SE | 7.8 | 11.4 | 6.4 | 0.14 | 0.16 |
| | | Al | l samples (n=36) | | |
| Minimum | 9.5 | 1.3 | 0.1 | 0.03 | 0.02 |
| Maximum | 59.0 | 55.0 | 25.1 | 5.88 | 4.06 |
| Mean | 31.2 | 22.1 | 7.9 | 1.04 | 0.48 |
| Median | 26.9 | 18.6 | 5.4 | 0.42 | 0.32 |
| SE | 2.3 | 2.2 | 1.2 | 0.24 | 0.11 |

| Table 5: Germination indices and total | Cd concentration accumulated in | perminated roots after 96 | hours of incubation |
|--|---------------------------------|---------------------------|---------------------|
| | ca concentration accamatacea in | Berninated roots after 50 | nours of incubation |

fractions in the soils with Cd toxicity to rice growth and Cd accumulation in germinated roots. The number of germinated seeds and %RSG were mainly significantly negatively and weakly correlated with the concentration of exchangeable Cd (F1) in the soils (p<0.05). The %RRG, %GI and length of root elongation were significantly negatively affected by the concentrations of total Cd and all Cd species in the soils, with the strengths of the correlations ranging from weak (correlation coefficient of 0.10 to 0.39) to moderate (correlation coefficient of 0.40 to 0.69), except for organic-bound Cd (F4) (p<0.05), which did not show a significant correlation with either %RRG or %GI (*p*>0.05). It is interesting that the correlation coefficients of %RRG, %GI and root length with F1 and F2 were higher than those of these three indices with the other Cd fractions (Table 6). These results were in good agreement with a previous study by Cataldo and Wildung (1978), who found that the soluble species of heavy metals strongly controlled the rate and amount of uptake as well as the toxicity in plants, as metals in soils have to be solubilized into the soil solution for root uptake. These conclusions are in accordance

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Fig. 4: Germination and length of rice roots germinated with (a) deionized water (control group) and (b) plant-available Cd extracted solution (test group)

with the results of this study (Fig. 5), which revealed significant strong positive correlations (p<0.05) between the total Cd concentration in the germinated roots and the plant-available Cd, exchangeable Cd (F1), carbonate-bound Cd (F2), and total Cd concentrations in soils, with correlation coefficients ranging from 0.77 to 0.89. Furthermore, the less mobile Cd fractions (F3 and F4) and residual Cd fraction (F5) showed positive correlations with the total Cd concentration accumulated in the germinated roots (Table 6). Similar results showing that the accumulation of Cd was principally affected by Cd availability and total Cd concentration were also reported by Li *et al.*, 2022; Nogueira *et al.*, 2022; McLaughlin *et al.*, 2021; Wang *et al.*, 2020).

Common characteristics of paddy soils causing different levels of Cd accumulation in rice

Cluster analysis was performed on the characteristics of soils, including Eh, pH, CEC, OM, total Cd concentration and percentages of

plant availability and mobility, and the total Cd concentration in germinated roots, to group together the paddy soil samples with similar patterns that caused different levels (low, moderate, and high) of Cd accumulation in germinated rice roots. A total of eight groups of samples with soil characteristics resulting in different levels of Cd accumulation in germinated roots were obtained (Fig. 6). Notably, the levels of Eh, pH, CEC, and OM were similar among the groups. The characteristics that caused different levels of Cd accumulation in germinated roots, which consequently caused Cd accumulation in rice grains, were the concentrations of total Cd, mobile Cd (F1 and F2) and plant-available Cd in soils. Based on the results (Fig. 6), it is possible to infer that high Cd accumulation in rice was mainly affected by a high percentage of plant availability of Cd in the soils regardless of the level of total Cd contamination. Thus, management options to mitigate the plant availability of Cd in soils should be applied to these soils to reduce Cd accumulation in rice.

