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Screening of potential hyperaccumulator for cadmium from contaminated soil

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ABSTRACT

In this study, cadmium hyperaccumulation potential of four species, Guinea grass (*Panicum maximum*), cosmos (*Cosmos sulphureus*), African marigold (*Tagetes erecta* L.) and sunflower (*Helianthus annuus*), was investigated in pot culture experiments in triplicate in a greenhouse. The concentration of cadmium solution was varied from 50 to 400 mg kg^{-1} of soil. Samples of different parts of plants after reaching flowering stage were harvested for cadmium analysis. The present study demonstrated that based on the total cadmium accumulation in plant, marigold showed higher potential, compared to other species. However, based on total biomass and total uptake, Guinea grass also showed high ability to uptake Cd from soil. Translocation factors (TFs) of marigold and cosmos were above one. Under all Cd treatments, the bioconcentration factor (BCF) of marigold was greater than one. Taking into consideration all factors, *Tagetes erecta* L. showed high potential for Cd uptake from contaminated soil.

Keywords: Cadmium contaminated soil; Marigold; Guinea grass; Translocation factor; Bioconcentration factor

1. Introduction

As a result of rapid industrialization in Asia, there is a growing concern over the contamination of soil, water and agricultural products by heavy metals. Several uses of heavy metals in various applications lead to their wide distribution in soil, sediment, air, and wastewater. Cadmium (Cd) is recognized as a significant pollutant due to its high toxicity and great solubility in water [1]. Soil contamination by toxic heavy metals is a great concern and environmental problem in Thailand and many Asian countries. Sources of soil contamination by cadmium include mostly industrial and agricultural activities such as electroplating, manufacturing of plastics, paint pigments and batteries containing cadmium, wastewater used for irrigation, mining and smelting of metalliferous ores, over application of pesticides and phosphate fertilizers and sludges [2,3]. Several conventional methods such as soil washing and flushing, solidification, vitrification, electrokinetic remediation etc., can be used to treat heavy metal contaminated soil. However, these technologies may destroy biological components in the soil and can drastically alter its chemical and physical characteristics. These methods are also labor-intensive and costly compared to phytoremediation.

Phytoremediation, which mainly uses hyperaccumulator and accumulator plants, has become a promising soil remediation technology as an alternative and ad-

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ditive approach to conventional methods, and can take up large amounts of excess heavy metals such Cd from contaminated soils. This is considered as a cost effective technology without major secondary environmental issues, especially for the remediation of large areas of contaminated soils with relatively low concentrations of heavy metals [4–6]. Phytoremediation takes advantage of the natural processes of plants, including chemical and water uptake as well as metabolism of the plants. Plant root systems can also influence the uptake of metals from the soil [7,8]. In addition, plants are considered as hyperaccumulators when they can accumulate high amount of heavy metals. The main characteristics of hyperaccumulators can be summarized as follows:

(1) Accumulating capacity; the threshold values (on dry biomass in the shoots) of various metals concentration in plants include 10,000 mg kg^{-1} for Zn and Mn, 1,000 mg kg^{-1} for Co, Cu, Ni, As and Se, and 100 mg kg^{-1} for Cd;
(2) Translocation factor (TF); the ratio of metal concentrations in shoots to roots should be greater than 1;
(3) Bioconcentration factor (BCF); the ratio of metal concentrations in plants to soil is greater than 1 [9–11].

Presently, the phytoremediation technique is not much used in practice due to slow growth rates, and long growing seasons as well as low biomass of the hyperaccumulators and accumulators. Screening of potential Cd-hyperaccumulating cultivars locally available may be useful for the remediation of contaminated soils.

The main objective of this study was to evaluate the cadmium hyperaccumulation potential of the four species: Guinea grass (*Panicum maximum*), cosmos (*Cosmos sulphureus*), African marigold (*Tagetes erecta* L.) and sunflower (*Helianthus annuus*). Cadmium uptake, translocation factor, bioconcentration factor were also investigated and compared among the different species studied.

2. Materials and methods

All reagents including concentrated acids and chemicals used for the experiments are analytical grades unless otherwise stated. Double de-ionized water (Milli-Q Millipore 18.2 MΩ/cm resistivity) was used for all solutions preparation. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg/l (Cd). All digestion vessels and glass wares were acid washed and rinsed with reagent water.

