SHORT REPORT

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Immobilization of cadmium in soil by cow manure and silicate fertilizer, and reduced accumulation of cadmium in sweet basil (*Ocimum basilicum*)

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ABSTRACT: This study was conducted to determine the effects of organic and inorganic soil additives in reducing Cd concentration in the edible parts of plants. A pot study was performed by growing *Ocimum basilicum* (sweet basil) in Cd contaminated soil (20 mg/kg Cd) and soils amended with cow manure and silicate fertilizer for 3 months. The results showed an increase in dry biomass production by factors of 4.7 and 1.7 in plants grown in soil supplemented with cow manure (20% w/w) and silicate fertilizer (20% w/w), respectively. Shoot Cd accumulation doubled in plants grown in cow manure treated soil. In contrast, the silicate fertilizer resulted in a 3-fold decrease in leaf Cd accumulation. The results of this investigation demonstrated the potential of silicate fertilizer in reducing Cd transport from roots to shoots, resulting in decreased Cd concentration in the edible parts of plants.

KEYWORDS: soil amendments, pot study, cadmium accumulation, dry biomass production

INTRODUCTION

The primary risk pathway associated with Cd contaminated soils has been identified as the soil-plant-human pathway and the consumption of the crop or by-products grown on these soils leads to its biomagnification in the food chain[1]. It is the Cd present in the soil and the transfer of this Cd to food plants together with the Cd deposited out of the atmosphere on edible plant parts which establishes the vast majority of human Cd intake[2]. There is considerable information in the literature regarding the Cd content of foods such as soybean (*Glycine max*)[3], wheat (*Triticum jurgiditum* var. *durum*)[4], rice (*Oryza sativa*)[5,6] grown in contaminated areas. In Thailand, the study in Mae Sod District, Tak Province, where elevated levels of Cd in paddy soils have been reported[6] is regarded as an important issue. Total soil Cd concentrations in these areas ranged from 0.5–284 mg/kg. Cadmium contamination in rice grains is associated with suspended sediment transported to rice fields via the irrigation supply. Consequently, this poses a significant public health risk to local communities[6].

Different actions can be undertaken in order to reduce the absorption of Cd by plants. The addition of amendments such as calcium carbonate, zeolite, and manganese oxide can reduce Cd uptake in rice and wheat. Chen et al[7] reported that zeolite was more effective in suppressing Cd uptake by rice and wheat than calcium carbonate or manganese oxide. They speculated that the increased pH and available silica arising from the zeolite contributed to the reduced Cd uptake in plants. Other evidence also suggested that silica possibly plays a significant role in decreasing Cd uptake in plants[8]. Farmyard manure and poultry are considered to be natural amendment used in tailings because the addition of organic matter can significantly improve the physical characteristics and the nutrient status of soil, and effectively lessen Cd uptake in plants[9]. For example, the addition of chicken and pig manure composts can decrease phytoavailability of Cd in cherry-red radish (*Raphanus sativus*)[10]. In general, it is commonly recognized that manure compost has positive effects on crop production[11].

Ocimum basilicum L. (sweet basil) is one of the Lamiaceae family members that has been traditionally grown as marketable crop in Europe, US, and SE Asia. Basil is cultivated for the freshmarket as a culinary herb, as a condiment or spice in the dried/frozen leaf form, and as a source of aromatic essential oils for use in foods, flavours, and fragrances. In addition, fresh herbage is exploited in medicinal treatment[12].

The objective of this study was to investigate the possibility of the use of organic (cow manure) and inorganic (silicate fertilizer) additives for the immobilization of Cd in contaminated soil in which *O. basilicum* is grown.

MATERIALS AND METHODS

Plant materials

Sweet basil seeds were purchased from Chia Tai Company, Bangkok. They were planted in 15-cm diameter plastic pots filled with agriculture soil purchased from Malee Kankaset, Bangkok and grown outdoors (30–35 °C, natural sunlight) for 2 months.