| Cd in | Total | Plant-available | F1ª | F2 ^b | F3° | $F4^{d}$ | F5 ^e | BCF | RSG | RRG | Ыğ | No. of seeds | Root |
|----------|---|---|-------------------------------------|------------------------|-------------|-------------------------|-----------------|-------|-------|-------|-------|--------------|--------|
| | Cd | 8 | | | | | | | (%) | (%) | (%) | germinated | length |
| | | | | | | | | | | | | | |
| | 1.00 | | | | | | | | | | | | |
| | 0.91* | 1.00 | | | | | | | | | | | |
| | *96.0 | *06:0 | 1.00 | | | | | | | | | | |
| | 0.94 [*] | 0.85* | 0.86* | 1.00 | | | | | | | | | |
| | 0.95* | 0.85* | 0.89* | 0.97* | 1.00 | | | | | | | | |
| | 0.93* | .79* | 0.88* | 0.88* | 0.91* | 1.00 | | | | | | | |
| | 0.91* | 0.82* | *06:0 | •0.79 | 0.82* | 0.83* | 1.00 | | | | | | |
| | -0.60* | -0.56* | -0.52* | -0.60* | -0.62* | -0.53* | -0.56* | 1.00 | | | | | |
| | -0.22 | -0.29 | -0.34* | -0.23 | -0.20 | -0.18 | -0.19 | -0.13 | 1.00 | | | | |
| | -0.39* | -0.47* | -0.51* | -0.44 * | -0.41* | -0.30 | -0.35* | 0.14 | 0.60* | 1.00 | | | |
| | -0.38 | -0.46* | -0.51* | -0.43* | -0.39* | -0.29 | -0.35* | 0.04 | 0.84* | 0.92* | 1.00 | | |
| * | -0.22 | -0.29 | -0.34* | -0.23 | -0.20 | -0.18 | -0.19 | -0.13 | 1.00* | 09:0 | 0.84* | 1.00 | |
| | -0.39* | -0.48* | -0.51* | -0.44 | -0.41* | -0.30 | -0.35* | 0.14 | 0.60* | 1.00* | 0.92* | 0.60* | 1.00 |
| <u>s</u> | rrelation at th ? Cd; ^b carbona | e 95% confidence leve ite-bound Cd; ^c Fe- and | el (<i>p</i> <0.05); 1 Mn-bound | Cd; ^d orgar | nic-bound C | d; ^e residua | l cd . | | | | | | |

Table 6: Correlations showing the effects of soil characteristics on the accumulation and toxicity of Cd to rice

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Fig. 5: Significant positive linear relationships between the Cd concentrations accumulated in germinated rice and the concentrations of (a) plant-available Cd, (b) exchangeable Cd, (c) carbonate-bound Cd, and (d) total Cd in the soils

Recommended remediation strategy for the study area

According to the authors' previous human health risk assessments of Cd exposure through rice consumption in the study area (Chanpiwat *et al.*, 2019; Suwatvitayakorn *et al.*, 2020), which found that locally grown and consumed rice contained Cd concentrations exceeding the national and Codex standards for Cd in polished rice and that the bioaccessibility of Cd in rice, which is readily absorbed by the human bloodstream, reached 84%, this study was conducted to assess the factors affecting the toxicity and accumulation of Cd in rice. The results showed that the concentrations of total Cd, exchangeable Cd (F1), carbonate-bound Cd (F2), and plant-available Cd were the main soil characteristics affecting the accumulation of Cd in rice, with plant-available Cd being the most important factor. Therefore, focus should be placed on reducing the plant-available Cd in soils to lower Cd accumulation and protect the public from Cd exposure. As the results further showed that the mobility and phytoavailability of Cd in the soils from the study area were mainly positively controlled by the total Cd and OM concentrations, two potential mitigation measures are i) to change the irrigation water to Cd-free water, as this measure would not add more Cd to the paddy soils, and ii) to add OMrich amendments to immobilize plant-available Cd in the soils. According to previous studies (Yavari et al., 2023, 2022; Haider et al., 2021; Hussain et al., 2021; Li et al., 2021a, 2021b; McLaughlin et al., 2021; Alam et al., 2020; Zhao and Wang, 2020), organic fertilizer, organic waste compost, organic manure, agrowaste-



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Rescaled Distance Cluster Combine

Fig. 6: Dendrogram showing the clustering of sampling sites with similar soil characteristics causing different levels of Cd accumulation in rice

derived biochar, and humic acid have shown a good capability to immobilize various soil pollutants, including metals, and reduce their accumulation in plant parts. However, the application rate of amendments can differ from those established in previous studies, as the soil characteristics and level of contamination can also differ. Considering the results of this study, which found a negative relationship between Eh and plant-available Cd, the maintenance of an oxidizing soil environment during cultivation would be another mitigation measure to simultaneously reduce the Cd mobility in soils and Cd accumulation in rice. Moreover, the cultivation of rice cultivars with low grain Cd accumulation, such as those tested in the study area and reported by Sriprachote *et al.* (2020), should be promoted.