2.1. Soil and plant preparation for pot experiment

Uncontaminated surface soils (0–20 cm) collected from the Asian Institute of Technology (AIT) agricultural farm were used in pot experiments. The collected soil samples were air-dried, ground, and homogenized through repeated mixing and analyzed for background concentrations of heavy metals as well as for pH, cation exchange capacity, electrical conductivity, and organic matter. For preparation of artificially Cd contaminated soil, the prepared soil was thoroughly spiked with the required amount of cadmium chloride solution (prepared by dissolving analytical grade $CdCl_2 \cdot 2.5H_2O$). A single application was conducted by spiking with cadmium chloride solution to achieve levels of Cd-spiking for various concentrations (50, 100, 200 and 400 mg kg^{-1}) into the potted soils. An uncontaminated soil was included as control treatment (CT). Each plastic pot (26 cm diameter, 24 cm height) was filled with 8 kg ground soil mixed with four levels of Cd solution. During the cadmium spiking process, cadmium spiked soils in these pots were incubated for about six weeks under the shade before they were used. All pots were watered with the amount of distilled water close to their field capacity of soil.

For plant preparation, seeds of studied plants were germinated in a material bed. After germination, for about two-three weeks, uniform seedlings were selected and transferred to artificially heavy metal contaminated soil pots under various cadmium concentrations as previously mentioned. After acclimatization for a few days in the shade, all transplanting pots were placed in the greenhouse with natural sunlight and relative humidity (60%). Three replicates were run for each treatment (three plants for each pot; nine plants for each treatment) and arranged in a completely randomized design. Water loss due to evaporation was replenished using tap water (no Cd detected). Each pot was watered daily throughout the experiment with an equal amount of tap water to maintain soil moisture content at 75% of field capacity.

2.2. Sampling

Before and after pot experiments, soil samples from each pot were collected for total heavy metal analysis to find out initial Cd concentration and concentration after plant harvesting. The soil samples were air dried, pulverized and kept in polyethylene bag before analysis in laboratory. For plant samples, at the end of experiments (flowering stage), studied plants were harvested and washed thoroughly with running tap water followed by distilled water before being divided into roots, leaves-stems, and flowers, and the fresh weight was determined. The fresh samples were dried at 60–70°C for 48–72 h in a hot air oven to obtain a constant weight. Dry weights (DW) of roots, leaves-stems and flowers were recorded.

2.3. Plant and soil analysis

Soil samples taken from each pot were air-dried at room temperature (until completely dry) and ground to pass through 2-mm stainless steel sieve. Soil samples (1.0 g) were digested by concentrated HNO_3 and HCl (1:3). The suspension was filtered through Whatman

No. 42 filter paper, and diluted to 50 ml with 1% nitric acid. A blank digestion was carried out in the same way. Plant samples were digested with an acid mixture containing 13 M HNO_3 [12]. Concentrations of Cd in plant and soil were determined by using inductively coupled plasma-optical emission spectrophotometry (ICP-OES), Perkin-Elmer ICP-OES model Optima 5000 DV series (detection limits of 0.1 μg/ L for Cd), at the Environmental Engineering Laboratory, Asian Institute of Technology (AIT), Thailand. The accuracy and sensitivity of heavy metals in plant and soil samples were checked by analyzing certified standard reference material (apple leaves 1515 and SRM 2709, respectively). Soil pH was measured using a pH meter with a soil to water ratio of 1:1 [12,13]. Organic carbon was determined by the Walkley-Black wet combustion method [14] (multiplied by 1.72 to convert to organic matter). Cation exchange capacity (CEC) was determined using the ammonium acetate method [15,16]. Electrical conductivity (EC) is determined in a 1: 2.5, soil: water suspension according to DOA [12].

2.4. Translocation factor and bioconcentration factor

Translocation factor (TF) is determined by the ratio of metal concentration in plant shoots to that in plant roots. Bioaccumulation factor (BCF) is defined as the ratio of metal concentration in plant roots or shoots to that in the soil. TF and BCF were used to evaluate the plants capacity to accumulate heavy metals.

