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Influence of organic amendments on phytostabilization of Cd-contaminated soil by *Eucalyptus camaldulensis*

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ABSTRACT: Greenhouse and field experiments were conducted to evaluate the influence of three different organic amendments (cow manure, pig manure, and organic fertilizer) on the Cd phytostabilization potential of *Eucalyptus camaldulensis* grown on Cd-contaminated soil. The application of all amendments, particularly organic fertilizer, improved plant growth (i.e., height and biomass production) when compared to the control (Cd-contaminated soil alone), in both greenhouse and field experiments. *E. camaldulensis* treated with organic fertilizer experienced the greatest height (39 cm and 3.8 m) and biomass production (2.0 g and 3.3 kg) in greenhouse and field experiments, respectively. Plants grown on amended soils had lower Cd accumulation than those grown on the Cd soil alone. Among the treatments, organic fertilizer resulted in a translocation factor < 1 and a bioconcentration factor for the root (BCFR) > 1, indicating the potential of this species to stabilize Cd in the roots. The results showed that *E. camaldulensis* is a promising species for phytostabilization of Cd-contaminated soil. The wood of the mature tree is used in the manufacture of commercial products; given the low Cd uptake by *E. camaldulensis*, it is feasible to harvest the wood grown on Cd-affected soils for making paper and furniture.

KEYWORDS: phytoremediation, excluder plant, pot experiment, field experiment

INTRODUCTION

Heavy metals are released to the environment via a number of avenues including metal smelting, land application of sewage sludge, and improper disposal to water and land, among others. Common sources of metals to the biosphere include mining and industrial activities. The release of heavy metals to soil and aquatic ecosystems can lead to metal accumulation in the local environment, including food chains[1].

Cadmium (Cd) is a notoriously toxic metal, as it is hazardous at low concentrations and its accumulation in organisms is linked with a number of nephrological and other diseases[2]. Cadmium has been detected in significant concentrations in soils of the Padaeng zinc mining area (Mae-Sot district, Tak Province, Thailand). Of more immediate importance, however, Cd occurs in soils of agricultural areas adjacent to and downstream of the mines. In the Mae Sot district, cultivation of various crops such as rice, corn, soya beans, garlic, and shallots is a common practice among local villagers as the soils are deep, fertile, and ideal for crop cultivation. Unfortunately, however, high Cd concentrations (range of 3.4–284.0 mg/kg) which are between 1 and 94 times higher than those allowed under the European Community limit of 3 mg/kg, pose a serious health risk to the public due to Cd uptake and translocation in agricultural crops[3]. The adverse health impacts of Cd exposure in population of the Mae Sot district have been reported in various studies. Reports indicate a high incidence of kidney disease among local populations, presumably resulting from consumption of agricultural crops cultivated on Cd-contaminated soil[4].

Despite the extent of Cd-contamination in the Mae Sot district, little research has been conducted to identify a viable and cost-effective method to remediate local soils. Among the existing remediation technologies, phytoremediation is considered an ecologically-friendly approach to treat metal-contaminated soil. The two primary phytoremediation strategies used to treat metal-affected soil are phytoextraction and phytostabilization[5]. The latter technology has gained significant attention in recent years by virtue of its success in various soil types, both upland and wetland. Phytostabilization is a well-established method for immobilizing metals in soil; extensive root systems sorb soil contaminants, thus preventing leaching or lateral migration. Plants in this strategy are termed 'excluders'. Plants enhance metal stabilization in the rhizosphere via the release of root exudates and by improving soil biological activity thus increasing organic carbon levels[6]. During phytostabilization soil structure is not disturbed and there are no hazardous by-products[7].

Phytostabilization is appropriate for long-term growth of timber for commercial purposes. However, proper selection of plants is critical in phytostabilization, as the species plays a key role with respect to the metal stabilizing potential of the root system and the quantity of vegetative cover for protection of soil from the effects of wind and water[8]. Excluder tree species used elsewhere include birch (*Betula pendula*), black locust (*Robinia pseudoacacia*), oak (*Quercus robur*, *Q. petraea*), Scots pine (*Pinus sylvestris*), and Douglas fir (*Pseudotsuga menziesii*)[9]. However, few reports exist regarding the utilization of excluder tree species in Thailand.

