Effects of aqueous Moringa oleifera leaf extract on growth performance and accumulation of cadmium in a Thai jasmine rice-Khao Dawk Mali 105 variety

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Abstract

The contamination of paddy fields and rice grains by cadmium (Cd) adversely affects human health. Thus, many approaches have been proposed to reduce the accumulation of Cd in rice. Here, we investigate the potential of aqueous Moringa oleifera leaf extract (AMOLE) in decreasing uptake and toxicity of Cd in a popular Thai jasmine rice variety, Khao Dawk Mali 105 (KDML105). Plants were grown in Petri dishes, a hydroponic system, and a pot system under different concentrations of Cd, in the presence and absence of AMOLE. In Petri dishes, Cd reduced the percentage of germination by 79%, but the treatment with 0.5 mg mL -1 AMOLE significantly increased the germination percentage. Moreover, AMOLE significantly decreased Cd accumulation in rice seedlings by 97%. In the hydroponics system, 0.5 mg mL -1 AMOLE decreased Cd content in shoots by 48%. Although no significant physiological changes in response to Cd treatments were observed in the pot system, a large amount of Cd was accumulated in rice roots. The AMOLE treatments significantly reduced Cd accumulation in rice shoots and decreased Cd content in milled grain by half compared to those without AMOLE treatment. We conclude that AMOLE reduced Cd toxicity, enhanced seedling growth, and reduced Cd accumulation in rice grains.

1. Introduction

Rapid urbanization and industrialization are among the major causes of pollutant contamination in the environment. In agricultural areas, plants can uptake toxic elements present in the soils and accumulate them in edible parts consumed by animals and humans. The intake of contaminated crops and vegetables has become a major source of public health problems in several countries (Chaney 2015). Rice is a staple food of more than half of the world's population. Unfortunately, the contamination of rice by toxic elements, particularly cadmium (Cd), has been shown to be an important factor contributing to acute and chronic diseases in humans (Chaney 2015; Wang et al. 2021). Cadmium contamination in soil occurs from both natural and anthropogenic sources. The major anthropogenic sources of Cd include mining activities, phosphate fertilizers, and industrial emissions (He et al. 2005). Cd enters freshwater from industrial sources and is mostly absorbed in sediments. In a paddy field, sediments and irrigating water originating from a contaminated water reservoir cause contamination, since the land is often continuously irrigated by local surface water (Charoenpanyanet and Huttagosol 2020).

In addition to its effects on human health, the presence of Cd in the soil causes many toxic symptoms in plants. The severity of Cd toxicity varies among plant species and varieties. In general, Cd interferes with photosynthesis by slowing down the activity of photosynthetic enzymes (Ying et al. 2010), by competing with Ca^{+2} entering guard cells and triggering stomata closure (Perfus-Barbeoch et al. 2002), and by inhibiting chlorophyll biosynthesis (Skrebsky et al. 2008). In addition, Cd also causes oxidative stress and interferes with nutrient uptake and carbohydrate metabolism, resulting in a reduction in biomass and yield (Kibria et al. 2006). Uptake of Cd in soil solution into the root occurs through symplasm, driven by the electrochemical potential gradient across the plasma membrane of root cells. After entering plant roots, most metals form phosphate, sulfate, or carbonate precipitates which are immobilized into extracellular and intracellular compartments such as cell walls and vacuoles. In rice, transporters such as

OsIRT1 (iron-regulated transporter1), OSIRT2, and OSNramp1 are potentially involved in Cd uptake (Uraguchi and Fujiwara 2012). Xylem is a major pathway of Cd translocation from root to shoot. The translocation of Cd is a relatively fast process by which substantial Cd can be detected in shoot tissues after Cd treatment of roots for only one hour. In contrast, phloem is the major Cd transport route into grains (Tanaka et al. 2007). Among rice cultivars, Cd accumulation in shoots and grains is potentially higher in indica than japonica cultivars. Moreover, some specific cultivars of indica rice accumulate much higher Cd in vegetative tissues and grains (Kato et al. 2010).

In Thailand, Cd contamination has been reported in Mae Sot District of Tak Province (Sriprachote et al. 2012a; Meeinkuirt et al. 2019). It has been demonstrated that the high level of Cd concentrations in the soil are linked with significant health problems for local villagers who consume crops grown in the area (Simmons et al. 2005; Sriprachote et al. 2012a). Agricultural soils in Mae Sot District have a diverse spatial distribution of Cd concentrations. Soil Cd concentrations there were found to range from 0.5 to 284.0 mg kg^{-1}. Rice grown in this region has been reported to accumulate Cd at a rate of more than 0.2 mg kg^{-1} (Saengwilai and Meeinkuirt 2021), classified as unsafe for human consumption according to the standards of the Codex Committee on Food Additives and Contaminants (CCFAC 2005). As a result, the local government has initiated several policies to reduce health risks by promoting the cultivation of alternative crops such as sugarcane and rubber trees. However, these efforts have been proven to be unsuccessful, and the farmers have returned to cultivating rice for their own consumption (Sriprachote et al. 2012a). It is, therefore, necessary to seek appropriate technologies to reduce Cd concentration in rice grain.