2.5. Statistical analysis

All the values expressed are mean ± S.D (standard deviation) of the three replicates calculated by Microsoft Office Excel 2003. Analysis of variance (one-way ANOVA) was carried out with SPSS 11.5. Multiple comparisons were made by the least significant difference (LSD). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Background soil characteristics

Characteristics of uncontaminated soil collected from AIT agricultural farm used in this experiment were as follows: soil pH value of 6.3, cation exchange capacity of 25.3 cmol kg^{-1}, electrical conductivity of 0.16 dS/cm at 25°C, organic matter and organic carbon of 3.31 and 1.92%, respectively. Background contents of heavy metals in the soil were 7.39, 61.11, 18.94, 28.11 mg kg^{-1} for As, Zn, Pb and Cu, respectively. Soil texture was found to be clay (clay 51.4, sand 30.5 and silt 18%). The pH is in the optimum range for plant growth. According to DOA [12], there will be no effect on the plants if the EC of the soil is in the range of 0–2 dS/cm. Cadmium in the soil was not detectable. Arsenic level in the soil is a bit higher than the recommended level of As for agricultural soil (not exceeding 3.9 mg kg^{-1}) according to Soil Quality Standards for habitat and agriculture [17].

3.2. Effects of cadmium on plant growth

It was found that individual plant heights differed under various Cd treatments as shown in Table 1. Heights of marigold, under all Cd treatments, differed significantly from control pots ($p < 0.05$). Heights of marigold under Cd concentration of 50 mg kg^{-1} differed significantly from those at other Cd treatments (100, 200 and 400 mg kg^{-1}). For other concentrations, the heights of marigold demonstrated no significant differences ($p > 0.05$) among these treatments. For cosmos, under all Cd treatments, except treatment of 50 mg kg^{-1}, plant heights significantly differed from control pots ($p < 0.05$). For Guinea grass, under all treatments, plant heights significantly differed from control pots. However, plant height under treatments of 50 and 100 mg kg^{-1} showed no significant differences ($p > 0.05$). For sunflower, height of plants under treatment of 50 and 100 mg kg^{-1} showed no significant difference from the control pots ($p > 0.05$) but plant height at Cd levels of

Table 1
Heights of studied plants under different cadmium treatments

Cd treatment (mg kg^{-1} soil)	Plant height (cm)			
	M_{ar}	C_{os}	S_{un}	G_{ui}*
CT	56.55^{c}±1.07	45.67^{d}±0.34	80.33^{c}±2.40	92.78^{d}±2.80
50	51.33^{b} ±1.53	43.33^{d}±2.33	82.17^{c}±0.93	70.78^{c}±1.83
100	32.89^{a}±1.50	40.39^{c}±1.92	79.11^{c}±0.70	63.44^{bc}±2.78
200	31.52^{a}±1.30	37.45^{b}±0.69	67.00^{b}±1.86	55.89^{b}±1.17
400	32.11^{a}±2.22	31.44^{a}±0.77	58.33^{a}±4.34	46.78^{a}±8.57

CT: control; M_{ar}: marigold; C_{os}: cosmos; S_{un}: sunflower; G_{ui}: Guinea grass. All data are presented as mean±S.D. ($n = 3$).
Means with different letters are significantly different from each other ($p < 0.05$).
*Length of the plant was measured from the above soil up to the longest part of the plant leaf.

200 and 400 mg kg^{-1} demonstrated significant difference from control pots and from each other ($p < 0.05$).

At the end of experiment (flowering stage), all studied plants grew well in control pots. However, under higher cadmium concentrations (200 and 400 mg kg^{-1}), significant decline in growth (based on plant heights) of four species was observed. The total biomass (dry weight, DW) of harvested plants from different species differed among treatments as shown in Table 2. For marigold, maximum total biomass was obtained from control pots as 22.51±2.37 g pot^{-1} and it significantly differed from other treatments. Under Cd levels of 100–400 mg kg^{-1}, no significant differences ($p > 0.05$) were observed for total biomass of marigold. For cosmos, total biomass of control pots and for treatment of 50 mg kg^{-1} significantly differed from other treatments ($p < 0.05$). However, at Cd treatments of 100, 200 and 400 mg kg^{-1}, total biomass of cosmos showed no significant difference ($p > 0.05$) from each other. For sunflower, at Cd levels of 50, 200 mg kg^{-1} and control pots, total biomass of sunflower significantly differed ($p < 0.05$) from other treatments. However, total biomass at Cd levels of 100 and 400 mg kg^{-1} illustrated no significant differences ($p > 0.05$).

For Guinea grass, significant differences of total biomass from control pots and other treatments were observed ($p < 0.05$), while differences were non-significant for the Cd treatments of 100 and 200 mg kg^{-1}.

