Growth performances of rice (*Oryza sativa* L. var. Chorati) grown in Cd-contaminated soils

Natthapong Pechampai[1], Weeradej Meeinkuirt[1,], Patompong Saengwilai[2], Malinee Sriariyanun[3], Theerawut Phusantisampan[4,*]

[1]*Mahidol University, Nakhonsawan Campus, Nakhonsawan 60130, Thailand*
[2]*Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[3]*Department of Chemical and Process Engineering, Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand*
[4]*Bioprocess Engineering and Biotechnology Research Group, Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand*
[*]*e-mail: phusantisampan.t@gmail.com*

Abstract

Cadmium contamination in soil can be translocated and accumulated to the plants that were cultivated in that area, and can cause toxicology effect on plant growth. At Mae Tao river basin in Tak province of Thailand, the rice crops that were cultivated in Cd-contaminated soil were found to uptake Cd in different levels, depending on spatial and temporal factors. *Oryza sativa* L. var. Chorati was the rice crop used in this study as it was a new rice variety and presumably suitable for rice cultivation in Thailand, even in Cd-contaminated cultivating area. This rice could adapt and grow well under high Cd-contaminated soils (15.4 mg kg^{-1}) and showed no visual toxicology symptom throughout the experiment. Organic amendments were encouraged to be supplied in Cd-contaminated soils at rice cultivation area because they could help support rice growth and diminish Cd toxicology effect on rice. Among different organic amendment options in this study, cow manure was most recommended one as it showed for dominant role on enhancing the rice growth (total dry biomass 125.8±9.4 g, number of tillers 38). Leonardite was another good organic amendment choice as a rice crops grown in Cd-contaminated soil that had leonardite supplementation having the highest organic matter content (6 %) along with the highest rice shoot (126.3±3.2 cm).

Keywords: *Oryza sativa* L. var Chorati, growth, cadmium (Cd), contaminated soil, organic amendment

Introduction

Cadmium is non-essential and poisonous element for living organisms. Human exposure to Cd could lead to severe health problems such as lung cancer (Alloway, 1995). Cd can rapidly migrate from the environment to living organisms by various pathways, including Cd uptake by plants and Cd accumulation through food chain cycle (Gallego et al. 2012; Grant et al. 1998). It can easily migrate from soil surface to deep soil layers and underground water, and thus it can distribute and accumulate in wide area, including agricultural and local community areas (Selvam and Wong, 2009). As the plant can uptake Cd from the Cd-contaminated soil, the uptake of Cd in the plant could have negative impacts on the plant growth. Severity levels of the impacts depend on the Cd uptake and the accumulation period.

In Mae Tao River Basin of Tak province, Thailand, two small creeks are the main irrigation supplied for the surrounded agricultural areas, in which Cd contamination in the soil has been detected for more than 20 years (National Research for Environmental and Hazadous Waste Management, 2005; Pollution Control Department, 2004). As a consequence of food chain effect, Cd accumulation has been carried over to living organisms, particularly to human being who consumed rice/vegetables grown in Cd polluted soil. Local people in this area

tended to have high risk having kidney and neuronal damages (Honda et al. 2010; Swuddiwudhipong et al. 2012). It was estimated that these local people might have encountered Cd accumulation for up to 94-210 μg kg^{-1} body weight weekly or about 14-30 times higher than the Cd tolerable weekly intake standard as suggested in the guideline of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Provisional Tolerable Weekly Intake (PTWI) (Padungtod et al. 2006).

It is imperative to utilize appropriate fertilizer which can enhance the ecological benefits and reduce the environmental hazards for sustainable agriculture. Organic fertilizers (i.e., manures, composts) are considered as an important component for improving soil properties and for enhancing plant growth. Animal manures are a good supply source for nitrogen and phosphorus to plant. Those major nutrients are the main limiting nutrients for agricultural soils (Gaind, 2014). Recently, leonardite (a special low rank coal) is considered to be used for agricultural purposes as it can help to enhance plant growth, increase fertilizer efficiency, and reduce soil compaction (Kalaitzidis et al. 2003; Sugier et al. 2013).