Eucalyptus (*Eucalyptus* spp.), a predominantly Australian native plant, consists of approximately 700 species. Plants of this genus provide substantial economic value for industrial markets via wood products and extraction of eucalyptus oil for pharmaceutical and medical markets[10]. Various species of eucalyptus have been investigated for their metal phytostabilization ability[11,12]. *Eucalyptus camaldulensis* is a moderately fast growing tree with high biomass production and wide adaptability to climatic and edaphic conditions. The species is well suited for plantation cultivation on degraded lands (e.g., waterlogged and saline soils) and helps improve soil quality[13]. Eucalyptus was found to accumulate low levels of heavy metals in various plant parts when compared to other species. Eucalyptus is rarely consumed by organisms, so any accumulated Cd has little risk of entering the food chain[12]. Hence this genus may serve as an effective phytostabilization agent in metal-enriched soils.

Although eucalyptus is an excluder of several heavy metals, soil amendments and/or fertilizer still must be added to contaminated soil to improve plant growth and enhance metal stabilization potential. Several organic soil amendments have been successfully used for phytotreatment of contaminated soil as they are non-toxic, inexpensive, and naturally degradable. Organic amendments such as animal manure and compost have been reported to enhance soil physical and chemical properties, ensure ecological biodiversity and decrease metal bioavailability[14]. The objective of this study was to evaluate the influence of organic amendments on phytostabilization of Cd-contaminated soil by *E. camaldulensis* via greenhouse and field experiments.

MATERIALS AND METHODS

Physicochemical properties of Cd-contaminated soil

The soils of interest were collected from agricultural fields, both Cd-contaminated and non-contaminated in the Mae Tao river basin, Mae Sot district, Tak province (N 16° 40′ 58.4″ E 98° 37′ 65.1″). Soil material was collected from the surface (< 20 cm depth) of plots measuring 12 m^2. Both types of soil material (i.e., contaminated and non-contaminated) were collected from five locations (four corners and one central location) and composited in the field. Soil samples were brought to the laboratory and oven-dried at 70 °C for 48 h. Dried samples were ground with an agate mortar and pestle and subsequently sieved through a 2-mm mesh sieve. After sieving, samples were analysed for total Cd concentration using a flame atomic absorption spectrophotometer (FAAS; AAnalyst 200, PerkinElmer) after HNO_3 digestion. Samples were tested for extractable Cd concentration using FAAS after extraction with 0.05 M diethylene triamine pentaacetic acid (DTPA)[15]. Total nitrogen (N) was determined using the Kjeldahl method. Extractable phosphorus (P) concentrations were measured using the Bray II method, and extractable potassium (K) using extraction with neutral NH_4OAc buffered to pH 7.0. Soil cation exchange capacity was determined after leaching with 1 N NH_4OAc buffer. Organic matter content was measured using the Walkley-Black titration[16]. Soil pH was measured on a 1:5 (w/v) suspension of soil : deionized water using a glass electrode pH meter (Hanna instruments HI 221), and electrical conductivity (EC) using an EC meter (Hanna instruments HI 993310). Soil texture was determined using the hydrometer method[17].

Plant cultivation

E. camaldulensis plants were germinated from seeds, grown in commercial soil material and placed in a greenhouse (27–29 °C, 70% relative humidity, 17 600 lx, 12/12 h photoperiod). After three months, healthy and homogenous plants (30 cm height) were selected for further study.

Greenhouse study

The greenhouse study was conducted under the same conditions as described in the previous section. Two kilograms of soil material were placed into plastic pots (20 cm diameter, 18 cm tall) fitted with a plastic screen at the base. All soil treatments were equilibrated following the method of Blaylock et al[18], i.e., deionized (DI) water was applied to soil overnight until water holding capacity attained approximately 80%. Healthy and uniform *E. camaldulensis* plants were transplanted into pots (one plant per pot). Treatment groups consisted of Cd-contaminated soil amended with cow manure, pig manure, and organic fertilizer (10% w/w). Cow and pig manure were obtained from lagoons on the university research farms. The organic fertilizer consisted of animal manures, plant debris, humic acid, and amino acids. The amendments were allowed to air-dry at 25 °C for 1 month. The dried amendments were sieved through a 2-mm mesh sieve and then applied. The control group was Cd-contaminated soil alone. Five replicates were established for each treatment.