Moringa oleifera Lam. is native to the north of India and grows well in tropical regions. It has been extensively studied because it is rich in amino acids, antioxidants, phytohormones, and minerals (Araújo et al. 2013). The content of compounds in Moringa extract depends on extraction technique. A solution of *M. oleifera* leaf extracted by 70% ethanol contains as many as 44 compounds, including pentacosane (17.41%), hexacosane (11.20%), (E)-phyton (7.66%), and 1-[2, 3, 6-trimethyl-phenyl]-2-butanone (3.44%). These compounds are beneficial for fighting against dermatophytes (Vongsak et al. 2014). In addition, Chuang et al. (2007) discovered that leaves of *M. oleifera* contain essential oils which have anti-fungal activities against dermatophytes. Apart from its medicinal benefits, Moringa extract has been shown to remove heavy metals and contaminants in water by a significant amount (Araújo et al. 2013). This is possibly due to some components such as thiol-containing proteins present in the extracts that interact with heavy metals, resulting in ion adsorption and charge neutralization. Recently, Kerdsomboon et al. (2021) reported that aqueous *Moringa oleifera* leaf extract (AMOLE) reduced intracellular reactive oxygen species (ROS) levels in yeast cells grown in the presence of As(III), Cd, Ni, and Pb. It was shown in that study that gallic acid, a component in the extracts, physically bound with As(III) via its hydroxyl and carboxyl groups, thereby preventing As(III) accumulation and As(III)-induced ROS generation (Kerdsomboon et al. 2021). In plants, the application of *Moringa oleifera* leaf extract improved the antioxidant enzyme system of wheat plants under salt stress, improving their performance significantly (Yasmeen et al. 2013). In a Cd contaminated environment, not only did Moringa coagulate Cd in the soil and thereby prevent its absorption and accumulation in wheat plants, but it also influenced the expression of genes associated with alleviation of metal toxicity (Hassanein et al. 2016).

The aim of the present study is to assess the effect of soluble AMOLE on toxicity and accumulation of Cd in Thai jasmine rice, Khao Dawk Mali 105 (KDML105). Plants were grown in Petri dishes, a hydroponic system, and a pot system under different Cd concentrations, with and without AMOLE treatments.

2. Materials And Methods

2.1. Plant Materials

The KDML105 rice variety was selected for all experiments because it is widely grown in Thailand and is popular due to its outstanding eating quality and unique aroma. KDML105 has been reported to accumulate a significant amount of Cd and is frequently planted in Cd contaminated areas in Tak Province, Thailand (Sriprachote et al. 2012a; Sriprachote et al. 2012b). It is photoperiod sensitive and thus can only produce a crop once a year. Seeds were obtained from the Rice Department, Ministry of Agriculture and Cooperatives, Thailand.

2.2. Preparation of aqueous M. oleifera leaf extract (AMOLE)

Aqueous *M. oleifera* leaf extract (AMOLE) was prepared according to Kerdsomboon et al. (2021) with some modifications. Specifically, 4.5 g of *M. oleifera* leaf powder was mixed with 30 mL distilled water in a 50 ml test tube. The mixture was vortexed for 30 sec and left at room temperature for 5 min. The solution was mixed again for 1 min and centrifuged at 2,600 g 30 ^{o}C for 30 min. The water-soluble part was collected and then centrifuged at 15,000 g for 20 min. The supernatants were sterilized by membrane filtration. The aqueous *M. oleifera* leaf extract (AMOLE*)* was lyophilized and stored at 4 ^{o}C until use.

2.3. Germination test

Seeds of KDML105 were surface-sterilized by 10% NaOCl for 1 min and rinsed five times with distilled water. The seeds were germinated in various concentrations of AMOLE (0.1, 0.3, 0.5, and 0.7 mg mL^{-1}) in the presence and absence of $CdCl_2$ (20, 40, 60, 80, 100 mg kg^{-1}) in sterilized Petri dishes. The range of Cd concentrations mimics polluted soils and is based on our preliminary studies (data not shown). Each Petri dish received 10 mL of the solution. The seeds were germinated in an incubator at 25 ^{o}C in the dark for seven days. The percentage of germination was then recorded once the radicle length had reached 2 mm (Thamayanthi et al. 2011). After that, the shoots and roots were placed in an oven at 70 ^{o}C for three days to determine dry weight and Cd content.

2.4. Growth conditions

2.4.1. Hydroponics

Seeds of KDML105 were surface-sterilized and pre-germinated in a roll-up of germination paper soaked with 0.5 mM $CaSO_4 \cdot 2H_2O$. The roll-up was incubated at 30 °C in the dark for seven days before exposure to supplementary light. At 10 days after germination, rice seedlings of similar size were moved to hydroponics chambers. Each seedling was placed in a hydroponic jar containing 150 mL of Yoshida solution (Yoshida et al. 1976; Meeinkuirt et al. 2019). The pH of the solution was adjusted to 5.5. The plants were acclimated for seven days before treatments. The seedlings were treated with 25 mg kg^{-1} $CdCl_2 \cdot H_2O$ in the presence of AMOLE at concentrations of 0, 0.1, 0.3, and 0.5 mg mL^{-1} for two weeks. The experiment was arranged using a randomized complete block design with five replications. The nutrient solution was renewed every four days until the plants were harvested. The number of leaves, shoot height, root length, the number of crown roots and lateral roots were determined. Finally, the plant samples were dried in an oven at 70°C for three days for dry weight and Cd content measurements.