CONCLUSION

This study is the first to report the factors affecting the plant availability and mobility of Cd in soils as well as the factors affecting the toxicity and accumulation of Cd in rice grown in paddy soils that were previously reported to produce rice grains with total Cd concentrations exceeding the acceptable level for human consumption of 0.4 mg/kg. Although the total Cd concentrations (0.20 to 89.87 mg/kg) in all soil samples (n=36) were lower than the national soil quality standard for agricultural activities of 762 mg/kg, 64% of the samples contained total Cd concentrations 1.2 to 52.9 times greater than the national background concentration in agricultural soils. The chemical fractions of Cd in the soils were found in the decreasing order F1>F5≈F2>F3>F4, with mobile Cd (F1+F2: 58.60%) composing the majority of species on average, which corresponds well to the mobile characteristics of Cd. A wide variation in plantavailable Cd (0.05 to 27.48 mg/kg) was found, with the highest mean percentage of plant availability (60%) found in the sandy loam soils, as sand particles do not have the capacity to absorb cations on their surface. Plant-available Cd exerted significant toxicity to both rice seed germination and root elongation, with approximately 69% and 48% reductions in the two indices, respectively. Cd accumulation in germinated roots (0.03 to 5.88 mg/kg) was observed in 44% of the test samples, with Cd levels exceeding the total Cd standard in rice (0.4 mg/kg). Therefore, the plant-available Cd concentrations in soils should be reduced to lower Cd accumulation in rice. Since the most important soil characteristics affecting the availability of Cd were the total concentration and speciation of Cd, the concentration of plant-available Cd in the soil solution can be decreased by i) reducing the total Cd contamination in the soils by stopping the use of Mae Tao Creek as the irrigation water and ii) increasing the OM concentrations in the soils by adding OM-rich amendments such as compost, manure, and biochar to immobilize plant-available Cd. By doing so, the concentration of Cd in locally consumed rice as well as the adverse human health impacts will be lessened. As this study did not include an investigation of the toxic effects of Cd throughout the rice cultivation period, it is highly recommended that future work include a study on the effects of local rice cultivation practices on the soil–plant system and the toxic effects throughout the rice growth cycle.

AUTHOR CONTRIBUTIONS

A. Numprasanthai, the corresponding author, contributed to conceptualization, investigation, methodology, resources, writing - review and editing, supervision, and funding acquisition. P. Chanpiwat, the first author, contributed to the investigation, methodology, data curation, validation, formal analysis, writing - original draft, visualization, and funding acquisition.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy, were observed by the authors.

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ABBREVIATIONS

| % | Percent |
|-----------------|---|
| μg | Microgram |
| μm | Micrometer |
| BCF | Bioconcentration factor |
| °C | Degree Celsius |
| Са | Calcium |
| Cd | Cadmium |
| CEC | Cation exchange capacity |
| ст | Centimeter |
| cm ² | Square centimeter |
| cmol | Centimole |
| DOA | Department of Agriculture |
| DPIM | Department of Primary Industries and Mines |
| DTPA | Diethylenetriaminepentaacetic acid |
| Eco-SGV | Ecological soil guideline value |
| Eh | Redox potential |
| ERIC | Environmental Research Institute, Chulalongkorn University |
| F1 | Exchangeable fraction |
| F2 | Carbonate-bound fraction |
| F3 | Fe- and Mn-bound fraction |
| F4 | Organic-bound fraction |
| F5 | Residual fraction |
| Fe | Iron |
| g | Gram |
| GI | Germination index |

| GIST | Gwangju Institute of Science and Technology |
|-------------|--|
| GRI | GIST Research Institute |
| HNO₃ | Nitric acid |
| H_2O_2 | Hydrogen peroxide |
| ICP–MS | Inductively coupled plasma–mass spectrometry |
| ICP–OES | Inductively coupled plasma–optical emission spectrometry |
| IWMI | International Water Management Institute |
| kg | Kilogram |
| KDML 105 | Khao Dok Mali 105 rice |
| L | Liter |
| LDD | Land Development Department |
| mg | Milligram |
| mL | Milliliter |
| mm | Millimeter |
| Mn | Manganese |
| mV | Millivolt |
| n | Sample size |
| NaOCl | Sodium hypochlorite |
| NIST | National Institute of Standards and Technology |
| ОМ | Organic matter |
| ORP | Oxidation-reduction potential |
| p value | Probability value |
| рН | Potential of hydrogen |
| PNEC | Predicted no-effect concentration |
| ррт | Part per million |
| RRG | Relative root growth |
| RSG | Relative seed germination |
| SE | Standard error of the mean |
| SPSS | Statistical Package for Social Science |
| SRM | Standard reference material |
| TSRI | Thailand Science Research and Innovation |
| v | Volume |
| W | Weight |
| Zn | Zinc |

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P. Chanpiwat and A. Numprasanthai

AUTHOR (S) BIOSKETCHES

Chanpiwat, P., Ph.D., Assistant Professor, ¹Environmental Research Institute, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand. ²Center of Excellence in Environmental Innovation and Management of Metals (EnvIMM), Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand.

- Email: Penradee.C@chula.ac.th
- ORCID: 0000-0001-7625-0952
- Web of Science ResearcherID: HJY-5028-2023
- Scopus Author ID: 35490058800
- Homepage: https://eric.chula.ac.th/profile/id/penradee.chanpiwat

Numprasanthai, A., Ph.D., Assistant Professor, ²Center of Excellence in Environmental Innovation and Management of Metals (EnvIMM), Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand. ³Department of Mining and Petroleum Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand.

- Email: Apisit.Nu@chula.ac.th
- ORCID: 0000-0001-6432-0650
- Web of Science ResearcherID: AAA-1335-2020
- Scopus Author ID: 54987315400

Homepage: https://www.eng.chula.ac.th/th/staff/asst-prof-apisit-numprasanthai-ph-d

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