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Effect of Cadmium Speciation on Vetiver Grass Uptake

ผลของรูปฟอร์มแคดเมียมต่อการนำเข้าของหญ้าแฝก

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ABSTRACT

The effects of chemical form and concentration of Cd taken up by vetiver grass in contaminated soil from Maesod District, Tak Province in Thailand was used a pot experiment in a greenhouse. The concentration of Cd in soil background was 0.788 mg kg-1dry weight. The chemical form of Cd contained in the soil was used by sequential extraction protocol for analysis Cd speciation. Cd was found about 34.10% and 30.80% in the residual and reducible fraction, respectively. The acid extractable fraction was less than the other fractions. The total Cd content of each pot was accumulated mainly in the roots. Cd concentration in the root of vetiver grass increased with time of experiment, it was significantly different in the plant root in all of treatments at the end of experiment ($p>0.05$). The reducible and residual and next in exchangeable fractions were bioavailable fraction comprised more than the other forms ($p>0.05$). There was no significant variation in absolute mobile fractions (oxidizable, and acid extractable) in the time of experiment.

บทคัดย่อ

ดินที่ปนเปื้อนแคดเมียมจากอำเภอแม่สอด จังหวัดตาก ได้ถูกนำมาใช้ทดลองปลูกหญ้าแฝกเพื่อหารูปฟอร์มของแคดเมียมและความเข้มข้นของแคดเมียมที่จะถูกดูดดึงเข้าสู่หญ้าแฝก แคดเมียมเบื้องต้นในดินมีความเข้มข้น 0.788 มิลลิกรัม/กิโลกรัม การวิเคราะห์หารูปฟอร์มของแคดเมียมในดินก่อนปลูกใช้วิธีการสกัดเป็นลำดับ รูปฟอร์มทางเคมีที่พบร้อยละ 34.10 และ 30.80 เป็นรูปฟอร์มของเศษเหลือและรูปฟอร์มการรับอิเล็กตรอนตามลำดับ ในขณะที่รูปฟอร์มที่ละลายในกรดพบได้น้อยกว่ารูปฟอร์มอื่น พบความเข้มข้นของแคดเมียมสะสมในรากเป็นส่วนมาก ความเข้มข้นของแคดเมียมในรากหญ้าแฝกเพิ่มขึ้นตามระยะเวลาที่ทดลองโดยมีแนวโน้มว่ามีความแตกต่างกันทางสถิติ ($p>0.05$) รูปฟอร์มของการรับอิเล็กตรอนและรูปฟอร์มเศษเหลือถัดมาคือรูปฟอร์มแลกเปลี่ยนไอออนเป็นรูปฟอร์มที่เคลื่อนย้ายเข้าสู่หญ้าแฝกได้อย่างว่องไวกว่ารูปฟอร์มอื่นโดยมีแนวโน้มว่ามีความแตกต่างกันทางสถิติ ($p>0.05$) และไม่มีนัยสำคัญทางสถิติกับรูปฟอร์มของการจ่ายอิเล็กตรอนและฟอร์มที่ละลายในกรด

Key Words : Cd speciation, Vetiver grass, Phytoremediation
คำสำคัญ : รูปฟอร์มแคดเมียม หญ้าแฝก พืชบำบัด

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Introduction

Status of contaminants such as heavy metals in soil will increase and become a major problem worldwide (Terry, 2003). Cd is considered a highly toxic heavy metal to plant, animal and microbes. Metal accumulation in plants has been of environmental concern because their uptake from contaminated soils is a process by which metal can enter the food chain (Ololade and Ologundudu, 2007). Thus paddy rice land in Japan, China, Korea, Vietnam and Thailand which has been shown to be contaminated by geogenic Zn and Cd sources is the largest area of soils requiring Cd remediation (Autumn S. Wang et al., 2006). In the Northwestern of Thailand, contamination of Cd in cultivated soil and rice grain found in Maesod District, Tak Province.