2.4.2 Pot experiment

Seeds of KDML105 were pre-germinated and transferred to 10-in pots containing sterilized soils, both with and without 0.5 mg mL^{-1} of AMOLE. The treatments were applied by adding 25 mg kg^{-1} Cd to the pots. The selected concentration of AMOLE was based on the results from the germination and hydroponic experiments. The experiment was arranged in a randomized complete block design with five replications. Before transplanting, each pot was saturated with water and supplemented with 250 mL of Yoshida solution. Plants were harvested four months after transplanting.

2.5 Measurement of growth parameters

Depending on the growing system, parameters including shoot traits, net photosynthetic rate, chlorophyll content, and yield were quantified. In general, shoot traits, including height, number of tillers, number of leaves, percentage of leaf senescence, days to flowering, and shoot dry weight, were measured at harvest. Net photosynthetic rate was measured using a portable photosynthetic system LI-6400XT (Li-Cor Biosciences, Lincoln, NE, US), with the following conditions: flow rate = 500 µmol/sec; temperature = 25°C; PAR = 1000 µmol/m^2/sec; CO_2 = 400 mg kg^{-1}. Yield was quantified as panicle number, panicle weight, and weight of 100 grains. For chlorophyll measurements, 100 mg of the leaf was cut into pieces of approximately 0.2 cm and extracted using acetone (95.5%). The amount of chlorophyll a and b and carotenoid were determined using a spectrophotometer at the wavelengths of 662, 644, and 470 nm, respectively. Root traits were measured according to Saengwilai et al. (2018). Number of adventitious roots, lateral root branching, and root length were measured manually by counting and using a ruler (Saengwilai et al. 2018).

2.6. Cadmium determination

For the measurement of Cd in plant materials, 0.5 g of each shoot or root sample was digested by 2:1 nitric acid and perchloric acid solution (HNO_3:$HClO_4$) with hydrochloric acid (HCl) in a heating block. For soil samples, 0.5 g of soil was put in a glass tube containing 6 mL of 3:1 hydrochloric acid and nitric acid solution (HCl:HNO_3). All samples were filtered with Whatman No. 42 filter paper and transferred into a volumetric flask. The sample volume was adjusted to 25 mL by distilled water. Cd content in the samples was then determined using atomic absorption spectrophotometer (Varian Spectra AA 55B). The translocation factors were calculated as the ratio of Cd concentrations in shoots to roots (Saengwilai and Meeinkuirt 2021).

2.7. Statistical analysis

Statistical analyses were performed using R version 3.1–152. Linear mixed effect models were fit using the lme function from the package nlme (Pinheiro et al. 2021). One-way and two-way ANOVA were used for comparisons among control, AMOLE, Cd, and Cd + AMOLE treatments and the interaction between these main effects.

3. Results

3.1. Effects of Cd and AMOLE on rice seed germination and growth performance of rice seedlings

The results showed that Cd significantly reduced seed germination and vigor of seedlings. Specifically, the presence of Cd alone reduced the germination rate to 20% compared to 100% germination in the control (Fig. 1a). In the presence of Cd, AMOLE treatments enhanced germination rate, particularly the treatments of 0.5 and 0.7 mg mL^{-1} (Fig. 1a). After seedlings germinated, severe Cd toxicity was observed in all plants. Without AMOLE supplementation, shoot mass was drastically reduced by 97% compared to the control. The treatments with 0.5 and 0.7 mg mL^{-1} AMOLE improved seedling weight by 4.7 and 7 times, respectively (Fig. 1b). Moreover, the treatments with AMOLE at all concentrations significantly decreased Cd content in rice seedlings (Fig. 1c). These findings suggest the potential use of AMOLE to mitigate Cd toxicity and accumulation in rice.

3.2. Effects of AMOLE on reducing Cd toxicity and accumulation in rice grown in the hydroponic system

After 14 days of the treatments, seedlings grown in the presence of Cd without AMOLE were senesced, while those treated with AMOLE stayed green and appeared healthy. In addition, shoot height, shoot dry weight, and the number of leaves of rice seedlings grown under Cd condition were significantly decreased ($P < 0.05$) by 38%, 13%, and 22%, respectively, compared with those of the control (without Cd) (Fig. 2). The addition of AMOLE at concentrations of 0.1, 0.3, and 0.5 mg mL^{-1} in Cd solution significantly reduced leaf senescence by 31%, 76%, and 85% compared with the treatment of Cd alone, respectively (Fig. 2d), particularly at the concentration of 0.5 mg mL^{-1} in which shoot dry weight was greatly enhanced (Fig. 2b). Interestingly, the treatments of AMOLE without Cd showed a significant increase in root development, i.e., increase in root number as well as root dry weight (Fig. 2).

Although the AMOLE did not affect Cd accumulation in the roots, Cd content in the shoots of plants treated with 0.3 and 0.5 mg mL^{-1} AMOLE was significantly reduced by 43% and 48%, respectively, compared with the Cd alone treatment (Fig. 3). These results reveal that the supplementation with 0.3

and 0.5 mg mL^{-1} of AMOLE significantly reduced translocation factor (TF) by 40% and 48%, respectively, compared with the Cd alone treatment (Fig. 3).