Soil preparation

The uncontaminated potting soil (51% sand, 31% silt, 18% clay) was air dried and ground to pass through a 2-mm sieve. Cadmium [$Cd(NO_3)_2 \cdot 9\,H_2O$] was added to the soil at the concentration of 20 mg/kg. The soil was mixed thoroughly and put in plastic pots each filled with 2 kg pretreated soil. The pots were watered with tap water daily for 2 weeks to avoid a heterogeneous distribution of Cd.

Two amendments were used, cow manure and silicate fertilizer. Fresh cow manure was collected from a local farm in Kanchanaburi province, Thailand. It was air dried for 1 week and ground before analysis and use. The source of silicate fertilizer was pumice from Lopburi province, Thailand. It was purchased from Thai Green Agro Company, Bangkok. The composition was (1) major oxides (%wt) : SiO_2 70, Al_2O_3 13.66, Fe_2O_3 1.26, P_2O_5 0.017, CaO 1.12, MgO 0.2, Na_2O 1.33, and (2) minor elements (ppm): As 5, Cu 33, Cd nil, Hg 0.05, Mn 294, Mo nil, Pb 15. Six treatments with three replicates each were investigated: CS (neither Cd nor additive was added), CdS (Cd added at 20 mg/kg), CdCw10 and CdCw20 (cow manure added to Cd soil at 10% and 20% w/w, respectively), CdSi10 and CdSi20 (silicate fertilizer added to Cd soil at 10% and 20% w/w, respectively). Cow manure and silicate fertilizer were mixed thoroughly with Cd soil and watered with tap water daily for 2 weeks before use. Soil without any additives served as control. Soil samples from each treatment and cow manure were characterized for their pH, electrical conductivity (EC), organic matter, total N, total P, total K[13], and total and DTPA extractable Cd[6].

Experimental procedures

Soils were transferred into 10-cm plastic pots at 1.5 kg/pot. Uniform-sized *O. basilicum* (two-month-old) were transplanted into pots (1 plant/pot as 1 replicate). Three replicates were performed for each treatment. A total of 54 plants were used in the 3-month pot experiment with a monthly harvest. Pots were placed in a complete randomized block design in a greenhouse (25–28 °C, 12/12 h photoperiod, 60% relative humidity). At each monthly harvest, plants were washed with tap water followed by deionized water, dried at 85 °C in the oven for 48 h, weighed for dry biomass, and separated into leaf, stem and root. Soil pH was measured before each harvest.

Metal analysis

Total concentration of Cd in soil was determined using an aqua regia (3:1 v/v HNO_3 : H_2O_2) open tube digestion method[14]. Cadmium availability in soil was attained by the DTPA extraction procedure[6]. Cadmium concentration in soil was determined by a flame atomic absorption spectrophotometer (FAAS, Variance Spectra AA55B).

Plant samples were digested in 2:1 v/v HNO_3 : $HClO_4$ using an open tube digestion technique[6]. Cadmium concentration in plant samples was determined by a flame atomic absorption spectrophotometer. The results were reported as concentrations of Cd in plants (mg/kg).

Data and statistical analysis

The translocation factor (TF) was calculated by dividing the concentration of metal in shoots by the concentration of metal in roots[15]. The TF values indicated the plant's ability to translocate heavy metals from root to shoot.

Two-way ANOVA was used to determine the variance of the data and means. The protected least square difference (LSD) was used to compare the means for each set of measurement.

RESULTS

Soil characteristics

The pH values of all soil mixtures were in the neutral range. The EC values of agriculture soil and the soil treated with Cd were 2.4 and 2.7 dS/m, respectively. In the soils amended with cow manure and silicate fertilizer, the EC values were 3.0 and 3.2 dS/m for 10% and 20% cow manure, and 3.9 and 3.7 dS/m for 10% and 20% silicate fertilizer, respectively (Table 1). The total Cd concentrations in soil spiked with 20 mg/kg Cd were in the range of 17.7–19.3 mg/kg (Table 1), indicating that the soil was quite homogeneous. Moreover, DTPA extractable Cd concentrations were in the range of 12.4–16.4 mg/kg.

