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RESEARCH ARTICLE

Soil amendments for cadmium phytostabilization by five marigold cultivars

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Abstract

In recent years, ornamental plants have come under investigation as phytoremediation agents. In addition to reducing contaminant concentrations in soil, such plants support local economies by serving social (e.g., religious) and decorative purposes. Greenhouse studies investigated the phytostabilization potential of soil cadmium (Cd) by five cultivars of marigold (*Tagetes erecta*), a common ornamental flower in Asia. The effects of organic (cattle manure and pig manure) and inorganic (leonardite and Osmocote®) amendments in supporting plant growth and enhancing Cd uptake were also examined. Marigold cultivars Babuda and Sunshine grown in soil supplemented with pig manure produced the greatest biomass and experienced greatest Cd accumulation and flower production. In all treatments, plant parts accumulated Cd in the following order: root > shoot ≈ flower. Furthermore, Babuda and Sunshine cultivars had a high phytostabilization potential as evidenced by translocation factors < 1 and bioconcentration factors > 1 for roots. It is proposed that Babuda and Sunshine marigold cultivars be applied toward Cd phytostabilization while enhancing local economies as an ornamental species.

Keywords Cadmium · Phytostabilization · Marigolds · Greenhouse experiment · Babuda · Sunshine

Introduction

The Mae Tao river basin of Tak Province in Western Thailand provides a well-documented example of the impacts of heavy metal contamination from anthropogenic activities. Over several decades, cadmium (Cd) from mining operations has contaminated agricultural soil; concentrations as high as 73.1 mg kg^{-1} are documented (Sriprachote et al. 2014). Such elevated Cd concentrations have been associated with acute and chronic Cd-related ailments (i.e., kidney and bone disease) in those consuming locally grown rice grain, corn, potato, and leafy vegetables (Putwattana et al. 2015; Swaddiwudhipong et al. 2012).

Marigold (*Tagetes erecta*) is important for its economic value as well as for its esthetic appeal (Haque et al. 2012). In Thailand, the marigold flower is used in Buddhist worship and for decorations on structures. The marigold is being cultivated on heavy metal–contaminated sites, as this species offers the potential to accumulate toxic elements such as As, Pb, and Cd in high concentrations (Chintakovid et al. 2008; Bosiacki 2008). Ornamental and other non-edible plants are considered an appropriate tool for removal of toxic metals from soil; their cultivation will improve local environmental conditions while providing economic benefits via marketing flowers (Nakbanpote et al. 2016).

Soil at many contaminated sites suffers from very low levels of essential nutrients, particularly nitrogen (N), phosphorus (P), and potassium (K). This may be accompanied by low pH and high metal concentrations, all of which can significantly limit plant growth (Cooke and Johnson 2002). Cadmium-affected agricultural soil in the Mae Tao river basin is documented as having pH ranging from near-neutral to 8.1; 0.02–0.18% total N; 9.4–53 mg kg^{-1} extractable P; 121.3–133 mg kg^{-1} extractable K; 15–29 g kg^{-1} organic matter (OM); and 9.2–15.2 cmol kg^{-1} cation exchange capacity (CEC) (Meeinkuirt et al. 2016; Phusantisampan et al. 2016; Putwattana et al. 2015; Saengwilai et al. 2017).

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Organic amendments improve both the physicochemical status and biological activity of contaminated soil. Repeated applications increase water retention capacity, reduce soil erosion, and affect metal speciation and plant bioavailability (Bot and Benites 2005; Shahid et al. 2014). Several organic materials including brown coal, biochar, animal manure, composts, and organic fertilizers have been evaluated as supplements for phytoremediation (Pichtel and Bradway 2007; Ogbonnaya and Semple 2013).

Recent studies have revealed that manure additions immobilize metals in rhizosphere soil, which may enhance metal phytostabilization and accumulation in roots (Elouear et al. 2016; Phusantisampan et al. 2016). Phytostabilization is an effective strategy in soil remediation as it uses root biomass to accumulate metals in high quantities while limiting accumulation in edible plant parts, resulting in no serious health risks from consumption. Soil amendments also convert the soluble and exchangeable metal forms to more geochemically stable solid phases, thus reducing the heavy metal pool for root uptake, resulting in reduced bioavailability (Cheng and Hseu 2002). In Thailand, organic amendments such as cattle, pig, and chicken manures are readily available, inexpensive, easy to apply, and free from heavy metals (Meeinkuirt et al. 2012, 2016).

In the reported study, five popular marigold cultivars were investigated in greenhouse experiments as potential phytostabilization agents on Cd-contaminated soil. In addition, selected organic and inorganic amendments were investigated for their potential to increase the efficiency of marigolds in Cd uptake and accumulation as well as in supporting plant growth.

Materials and Methods

Greenhouse study

Greenhouse experiments were carried out at Mahidol University, Nakhonsawan campus. Composite soil samples were collected from the surface 0–20 cm in fields documented as Cd-contaminated (N16° 40′ 35.9″ E98° 37′ 37.4″) (Meeinkuirt et al. 2016). Soil material was composited in the field, air-dried, and sieved to pass a 2-mm mesh sieve. Non-contaminated soil was purchased from commercial sources. Cattle and pig manure, leonardite, and Osmocote® were evaluated as soil amendments. Manures were obtained from lagoons on the University research farms, whereas leonardite and Osmocote® were purchased from an agricultural supply store. Cattle and pig manures were allowed to air-dry and subsequently sieved through a 2-mm mesh sieve. Treatments are designated as follows: Soil containing Cd: Ctrl (control soil); Cd soil + pig manure: Cdpig; Cd soil + cattle manure: CdCow; Cd soil + leonardite: CdLeo; Cd soil + Osmocote®: CdOsm; and commercial soil + Osmocote®: ComOsm.

Soil was placed into 3.5-L plastic pots with 5 replications (5 pots per treatment). Treatments consisted of Cd-contaminated soil with no amendment, and Cd-contaminated soil (3.15 kg) amended with pig manure, cattle manure, leonardite (0.35 kg) (10% *w*/*w*), or Osmocote® (0.15%). Commercial soil was amended with Osmocote® at the same rate. Pots were supplemented monthly with 100 mL of Hoagland's solution with low phosphate (0.01 mM KH_2PO_4) to maintain adequate levels of essential nutrients. Marigold seeds were allowed to germinate in acid-washed sand for 1 week, following which young marigolds of the same size (8.3 ± 2.4 cm height) and uniform shape were transferred to the pots and moved to the greenhouse. Temperature, humidity levels, and light intensity were similar to those of the local outdoor environment (27–30 °C; ~ 70% relative humidity; ~ 18,000 lx).