Plant pots were arranged in the greenhouse using a randomized complete block design. Pots were watered daily (250 ml/pot) and supplemented weekly with 100 ml concentrated Hoagland's nutrient solution (low phosphate concentration; 0.01 mM KH_2PO_4). Two grams of organic amendment were added before planting and every other month. After three months all plants were harvested by carefully removing the entire plant from the pots, washed thoroughly with tap water to remove excess soil, and rinsed with DI water. Plants were subsequently separated into shoots and roots, and oven-dried at 70 °C for two days prior to determination of the dry weight.

Field study

The field study took place from February to October 2014. Total rainfall and average air temperature were 1429.8 mm and 27.3 °C, respectively. Plots measuring 3×3 m were established; each plot had three rows containing nine plants with a spacing of 1 m^2. Plots received either cow manure, pig manure, or organic fertilizer, or no treatment. Each treatment consisted of three replicates. Young plants were transplanted into the plots and covered by plastic nets to shade them against the intense summer sunlight. Prior to transplanting and every three months afterwards, 12 g of organic amendment was applied as a soil treatment. The amendments were incorporated to a 25-cm depth with a steel shovel. All plots were watered daily using equal amounts of water (500 ml and 1 l for the young and adult plants (> 6 m), respectively). At the end of the experiment, three plants were removed from each plot, thoroughly washed, and separated into shoots and roots. Plant tissue was oven-dried at 70 °C for 48 h for subsequent chemical analysis.

Plant growth performance

At harvest, plant growth performance criteria including survival rate, growth rate, plant height and dry biomass production were determined. Survival rate is defined as the fraction of plants surviving per treatment during the experimental period, i.e., the number of remaining plants after the experiment divided by the total number of plants at the start of the experiment. Plant growth rate is a vital tool for remediation studies as it indicates the capability of plant to grow under stressed conditions[19]. Growth rate was calculated as $(\mathrm{DBP_a} - \mathrm{DBP_i})/\Delta t$ or as $(H_\mathrm{a} - H_\mathrm{i})/\Delta t$, where $\mathrm{DBP_a}$ and H_a are the dry biomass and height for plants after harvesting, respectively, while $\mathrm{DBP_i}$ and H_i are the dry biomass and height for plants before planting, and Δt is the time interval before plant collection.

Cd analysis in plant tissue and soil

Dried plant material was ground with an IKA MF 10 basic microfine grinder drive, sieved through a 2-mm mesh sieve and weighed. Approximately 0.5 g of plant tissue was placed into a Pyrex test tube. Samples were acid digested using 70% HNO_3 and 37% HCl in a microwave oven (ETHOS One; Milestone Inc.). Digested samples were analysed with either FAAS or a graphite furnace atomic absorption spectrophotometer (GF-AAS; AAnalyst 600, PerkinElmer), depending on metal concentration. Soil samples were digested in a microwave with concentrated 70% HNO_3 and 30% H_2O_2. Cadmium concentrations in digested soil samples were determined using either FAAS or GF-AAS, depending on Cd concentration.

Table 1 Selected physical and chemical properties of the soil before planting.

Parameter	Cd*	CdOrg	CdPig	CdCow
pH	7.2	7.4	7.7	7.3
EC (dS/m)†	0.20	0.34	0.48	0.39
CEC (cmol/kg)	10.0	13.6	14.3	13.1
OM (g/kg)	23.4	43.9	58.2	49.1
Total N‡	2823	3446	4943	3828
Extractable P‡	10.0	6.0	14.0	12.0
Extractable K‡	140.0	490.0	1030.0	710.0
Total Cd‡	19.6	19.4	19.5	19.5
Extractable Cd‡	3.6	5.1	5.8	5.9

* Cd: Cd-contaminated soil (control); Org: organic fertilizer; Pig: pig manure; Cow: cow manure.
† EC: electrical conductivity; CEC: cation exchange capacity; OM: organic matter.
‡ (mg/kg)

Table 2 Physicochemical properties of the amendments at the initiation of the study.