Table 1 Physical and chemical characteristics of agricultural soil spiked with 20 mg/l Cd and Cd with cow manure (10%, 20%) and silicate fertilizer (10%, 20%) before the experiment.

Parameter	Cow manure	Control soil	Cd soil	CdCw10 soil	CdCw20 soil	CdSi10 soil	CdSi20 soil
pH	7.5	7	7	7.2	7.3	7.1	6.9
Organic matter (%)	29.2	6.9	12.7	10	10.8	10.8	10.9
Total N (%)	1.6	0.1	0.2	0.2	0.3	0.2	0.2
EC (dS/m)	1.4	2.4	2.7	3	3.2	3.9	3.7
Total Cd (mg/kg)	3.3	0	19.3 ± 0.7^{a}	18.2 ± 1.0^{a}	17.9 ± 1.7^{a}	17.7 ± 1.7^{a}	18.2 ± 1.2^{a}
DTPA extractable Cd (mg/kg)	-	0	16.4 ± 0.7^{a}	14.3 ± 0.3^{a}	14.8 ± 0.2^{a}	12.7 ± 0.2^{b}	12.4 ± 0.6^{b}
Total P (mg/kg)	6600	272	263	501	471	310	263
Total K (mg/kg)	10 500	1000	1100	1500	1500	1000	900

Means followed by the same letters within the same experiment are not significantly different by LSD test at ($P < 0.05$).

Table 2 Dry biomass production of *O. basilicum* grown in 20 mg/kg Cd and cow manure (10%, 20%) and silicate fertilizer (10%, 20%) under greenhouse conditions for 3 months.

Soil treatment	Dry biomass (g/plant)		
	Month 1	Month 2	Month 3
CS	7.6 ± 1.5^{a}	9.2 ± 1.3^{a}	9.3 ± 3.1^{a}
CdS	7.1 ± 2.1^{a}	5.9 ± 1.3^{a}	5.7 ± 2.2^{a}
CdCw10	8.4 ± 2.8^{a}	10.1 ± 1.6^{a}	10.2 ± 1.9^{a}
CdCw20	5.7 ± 0.3^{a}	16.8 ± 7.9^{b}	27.0 ± 10.0^{b}
CdSi 10	9.1 ± 1.3^{a}	6.6 ± 0.2^{a}	$5.3 \pm 0.7^{a*}$
CdSi20	5.2 ± 1.8^{a}	7.2 ± 1.3^{a}	$9.9 \pm 1.5^{a*}$

Means followed by the same letters within the same experiment are not significantly different by LSD test at ($P < 0.05$). * indicates the significant difference between exposure time in the same soil treatments.

The addition of silicate fertilizer did not significantly change the total Cd concentration ($P > 0.05$) but significantly reduced the extractable Cd concentration ($P < 0.05$) (Table 1).

Growth performance and survival rate

The survival rate was 100% in all treatments indicating that plants were tolerant to relatively high concentrations of Cd (Table 2). Various soil additives did not significantly change the dry biomass production in the first month ($P > 0.05$) (Table 2). However, the addition of 20% cow manure resulted in the highest biomass production in the second (16.8 g/plant) and third month (27.0 g/plant). The lowest dry biomass production was observed in plants grown in soil with Cd and 10% silicate fertilizer (5.3 g) after 3 months of exposure (Table 2).

Cadmium accumulation and distribution

Cadmium accumulated more in roots > stems > leaves (Table 3). It was increased with the increase in exposure time. In shoots, the accumulation of Cd did not change in the first month when cow manure and silicate fertilizer were added to the soil. The application of 10% and 20% silicate fertilizer significantly reduced Cd accumulation in the second month ($P < 0.05$). The highest Cd accumulation in shoots (26.8 mg/kg) was noticed in the third month when 10% cow manure was applied to the soil (Table 3). Cadmium accumulation in roots did not significantly change among soil treatments throughout the exposure time ($P > 0.05$). The highest Cd accumulation in roots (32.0 mg/kg) was observed in soil amended with 10% and 20% cow manure in the third month (Table 3).