Five marigold cultivars including *T. erecta* (American, Babuda, Honey, and Sunshine), and *T. patula* (French marigold) were selected for the study. Plants were cultivated on benches in a randomized complete block design. Plants were irrigated twice daily with 150 mL 2 mg L^{-1} Cd solution. Entire plants, including aboveground shoot tissue, flowers, and roots were harvested 3 months after planting. Rhizosphere soil was collected using a plastic spatula for determination of total Cd and extractable Cd concentrations.

Plant, soil, and amendment analyses

Plant materials were rinsed carefully with distilled water to remove attached soil and debris. Plants were separated into roots, shoots, and flowers following which they were oven-dried at 70–80 °C for 3 days. Dried tissue was ground using an agate mortar and pestle and passed through a 1-mm mesh sieve. One-half gram of shoot and flower tissue was digested with 70% HNO_3 and 37% HCl, while 0.5 g of root material was digested with 65% HNO_3 and 40% HF using a microwave digestion system (ETHOS ONE®; Milestone Inc., Shelton, CT, USA) (Richter et al. 2016).

Soil samples were oven-dried at 80 °C for 24 h. Soil pH was measured on a 1:5 soil:water (*w*/*v*) suspension using an Accumet® AP115 pH meter. Cation exchange capacity (CEC) was determined by leaching with 1 N ammonium acetate adjusted to pH 7 followed by distillation (Sparks et al. 1996). Electrical conductivity (EC) was determined by an EC meter (Hanna instruments; HI 993310). Total N was determined by the Kjeldahl method (Black 1965). Extractable P was recovered using the Bray II method (Bray and Kurtz 1945), followed by analysis on an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Varian® 720-ES). Extractable K was determined by ICP-OES after extraction with NH_4OAc at pH 7.0. Organic matter content was determined by Walkley-Black titration (Walkley and Black 1934). Soils were extracted for Ca and Mg with diethylenetriaminepentaacetic acid (DTPA) and analyzed via flame atomic absorption spectrometry (FAAS) (APHA, AWWA & WEF 2005). Total Cd was determined using microwave digestion (ETHOS ONE®) with concentrated 70% HNO_3 and 30% H_2O_2 followed by FAAS. DTPA-extractable Cd was determined by FAAS or GF-AAS after DTPA extraction (APHA, AWWA & WEF 2005). NIST 1515 apple leaves and 2711a Montana soil were used for quality control in plant and soil analysis (90–110% recovery, respectively). Soil texture was determined using the hydrometer method (Allen et al. 1974).

Analysis of the animal manures and leonardite was as follows: The amendment was shaken in distilled water in a 1:2.5 (*w*/*v*) solid-water suspension for 1 h, and pH was analyzed using an Accumet® AP115 pH meter. Electrical conductivity was measured using an EC meter (Hanna instruments; HI 993310). Organic matter was measured using the potassium dichromate wet digestion method (Schnitzer 1982). Total N was determined by the Kjeldahl method (Bremmer and Mulvaney 1982). Extractable P was extracted by 0.5 N $NaHCO_3$ at pH 8.5 (Olsen and Sommers 1982), followed by analysis by ICP-OES. Extractable K was determined by ICP-OES after extraction with 1 N ammonium acetate at pH 7.0 (Knudsen et al. 1982). Calcium and Mg were extracted with 1 N NH_4OAc followed by FAAS analysis (Han et al. 2016). Total Cd was determined using microwave digestion (ETHOS ONE®) with concentrated 70% HNO_3 and 30% H_2O_2 followed by FAAS. The physicochemical properties of Osmocote® fertilizer were determined at Central Lab Company, Bangkok.

Data analysis

Plant growth performance attributes including percent survival rate, dry biomass production, plant height, root length, flower number, flower diameter, growth rate, and root/shoot ratio were recorded (Saengwilai et al. 2017).

$$\text{Growth rate (GR)} = \frac{\text{total dry biomass after harvest} - \text{total dry biomass before harvest}}{\text{total months in greenhouse experiment}}$$

$$\text{Root/shootratio (R/S ratio)} = \frac{\text{total dry biomass of root}}{\text{total dry biomass of shoot}}$$

Cadmium translocation and accumulation indices of the plants were calculated as follows (Saengwilai et al. 2017):

$$\text{Translocation factor (TF)} = \frac{\text{Cd concentration in shoot tissue } \left(\text{mg kg}^{-1}\text{ DW}\right)}{\text{Cd concentration in root tissue } \left(\text{mg kg}^{-1}\text{ DW}\right)}$$

$$\text{Bioconcentration coefficient (BCF)} = \frac{\text{Cd concentraion in shoot or root tissue } \left(\text{mg kg}^{-1}\text{ DW}\right)}{\text{extractable Cd concentration in soil } \left(\text{mg kg}^{-1}\text{ DW}\right)}$$

$$\text{Cd uptake} = C_{\text{shoot/root}} \text{ x total dry biomass of plant (shoot/root)}$$

where C is the Cd concentration in plant tissue.

Data was subjected to analysis of variance (ANOVA) and least significant difference (LSD) post hoc comparison using SPSS® (SPSS, Chicago, IL) on a Windows-based PC. A probability of $p < 0.05$ was considered statistically significant.

Results

Physicochemical properties of soils and amendments

The contaminated soil contained a relatively low concentration (5 mg kg^{-1}) of Cd and had a near-neutral pH (Table 1). Soil in all treatments became slightly acidic after amendment application with the exception of the CdCow treatment, which became slightly alkaline (pH 7.3). The control soil texture was clay but soils in the CdCow, CdLeo, and CdOsm treatments were loam. Levels of N and P were markedly higher in the CdLeo and CdPig treatments–concentrations were 3× and 4× compared to the control soil, respectively. Increases in EC and OM values occurred in most amended treatments; however, CEC values in the soils were similar, except for the ComOsm treatment, where CEC decreased. The ComOsm treatment had the lowest EC and CEC values, as well as the lowest P, K, Ca, Mg, total Cd, and extractable Cd concentrations.