Cd can enter the environment in several ways (ATSDR, 1993). Most of the Cd in the environment comes from man-made sources, especially from non-ferrous smelting, fuel combustion, disposal of cadmium-containing products and application of phosphate fertilizer (Toronto Public Health, 1993). Cd does not break down in the environment but can change into different forms. Most Cd stays where it enters the environment for a long time (ATSDR, 1993). Cd affects the growth of plants in experimental studies, although no field effects have been reported. The metal is taken up into plants more readily from nutrient solutions than from soil; effects have been mainly shown in studies involving culture in nutrient solutions (WHO, 1992).

Distribution of Cd is relatively independent of the route of exposure. It is distributed throughout the body, with particularly high levels in the liver and kidneys (Toronto Public Health, 1993). The U.S. Department of Health and Human Services (DHHS) has determined that Cd and Cd compounds are known human carcinogens. The International Agency for Research on Cancer (IARC) has determined that Cd is carcinogenic to humans (ATSDR, 2008).

Phytoremediation is a method of remedying environments contaminated with hazardous of plants. Phytoremediation is the use green plants to detoxify a degraded or polluted environment (Kirkham, 2006). The mechanisms that control the uptake of Cd by plant roots and accumulation in edible plant foods are not well understood (Vassilev et al., 2002). Plants can extract Cd from the soil and transport it via the xylem into shoots and leaves where it can accumulate (Sam, 2007). Vetiver grass is an “ecological-climax” species. It outlasts its neighbours and seems to survive for decades showing no aggressiveness or colonization ability. It withstands drought and high levels of flooding (TVNI, 2007). It is tolerant to high levels of pesticides and herbicides and also to a wide range of toxic and heavy metals.

In fact, plant Cd uptake and translocations of Cd from root to shoot had been studied for a long time in several species, but most research studies on a Cd removal by vetiver grass were made in its totality only. The main objectives of this study were focused on the Cd speciation in soil contamination and investigate the Cd speciation uptake ability of the roots and shoots of vetiver grass in Cd-contaminated soils.

Materials and methods

Soil samples were collected from the 0-25 cm surface layer of the Cd-contaminated areas in Maesod District, Tak Province in Thailand. The soil samples were mixed thoroughly and homogenized, air-dried and passed through 2 mm diameter sieve. The soil was divided into 2 parts. The first part was used to grow vetiver grass in pot experiment. The second part was used to analysis for both physical and chemical characteristics. The soil's pH was measured by soil-water ratio equal 1:1 using a pH meter. Organic matter content was measured using Walkley-Black method and cation exchange capacity (CEC) of the soil was determined using the ammonium acetate saturation method. The soil texture, total N and Soil Moisture were measured by the procedures described by Land Development Department, 2001. The background concentrations of Cd were determined by the Flame Absorption Atomic Spectrometry (FAAS) on a Varian SpectrAA 220FS after strong acid digestion (conc. HNO_3) in Microwave digester (MARSX model XP 1500).

The chemical forms of Cd were investigated by the sequential extraction procedures (SEPs) used are modified from Tessier et al., (1979). The extractants and operationally described in chemical fractions were as follows:

(1) One gram of soil sample extracted with 8 mL of 1 M $MgCl_2$ at pH 7, Shaken 1 hr at room temp.

(2) The residue from (1) extracted with 8 mL of 1 M NaOAc, pH 5, Shaken 5 h at room temp.

(3) The residue from (2) extracted with 20 mL of 0.04 M $NH_2OH.HCl$ in 25%(v/v) HOAc at pH 2 (adjusted with HNO_3), Shaken 5 h at 90 °C in a water bath.

(4) The residue from (3) extracted with 3 mL of 0.02 M HNO_3 and 5 mL of 30%. (v/v) H_2O_2 (adjusted to pH 2 with HNO_3), Shaken 30 min at 85 °C in a water bath.

(5) The residue from (4) extracted with HNO_3 and HF mixture in microwave digester.

Concentration of Cd in all extracts was measured by FAAS. Extracts were replicated, with mean values and standard deviation (SD) of three replications.