3.3. Effects of AMOLE on reducing Cd toxicity and accumulation in rice grown in the pot system

No visible Cd toxicity symptoms were detected in any of the treatments. In addition, statistical analyses indicated that shoot height, panicle number, photosynthesis rate, and pigments (chlorophyll a, chlorophyll b and carotenoid) of rice from all conditions were not significantly different (Table 1). However, Cd accumulation in shoots was significantly reduced in the AMOLE treatment. Interestingly, most Cd was accumulated in roots, and AMOLE treatment enhanced the Cd content in rice roots (Fig. 4). The translocation factor was below 1 and was reduced by approximately 50% in the AMOLE treatment. The AMOLE treatment also decreased Cd content in milled grain by half of that in the rice grown in the Cd without AMOLE treatment (Fig. 4).

Table 1
Morphological and physiological traits of rice grown in the pot system under different conditions. The results showed that there was no significant difference in all treatments. Data show average values ± SE. NS abbreviates for Not Significant at P = 0.05.

Treatment	Shoot height (cm)	Panicle number	Photosynthesis (μM/s)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)
Control	145.25 ± 4.64	3.50 ± 0.96	8.87 ± 1.19	2.03 ± 0.14	0.89 ± 0.06	0.63 ± 0.00
AMOLE	141.75 ± 5.44	3.50 ± 0.96	9.00 ± 1.87	2.00 ± 0.10	0.90 ± 0.06	0.60 ± 0.04
Cd	145.50 ± 3.80	2.75 ± 0.95	11.17 ± 0.57	1.91 ± 0.08	0.81 ± 0.03	0.62 ± 0.01
Cd + AMOLE	145.75 ± 4.89	3.25 ± 1.18	8.52 ± 1.13	1.54 ± 0.36	0.71 ± 0.16	0.61 ± 0.05
Significance	NS	NS	NS	NS	NS	NS

4. Discussion

Cadmium (Cd) is one of the most toxic metals that cause adverse effects in living organisms. Consumption of agricultural products, particularly rice, contaminated with Cd is the major cause of health problems related to heavy metals. In the present study, we demonstrated that the aqueous extract from *Moringa oleifera* leaf (MOLE) could enhance the germination rate, survival, and growth performance of seedlings, while substantially reducing the accumulation of Cd in plant tissues and grain yield across the growing systems.

The presence of Cd in soils adversely affects seed germination and the survival of seedlings (He et al. 2008; Subin and Francis 2013) In our study, the germination rate of KDML105 rice was substantially reduced to only 20%. The severe reduction of plumule and radicle growth is consistent with other studies in rice (He et al. 2008). Heavy metals have been reported to physically inhibit the absorption of water by seeds (Azmat et al. 2006) and accelerate the breakdown of reserved food materials in the seed embryo; thus, seed germination is decreased as the heavy metal concentration increases. The addition of AMOLE, particularly at high concentrations, could completely protect KDML105 seeds from cadmium's effect on germination. However, the germinating seeds still suffered from Cd toxicity despite a substantial reduction in the Cd concentration in their tissues. It has been reported that *M. oleifer*a leaf contains quercetin-3-O-glucoside and kaempferol-3-O-glucoside, along with 3-caffeoylquinic acid and 5-caffeoylquinic acid. Glucosinolate contains a central carbon atom that is bonded to the thioglucose group via a sulfur atom, and to a sulfate group via a nitrogen atom. These functional groups are good metal sequesters from an aqueous solution (Bennett et al. 2003). Additionally, Kerdsomboon et al. (2021) reported that the hydroxyl and carboxyl groups of gallic acid found in AMOLE play a critical role in chelating As(III) and potentially bind with cadmium and other heavy metals as well. We hypothesized that AMOLE contents could effectively act as Cd chelators preventing Cd ions from interacting with seeds, resulting in reduced toxicity on rice seed germination. Still, once the seeds germinated, the presence of free Cd ions largely inhibited root growth, which reduced the acquisition of water and nutrients, and Cd. This caused a drastic decrease in overall biomass and Cd content in plant tissues compared to the control treatment.

In the hydroponic system, Cd reduced overall growth performance and promoted leaf senescence. The toxicity of Cd on plant growth and development is well documented (Dong et al. 2005; Haider et al. 2021). The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll and photosynthesis, possibly by the production of reactive oxygen species (ROS). The degree of damage due to ROS depends on metal stress level, its type and form, plant nature, and time of exposure, etc. ROS reacts with cell membranes, organelles and biomolecules, resulting in diminished normal cell functions, induced cell abnormalities, and death (Sanita` di Toppi and Gabbrielli 1999). Our results show that plants treated with MOLE in the presence of Cd improved shoot dry weight and maintained green leaves (Fig. 2). The application of AMOLE has been shown to increase the production and activity of proteins coping with ROS, which enhances a plant's tolerance to environmental stresses. For example, Latif and Mohamed (2016) reported that a foliar spray of *M. oleifer*a extracts sufficiently enhanced the glutathione content, which reduced ROS contents such as malondialdehyde, oxygen radicals, and hydrogen peroxides, and increased salt tolerance in common beans. It is also important to note that the treatment of AMOLE alone without Cd supplementation significantly promoted root growth, particularly root number, resulting in an overall increase in root dry weight. It has been shown that *M. oleifer*a leaf also contains plant growth enhancers such as cytokinin, which has a critical role in enhancing cell division, cell elongation, chlorophyll biosynthesis, as well as modification of apical dominance in plants. Furthermore, treatment with *M. oleifer*a extract has been shown to enhance levels of growth hormones such as auxin and gibberellins in other plant species (Latif and Mohamed 2016). The influence of AMOLE on ROS and phytohormones could be the key factor for improving rice tolerance to Cd.