Among the amendments, only leonardite was acidic (pH 2.6), while pig manure contained the highest EC, OM content, and extractable P (Table 2). All amendment materials had similar total N contents except for leonardite, which had approximately 2× lower N. Furthermore, leonardite had the

Table 1 Physicochemical properties of tested soils before the experiments

Parameter	Ctrl	ComOsm	CdPig	CdCow	CdLeo	CdOsm
pH	7.0	5.8	6.6	7.3	6.0	6.9
EC (dS m^{-1})	1.0	0.8	1.7	1.7	3.2	1.2
CEC (cmol kg^{-1})	26.3	18.7	27.6	24.9	32.2	25.0
OM (%)	4.9	8.6	7.4	6.2	12.1	4.5
Sand (%)	19	31	15	40	37	26
Silt (%)	23	28	32	36	51	34
Clay (%)	58	42	54	25	12	40
Soil texture	Clay	Clay	Clay	Loam	Silt loam	Clay loam
Total N (%)	0.2	0.4	0.4	0.3	0.6	0.2
Ext. P (mg kg^{-1})	334.4	337.8	1346.9	397.1	304.1	425.3
Ext. K (mg kg^{-1})	695.6	1223.6	1261.1	2416.1	520.6	643.6
Ext. Ca (mg kg^{-1})	5283	1559	4357	4468	7725	5228
Ext. Mg (mg kg^{-1})	708	579	920	909	886	628
Total Cd (mg kg^{-1})	5.0	2.5	7.8	9.3	6.1	5.4
Ext. Cd (mg kg^{-1})	3.0	0.4	5.1	4.6	3.8	2.5

Ctrl control, *Com* commercial soil (low Cd), *Osm* Osmocote®, *Cd* cadmium, *Pig* pig manure, *Cow* cattle manure, *Leo* leonardite, *EC* electrical conductivity, *CEC* cation exchange capacity, *OM* organic matter, *Ext* extractable

lowest extractable P and K concentrations. All amendments had similar contents of Ca, Mg, and total Cd, except for Osmocote, where the Cd value was below detection limits.

After amendment application, total Cd levels increased approximately 1.1–1.9×, except for the ComOsm treatment, where Cd concentration decreased by approximately 50%. Extractable Cd concentrations increased approximately 1.3–1.7× in CdLeo, CdCow, and CdPig treatments, respectively, and extractable Cd decreased approximately 1.2–7.5× in the CdOsm and ComOsm treatments, respectively.

After harvest, higher levels of total and extractable Cd were noted in various treatments. However, slight increases in total Cd were found in ComOsm treatments for all marigold cultivars, except for Honey marigold whose value was higher than Cd soil before experiment or approximately 1.8×.

Table 2 Physicochemical properties of amendments used in greenhouse study

Parameter	Osm	Pig	Cow	Leo
pH	7.4	8.3	8.6	2.6
EC (dS m^{-1})	3.9	4.8	3.5	3.9
OM (%)	–	63.2	37.4	20.1
Total N (%)	1.3	1.2	1.4	0.6
Ext. P (%)	1.4	4.3	< 0.5	BDL
Ext. K (%)	1.3	1.3	1.5	0.17
Ext. Ca (%)	–	1.3	1.5	1.7
Ext. Mg (%)	–	0.34	0.64	0.28
Total Cd (mg kg^{-1})	BDL	2.2	2.3	2.4

BDL below detectable limits, *Osm* Osmocote®, *Pig* pig manure, *Cow*, cattle manure, *Leo* leonardite

Growth performance of marigolds

Plant survival rate was 100% and no toxicity symptoms were observed throughout the experimental period. Application of amendments, particularly pig manure, enhanced plant growth in the Cd-contaminated soil (Table 3). Pig manure contained the highest concentrations of OM and extractable P among the amendments (Table 1). Plant height was greatest in the CdPig treatment, which also had the greatest number of flowers, and had the greatest total dry biomass in shoots, roots, and whole plant ($p < 0.05$). Pig manure also had a positive effect on flower diameter across all cultivars. The CdPig treatment resulted in the highest growth rate in biomass for all cultivars ($p < 0.05$), which was also consistent with total dry biomass production. The order of total dry biomass production was as follows: Sunshine > American > Babuda > Honey > French.

Highest root/shoot (R/S) ratios among the marigold cultivars occurred in the ComOsm treatment (0.2–0.38) ($p < 0.05$) (Fig. 1). The order of R/S ratio in the ComOsm treatment was: American > French > Babuda ≈ Honey > Sunshine. Significant R/S values were recorded for French and American cultivars in the CdPig treatment. R/S ratios in other Cd soil treatments ranged from 0.06–0.12.

Cd uptake and accumulation in marigold tissue

In the organic amendment treatments, marigolds accumulated Cd primarily in roots, compared to shoots and flowers ($p < 0.05$) (Table 4). Significant values ($p < 0.05$) were measured in French and American cultivars, respectively. Flowers in the

Table 3 Growth performance of five marigold cultivars (*n* = 5)