Parameter	Org*	Pig	Cow
pH	8.1	8.3	8.2
EC (dS/m)	4.3	4.8	4.5
CEC (cmol/kg)	34.8	41.2	37.9
OM (g/kg)	63.2	72.5	65.9
Total N (mg/kg)	4355	6323	5133
Extractable P (mg/kg)	23.3	43.4	34.3
Extractable K (mg/kg)	734.1	983.2	773.8
Total Cd (mg/kg)	0.2	0.2	0.1
Extractable Cd (mg/kg)	BDL†	BDL	BDL

* Abbreviations as in Table 1.
† BDL: below detectable limit.

Analytical accuracy and precision for FAAS analysis were checked by running standard solutions after every 20 samples. $Cd(NO_3)_2$ (Merck) was used as the internal standard. A method blank and certified reference materials (NIST SRM 2710a Montana soil, and NIST SRM 1515 apple leaves, for soils and plants, respectively) were included for quality control.

Data analysis for Cd uptake

Metal uptake was calculated as $C \times \text{DBP}$, where C is the metal concentration in the plant part (shoot or root) and DBP is the dry biomass of plant. Metal uptake indicates metal concentration in the plant part which is sequestered by translocation processes[11]. The translocation factor (TF), which indicates the degree of metal movement within the plant[20], was calculated as $\text{TF} = C_{\text{shoot}}/C_{\text{root}}$, and the bioconcentration factor for the root (BCFR), which provides an indication of the root accumulation potential[21], was calculated as $\text{BCFR} = C_{\text{root}}/C^*_{\text{soil}}$, where C^*_{soil} is the metal-extractable concentration in the soil.

Statistical analysis

All data were analysed using SPSS 18.0. ANOVA was performed using least significant difference (LSD) post hoc comparisons, with 95% confidence level ($p < 0.05$).

RESULTS

Properties of soil and amendments

The contaminated soil had a neutral or slightly alkaline pH (range 7.2–7.7) (Table 1). Levels of plant-available N, P, and K were within acceptable limits. Total and extractable soil Cd concentrations were 19.6 and 3.6 mg/kg, respectively. Soil Cd concentrations in excess of about 1 mg/kg are considered to be evidence of anthropogenic pollution[22]. Soil texture was classified as loam.

All amendments contained medium to high levels of total N, extractable P and K (Table 2). The high extractable P values in the animal manure may be the result of elevated P concentrations in animal feed. Total Cd concentrations in all amendments were < 0.2 mg/kg (Table 2). Many organic and inorganic fertilizers have been found to contain toxic elements such as Cd, Pb, or As. The average Cd concentration in European fertilizers is 138 mg/kg[23]. Organic and inorganic P fertilizers have increased Cd concentrations in surface soil up to 300 mg/kg[24]. Hansen[25] stated that average Cd levels in fertilized soil were 0.18–0.24 mg/kg, depending on fertilizer type. Organic amendments are a main source of both macro- and micro-nutrients in agricultural soil, especially those used by farmers in developing countries; however, there is still no established regulatory Cd limit for organic amendments, particularly in animal manures. Thus it is desirable to ensure that organic fertilizers contain low Cd concentrations before plant cultivation.

The amended treatments had increased EC, CEC, OM and nutrient concentrations compared with Cd-contaminated soil (Table 3). Total and DTPA-extractable Cd concentrations were relatively low, but still exceeded the standard for non-contaminated soil (> 3 mg/kg); such levels are toxic to many plant species[26]. The DTPA-extractable Cd concentrations in all amended soils were slightly higher than that of the Cd-contaminated soil alone (Table 3).

After plant harvest in both greenhouse and

Table 3 Total and DTPA extractable Cd concentration in soils of various treatments in greenhouse and field experiments, before and after plant harvest.