Vetiver grass (*Vetiveria nemoralis* A.Camus (Nakhonsawan ecotype)) was obtained from Huai Sai Royal Development Study Center, Cha-am district, Phetchaburi Province, Thailand. The vetiver grass stage about one year old was used. In a greenhouse experiment, the vetiver grass was grown in 25 cm high and 30 cm diameter the plastic pots filled with 2.5 kg air-dried soil. The design for the control group was the same with no planting. A 4 × 6 factorial experiments as a completely randomized design. Each pot was watered 2 times daily during experimental period and three replicates were used for each treatment. The vetiver grass at the end of each experiment periods (15, 30 and 45 days growth) were harvested. Root and shoot were separated and thoroughly washed, including a final rinse with distilled water, and kept in a refrigerator until analysis. Soil sample beneath each plant root were collected after harvesting the plant.

For determination of total Cd content of the soils, 0.5 g of each soil sample (oven-dried at 70°C for 48 h) was accurately into a digestion tube and 10 ml of concentrated nitric acid added. The sample was then heat and pressure at 210 °C and 200, respectively on Microwave digester for 30 minutes. After cooling, the soil sample was filtered through a Whatman No.42 filter paper into a 50 ml volumetric flash with distilled water, and then analyzed for Cd concentration by using FAAS. Sequential extraction procedure was used for Cd assay.

Into the digestion tube, 0.5 g (accurately weighed) of plant sample (separated shoot and root) (oven-dried at 70°C for 48 h) were placed and 10 ml of concentrated HNO_3 added. The sample was then heat and pressure at 150 °C and 170, respectively on Microwave digester for 30 minutes. Finally, the digest was cooled and filtered through a Whatman No.42 filter paper into a 50 ml volumetric flash with distilled water. Cd was determined in the digest solutions by using FAAS. The chemical forms of Cd were investigated by the sequential extraction procedures used are modified from the schemes developed by Tessier et al., (1979).

Results and discussion

In this study, the contaminated soil taken from Maesod District, Tak Province in Thailand contains the Cd speciation that was used for planting. The chemical and physical characteristics of soil were analyzed before experiment. The results are shown in table 1. Soil chemical and physical characteristics, the soil pH and composition, particularly the nature of soil clays, the organic matter content, and, obviously, the soil cadmium level, affect of Cd availability (WHO, 1992).

Table 1 The chemical and physical characteristics of soil from Maesod District, Tak Province in Thailand.

Parameters	Unit	Values
Soil pH		7.96
Organic Matter (OM)	g/kg	2.49
Soil Texture		
Sand	%	22.6
Silt	%	34.6
Clay	%	42.8
Cation Exchange Capacity	cmol/kg	9.7
Soil Moisture	%	5.84 %
Total Nitrogen	%	0.12 %
Available Phosphorus	mg/kg	7.11
Available Potassium	mg/kg	42

All results of the soil characteristics in this study were very effective on mobility and phytoavailability of Cd uptake by plant, and it contains bioregulators which affects plant growth in pot experiments. The analyzed results regarding the amount of initial Cd in the soil samples was 0.788 mg kg^{-1}dry dry weight. The experimental results showed that contaminated soil that was taken from the agricultural areas had very low concentration of Cd. Cd generally decreased from the topsoil to the subsoil and varied with soil type (Onyatta and Huang, 1998). Cd might have disturbed metabolic pathways and plant mineral metabolism, especially in this soil where rice was planted for a long time. According to the study of the joint investigation of International Waste Management Institute (IWMI) and the Department of Agriculture (DOA) in 2003 (NRCE, 2005), reported that contaminated of Cd in cultivated soil and rice grain found in Maesod

District, Tak Province, the Northwestern of Thailand. The IWMI survey showed that about 2,000 hectares in the Mae Ku area and its vicinity had been polluted with Cd.