Genotype	Treatment	Height (cm)	Root (cm)	Flower		Biomass (g plant^{-1})	Growth rate
				Number of flower	Diameter (cm)		
American	Ctrl	35.7 ± 9.3 dB	17.1 ± 6.3cA	-	-	2.5 ± 1.5cB	1.2 ± 0.7cB
	ComOsm	51.7 ± 3.0bcC	56.9 ± 6.1aA	5.8 ± 1.1aA	6.1 ± 2.5aAB	13.4 ± 2.6bA	6.7 ± 1.3bA
	CdPig	76.2 ± 7.9aAB	39.9 ± 15.2bA	5.2 ± 0.8abB	6.3 ± 1.2aBC	45.3 ± 5.4aA	22.6 ± 2.7aB
	CdCow	42.6 ± 8.4cdC	12.3 ± 1.9cBC	1.3 ± 0.6cB	4.3 ± 1.2aBC	4.9 ± 2.8cB	2.5 ± 1.4cC
	CdLeo	41.9 ± 6.2cdCD	12.3 ± 3.6cB	1.7 ± 0.6cB	4.3 ± 1.4aBC	2.7 ± 1.2cB	1.3 ± 0.6cB
	CdOsm	55.7 ± 18.4bAB	22.6 ± 15.0cA	3.3 ± 2.9bcA	5.6 ± 0.4aB	16.1 ± 12.3bA	5.4 ± 2.3bA
French	Ctrl	35.2 ± 8.2bB	20.7 ± 8.9bcA	1.4 ± 0.5cB	4.1 ± 0.9bA	2.7 ± 1.2bB	1.4 ± 0.6bB
	ComOsm	39.4 ± 2.0bD	34.0 ± 7.9aC	3.6 ± 1.1bcB	4.1 ± 0.9bB	7.6 ± 0.8bB	3.8 ± 0.4bB
	CdPig	52.7 ± 6.8aB	24.5 ± 8.3abB	10.6 ± 4.1aA	5.6 ± 0.4aC	34.5 ± 15.7aA	17.2 ± 7.8aB
	CdCow	32.3 ± 5.8bC	11.6 ± 6.8cBC	4.8 ± 3.1bAB	4.1 ± 1.1bC	4.0 ± 2.3bB	2.1 ± 1.2bC
	CdLeo	31.7 ± 3.8bD	12.1 ± 3.7bcB	2.8 ± 1.7bcB	3.9 ± 0.4bC	2.4 ± 1.7bB	1.4 ± 0.8bB
	CdOsm	40.7 ± 11.3bC	22.2 ± 16.8abcA	2.5 ± 1.3bcA	4.3 ± 0.7bC	6.0 ± 3.3bB	3.7 ± 0.4bB
Babuda	Ctrl	48.1 ± 28.3dAB	14.7 ± 2.5cA	3.0 ± 1.7bcA	5.3 ± 2.2aA	1.8 ± 1.0 dB	1.0 ± 0.6bB
	ComOsm	61.4 ± 4.6bcdB	43.4 ± 7.4aB	3.0 ± 0.7bcB	5.7 ± 0.7aAB	10.0 ± 0.7cdB	5.0 ± 0.3bB
	CdPig	89.5 ± 5.7aA	17.6 ± 3.3cB	8.8 ± 2.1aAB	7.0 ± 1.0aAB	44.6 ± 14.9aA	22.3 ± 7.5aB
	CdCow	71.4 ± 22.3abcAB	17.3 ± 3.8cAB	5.8 ± 3.9abA	6.7 ± 0.8aA	23.6 ± 19.7bcA	18.6 ± 4.7aA
	CdLeo	80.9 ± 14.8abA	31.3 ± 11.3bA	6.6 ± 4.4abA	6.0 ± 0.6aA	35.6 ± 18.4abA	17.8 ± 9.2aA
	CdOsm	51.1 ± 1.9cdBC	17.3 ± 7.5cA	1.8 ± 1.0cA	5.8 ± 1.0aB	7.2 ± 1.3 dB	3.7 ± 0.7bB
Honey	Ctrl	57.0 ± 2.6aA	20.2 ± 3.5bcA	1.0 ± 0.0cB	4.5 ± 1.1dA	6.5 ± 2.4bcA	3.2 ± 1.2bcA
	ComOsm	63.4 ± 6.1aB	50.1 ± 6.4aAB	3.4 ± 2.1bB	6.9 ± 1.5abA	15.4 ± 2.4bA	7.7 ± 1.2bA
	CdPig	75.9 ± 35.9aAB	26.5 ± 10.0bB	5.4 ± 0.9aB	6.1 ± 0.5abcBC	40.7 ± 7.4aA	20.3 ± 8.7aB
	CdCow	49.1 ± 37.3aBC	8.6 ± 4.2dC	2.4 ± 1.5bcAB	5.2 ± 1.5cdABC	1.5 ± 0.5cB	0.9 ± 0.2cC
	CdLeo	52.9 ± 4.6aBC	15.6 ± 1.8cdB	2.0 ± 0.7bcB	5.4 ± 1.4bcdAB	4.2 ± 1.0cB	2.1 ± 0.5cB
	CdOsm	54.8 ± 4.1aB	26.9 ± 12.5bA	1.6 ± 0.5cA	7.6 ± 0.8aA	10.2 ± 1.5bcAB	5.1 ± 0.8bcAB
Sunshine	Ctrl	58.8 ± 9.8cA	13.4 ± 4.9cA	3.3 ± 0.6bcA	4.5 ± 1.7bA	5.4 ± 2.3bA	2.7 ± 1.2bcA
	ComOsm	71.2 ± 3.5bcA	42.3 ± 3.7aBC	2.4 ± 0.9cB	6.2 ± 1.4abA	14.6 ± 2.5bA	7.3 ± 1.3bcA
	CdPig	103.4 ± 37.8aA	21.0 ± 8.4bcB	7.0 ± 4.2aAB	7.7 ± 0.9aA	55.2 ± 35.6aA	34.1 ± 11.8aA
	CdCow	91.0 ± 19.4abA	24.5 ± 11.2bA	5.7 ± 0.6abAB	6.3 ± 1.7abAB	19.2 ± 12.0bA	9.6 ± 6.0bB
	CdLeo	65.3 ± 12.4cB	12.9 ± 4.9cB	1.7 ± 0.6cB	5.7 ± 0.8bAB	4.0 ± 2.2bB	2.0 ± 1.1cB
	CdOsm	69.2 ± 5.2bcA	18.3 ± 5.6bcA	2.8 ± 1.5bcA	7.7 ± 0.5aA	11.5 ± 0.8bAB	6.0 ± 0.7bcA

Values followed by the same letter are not significantly different; small letters show the difference of treatments of the same cultivar (LSD: $p < 0.05$); capital letters indicate the difference of plant growth performance among cultivars within the same treatment (LSD: $p < 0.05$)

organic treatments, particularly the CdCow treatment for American, French, and Honey, contained significant Cd concentrations (34.2–71.5 mg kg^{-1}, respectively). Highest Cd values in entire plants occurred in the control treatment for the American cultivar (> 100 mg kg^{-1}), while CdCow and CdOsm treatments for French and Babuda were also high in Cd.

BCF and TF values are important indices for predicting the phytoremediation potential of the tested plants (Saengwilai et al. 2017). BCF values were > 1 for both shoots and roots for all cultivars across all treatments (Table 5). High BCF values for both shoots and roots were found in all cultivars in the ComOsm treatment; BCF values for French cultivars in the CdCow treatment were also high (12.9).

Discussion

The highest soil pH value (7.3) was measured in soil amended with cattle manure. Cattle manure as an amendment is effective in increasing soil pH (Lai et al. 2010). Soil pH is considered a key parameter affecting metal bioavailability (Grant et al. 1999). In alkaline soils, the monovalent species (e.g., $CdOH^+$) will not be adsorbed by cation exchange processes and may be available for plant uptake (Laxen 1985; Kabata-Pendias 2001).