The objective of this study was to determine the effects on rice growth from supplementation of different types of organic fertilizers (pig and cow manures, leonardite) mixed in Cd-contaminated soil.

Methodology

Soil samples

Soil samples were collected from five sub-samples (0-30 cm depth) in Mae Tao river basin agricultural area of Tak province, Thailand. Soil sampling locations were near Zn mine, which was the main source for Cd contamination.

Physico-chemical properties of the soil samples were characterized before the experiment. All Cd-contaminated soil samples were mixed thoroughly for gaining the composite soil, and then air-dried in an oven at 75°C for 3 days. pH value was determined on a 1:5 soil to solution ratio using a pH meter. Electrical conductivity (EC) was determined by an EC meter. Soil texture was determined by a hydrometer (Allen et al. 1974). Organic matter was determined by using Walkley titration method (Walkley and Black, 1934). Total nitrogen value was determined by using Kjedahal method (Black, 1965). Extractable phosphorus was determined by using Bray II method (Bray and Kurtz, 1945). Extractable potassium was determined by using a Flame Atomic Absorption Spectrophotometer (FAAS) after extraction with NH_4OAc. Total Cd content was determined by FAAS after soil digestion with microwave digestion system (ETHOS ONE®)

Columns were containers in which rice were cultivated in this study (column diameter 10.5 cm, column height 30 cm). All columns were pre-cleaned with 10% nitric acid and deionized water several times. The column treatments include 1) control soil (non-contaminated soil) 2) Cd-contaminated soil 3) 10% leonardite in Cd-contaminated soil (w/w) 4) 10% pig manure in Cd-contaminated soil (w/w) 5) 10% cow manure in Cd-contaminated soil (w/w) Three replications were conducted for each treatment.

Plant materials, sampling and data analyses

Rice seeds (*O. sativa* L. var. Chorati), a new rice variety, were obtained from a rice farm demonstration school in Nakhonsawan province. The rice seeds were germinated in saturated sand until they reached up to 10 cm. Only the healthy plants were selected and transplanted into the soil columns (Figure 1). Deionized water (DI water) was added into the columns to maintain water level at 2-3 cm above soil surface.

After ripening of the rice grain, rice plant was determined height, root length and weigh for total dry biomass. Number of tillers was also recorded.
Analysis of variance (one way ANOVA; SPSS 17.0 computer software) were used to determine the effect of the treatments on plant growth performances. Mean values were compared with least significant difference (LSD).

Figure 1: Various treatments of *Oryza sativa* L. var. Chorati grown in PVC column

Results

Soil characterization
Characterization of the five soil samples were compared and shown in Table 1. The pH values of samples were slightly basic (7.1-7.8), except for soil in CdLeo (5.8) and Ctrl treatments (6.9). The EC values were in the range of 0.3-2.1 dS m^{-1}, which exceeded the normal levels of soil range from 0-0.2 dS m^{-1} (Shu et al. 2001; Suhardiyanto et al. 2009). Cd soil had the lowest organic matter content and nutrient content in the Cd-contaminated soil was found to increase after applying fertilizers. The texture of the soil samples was loam. Cd treatments in this study represented the soil samples that had Cd concentration exceeded standard level (0.3 mg kg^{-1}) and could be toxic to plant (Kabata-Pendias and Pendias, 1984). The Cd contaminated soil samples that had organic fertilizer supplementation contained lower Cd concentration.

Table 1 Soil characterizations of five soil treatments

Parameters	Ctrl	Cd	CdLeo	CdPig	CdCow
pH	6.9	7.8	5.8	7.1	7.6
EC (dS m^{-1})	0.3	0.3	2.1	0.7	0.6
OM (%)	4.0	3.4	6.0	4.9	5.3
Total N (%)	0.2	0.2	0.3	0.3	0.3
Extractable P (mg kg^{-1})	26.9	26.1	26.8	233.4	80.4
Extractable K (mg kg^{-1})	340.2	116.4	96.1	603.9	686.4
Total Cd (mg kg^{-1})	0.0	15.4	12.3	12.8	12.1
Soil texture	loam	loam	loam	loam	loam