The results of Cd elemental analysis revealed that Cd was mostly accumulated in the roots of rice grown in both hydroponics and pot systems. Our results are consistent with many other reports in rice (Meeinkuirt et al. 2019; Saengwilai and Meeinkuirt 2021), emphasizing the role of rice as an excluder for Cd. The high retention of Cd in roots could be associated with several metal-binding ligands through intracellular heavy-metal detoxification mechanisms such as phytochelatins (PCs). Phytochelatins chelate Cd and then the low molecular weight Cd-PC complex is translocated across the tonoplast and transported into the central vacuoles of root cells by means of the ATP-binding cassette (ABC) transporter (Song et al. 2014). In our study, the AMOLE treatment did not alter the Cd content in root tissues, but effectively decreased the translocation of Cd from root to shoot, resulting in the reduction of Cd content in the shoot. These results suggest that AMOLE does not only act as a heavy metal sequester outside the plant's roots but may also promote the production and activity of proteins and enzymes coping with ROS and Cd sequestration in plants.

Unlike plants grown in the hydroponic system, rice plants grown in soils enriched with Cd exhibited no Cd toxicity symptoms. Growth and physiological parameters, including shoot height, biomass, chlorophyll contents, and photosynthetic rates appeared to be comparable with the control and the AMOLE treatment (Table 1). However, AMOLE significantly reduced shoot Cd content. Along with these results, we observed that AMOLE enhanced the accumulation of Cd in the roots and thus largely decreased translocation of Cd to the shoot, which is consistent with the results from the hydroponic experiment, and eventually led to lower Cd content in the milled grains. We suggest that further experiments in the field are needed to validate the potential of AMOLE in reducing Cd contamination in rice grains. The biochemical and molecular mechanisms should also be elucidated to expand our understanding and utilization of this beneficial plant to its maximal potential.

5. Conclusions

In this study, we demonstrated that AMOLE treatment increased seed germination, enhanced plant growth, and reduced Cd accumulation in rice plants and grain. Moringa plants are easy to grow and have been used widely in developing countries. Our findings suggest that a water extraction of *M. oleifer*a leaf can effectively reduce Cd contamination in rice, which provides a sustainable, accessible, environmentally friendly option to farmers growing rice in Cd contaminated areas. We propose that this technology may be applied to other crop species and different inorganic contaminants with further testing.

Declarations

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Authors contributions:

Conceptualization: Choowong Auesukaree, Patompong Johns Saengwilai; Methodology: Jutamas Bussarakum, Sirin Sirirakphaisarn; Formal analysis and investigation: Choowong Auesukaree, Patompong Johns Saengwilai, Jutamas Bussarakum, Sirin Sirirakphaisarn; Writing-original draft preparation: Choowong Auesukaree, Jutamas Bussarakum, Sirin Sirirakphaisarn; Writing-review and editing: Choowong Auesukaree, Patompong Johns Saengwilai; Funding acquisition: Choowong Auesukaree, Patompong Johns Saengwilai; Resources: Patompong Johns Saengwilai; Supervision: Patompong Johns Saengwilai.

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The authors declare that they have no competing interests.