In this study, Cd-contaminated soil contained sufficient N, P, and K to support vigorous plant growth (Karamanos 2013; Phusantisampan et al. 2016). This is particularly important as macronutrients are often limiting in contaminated soils (Brady

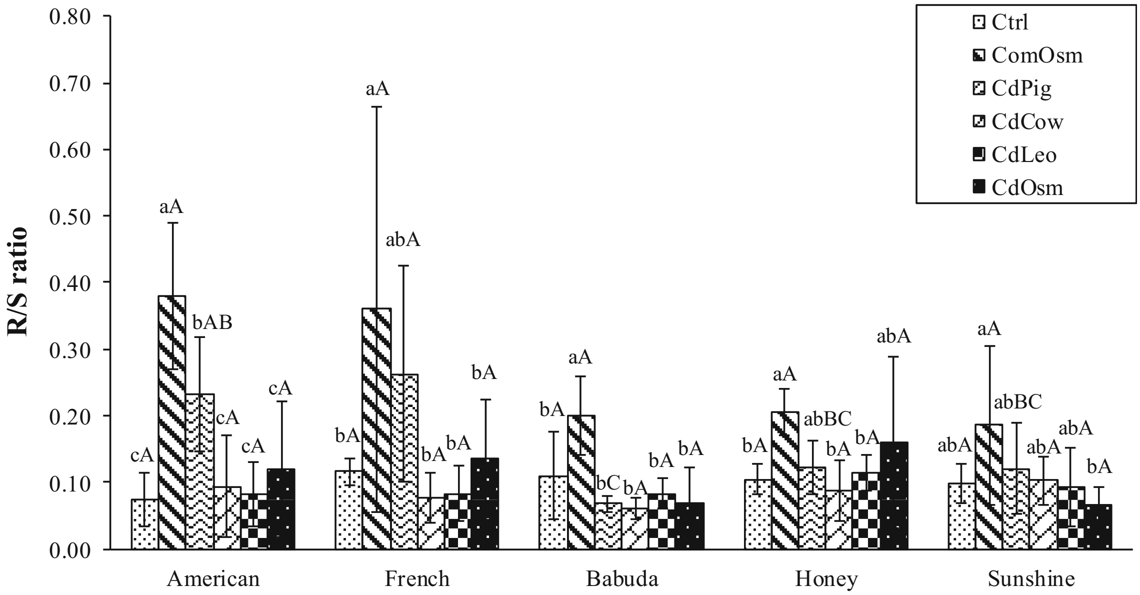

Fig. 1 Root/shoot ratios of Cd in marigold cultivars. Values followed by the same letter are not significantly different; small letters show the difference between amendment effects on each cultivar (LSD: $p < 0.05$). Capital letters indicate the difference between amendment effects within the same treatment (LSD: $p < 0.05$)

and Weil 2002). Desired environmental parameters for marigold cultivation include soil pH of 5.5–7.0, well-drained sandy loam to clay loam texture, and high OM content (Singh et al. 2003). The soil textures in this study did not pose an obstacle to plant growth.

The contaminated soil initially contained a low Cd concentration (5 mg kg^{-1}). However, soil Cd concentrations in excess of approximately 1 mg kg^{-1} are considered evidence of anthropogenic pollution (Uminska 1993); the reported values exceed the Canadian Guidelines limit of 1.4 mg kg^{-1} for agricultural soil (TRC 2005). The key Cd input to agricultural soils in the Mae Tao river basin is irrigation water derived from runoff from nearby Zn mines that contains both Cd and Zn due to decades-long recovery and processing of ores (Sricoth et al. 2018). To some extent, the amendments themselves were a source of some Cd to the tested soils, as Cd concentrations in all amended soils increased slightly. Fertilizer and amendment materials must, therefore, be evaluated for chemical properties prior to application to agricultural fields. Soil Cd contamination events caused by fertilizer application have been reported elsewhere (Mendes et al. 2006; Dharma-Wardana 2018).

All plants exposed to low Cd concentrations demonstrated survival under harsh conditions. Increased plant growth was consistent over the growing period, indicating adequate tolerance to Cd contamination. Plants contained high tissue Cd concentrations (> 14 mg kg^{-1} whole plant), which exceeds the tolerance level of most plants, reported as 0.2 mg kg^{-1} (Zhang et al. 2014).

Total dry biomass production and growth rate in biomass serve as useful indicators of plant growth performance (Meeinkuirt et al. 2016). Each cultivar experienced similar trends at harvest. Highest total dry biomass production and growth rate in biomass, found in Sunshine and American cultivars in the CdPig treatment, were consistent with the highest soil P and Mg concentrations.

Application of amendments, particularly pig manure, enhanced plant growth in Cd-contaminated soil. Pig manure contained the highest P and Mg concentrations and moderate concentrations of OM, N, K, and Ca. In Thailand, organic wastes are commonly used in agricultural fields as they are readily available and easily handled and applied. To some extent, leonardite may not be an appropriate soil amendment as some cultivars experienced low total dry biomass and growth rate values in the CdLeo treatment. However, a mixture of leonardite and zeolite increased barley yield, compared to compost alone (Moreno et al. 2017).

Flower yield is included in this study as this product offers substantial economic value to local economies. In a study by Hladun et al. (2015), the average number of flowers and other floral morphological traits were not affected by Cd; however, at 0.25 mM $CdSO_4$, flowering was delayed and the head size of sunflower (*Helianthus annuus*) was smaller in Cd-treated plants (Gopal and Khurana 2011). In the current study, flower production of plants grown on amended soil was higher than those in Cd-contaminated soil alone. The adverse effects of Cd were noted in American marigold grown in the control treatment, where flowers did not develop. Thus, while Cd in soil could impact plant growth, the severity of Cd on plant health may depend upon cultivar specificity and plant tolerance to metal toxicity (Chandra et al. 2010).