In this study was to investigate the partitioning of chemical form of Cd contains in the contaminated soil was used by sequential extraction protocol for analysis Cd speciation in soil method. Result for the sequential extraction of a certified reference material by using the modified from Tessier et al., (1979). The percentage of Cd fraction calculated by the summation of sequential fractions in relation to the total Cd content extracted with aqua regia and shown in figure 1. The chemical form of Cd concentration, order of sequential fraction was F5 > F3 > F1 > F4 > F2. The percentage of Cd fractions were 34.1, 30.8, 26.5, 8.1 and 0.4 percent, respectively.

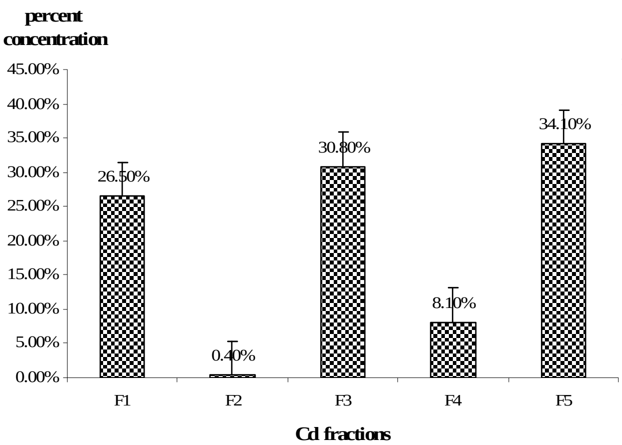


Figure 1 The percentage of each fraction of Cd in soil extracted by the sequential extraction protocol.

The results from Figure 1 found that chemical form of the analysis of Cd from soil samples background from the 5 fractions have the following sequence were F1 : exchangeable (exchangeable fraction), F2 : bound to carbonate (acid extractable fraction), F3 : bound to Fe-Mn oxides (reducible fraction), F4 : bound to organic matters (oxidizable fraction), and F5 : residual (residual fraction), respectively. Results of this experiment showed that the chemical form of Cd was the most concentration was reducible fraction and residual fraction while other fraction was not dominant. The experimental results was similar to study of Chusai (2006) reported reducible fraction and residual fraction were the most concentration of Cd in sediment samples from Maetao River in Maesod District, Tak Province.

At the end of the 45 days experiment, after the vetiver grass was transplanted, the plant morphology was as follows: green leaves remained unchanged and a very long branching root system was noticed. The vetiver grass can grew well on the soils contaminated with Cd. Vetiver grass harvested yielded higher dried shoot weight than those harvested 15 and 30 days after planting. In comparison, the length of vetiver grass leaves showed that more time is needed to make the leaves longer than the original. Therefore, Cd in contaminated soil used in experiments has no serious effects on plant morphology.

The average of total wet weight of the initial experimental plants was 15.08 g. The average total dry weights of shoots and roots for 15, 30 and 45 days after planting were 12.73, 12.97 and 14.60 g, respectively.

The mean of the shoot height between initial experiment and final experiment were 24.63 and 104.8 cm, respectively. The different initial root length was 4.87 cm and the end of experiment was 13.17 cm. The vetiver grass was monocotyledon plant. Monocotyledon plant has more Cd tolerance than dicotyledon plant (Agrawal and Agrawal, 2000). These results confirmed the strong biomass productivity of vetiver grass until the end of experiment and Cd was not essential for plant growth regulator.

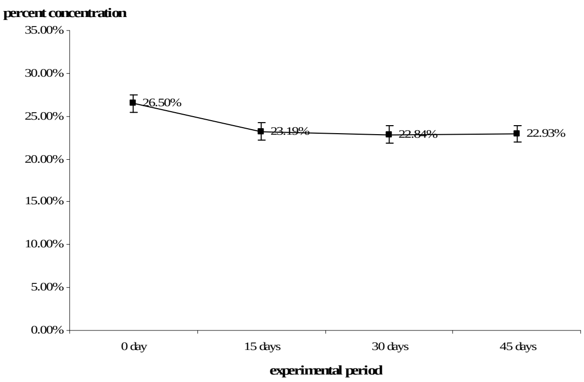
In this experiment was to investigate the chemical form of Cd contains in plant organ was used by sequential extraction protocol for analysis Cd speciation. It was found that the analyzed concentration of Cd among various fractions determined by any sequential extraction methods could not analysis because the Cd concentrations in plant organ sample were lower than detection limit of FAAS. However, the analysis of total Cd in plant sample conducted in laboratory experiment. Extracts were replicated, with mean values and standard deviation (SD) with the experiment times reported and shown in Table 2.