Soil	Start		Greenhouse		Field	
	Total	Extractable	Total	Extractable	Total	Extractable
Cd	19.6 ± 2.4^{a}	5.6 ± 0.4^{ab}	18.1 ± 1.5^{a}	2.4 ± 0.8^{ab}	19.0 ± 1.3^{a}	6.0 ± 0.5^{a}
CdOrg	19.4 ± 1.3^{a}	5.1 ± 1.1^{a}	18.7 ± 0.4^{a}	2.1 ± 1.4^{a}	18.0 ± 1.0^{a}	4.6 ± 0.7^{a}
CdPig	19.5 ± 1.1^{a}	5.8 ± 1.2^{a}	18.7 ± 1.3^{a}	1.9 ± 0.8^{a}	18.7 ± 1.5^{a}	5.3 ± 0.6^{a}
CdCow	19.5 ± 0.6^{a}	5.9 ± 1.4^{a}	18.1 ± 1.4^{a}	2.3 ± 0.3^{a}	8.9 ± 1.8^{a}	5.3 ± 0.3^{a}

Data are mean ± SD. Different letters within the same column show significant different data (LSD: $p < 0.05$)

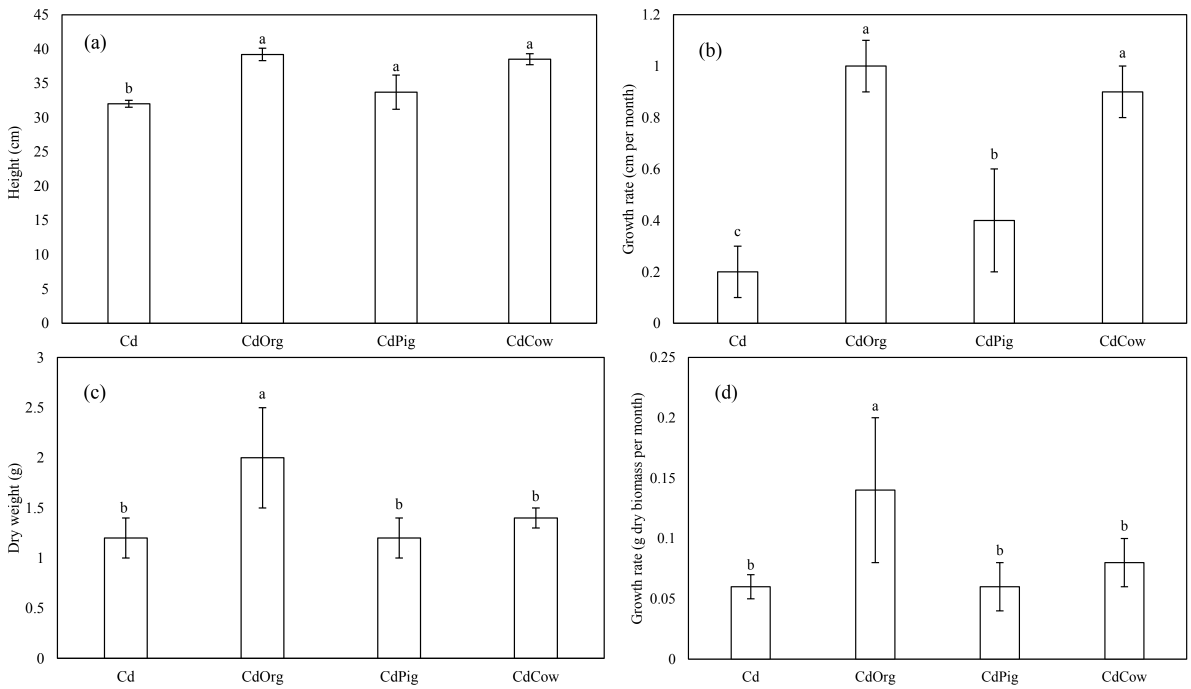

Fig. 1 Growth performance of *E. camaldulensis* in greenhouse experiment: (a) height; (b) growth rate in height; (c) dry biomass; (d) growth rate in dry biomass. Small letters show the difference between amendment effects (LSD: $p < 0.05$).

field experiments, total and DTPA-extractable Cd concentrations in all amended treatments were not significantly different ($p > 0.05$) from those of non-amended soil, indicating that the organic amendments did not increase soil Cd solubility (Table 3).

Plant growth performance

All plants in both greenhouse and field experiments survived throughout the respective experimental periods (Fig. 1, Fig. 2) with no observable toxic effects. The amendments markedly increased plant growth in terms of total dry biomass and height ($p < 0.05$). Dry biomass production increased from 1.2 g/plant to 2.0 g/plant, and from 1.24 kg/plant to 3.34 kg/plant in the greenhouse and field studies, respectively. Without amendment supplementation, *E. camaldulensis* survived but total dry biomass production and height were lower than those in the amended treatments. The relative order of plant height for the greenhouse and field studies was CdOrg ∼ CdCow > CdPig > Cd and CdOrg ∼ CdPig > CdCow > Cd, respectively. The relative order for biomass production from both greenhouse and field studies was CdOrg > CdCow ∼ CdPig > Cd (Fig. 1, Fig. 2).