Results indicated that control pots provided the highest total biomass for all species. For Guinea grass, total biomass was high at all treatment conditions, as compared to other species. It can be seen that plant growth (based on the height and total biomass of four species) was reduced as a result of Cd toxicity at higher concentrations. Previous researchers [18–22,] also reported that cadmium can reduce plant growth, photosynthesis, chlorophyll content, induce oxidative stress, and can cause various changes in biological activities. Chlorosis, leaf rolls and stunting are the main symptoms of Cd toxicity in plants. Cadmium has been shown to interfere with the uptake, transport and use of several elements and water by plants [20,23]. Moreover, at the highest Cd treatment of 400 mg kg^{-1}, the flowering stage was delayed (by about two to three weeks as compared to the control pots) for marigold, cosmos and sunflower. For Guinea grass under all cadmium treatments, no flowering stage was observed during the time of investigation. A reduction in total biomass and leaf size as well as stem size of marigold, cosmos and sunflower was clearly noticed at higher concentrations of 200 and 400 mg kg^{-1}, indicating phytotoxicity by cadmium. Alloway [24] stated that an excess of both essential and non essential metals results in phytotoxicity, and also acute Cd toxicity manifests as leaf chlorosis, wilting, and stunted growth.

3.3. Cd accumulation in harvested plants

After reaching the flowering stage, studied species were harvested and then analyzed for total cadmium contents in whole plant. From results obtained (Fig. 1.), it was found that at Cd treatments of 50, 100, 200 and 400 mg kg^{-1}, total Cd concentrations in marigold were 431.19 ± 27.35, 769.47 ± 179.64, 958.29 ± 98.40, 1361.79 ± 263.30 mg kg^{-1} of dry biomass, respectively. For Guinea grass, total Cd concentration was lower, compared to other species, even at 400 mg kg^{-1}. In general, the mean levels of Cd in harvested plants increased with an increase of Cd in the soil. Based on total cadmium concentration accumulated in studied plants, marigold showed higher potential of cadmium accumulation in whole plant tissues.

Under all Cd treatments (50, 100, 200 and 400 mg kg^{-1}), marigold has a great ability to accumulate Cd in the above ground parts (222.19 ± 24.04, 382.94 ± 131.07, 366.45 ± 78.63, 612.05 ± 20.95 mg kg^{-1}, respectively). The maximum Cd accumulation of marigold shoots reached the threshold value, 100 mg kg^{-1} dry weight, meeting one of the criteria for hyperaccumulator as shown in Fig. 2. Moreover, Cd contents in shoots of marigold differed significantly from other species ($p < 0.05$). It was found that contents of Cd in shoots increased with Cd concentrations in soils. Similar results were observed by Xiong [25]. It was illustrated that heavy metal concentrations

Table 2
Total biomass of studied species under different Cd treatments

Cd treatment (mg kg^{-1} soil)	Total biomass (g pot^{-1}, dry weight)			
	M_{ar}	C_{os}	S_{un}	G_{ui}
CT	22.51^{c}±2.37	12.98^{c}±0.13	12.69^{d}±0.34	81.43^{d}±3.35
50	8.93^{b}±3.77	5.26^{b}±1.98	10.06^{c}±0.84	68.58^{c}±4.28
100	4.13^{ab}±3.45	2.36^{a}±0.93	2.90^{a}±1.38	46.57^{b}±9.11
200	1.31^{a}±0.68	1.65^{a}±0.11	6.07^{b}±1.67	46.16^{b}±4.89
400	1.73^{a}±0.51	1.87^{a}±0.26	2.06^{a}±2.01	15.98^{a}±4.59

CT: control; M_{ar}: marigold; C_{os}: cosmos; S_{un}: sunflower; G_{ui}: Guinea grass. All data are presented as means ± S.D. ($n = 3$). Means with different letters are significantly different from each other ($p < 0.05$).