Table 2 Summary of concentration and fractionation of Cd in plant organ with experimental period after planting.

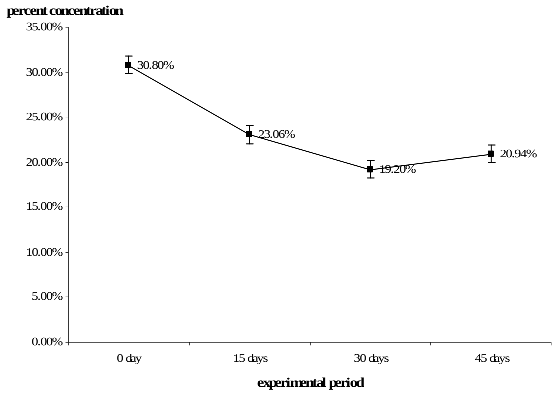
Experimental periods (Treat)	**Cd fractions**	**Cd concentration**	
		Root	**Shoot**
15 days	F1	DL	DL
	F2	DL	DL
	F3	DL	DL
	F4	DL	DL
	F5	DL	DL
	Sum of fractions	0.0430±0.0034	DL
30 days	F1	DL	DL
	F2	DL	DL
	F3	DL	DL
	F4	DL	DL
	F5	DL	DL
	Sum of fractions	0.0477±0.0118	DL
45 days	F1	DL	DL
	F2	DL	DL
	F3	DL	DL
	F4	DL	DL
	F5	DL	DL
	Sum of fractions	0.1133±0.0507	DL

Remark : Values expressed as mean ± SD (mg kg^{-1} dry weight, n = 3)

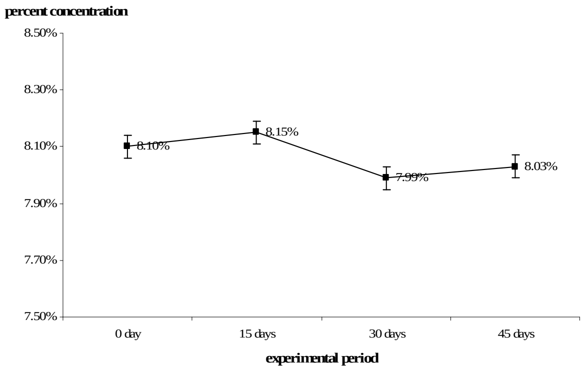
DL : Below detection limit (DL) of FAAS



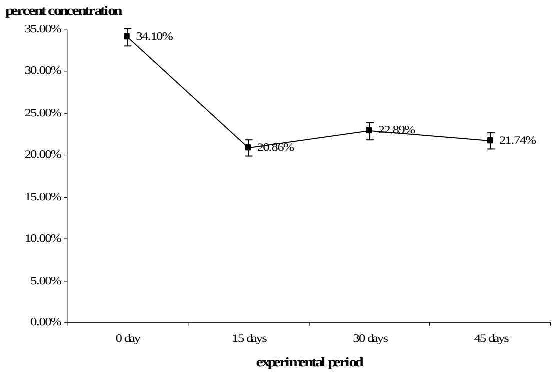
F1



F3



F4



F5

Figure 2 The percentage of each fraction (F1, F2, F3 and F4 fractions) of Cd in soil with time of experiment.