Table 4 Cd uptake and accumulation by five marigold cultivars ($n = 5$)

Genotype	Treatment	Cd accumulation in plant (mg kg^{-1})				Cd uptake (mg plant^{-1})
		Shoot	Root	Flower	Whole plant	
American	Ctrl	50.3 ± 9.2aA	51.6 ± 20.4abAB	–	103.4 ± 58.1aA	197.3 ± 74.5bcdA
	ComOsm	44.9 ± 12.7aA	20.0 ± 5.1bcB	13.3 ± 1.3bB	22.9 ± 4.1bC	314.3 ± 73.2bB
	CdPig	18.5 ± 3.1bABC	13.9 ± 1.9cA	12.1 ± 0.5bA	14.0 ± 3.9bC	624.9 ± 149.6aB
	CdCow	23.8 ± 20.7bAB	63.8 ± 42.5aAB	71.5 ± 64.0aA	30.4 ± 24.6bB	149.9 ± 84.9cdBC
	CdLeo	22.4 ± 2.9bAB	56.0 ± 32.6aA	19.3 ± 8.8bB	20.8 ± 6.4bB	51.3 ± 18.0 dB
	CdOsm	17.9 ± 4.0bC	23.5 ± 14.7bcB	17.7 ± 7.8bAB	16.4 ± 2.8bD	264.1 ± 186.1bcB
French	Ctrl	42.1 ± 5.2abAB	32.8 ± 22.2bAB	31.2 ± 3.8abA	55.2 ± 9.1abB	144.0 ± 40.5cA
	ComOsm	50.2 ± 10.0aA	38.9 ± 17.3bA	31.8 ± 23.6abA	56.0 ± 8.0abA	421.8 ± 42.5bAB
	CdPig	27.0 ± 12.8bcA	29.6 ± 25.4bA	14.1 ± 3.8bA	31.7 ± 6.4bA	1024.8 ± 418.3aAB
	CdCow	25.4 ± 19.4cAB	87.0 ± 64.3aA	34.2 ± 19.0aAB	70.4 ± 61.3aA	210.7 ± 175.2bcBC
	CdLeo	28.9 ± 5.1bcA	41.8 ± 10.0abAB	24.9 ± 12.0abB	46.5 ± 11.2abA	116.7 ± 102.8cB
	CdOsm	29.1 ± 10.9bcAB	22.6 ± 6.6bB	26.7 ± 9.8abA	51.3 ± 7.1abAB	331.2 ± 187.1bcAB
Babuda	Ctrl	31.9 ± 10.4abBC	55.8 ± 42.9aA	19.6 ± 9.5aAB	52.3 ± 16.9abB	122.0 ± 64.2dA
	ComOsm	19.7 ± 5.5bcB	36.6 ± 8.5abAB	15.3 ± 2.2abB	31.3 ± 10.2bcB	314.7 ± 115.1cdB
	CdPig	21.1 ± 7.3bcAB	20.8 ± 3.4bA	12.8 ± 1.7abA	30.1 ± 4.0bcAB	1342.8 ± 473.7aA
	CdCow	27.0 ± 10.2bA	30.1 ± 27.3abBC	17.2 ± 4.9abB	49.5 ± 25.1abAB	812.1 ± 640.7abcA
	CdLeo	15.7 ± 4.8cC	12.9 ± 5.0bC	12.8 ± 1.9abB	27.3 ± 4.9cB	897.6 ± 522.4abA
	CdOsm	38.8 ± 19.4aA	56.1 ± 18.2aA	19.0 ± 6.7aAB	60.1 ± 16.7aA	426.4 ± 118.2bcdAB
Honey	Ctrl	24.5 ± 8.9aC	20.4 ± 5.3bcB	20.6 ± 13.3abAB	32.9 ± 8.3abcB	218.1 ± 119.0bA
	ComOsm	21.2 ± 9.4abB	42.1 ± 21.5abA	11.8 ± 0.9bB	35.6 ± 12.3abcB	542.3 ± 187.4cA
	CdPig	9.8 ± 1.3bC	18.5 ± 5.0cA	14.1 ± 4.9abA	22.5 ± 9.9cBC	786.2 ± 227.4aAB
	CdCow	22.6 ± 13.2aAB	46.7 ± 24.1aBC	33.9 ± 31.2aAB	40.2 ± 16.1abB	66.8 ± 41.8cC
	CdLeo	17.4 ± 7.7abBC	33.1 ± 19.2abcABC	20.0 ± 17.3abB	30.3 ± 7.7bcB	123.1 ± 22.2cB
	CdOsm	15.2 ± 3.8abC	21.9 ± 2.0bcB	13.5 ± 2.4bB	46.5 ± 9.9aBC	484.7 ± 148.5bA
Sunshine	Ctrl	12.9 ± 5.5cD	40.8 ± 11.6aAB	15.1 ± 1.2bB	29.1 ± 9.3aB	153.8 ± 98.2bA
	ComOsm	10.5 ± 2.7cB	43.4 ± 24.6aA	14.2 ± 2.5bB	29.8 ± 6.1aBC	444.9 ± 149.9bAB
	CdPig	13.8 ± 2.3bcBC	19.3 ± 11.3bA	10.4 ± 0.3bA	25.5 ± 7.3aAB	1261.8 ± 845.4aAB
	CdCow	14.5 ± 5.2bcB	22.6 ± 16.4bC	11.2 ± 0.8bB	38.1 ± 21.3aB	601.9 ± 391.5bAB
	CdLeo	19.1 ± 2.3abBC	18.9 ± 4.4bBC	46.3 ± 12.8aA	29.3 ± 8.4aB	123.4 ± 88.0bB
	CdOsm	22.5 ± 5.8aBC	41.6 ± 9.0aA	16.4 ± 4.1bB	37.1 ± 5.7aC	428.2 ± 82.9bAB

Values followed by the same letter are not significantly different; small letters show the difference of treatments of the same cultivar (LSD: $p < 0.05$); capital letters indicate the difference in Cd accumulation and uptake performance among cultivars within the same treatment (LSD: $p < 0.05$)

In general, shoots (including flowers) of plants in the amended treatments had lower tissue Cd concentrations as compared with those in non-amended soil (control). In general, the greatest Cd uptake and accumulation were detected in roots, followed by shoots (≈ flowers). Marigold roots accumulated substantial Cd, thus indicating the potential for phytostabilization. Some reports indicate that organic acids (i.e., carboxylic acid and amino acids) in root exudates form complexes with metals, which promote stabilization within roots. This mechanism has been noted for chromium (Coelho et al. 2017; Srivastava et al. 1999). Considering the excluder potential of marigolds, plants accumulated Cd primarily in roots, particularly in French and American marigolds in the CdCow treatment; however, when considered by Cd uptake (considering Cd in whole plant mass), Babuda, Sunshine, and French marigolds in the same treatment were considered as very high potential for phytoremediation (> 1000 mg kg^{-1}). Recently, some ornamental plant species including landscape shrubs such as *Osmanthus fragrans*, *Ligustrum vicaryi*, *Loropetalum chinense* var. rubrumsince, and *Euonymus japonicas* cv. Aureo-mar were reported as excluders. However, soil Cd concentrations > 24.6 mg kg^{-1} could adversely affect plant growth, microbial community composition, and ultimately Cd phytostabilization potential in the plant (Zeng et al. 2018).