Cd accumulation and uptake

Cadmium uptake and accumulation by *E. camaldulensis* showed similar trends in both greenhouse and field experiments. *E. camaldulensis* accumulated

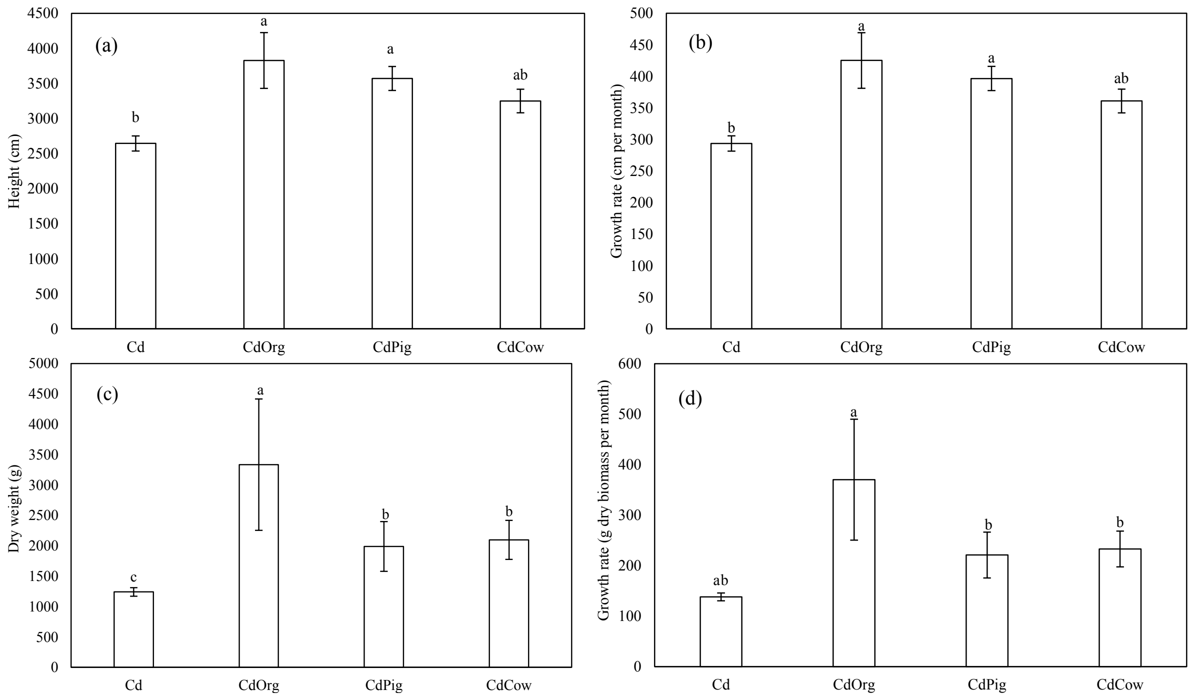

Fig. 2 Growth performance of *E. camaldulensis* in field experiment: (a) height; (b) growth rate in height; (c) dry biomass; (d) growth rate in dry biomass. Small letters showed differences between amendment effects (LSD: $p < 0.05$).

Table 4 Dry biomass production (DBP), Cd accumulation (Cd-A), Cd uptake (Cd-U), translocation factor (TF) and bioconcentration factor of root (BCFR) of *E. camaldulensis* in greenhouse and field experiments for 3 and 9 months, respectively.