Germination test and growth performances of the rice sample

Rice seeds exhibited 100% germination rate, indicating their proper germination condition over the germination period given and could imply their good health and success in growth performance when they grow up. All mature rice also survived successfully (100%) and grew fairly quickly, depending on the duration exposure. Among five soil treatments, rice samples in Cd treatment showed the lowest growth performances ($p \leq 0.05$) based on plant height, root length, total dry biomass and number of tiller (Figure 2). Total dry biomass content of plant is the mass of organic matter and normally used to compare growth performance of the plant species in heavy metals-contaminated soil (Meeinkuirt et al. 2012b, 2013a). The results indicated that supplementation of any type of all organic fertilizers to the Cd-contaminated soil could increase total dry biomass content of rice ($p \leq 0.05$). Results also showed that cow manure was the best amendment fertilizer for Cd-contaminated soil as seen in plant height and total dry biomass content.

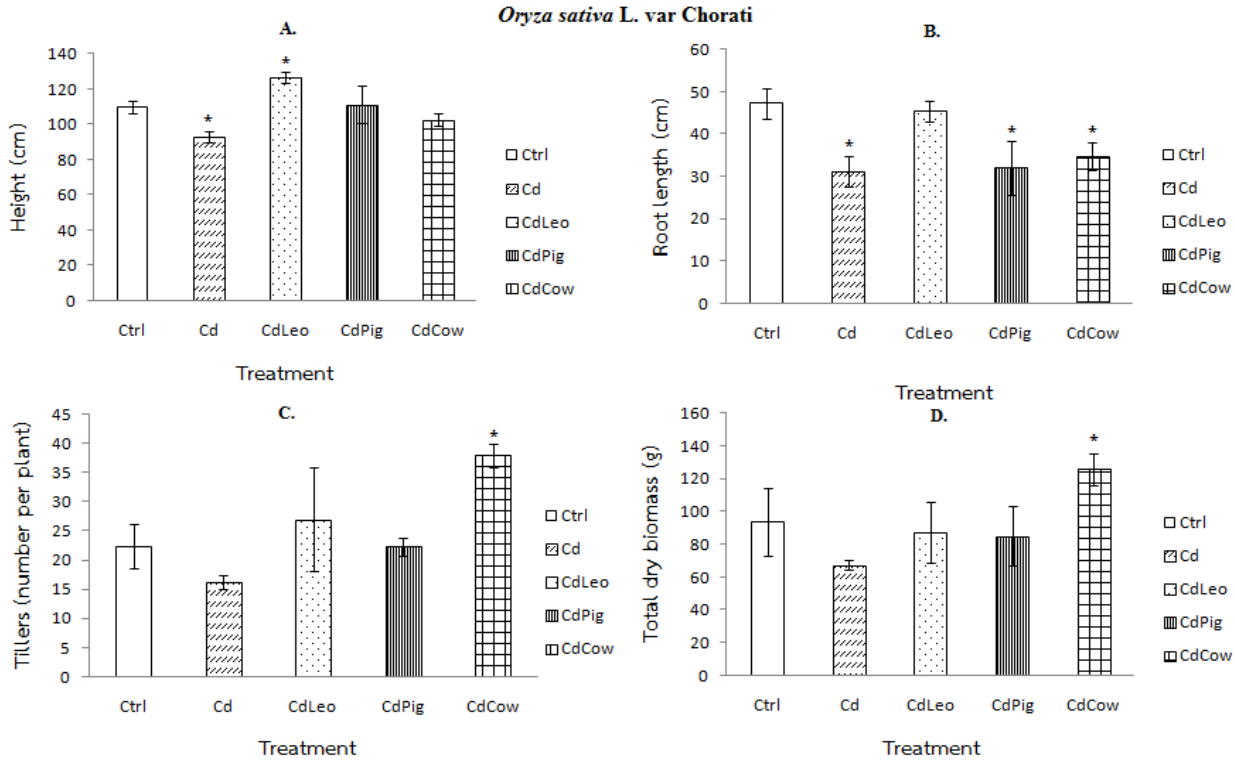

Figure 2: Height, root length, number of tillers and total dry biomass of the rice samples
* indicate the different amendment effect when compared with control group (LSD, $p \leq 0.05$)