The Cd bioconcentration factor and translocation factor for roots of the marigold cultivars were > 1 which confirms its

Table 5 Bioconcentration coefficients for marigold shoots and roots, translocation factors, and Cd accumulation in soil after harvest ($n = 5$)

Genotype	Treatment	BCF		TF	Cd accumulation in soil	
		Shoot	Root		Total	Extractable
American	Ctrl	6.4 ± 2.4bA	6.9 ± 2.9abcAB	1.1 ± 0.3bcAB	8.5 ± 2.0aAB	4.1 ± 1.0bcA
	ComOsm	23.1 ± 7.7aA	10.1 ± 2.5aB	2.3 ± 0.8aA	2.0 ± 0.1cB	0.5 ± 0.03eB
	CdPig	2.3 ± 0.7bAB	1.7 ± 0.1cB	1.4 ± 0.4bA	8.2 ± 1.2aA	2.4 ± 0.3dA
	CdCow	3.3 ± 2.7bAB	8.0 ± 6.2abAB	0.5 ± 0.3 dB	8.7 ± 0.8aA	5.6 ± 1.6aA
	CdLeo	3.5 ± 0.8bAB	8.4 ± 5.0abA	0.6 ± 0.5cdBC	6.7 ± 1.3bAB	5.0 ± 0.7abA
	CdOsm	2.9 ± 0.6bBC	4.0 ± 2.6bcBC	1.0 ± 0.4bcdB	6.0 ± 0.5bC	3.9 ± 0.4cB
French	Ctrl	5.0 ± 1.2bA	3.7 ± 1.8bcAB	1.6 ± 0.7aA	8.7 ± 2.0abAB	3.9 ± 0.9aA
	ComOsm	25.3 ± 5.3aA	19.8 ± 10.4aA	1.5 ± 0.6abB	2.0 ± 0.2cB	0.6 ± 0.2bB
	CdPig	3.6 ± 2.2bA	5.2 ± 4.3bcA	1.2 ± 0.7abA	11.0 ± 4.7aA	3.1 ± 1.3aA
	CdCow	5.2 ± 4.5bA	12.9 ± 10.8abA	0.5 ± 0.4cAB	7.1 ± 1.6bA	4.6 ± 1.6aAB
	CdLeo	5.1 ± 1.9bA	7.3 ± 2.3bcAB	0.7 ± 0.3bcBC	6.6 ± 1.8bAB	3.9 ± 1.0aA
	CdOsm	4.2 ± 0.9bAB	3.2 ± 1.9cBC	1.5 ± 0.7abA	7.7 ± 1.5bBC	4.6 ± 1.3aAB
Babuda	Ctrl	5.4 ± 2.9bA	9.3 ± 7.8bA	0.7 ± 0.3bcBC	6.6 ± 1.8abB	4.5 ± 0.02abA
	ComOsm	13.8 ± 6.0aB	24.7 ± 8.0aA	0.5 ± 0.1cC	1.6 ± 0.4cB	0.4 ± 0.1cB
	CdPig	2.9 ± 1.5bAB	2.8 ± 1.2bAB	1.0 ± 0.3abAB	7.3 ± 2.3abA	3.3 ± 1.2bA
	CdCow	3.7 ± 1.6bAB	2.6 ± 0.7bB	1.0 ± 0.3abAB	7.8 ± 1.3aA	5.5 ± 2.0aA
	CdLeo	3.2 ± 1.5bB	2.5 ± 0.7bC	1.2 ± 0.3aA	5.6 ± 1.7bB	3.7 ± 1.7bA
	CdOsm	5.7 ± 3.0bA	8.2 ± 3.1bA	0.7 ± 0.4bcB	6.8 ± 0.4abBC	5.8 ± 1.4aA
Honey	Ctrl	2.5 ± 1.1bB	2.1 ± 0.9cB	1.2 ± 0.4aAB	10.2 ± 2.7aA	4.7 ± 0.9aA
	ComOsm	5.5 ± 3.1aC	10.4 ± 6.0aB	0.5 ± 0.1bC	4.6 ± 2.6bA	3.0 ± 3.2abA
	CdPig	1.3 ± 0.5bB	2.5 ± 1.5bcAB	0.6 ± 0.2bB	8.8 ± 3.3aA	2.3 ± 1.0bA
	CdCow	3.9 ± 2.9abAB	6.8 ± 5.0abAB	0.5 ± 0.3bB	9.8 ± 4.8aA	5.0 ± 1.8aAB
	CdLeo	2.2 ± 1.2bB	4.2 ± 2.9bcBC	0.6 ± 0.1bC	8.7 ± 2.4aA	4.7 ± 1.3aA
	CdOsm	1.6 ± 0.6bC	2.2 ± 0.4cC	0.7 ± 0.2bB	10.3 ± 2.0aA	4.3 ± 0.4abB
Sunshine	Ctrl	2.2 ± 0.9bB	9.8 ± 7.3bA	0.4 ± 0.2aC	6.4 ± 2.1aB	5.5 ± 2.6aA
	ComOsm	5.7 ± 1.9aC	21.1 ± 4.6aA	0.3 ± 0.1aC	1.9 ± 0.3bB	0.6 ± 0.02dB
	CdPig	1.7 ± 0.6bB	2.0 ± 0.6cB	0.9 ± 0.4aAB	8.9 ± 3.5aA	2.8 ± 1.0cA
	CdCow	1.9 ± 1.0bB	1.3 ± 0.6cB	1.1 ± 1.3aA	8.2 ± 2.8aA	3.3 ± 1.2bcB
	CdLeo	2.6 ± 0.4bB	2.7 ± 0.6cC	1.0 ± 0.3aAB	7.4 ± 0.9aAB	4.6 ± 1.0abcA
	CdOsm	2.9 ± 1.2bBC	5.5 ± 2.5bcAB	0.6 ± 0.3aB	8.5 ± 3.1aAB	4.7 ± 1.3abAB

Values followed by the same letter are not significantly different; small letters show the difference of treatments of the same cultivar (LSD: $p < 0.05$); capital letters indicate the difference of BCF (for root and shoot), TF, and Cd accumulation in soil among cultivars within the same treatment (LSD: $p < 0.05$)

phytostabilization potential in Cd-contaminated soil. Marigolds are suitable for phytostabilization since they grow fast, have a well-developed root system and accumulate high metal concentrations in vacuoles and nuclei of roots. They furthermore act as pioneer species in poor and harsh soil environments (Das and Maiti 2007; Lux et al. 2011).