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Figures

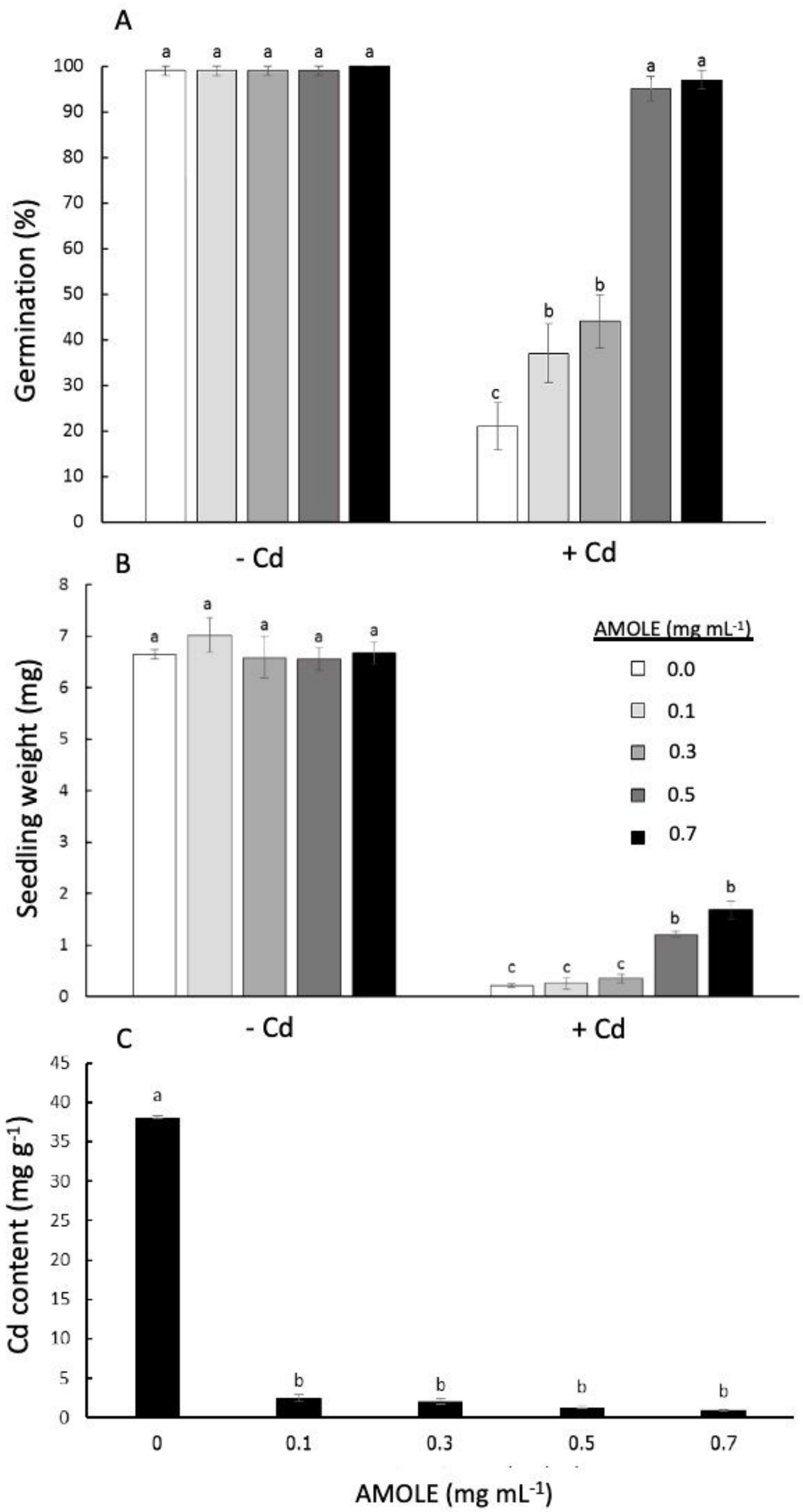

Figure 1

Percentage of germination (A), seedling weight (B), and Cd content (C) of rice grown in Petri dishes containing various concentrations of AMOLE with (+Cd) and without (-Cd) cadmium. Seedlings were harvested at 14 days after germination. Error bars represent ± SE. (n=5). Different letters indicate significant differences at P<0.05.