	DBP (g/plant)	Cd-A in plant (mg/kg)			Cd-U (μg/plant)	TF	BCFR
		Shoot	Root	Whole plant			
Greenhouse experiment							
Cd	1.2 ± 0.2^{b}	0.6 ± 0.1^{b}	1.3 ± 0.3^{b}	0.8 ± 0.2^{b}	1.0 ± 0.1^{b}	0.2 ± 0.0^{b}	2.3 ± 0.6^{a}
CdOrg	2.0 ± 0.5^{a}	0.8 ± 0.1^{b}	3.0 ± 0.7^{a}	1.4 ± 0.2^{a}	2.6 ± 0.5^{a}	0.3 ± 0.1^{b}	2.0 ± 1.3^{a}
CdPig	1.2 ± 0.2^{b}	1.5 ± 0.2^{a}	2.6 ± 0.3^{ab}	1.6 ± 0.4^{a}	2.0 ± 0.6^{ab}	0.6 ± 0.1^{a}	1.6 ± 0.6^{b}
CdCow	1.4 ± 0.1^{ab}	0.7 ± 0.2^{b}	4.0 ± 0.4^{a}	1.8 ± 0.4^{a}	2.7 ± 0.8^{a}	0.2 ± 0.1^{b}	1.8 ± 0.3^{a}
Field experiment							
Cd	1240 ± 70^{c}	11.4 ± 3.1^{a}	21.8 ± 4.3^{a}	14.5 ± 1.7^{a}	$18\,000 \pm 1200^{c}$	0.5 ± 0.1^{b}	3.8 ± 1.3^{a}
CdOrg	3340 ± 1080^{a}	9.5 ± 2.4^{a}	17.2 ± 3.8^{bc}	11.6 ± 1.6^{a}	$38\,600 \pm 12\,100^{a}$	0.6 ± 0.2^{b}	3.4 ± 0.7^{b}
CdPig	1990 ± 410^{b}	10.8 ± 0.6^{a}	18.1 ± 1.6^{bc}	12.1 ± 2.2^{a}	$24\,600 \pm 9400^{b}$	0.6 ± 0.1^{a}	3.5 ± 0.8^{ab}
CdCow	2100 ± 320^{b}	9.9 ± 1.0^{a}	17.6 ± 1.0^{b}	11.4 ± 2.5^{a}	$23\,500 \pm 1200^{b}$	0.6 ± 0.1^{b}	3.5 ± 0.5^{ab}

Data are mean ± SD. Different letters within the same column show significant different data (LSD: $p < 0.05$)

higher Cd concentrations in roots compared with shoots. In the field experiment, addition of organic amendments resulted in increased Cd uptake in the order: CdOrg > CdCow ∼ CdPig > Cd (Table 4). All treatments exhibited BCFR values > 1 (Table 4), indicating the potential of this species as an excluder. The highest BCFR values were determined in the Cd-contaminated soil alone. All TF values were smaller than 1 (Table 4).

Tissue Cd concentrations significantly increased with increase of exposure time ($p < 0.05$) i.e., there were greater tissue Cd levels in the field-grown plants (9 months) compared with the greenhouse-grown plants (3 months).

DISCUSSION

Influence of organic amendments on plant growth and metal accumulation

Many agricultural fields in the Mae Sot district of Thailand have experienced substantial contamination with Cd; soil collected from fields for the current study contained 19.6 mg/kg total Cd. This concentration is categorized as moderate in terms of potential detrimental effects to plant growth on agricultural soil[3]. The organic amendment supplied are not expected to increase soil Cd content. Regardless, however, the soil contained adequate levels of plant nutrients. Furthermore, relevant soil physical and chemical properties (neutral pH, acceptable levels of EC, CEC, OM) were sufficient to support plant growth.

Utilization of organic amendments is beneficial for soil health as it improves soil physicochemical properties. Organic sources can also reduce the dependency on costly commercial fertilizers by providing nutrients, both soluble and slow-release organic forms. Organic amendments can also modify the degree of heavy metal availability[27].

In the reported study the addition of organic amendments (cow manure, pig manure, organic fertilizer) to Cd-contaminated soil resulted in slight increases in pH and total N concentration, moderate increases in EC, CEC, OM, and total P concentrations, and a marked increase in total K when compared to non-amended soil. Higher nitrogen content after organic amendment application, particularly in the organic fertilizer treatment, could help to increase plant growth, as the nitrogen is available in slow-release form[28]. Nitrogen is essential for protein formation and photosynthesis[29]. Soil pH values are considered appropriate for *E. camaldulensis* cultivation, whose recommended pH ranges from 4.0–8.5[30]. Plant nutrient availability is maximal at near-neutral pH values, and metal toxicity is minimal[31]. It has been documented that these pH values can improve plant growth performance and increase sorption of metal cations, resulting in low mobility of metals and metalloids in contaminated soil[32]. EC values were addressed because all samples possessed EC values > 0.2 dS/m which exceeds the normal standard soil range[33]; furthermore, values $\geqslant 1.4$ dS/m may impair root development, decreasing plant growth and yields[33]. In the reported study, however, *E. camaldulensis* retained normal growth under the elevated EC values (0.3–0.5 dS/m).