Several marigold cultivars have been considered for use in remediation of Cd-contaminated and other derelict sites, such as American (*T. erecta*), French (*T. patula*), and nugget (triploid hybrid between *T. erecta* and *T. patula*). In Thailand, these species are commonly grown on large-scale plantations for economic purposes (Prasad et al. 2015). *Tagetes patala* might serve as another species on Cd-contaminated soil as it has high tolerance to Cd-induced toxicity by activation of its antioxidative defense system (Liu et al. 2011). The highest flower production (flower number, flower diameter, and biomass) was found in the Babuda and Sunshine cultivars, which is consistent with the highest Cd accumulation in whole plant tissues of the same soil treatments (CdPig treatment). This implies that the two cultivars may be considered as alternatives to replace edible crop species in Cd-contaminated fields.

Adding Cd solution throughout the study is presumably the main source of high Cd concentrations in marigold tissue. The data imply that the tested plants had a high potential for Cd uptake and accumulation.

Previous studies reported that application of manure significantly reduced extractable Cd concentrations as it contains high quantities of OM and P which stabilize metals in soil.

Cadmium binds readily with organic matter to form stable complexes (He and Singh 1993; Wenzel et al. 1996). Application of manure to soil increased the percentage of organically bound and residual metals but decreased exchangeable levels (Pierzynski et al. 2002). The level of soil organic matter is key to ensuring optimal soil physical properties and improving fertility and microbial activities, thereby improving crop growth and yield. The effects of organic amendments on reducing metal mobility and bioavailability are ultimately a function of the composition, quantity, type, and maturation of OM, microbial degradability, soil physicochemical properties, soil type, and metals present (Hattab et al. 2015). Chang et al. (2007) reported that 540 kg N ha^{-1} of the organic amendment is suitable for maintaining high organic matter in soil, which is the basis for optimizing crop yields and soil chemical, biochemical, and enzymatic activities. Manure amendments should be composted and/or dried prior to application to soil because high concentrations of organically bound N may be converted rapidly to nitrate-nitrogen (NO_3-N). This N species is readily leachable in the profile which can thereby lead to deleterious environmental and health problems (Ahmad et al. 2016).

A low R/S ratio indicates generally healthy plants (Meeinkuirt et al. 2012). Among the soil treatments, elevated Cd levels did not have affect plant growth because R/S ratios generally followed similar trends. Although the R/S ratios in French and American cultivars were highest, growth was high when compared with plants in different treatments.

High EC values in the CdPig treatment were noted. Elevated soil EC values (approximately > 2 dS m^{-1}) can affect the growth performance of some plants. Certain tolerant crops, for example, barley, can grow in soil with EC values up to 16 dS m^{-1} (Richards 1954; Rhoades and Loveday 1990). The EC values in this study did not impart any deleterious effects on the tested plants.

Soil treatments were slightly acidic, except for the CdPig treatment. Acidic conditions can increase metal mobility in soil (Loosemore et al. 2004). Acidic soil in the rhizosphere may also enhance Cd uptake and accumulation in plants (Hu et al. 2013); thus, high uptake and accumulation rates in low soil pH can be useful for many hyperaccumulators. Increased biomass production after fertilization may further improve phytoextraction efficiency (Wei et al. 2010; Paz-Ferreiro et al. 2014). In this study, Cd concentrations in aboveground plant parts did not achieve the criteria for hyperaccumulators (i.e., ≥ 100 mg kg^{-1}) (Baker and Brooks 1989). However, the tissue Cd concentrations reported in this study can potentially increase health risks to consumers. Cadmium concentrations were > 0.15 mg kg^{-1} in flowers of plants grown in all soil treatments, indicating that they pose some risk for human consumption (Saengwilai et al. 2017). In several countries, marigold flowers are commonly used in food products such as cola and alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments, and relishes (Meshkatalsadat et al. 2010). Thus, it is suggested that all edible plant parts be evaluated for Cd content, to ensure safety both for the environment and for organisms.

Various authors have reported the potential Cd excluder capacity of species such as *Oryza sativa*, *Eucalyptus camaldulensis*, and *Vetiveria zizanioides* in contaminated areas of the Mae Tao River Basin, as they accumulate Cd mainly in roots and possess TF values < 1 (Prasad et al. 2015; Meeinkuirt et al. 2016; Phusantisampan et al. 2016; Saengwilai et al. 2017). The above species possess inherent Cd tolerance and high Cd uptake and accumulation capabilities; they, along with marigolds, also offer substantial commercial benefits. In contaminated areas, marigolds can grow well and produce flowers throughout the year. More importantly, they produce substantial aboveground biomass and experience high propagation rates, and shoots remain upright, allowing for easy harvest. Thus, marigolds could serve both as an excluder and commercial ornamental plant in Cd-contaminated areas. It should be noted, however, that some marigold cultivars (e.g., Pusa narangi and Ritu raj) are known to accumulate metals mainly in shoots (Saxena et al. 2012); thus, plant genotypes must be carefully screened for phytostabilization potential.

Conclusions

Various marigold cultivars should be encouraged for cultivation in Cd-affected fields in Tak province, Thailand, as they can replace edible plants. Enhancing marigold quality and controlling costs of planting are important for farmers. These goals may be attained via sustainable crop management in Cd-contaminated areas. The five marigold cultivars tested herein demonstrated a high potential for Cd phytostabilization. In greenhouse experiments, plants grew and adapted well to soil contaminated with Cd as evidenced by a 100% survival rate, and with TF < 1 and BCFR values > 1. In terms of phytomanagement, Babuda and Sunshine marigolds are an alternative option for phytostabilization strategy because they possess excluder characteristics with high Cd accumulation in roots and excellent flower production. In addition, certain organic amendments, particularly pig manure, improved plant growth and are recommended for Cd stabilization.

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