Discussion

Different Cd concentrations have been discovered in heterogeneous contaminated areas of Mae Tao river basin in Tak province, Thailand for over three decades (Simmons et al. 2005). High levels of Cd were discovered in tailing soil in Zn mine (>1,000 mg kg^{-1}), and also found in agricultural contaminated areas of Padae sub-district (542.6-894 mg kg^{-1}) (Phaenark et al. 2008). The unusual high Cd concentrations were observed in the experimental soils, except for control soil. Alloway et al. (1990) stated that the normal Cd concentration in soil should be in the range of 0.01-2.0 mg kg^{-1}. Edible plants have been cultivating in such areas i.e., rice, sugar cane, potato etc. which can accumulate Cd content in the plant parts. Despite low Cd concentration detection in edible plants, human who consumed those plants can have risk on their health problem due to Cd accumulation in the food chain (Pinto et al. 2004). Thus, Cd-contaminated soil needs to be rehabilitated before doing any plantation. Various organic fertilizers were reported to be the best amendments to the heavy metals-contaminated soil due to their reasonable cost, stabilization effectiveness for heavy metals in soil, and their capacity in enhancing the plant growth (Chaiyarat et al. 2011). Organic matter contents in Cd soils were relatively fertile (>1%). In particular, CdLeo treatment had the highest organic matter content (6%)

It was apparent that all rice samples could survive and grow well under harsh environment, probably because the nutrients and organic matter could support plant growth and reduce Cd toxicity. Total dry biomass content is a good indicative parameter to estimate plant's health in this study. The highest total dry biomass content in CdCow treatment was related to the number of tillers in this study. Chaiyarat et al. (2011) reported that cow manure could increase total dry biomass content and reduce Cd accumulation in the plant part. To some extent, soil properties in Cd treatment contained the lowest organic matter content as well as

total nitrogen content. Nitrogen was the main parameter for enhancing plant photosynthesis (Meeinkuirt et al. 2013a). Organic fertilizers containing high organic matter and nitrogen contents could reduce metal toxicity and leachability, support plant growth and improve water and nutrient holding capacity in contaminated soil (Tordoff et al. 2000).

Conclusions

Thai rice is recognized the national main economic plant and a major export product of the country. At Mae Tao river basin in Mae Sot of Tak province, there were rice cultivating areas, which supplied rice for local food, and export to local areas or overseas. However, Cd contamination in the rice cultivation areas at Mae Tao river basin was found to be one of the obstacles for promoting world-class rice export of Thailand. Cadmium could affect the rice growth, meaning that the rice could not reach up the proper growth and its flowering stage. Resulting from this study showed that supplementation of organic amendments in the soil could help in reducing the Cd mobility and thus enhancing plant growth. Among various organic amendments, cow manure was highly recommended as alternative organic fertilizer to support plant growth, based on the number of tillers and total dry biomass content along with its reasonable cost. However, further investigation should be conducted to determine the Cd accumulation in rice and to evaluate on the potential of organic amendments for stabilizing or reducing Cd content in soil that might later be translocated to the plant parts.

Acknowledgement

This research was funded by King Mongkut's University of Technology North Bangkok. Contract no. KMUTNB-NEW-57-03. The authors would like to thank the reviewers for their time to help improve this paper.

References

Allen S.E., Grimshaw H.M., Parkinson, H.M., Quarmby J.A. (1974) Chemical Analysis of Ecological Materials. Oxford (UK): Blackwell. P.565.

Alloway B.J. (1990) Heavy metals in soils. Glasgow: Blackie & Son.

Alloway B.J. (1995b) Heavy metals in soils. 2nd Ed. Blackie Academic & Professional, An imprint of Chapman & Hall, U.K.

Black G.R. (1965) *Bulk Density: Method of Soil Analysis*. Monograph No.9 Part I. American Society of Agronomy Inc., Madison, Wisconsin.

Bray R.H., Kurtz L.T. (1945) Determination of total, organic and available forms of phosphorus in soil. Soil Science 59:39-45.