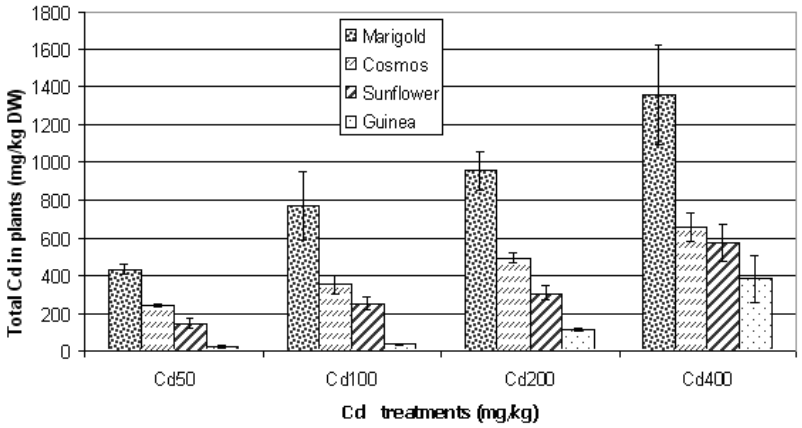

Fig. 1. Total Cd in studied plants (whole plants) under various Cd treatments. All data are mean ± S.D. (n = 3). One way ANOVA (one factor: different Cd treatments) was performed for total cadmium accumulation in plants.

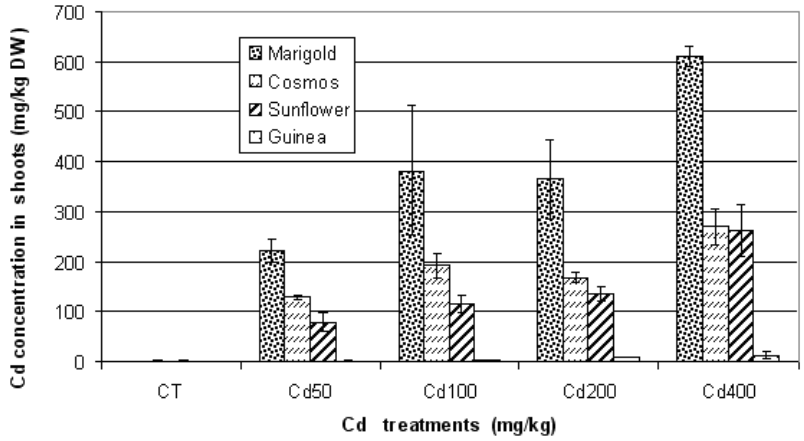

Fig. 2. Cadmium concentrations in shoots of studied plants under various Cd treatments. All data are means ± S.D. (n = 3). One way ANOVA (one factor: different Cd treatments) was performed for total cadmium accumulation in shoot. CT: control.

in plants are a function of heavy metal contents in the environment.

Furthermore, accumulation of cadmium displayed the same pattern in all species that is root > leaf-stem > flower (data not shown). Concentrations of Cd in root, leaf-stem and flower of individual species increased with an increase in Cd content in the soil. Several researchers [26,27] reported that cadmium concentrations were higher in roots than that in the shoots.

This study focused mainly on the screening of the species which show best uptake of Cd from artificially spiked soil with various Cd concentrations without considering the adsorption capacity of the soil influencing the uptake of Cd by plants. Unadsorbed Cd in the soil which is in bioavailable fraction (available form of Cd for plant uptake) is expected to be taken up by plants [3].To understand in more details, speciation studies are required. Thus, in ongoing experiments, Cd pools in the soil after plant harvesting will be determined to evaluate Cd speciation and uptake by plants.

Although Guinea grass accumulated the lowest Cd concentrations, the total Cd uptake per pot reached maximum with Guinea grass under Cd treatment of 400 mg kg^{-1} (Fig. 3) due to higher biomass. Some researchers [28,29] found that higher biomass producing species usually contained low to average heavy metal concentration. However, higher biomass can compensate for their lower Cd contents, when compared to hyperaccumulating species producing lower biomass. Due to higher biomass, Guinea grass could be used for long term remediation of the contaminated areas, where mild contaminations (50–200 mg kg^{-1}) are observed. Based on the results obtained, the removal rate of Cd from the soil by marigold was found to be 2.384 g/m^2 per crop. If the total weight of the top soil 0–20 cm is around 195 kg per m^2 area (based on Department of Agriculture, Bangkok,