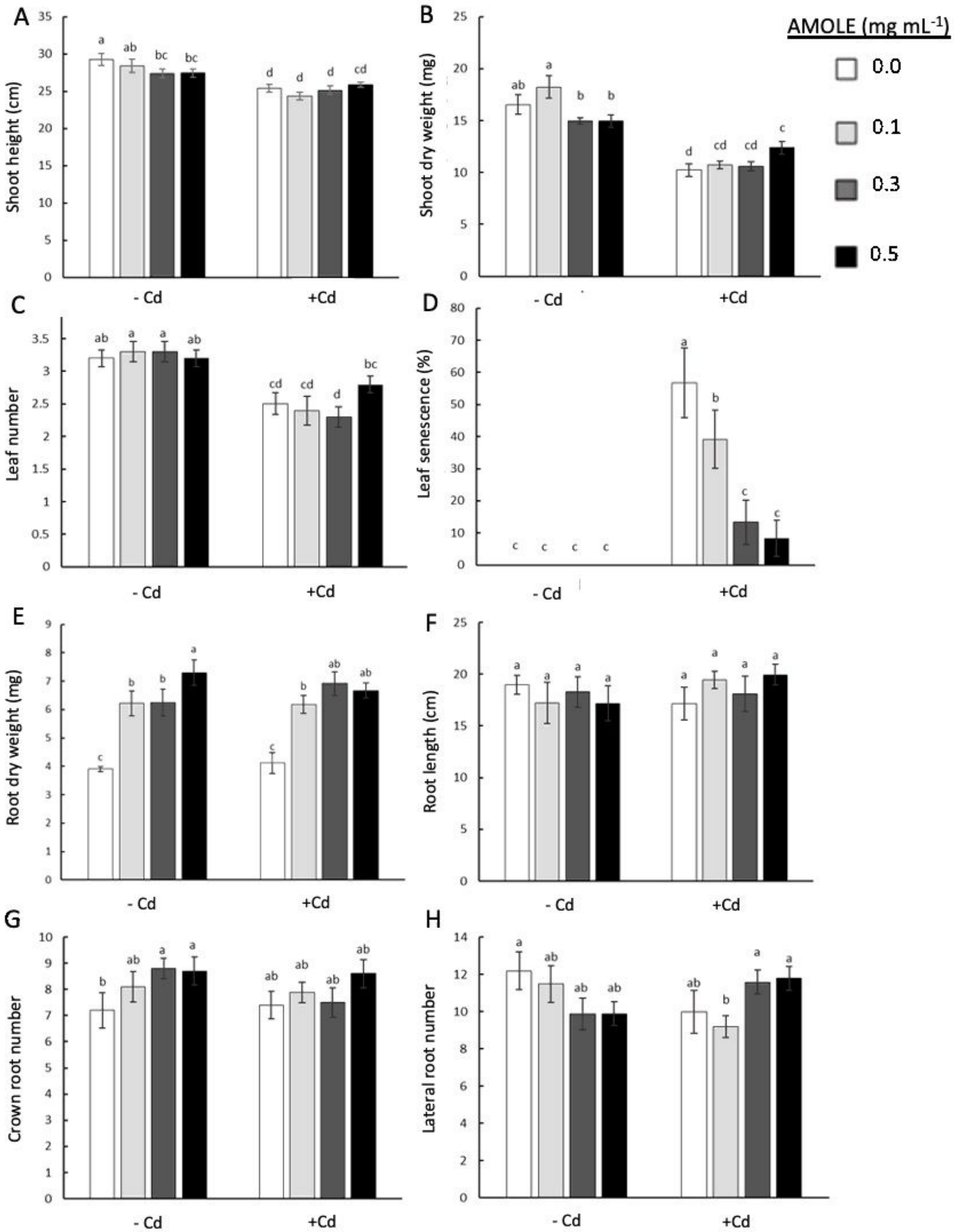

Figure 2

Effect of AMOLE on shoot height (A), shoot dry weight (B), leaf number (C), leaf senescence (D), root dry weight (E), root length (F), crown root number (G), and lateral root number (H) of rice seedlings in the hydroponic systems supplemented with 0−0.5 mg mL-1 AMOLE in the presence (+Cd) and absence (-Cd) of 25 mg Kg-1 Cd. Plants were evaluated 14 days after treatment. Error bars represent ± SE. (n=10). Different letters indicate significant differences at P<0.05.