E. camaldulensis grown in amended soil exhibited improved growth performance, as demonstrated by plant height and dry biomass, compared with plants grown in non-amended soil. Organic fertilizer was the most effective amendment, resulting in the highest growth rates in both greenhouse and field experiments, followed by cow manure and pig manure. The results are in good agreement with previous studies which showed that organic fertilizer increased vegetative growth and biomass production[14,34]. The OM present in the organic manure or fertilizer provides sources of slow-release nutrients, while at the same time increases the soil buffering capacity for acidity, cations, and water[35]. Besides acting as nutrient sources for plants, organic amendments also reduce metal toxicity by decreasing bioavailability of heavy metals[27].

Different soil organic amendments including compost, sewage sludge, animal manures, and organic fertilizer have been used for phytoremediation of heavy metal-contaminated soil[36]. Most studies have demonstrated that organic amendments having a high humified OM content decrease bioavailability of metals in soil via adsorption and formation of stable complexes with humic substances[37]. Application of organic amendments did not result in a significant decrease of extractable Cd concentrations in the greenhouse and field studies. There was however a significant decrease in Cd accumulation in *E. camaldulensis* grown in organic-amended soil after 9 months. The order of Cd uptake was CdOrg $>$ CdCow $\sim$ CdPig $>$ Cd. It has been suggested that increasing yields have resulted in decreased Cd concentrations in plants because of a dilution effect caused by plant growth rates exceeding the ability of plants to acquire Cd[38].

The reported study demonstrates that organic fertilizer was the most effective amendment in increasing biomass of *E. camaldulensis* grown in Cd-contaminated soil. Similar findings were reported for *Acacia mangium* and *Vetiver zizanioides* grown in Pb-contaminated mine tailings[11,29]. Much evidence indicates that soil microbial activity in organic fertilizers enhances organic matter decomposition, resulting in increased availability of plant nutrients[39]. Organic fertilizers are used worldwide by virtue of their benefits to soil physical and chemical properties, low cost, and easy field application.

Suitability for phytostabilization

The desirable features of plant species selected for phytostabilization are tolerance to high concentrations of contaminants, the ability to develop an extensive root system, accumulation of pollutants in non-edible underground parts, low maintenance requirements, relatively high transpiration rates, relatively long growing period or ability for self-propagation, and adaptation to local climate[40]. *E. camaldulensis* possesses most of these attributes. In the present study, *E. camaldulensis* grew well in contaminated soil, indicating its Cd tolerance. The plant has an extensive root system, is fast-growing, and produces substantial biomass. It accumulates Cd mainly in the roots and limits Cd translocation from roots to shoots (TF < 1), thereby limiting the distribution of Cd into the food chain. The plant high BCFR values reflect its ability to uptake Cd. Thus the higher BCFR and Cd uptake values in the field, compared with those in the greenhouse, might be due to the higher growth rate of plants under field conditions.

CONCLUSIONS

E. camaldulensis shows significant potential for phytostabilization of Cd-contaminated soil. The plant is tolerant of high soil Cd concentrations and accumulated little Cd in aboveground biomass. All organic amendments, particularly organic fertilizer, were effective in enhancing plant growth. The organic fertilizer treatment resulted in a TF < 1 and a BCFR > 1, indicating the potential of this species to stabilize Cd in the roots. Plants grown on amended soils had lower Cd accumulation compared to those grown on the Cd soil alone. Eucalyptus, even though indigenous to Australia, is widely cultivated worldwide because of its rapid growth, broad adaptability, and low maintenance requirements, in addition to the demand for wood products and oils. The plant is useful for commercial purposes such as paper and furniture industries; cultivation of this species can be successfully carried out on metal-enriched soil.

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