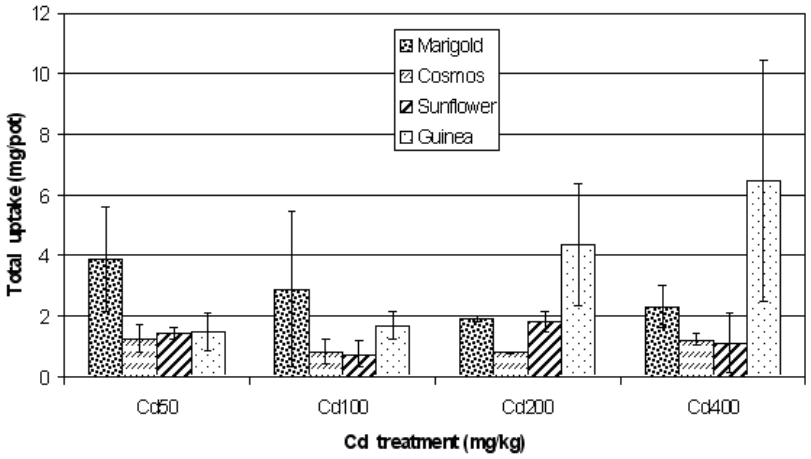

Fig. 3. Total Cd uptake by various plants under different Cd treatments per pot. All data are means ± S.D. (n = 3). One way ANOVA (one factor: different Cd treatments) was performed for total uptake of Cd.

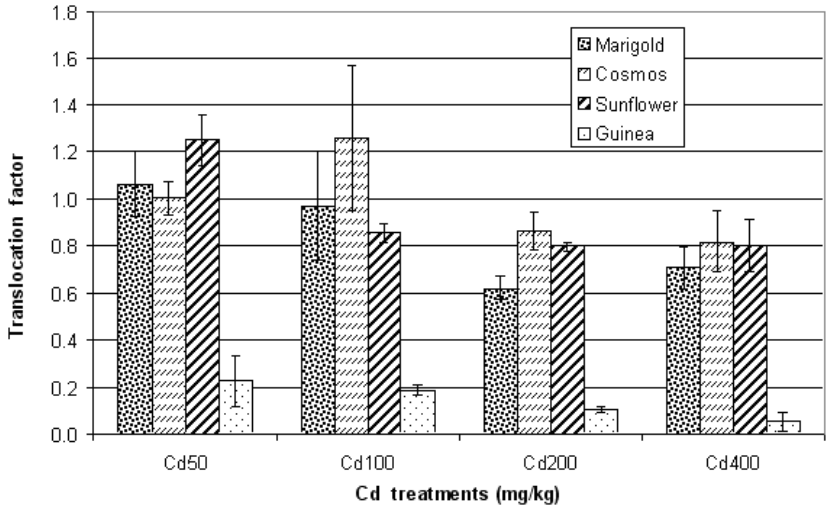

Fig. 4. Translocation factor (TF) of harvested plants under various Cd treatments. All data are means ± S.D. (n = 3). One way ANOVA (one factor: different Cd treatments) was performed for translocation factor.

Thailand), then the total Cd concentration in the soil is around 9.750 g/m^2 when the Cd contamination is 50 mg of Cd/kg of soil. It is estimated that about four crops of marigold will be required to clean the contaminated area (assuming that the removal rate is constant and one crop takes about 80–90 d). This will depend also on the amount of biomass.

It can be seen from Fig. 4 that at Cd treatment of 50 mg kg^{-1}, the maximum translocation factor (TF) was obtained by sunflower followed by marigold, cosmos and guinea grass with TF of 1.25 ± 0.11, 1.07 ± 0.14, 1.00 ± 0.07 and 0.22 ± 0.11, respectively. However, no significant difference between the TF of sunflower and marigold (p > 0.05) and also no significant difference between TF of marigold and cosmos (p > 0.05) were observed. Moreover, there were significant difference for TFs of sunflower and cosmos (p < 0.05). TFs of marigold, cosmos and sunflower were greater than one, indicating the ability of the plants to translocate Cd from roots to shoots [9]. Thus, marigold showed the potential to be a Cd hyperaccumulator (Cd in shoots is greater than 100 mg kg^{-1}) based on the criteria proposed by [9]. In addition, at Cd treatment of 100 mg kg^{-1}, there was no significant difference between the TFs of cosmos and marigold (1.26 ± 0.31 and 0.97 ± 0.23), (p > 0.05). At highest concentration (400 mg kg^{-1}), there were no significant differences in TF of cosmos, sunflower and marigold. Higher TF values in the plants are crucial for phytoextraction of heavy metals from contaminated soil because it enables phytoremediation by harvesting only the above ground parts of the plants.