From Table 2, Plant-soluble fraction of Cd was below detection limits for all fractions, both in root and shoot after planting. The total Cd content of each pot was determined by digesting with nitric acid and it was observed that Cd was accumulated mainly in the roots, and only a small portion was transferred to the shoots. The accumulation of Cd in plant root on 15 days was less than 30 days after harvesting from contaminated soil. The highest level of Cd accumulation was found at the end of experiment. Cd availability to plants was affected by both biotic factors and non-biotic factors (McLaughlin and Singh, 1999). The effects of land used changes on Cd mobility (Römkens and Salomons, 1998). Major factors affecting the mobility and availability of Cd were soil pH. Soil contamination was taken from the agricultural areas had very low concentration of Cd, and soil pH was slightly alkaline. Supamart (2540), reported that Cd mobility in soil pH value range 4.5-5.5, whereas in alkaline soil Cd was rather immobile, and this may have led to decreased Cd mobility in soils including transfer of Cd from soil to plants.

All results presented and discussed were based on mean values and standard deviation (SD) of three replications. Cd concentration in the root of vetiver grass increased with time of experiment, it was significantly different in the plant root in all of treatments at the end of experiment ($p>0.05$).

From the results in Figure 2, only F1, F3, F4 and F5 fractions showed the highest share of the available fractions in experimental group, whereas Cd form in F2 fraction was not present. The percentage of each fraction (F1, F3, F4 and F5) of Cd in soil with time of experiment, it were decreases during the 15-45 days of experiment. In the sequential extraction method used in this study, the mobility and bioavailability of Cd fractions were low all fraction in the contaminated soil before planting. In comparison, the percentage of all fractions in experimental group was less than in the soil control under the same conditions. This results of experiment show that there were significant differences in Cd concentration of each fraction between the beginning and end of the experiment.

At the end of experiment, comparison of the Cd fraction between the initial fractions before planting and all fractions after the vetiver grass have been harvested was presented in figure 2, it was found that, the predominant form of Cd and less in the soil at first fifty-thirty days was the reducible fraction, residual followed by exchangeable and oxidizable fraction. At the end of the 45 days experiment, after the vetiver grass was transplanted, the percentage values for the Cd forms in residual fraction contained significant amounts of available fractions.

The reducible and residual fractions were easily bioavailable of Cd in contaminated soil. The metal fraction bound to Fe - Mn oxides and organic matter (reducible fraction) can be mobilized when environmental conditions become increasingly reducing or oxidizing (Bryan and Langston, 1992). This experiment result was similar to study of Otte, et al., (1995) who reported that the residual fraction could be converted to reducible fraction by the activity of plant roots. There was no significant variation in absolute mobile fractions (oxidizable, and acid extractable) in Cd concentration of each fraction between the beginning and end of the experiment. Bioavailability is the dynamic process, which include exposure and uptake route of Cd, chemical fluxes for specific biological species and redistribution processes within the species (CPCB, 2009).

Conclusions

The contamination degree for soil sample in this study area was low and non toxicity to vetiver grass. The higher concentrations of residual fraction and reducible fraction were found in contaminated soil background. Cd was found dominantly in residual fraction 34.10%, and next in reducible fraction 30.80% of initial soil. The acid extractable fraction was the lowest concentration in soil samples analyzed. The vetiver grass used for experiment was young and non-contaminated with any heavy metals. The vetiver grass can grew well on the soils contaminated with Cd since the beginning of the experiment until the end of experimental period. The total Cd uptake was found in all root samples during the experiment duration (45 days). Cd accumulation was higher in roots than shoots. All each pot of vetiver grass could not determine chemical form of Cd by using FAAS. Cd concentration in all fractions of roots and shoots were below detection limit.

At the end of experiment, it was significantly different in the reducible and residual fractions were bioavailable fraction comprised more than the other forms ($p>0.05$). There was no significant variation in absolute mobile fractions (oxidizable, and acid extractable) in the time of experiment. These results indicated that, the different fraction patterns of Cd reflect the sources of Cd contamination and the physicochemical properties of soil that effect on the efficiency of Cd fraction for phytoavailability.

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