When the total Cd uptake of plant (Cd concentration × plant total biomass production) was considered after 3 months of treatment, the 20% cow manure treatment resulted in the highest total Cd uptake (1.3 mg/plant), followed by the 10% cow manure treatment (0.6 mg/plant). On the other hand, both Cd alone and 10% and 20% silicate treatments showed the lowest total Cd uptake (0.1–0.2 mg/plant).

O. basilicum grown in Cd soil and soil amended with cow manure showed increases in TF values with the exposure time (Table 3). The highest values of TF (0.99) was found in plants grown in 10% cow manure treatment after 3 months. In contrast, the addition of silicate fertilizer resulted in the lowest TF values (0.11–0.16) (Table 3).

DISCUSSION

Both silicate fertilizer and cow manure influenced the biomass production of *O. basilicum*. The addition of 20% silicate fertilizer improved the growth inhibition induced by the toxicity of Cd. There is much evidence that Si has many direct and indirect beneficial effects on plant growth and development[8]. Silica induces plant growth by alleviating the toxic effect of Cd[16]. In addition, Si can enhance resistance and tolerance of plants to toxic elements such as Mn, Al, and Cd[17]. When compared to silicate fertilizer, the addition of 20% cow manure greatly enhanced the dry biomass production of *O. basilicum*. Manure compost contains

Table 3 Cadmium accumulation in plants exposed to control soil and soil with Cd, cow manure, and silicate fertilizer during the 3-month exposure.

Month	Soil treatment	Cd accumulation (mg/kg)				TF
		Leaves	Stem	Shoot	Root	
1	CS	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	CdS	1.9 ± 0.9^{a}	3.4 ± 0.2^{a}	5.3 ± 0.9^{ab}	25.5 ± 2.3^{a}	0.21
	CdCw10	2.0 ± 0.8^{a}	2.2 ± 1.7^{a}	4.1 ± 2.5^{a}	15.8 ± 2.8^{a}	0.35
	CdCw20	2.8 ± 0.4^{a}	5.3 ± 1.6^{a}	8.1 ± 2.1^{b}	19.3 ± 5.8^{a}	0.42
	CdSi10	3.5 ± 1.6^{a}	4.1 ± 1.1^{a}	6.2 ± 0.2^{a}	28.3 ± 1.8^{a}	0.28
	CdSi20	2.2 ± 1.1^{a}	3.3 ± 0.4^{a}	5.5 ± 1.3^{a}	23.5 ± 4.9^{a}	0.23
2	CS	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	CdS	$6.6 \pm 2.5^{a*}$	$5.8 \pm 1.1^{a*}$	$12.3 \pm 4.4^{a*}$	23.1 ± 1.8^{a}	0.64
	CdCw10	3.5 ± 0.8^{a}	$5.7 \pm 1.7^{a*}$	9.2 ± 2.4^{ab}	$20.0 \pm 3.7^{a*}$	0.46
	CdCw20	4.5 ± 0.7^{a}	8.4 ± 0.8^{a}	12.8 ± 1.4^{a}	$27.2 \pm 5.2^{a*}$	0.47
	CdSi10	1.8 ± 0.2^{b}	5.3 ± 0.7^{a}	7.0 ± 0.6^{b}	27.9 ± 1.0^{a}	0.25
	CdSi20	2.5 ± 0.9^{b}	3.7 ± 2.4^{a}	6.2 ± 3.3^{b}	21.9 ± 5.3^{a}	0.2
3	CS	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	CdS	$5.7 \pm 0.3^{a*}$	$7.9 \pm 1.3^{a*}$	$13.7 \pm 1.1^{a*}$	29.0 ± 2.3^{a}	0.72
	CdCw10	$8.5 \pm 0.5^{a*}$	$16.8 \pm 3.9^{b*}$	$26.8 \pm 2.9^{b*}$	$32.0 \pm 11.1^{a***}$	0.99
	CdCw20	$6.8 \pm 2.7^{a*}$	$11.0 \pm 4.4^{a*}$	$17.9 \pm 7.1^{a*}$	$32.0 \pm 8.5^{a*}$	0.65
	CdSi10	1.6 ± 1.2^{a}	2.6 ± 0.6^{c}	4.3 ± 2.1^{c}	26.3 ± 4.4^{a}	0.16
	CdSi20	0.7 ± 0.4^{a}	2.6 ± 1.1^{c}	3.4 ± 1.5^{c}	23.1 ± 11.9^{a}	0.11