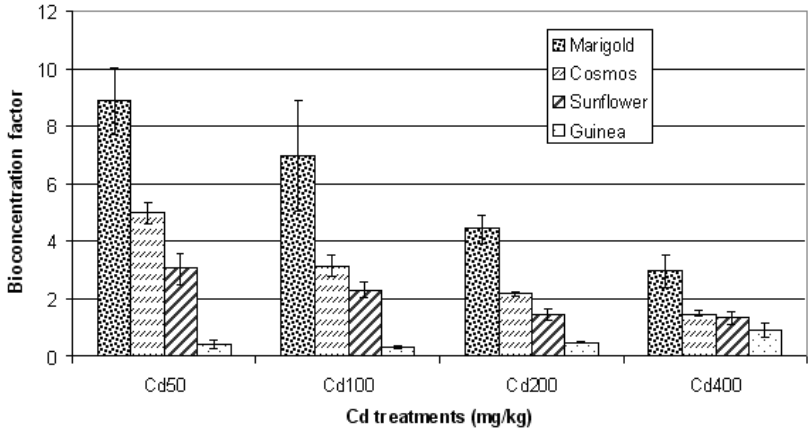

Fig. 5. Bioconcentration factors (BCFs) of harvested plants under various Cd treatments. All data are mean ± S.D. (n = 3). One way ANOVA (one factor: different Cd treatments) was performed for translocation factor.

The maximum bioconcentration factor (BCF) was obtained for marigold followed by cosmos, sunflower and Guinea grass for all Cd treatments, as can be seen from Fig. 5.

The BCF for marigold was significantly different ($p < 0.05$) from other species. The maximum BCFs of 8.86 ± 1.17, 6.96 ± 1.93, 4.41 ± 0.49 and 2.96 ± 0.57 were obtained for marigold under Cd treatments of 50, 100, 200 and 400 mg kg^{-1}, respectively. At Cd treatment of 100 mg kg^{-1}, the BCF of marigold was higher than those of other species, and also showed significant differences from other species ($p < 0.05$). However, there were no statistical differences for cosmos and sunflower ($p > 0.05$). From the result obtained, it is noticed that under all Cd treatments, the BCF of marigold was greater than one, indicating that more cadmium is accumulated in the plants as compared to that in the soil.

From the results obtained, based on Cd accumulation in above ground parts, marigold showed greater ability to accumulate more Cd in shoots (under all Cd treatments) as compared to other species. Regarding the TF values, although TF of sunflower (at Cd level of 50 mg kg^{-1}) was higher than that of marigold and cosmos, there were no significant differences ($p > 0.05$) among the TFs of marigold and sunflower as well as the TFs of marigold and cosmos.

Moreover, according to BCF values, under all Cd treatments, marigold possessed a higher BCF value as compared to other species studied. Although, TF and BCF values of Guinea grass were lowest as compared to other species, total uptake of Cd from contaminated soil is higher due to higher biomass production. As a result, marigold and Guinea grass have potential as alternative plant species for phytoremediation of Cd contaminated soil, and further investigations need to be carried out for these two species. Marigold is an ornamental plant. *Tagetes erecta* L. flowers are rich sources of pigments, mainly carotenoid and flavonoid, which can be used as active ingredients in textile coloration. Jothi [30] and Vankar et al. [31] reported that marigold flower had been shown to have good dyeing prospects. However, the potential use of marigold as a natural textile colorant on an industrial scale needs to be further investigated. Harvested plants can be incinerated in incineration plants. With modern flue gas cleaning technologies, metal-containing dust can be captured. For final disposal, ashes and dusts produced from incineration can be solidified and disposed on secured landfill site. Incineration can reduce more than 90% of contaminated dry biomass [32]. In the case the biomass produced is high enough, heat produced from the incineration process can be used for power generation and also other purposes.

4. Conclusions

Based on the total cadmium accumulation in whole plants and shoots, marigold showed higher potential. However, due to higher biomass and numerous roots, the total uptake of Cd from the soil by Guinea grass could be maximized. Cadmium accumulation in marigold shoots was > 100 mg kg^{-1}, TF > 1 (Cd treatment of 50 mg kg^{-1}) and BCF was found to be > 1 for all Cd treatments. The study shows that T*agetes erecta* L. has the basic properties of a hyperaccumulator and possesses a great potential to be raised as an alternative flower crop for phytoremediation. Although this is a slow process to clean the heavy metal contaminated soil, it has several advantages. This technology is inexpensive, environmental friendly reducing soil erosion, enriching soil organic matter leading to enhance soil fertility and also help in decreasing the Cd contamination of surface and ground water. It also helps in removing the CO_2 from the air during the photosynthesis process.

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