Chaiyarat R., Suebsima R., Putwattana N., Kruatrachue M., Pokethitiyook P. (2011) Effects of soil amendments on growth and metal uptake by Ocimum gratissimum grown in Cd/Zn-contaminated soil. Water Air and Soil Pollution 214(1-4):383-392.

Gaind S. (2014) Effect of fungal consortium and animal manure amendments on phosphorus fractions of paddy-straw compost. International Biodeterioration and Biodegradation 94:90-97.

Gallego S.M., Pena L.B., Barcia R.A., Azpilicueta Lannone M.F., Maria F. (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environmental and Experimental Botany 83:33-46.

Grant C.A., Buckley W.T., Bailey L.D., Selles, F. (1998) Cadmium accumulation in crops. Canadian Journal of Plant Sciences 78:1-17.

Honda R., Swaddiwudhipong W., Nishijo M., Mahasakpan P., Teeyakasem W., Ruangyuttikarn W., Satarug S., Padungtod C., Nakagawa H. (2010) Cadmium induced renal

dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. Toxicology Letters 198:26-32.

Kabata-Pendias A., Pendias H. (1984) Trace elements in soils and plants. CRC Press, Boca Raton, U.S.A.

Kalaitzidis S., Papazisimou S., Giannouli A., Bouzinos A., Christanis K. (2003) Preliminary comparative analyzes of two Greek leonardites. Fuel 82:859-861.

Meeinkuirt W., Kruatrachue M., Tanhan P., Chaiyarat R., Pokethitiyook P. (2013a) Phytostabilization potential of Pb mine tailings by two grass species, *Thysanolaena maxima* and *Vetiveria zizanioides*. Water Air and Soil Pollution 224:1750.

Meeinkuirt W., Pokethitiyook P., Kruatrachue M., Tanhan P., Chaiyarat R. (2012b) Phytostabilization of a Pb-contaminated mine tailing by various tree species in pot and field trial experiments. International Journal of Phytoremediation 14: 925-938.

National Research for Environmental and Hazardous Waste Management, Chulalongkorn University. (2005) Distribution of cadmium and absorption by rice plants in areas nearby the zinc mine in Mae Sot district. Chulalongkorn University, Bangkok, Thailand.

Patungtod C., Swaddiwudhipong W., Nishijo M., Ruangyuttikarn W., Inud T. (2006) Health risk management for cadmium contamination in Thailand-are challenges overcome? In: Proceeding of the fifth session of the intergovernmental Forum on Chemical Safety (IFCS), Forum V, Budapest, Hungary, pp.25-26.

Phaenark C., Pokethitiyook P., Kruatrachue M., Ngernsansaruay C. (2009) Cd and Zn accumulation in plants from the Padaeng zinc mine area. International Journal of Phytoremediation 11(5):479-495.

Pinto A.P., Mota A.M., de Varennes A., Pinto F.C. (2004) Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. Science of the Total Environment 326:239-247.

Pollution Control Department. (2004) Cadmium contamination in Mae Tao Creek, Mae Sot district, Tak province, Thailand. Ministry of Natural Resources and Environment, Bangkok.

Selvam A., Wong J.W.C. (2009) Cadmium uptake potential of *Brassica napus* cocropped with *Brassica parachinensis* and *Zea mays*. Journal of Hazardous Materials 167:170-178.

Shu W.S., Ye Z.H., Lan C.Y., Zhang Z.Q., Wong M.H. (2001) Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. Environmental International 26:389-394.

Suyantohadi A. Purnomo M.H., Hariadi M. (2009) Identification of soybean plant growth on statics neural network. Agricultural Technology Agritech, University of Gadjah Mada, pp.16-23.

Swaddiwudhipong W., Limpatanachote P., Mahasakpan P., Krintratun S., Punta B., Funkhiew T. (2012) Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: A five-year follow-up. Environmental Research 112:194-198.

Tordoff G.M., Baker A.J.M., Willis A.J. (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41:219-228.

Walkley A., Black C.A. (1934) An examination of degradation method for determining soil organic matter: a proposed modification of the chromic acid titration method. Soil Science 37:29-35.