Means followed by the same letters within the same experiment are not significantly different by LSD test at ($P < 0.05$).
* indicates the significant difference between exposure time in the same soil treatments.
TF = Translocation Factor; $*P < 0.05$; $***P < 0.001$

large amounts of macronutrients (N, P, K) and organic carbon content[11]. The macronutrients supplied by the manure and improved soil conditions may have contributed to the vast increase in growth in this treatment. The organic carbon content from manure acts as a nutrient pool, improves nutrient cycling, increases CEC and buffer capacity, reduces compaction, and improves soil physical properties[18].

In general, organic amendment such as cow or pig manure which contains a high proportion of humified organic matter (OM) can decrease the bioavailability of heavy metals in soil[19]. The OM forms strong complexes with Cd which results in immobilization of Cd in the soil. Several studies have shown that the application of OM (farmyard manure, compost, peat soil) resulted in a decrease in Cd concentration in some crop plants (corn, wheat, radish)[20,21]. On the contrary, OM has been reported to show different effects on metal extractability, which may depend on the sources of OM and plant species. Haghiri[22] found no relation between OM and Cd accumulation in oats. Narwal and Singh[23] reported a significant increase in Cd concentration in wheat when pig manure was the OM source. The present study found a significant increase in shoot Cd concentration and the total Cd uptake of plants when cow manure was applied. There are several possible explanations for this result. Firstly, it was observed that by the application of cow manure, the soil pH was increased slightly (from 7 to 7.3) which may have caused the formation of soluble Cd-organic complexes which can increase metal solubility[24]. Secondly, Cd may form weak complexes with OM and can be easily removed[25]. Thirdly, application of OM increases Cd solubility, due to the provision of soluble organic compounds complexes[26]. High concentrations of dissolved organic compounds may promote the trace metal uptake to the root surface[27]. It was found that the mobilization of Cd was increased by naturally occurring dissolved organic carbon[26]. Lastly, the high biomass productivity in the plants grown in cow manure soil possibly resulted in the increase of shoot Cd accumulation and total Cd uptake[26].

The application of 20% silicate fertilizer significantly reduced shoot Cd accumulation (from 13.7 to 3.4 mg/kg Cd), resulting in the restriction of Cd transfer from roots to shoots. Similar results were shown in previous studies. A reduction in Cd uptake was shown in rice seedlings treated with silicon. Liang et al[16] reported that the application of silicon as sodium metasilicate (50 and 400 mg/kg) resulted in a decrease in root and shoot Cd concentration. The reduced availability in the 400 mg/kg Si treatment was a consequence of the pH rise which reduced the phytoavailability of Cd[16]. However, silicate fertilizer did not cause a pH change and there was no change in root Cd concentration in the present study. Previous research identified the possible mechanism of silicates in heavy metal restriction from roots to shoots. It is possible that silicate decreases Cd concentration in the symplasm (cytoplasm) by generating coprecipitation of Cd and silicate in the cell wall[28]. In addition, there is more supportive evidence that Si partially blocks apoplast (cell wall) bypass flow across the roots and inactivates Cd apoplastic transport from roots to shoots. As a result, Cd accumulation in the shoot is significantly reduced.

CONCLUSIONS

The results of the present study showed that silicate fertilizer can effectively immobilize Cd in the soil. It has a potential to reduce shoot Cd accumulation in sweet basil. Although cow manure application resulted in higher growth of plants than Si, it also caused an increase in shoot Cd accumulation. A more detailed study is required to grow sweet basil or other crops in Cd contaminated areas and evaluate their growth and Cd distribution. The application of silicate fertilizer to the soil possibly reduces Cd in the edible parts of plants and helps to reduce the risk to the health of people living in the contaminated area.

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