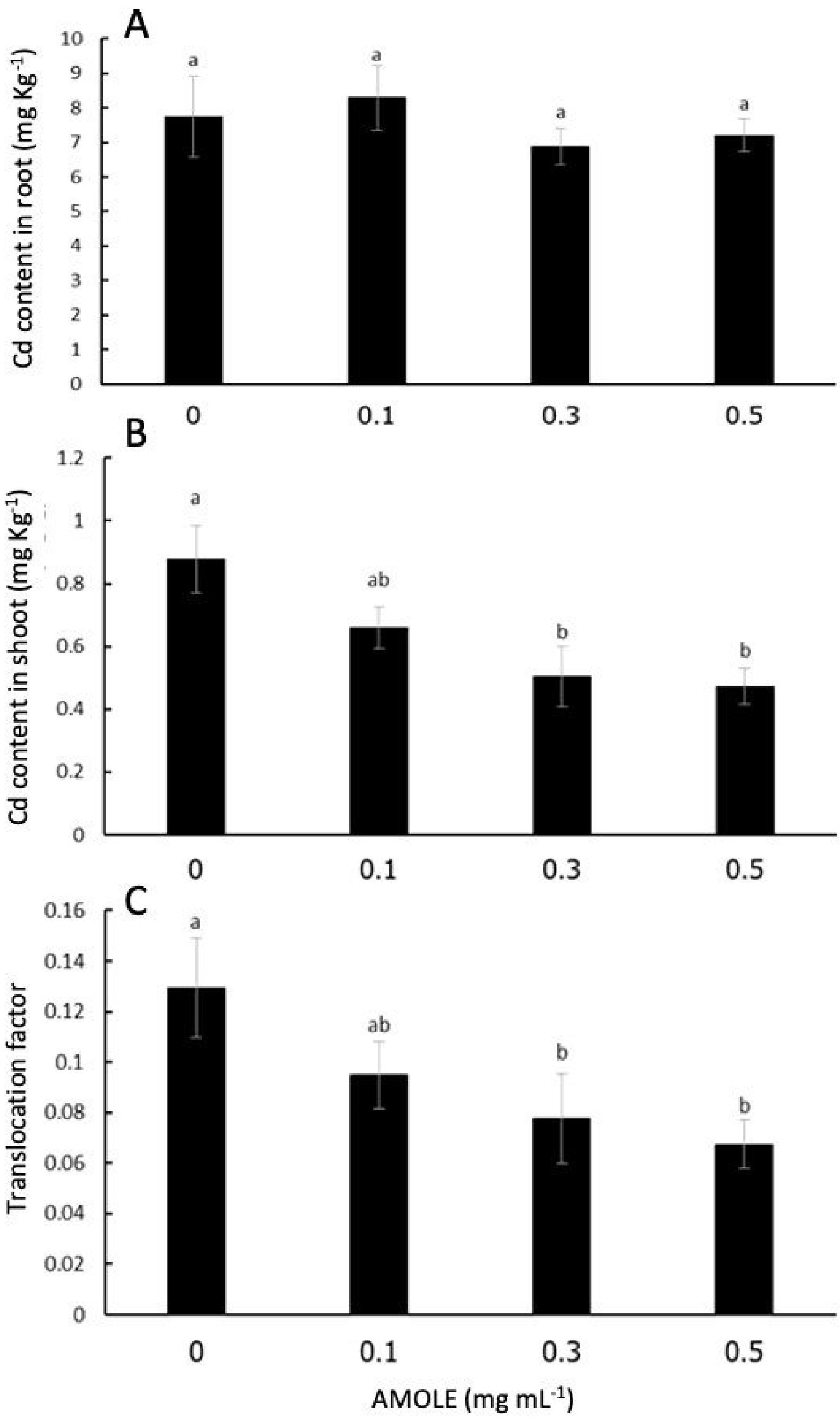

Figure 3

Cd contents (mg Kg-1) in roots (A) and shoots (B), and translocation factor (C) of rice seedlings grown in the hydroponic systems supplemented with 0–0.5 mg mL-1 AMOLE in the Cd treatment. Error bars represent ± SE. (n=10). Different letters indicate significant differences at P<0.05.

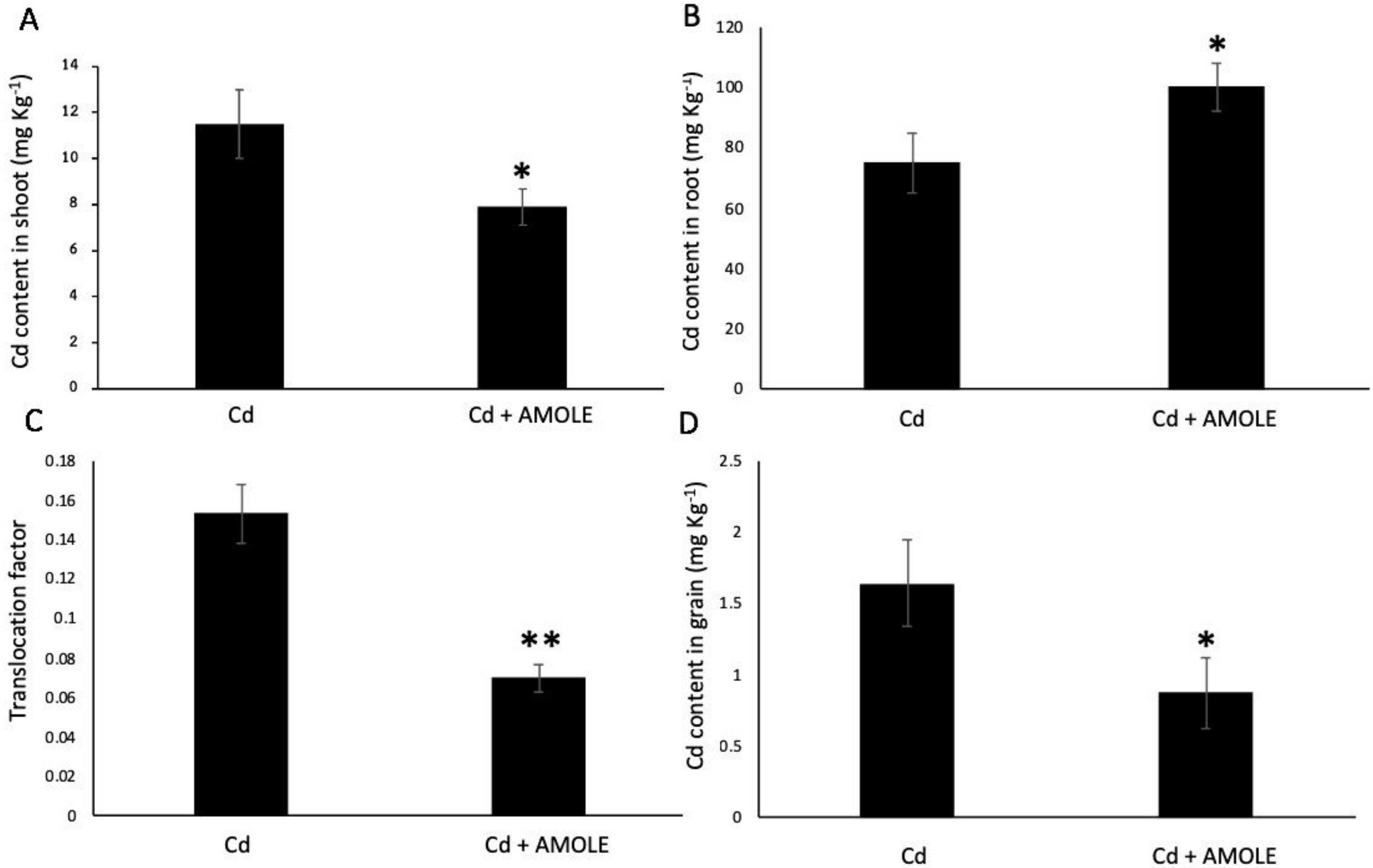

Figure 4

Cd accumulation in shoots (A), roots (B), translocation factor (C), and Cd content in milled grain (D). Error bars represent ± SE. (n=10). Asterisks represent significance at $P<0.05$ (*) and $P<0.001$ (**).