

Full Length Research Paper

## Evaluation of cadmium bioaccumulation and translocation by *Hopea odorata* grown in a contaminated soil

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Cadmium (Cd) contamination has an adverse effect on soil productivity and crop production. Phytoremediation is a long term and environmental friendly technology to remediate Cadmium polluted areas. This study was conducted to evaluate the potential of *Hopea odorata* for remediation of soils contaminated with Cd. Plant seedlings were planted in a clayey soil spiked contaminated with Cd in the amount of 0, 25, 50, 75, 100 and 150 mg kg<sup>-1</sup> named as; Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub> for a period of five months. The highest growth performance was recorded in the control (Cd<sub>0</sub>). Cd concentrations among plant parts were in the following trend: roots>stems>leaves. In order to evaluate the potential of species selected as phytoremediator, three indicators were used namely, bioconcentration factor (BCF, the metal concentration ratio of plant roots to soil), translocation factor (TF, the metal concentration ratio of plant shoots to roots) and removal efficiency (RE, total concentrations of metal and dry biomass of plants to total loaded metal in growth media). The highest total Cd concentration (290.23 ± 13.38 mg kg<sup>-1</sup>) and Cd removal efficiency (0.81 ± 0.06%) were found in Cd<sub>5</sub> and Cd<sub>1</sub>, respectively. Cd<sub>2</sub> exhibited the maximum total dry biomass (60.88 ± 1.78 g). *H. odorata* showed high BCFs (>1) and low TFs (<1). It can be concluded that this species is suitable to be used in phytoremediation of Cd-contaminated. For further confirmation, an evaluation under field condition will be needed.

**Key words:** Phytoremediation, *Hopea odorata*, heavy metals, soil pollution, removal efficiency

### INTRODUCTION

Contamination of soils with heavy metals has an adverse effect on soil fertility and crop production (Alkorta and Garbisu, 2001; Odoemelam and Ukpé, 2008). Agricultural and industrial activities are the sources of heavy metals by which Cd release into the environment and leave toxic effects (Nabulo et al., 2006; He et al., 2008). Cadmium is

a non-essential element and due to its high mobility and solubility in biological systems, it is known as one of the most hazardous element (Pinto et al., 2004; Dickinson and Pulford, 2005). It is listed as one of 126 priority pollutants (Nordberg, 2009). The widespread release of cadmium has reached 22,000 t (metric ton) over the past five decades (Jadia and Fulekar, 2009). The normal concentration of Cd in soil ranges from 0.01 to 2.0 mg kg<sup>-1</sup>; however, in urban and agricultural soils, Cd levels exceed the thresholds set in guidelines (Alloway, 1995; Sahibin

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et al., 2002). Conventional method for extracting heavy metals from soils such as *ex situ* excavation, landfill of the top contaminated soil, soil flushing and physico-chemical remediation are expensive, time consuming and labor exhaustive (Manousaki et al., 2008; Danh et al., 2009; Liu et al., 2010). Therefore, these remediation techniques are not technically and financially suitable for large contaminated areas (Soleimani et al., 2010). In contrast, phytoremediation is a low cost, longer lasting, environmental friendly new promising technology. It is applied to immobilize, degrade, remove, or detoxify contaminants including metals, pesticides, hydrocarbons, and chlorinated solvents (Zhang et al., 2010). Many studies have been done on phytoremediation of contaminated soils using weeds and leafy wild vegetables and ornamental plants, but information is lacking regarding the potential of tropical plant species to remediate Cd-contaminated soils. The suggested ideal plant for successful phytoremediation should have rapid growth rate, high biomass, an excessive root system, accumulate high concentration of heavy metals and high tolerance when expose to high concentrations of heavy metals (Garbisu and Alkorta, 2001). Use the woody plant species that grow locally near the site is feasible for this process. These species are less competitive under local conditions and will reduce the metal concentration to an acceptable level for normal plant growth (Rajakaruna et al., 2006). One of potential woody plant species is *H. odorata* which is a fast growing species, and can grow up to 45 m of height (Orwa et al., 2009). The objective of this study was to assess the growth performance and the phytoremediation potential of *H. odorata* to absorb Cd in Cd-contaminated soil.

## MATERIALS AND METHODS

This study was conducted at the greenhouse of Faculty of Forestry, Universiti Putra Malaysia (20°59'18.24" N latitude and 101°42'45.45" E longitude). The average temperature in the greenhouse was 27, 36 and 32°C in the morning, afternoon and evening, respectively. Relative humidity was 65%. The period of study was five months from February to June 2010. Healthy seedlings of the same age and similar form were selected for every species collected from Malaysia Agriculture Research Institute (MARDI), Serdang, and Selangor. A clayey soil (munchong series) which belongs to Ultisols was used in this experiment. The soil was air-dried until it could be crushed to pass through a 4mm-sieve for soil growing media. Stainless sieve was used to supply a homogenous soil composite as a growing media. Seedlings were transplanted into proper plastic pots (32.0 cm height, 106.0 cm upper diameter and 69.0 lower diameters) gently without damaging the root system. A completely randomized design (CRD) was followed with six treatments replicated four times. The growth media was prepared using soil thoroughly mixed with different levels of Cd including: Cd<sub>1</sub> (soil; 25 ppm Cd), Cd<sub>2</sub> (soil; 50 ppm Cd), Cd<sub>3</sub> (soil; 75 ppm Cd), Cd<sub>4</sub> (soil; 100 ppm Cd) and Cd<sub>5</sub> (soil; 150 ppm Cd) and control (Cd<sub>0</sub> =100% soil). To provide different concentrations of Cd, cadmium chloride hydrate (CdCl<sub>2</sub> · 2.5H<sub>2</sub>O) was applied. A total of 24 seedlings were used in this experiment. The basal diameter, height

and number of leaves were measured every month. Soil samples were air dried until it could be crushed to pass through a 2 mm-sieve for analysis of physico-chemical properties in the laboratory. Soil texture was determined using the pipette gravimetric method (Tan, 2005). The pH of the soil was measured in the suspension of a 1:2.5 soil: liquid mixture. The CEC and exchangeable cations (Ca, Mg, K) were determined by leaching method using 1 M ammonium acetate at pH 7. Exchangeable Al and H determined by the NaOH titration method. Available P (ppm) was extracted using Bray II with a mixture including 0.03M ammonium fluoride (NH<sub>4</sub>F) and 0.1 M hydrochloric acid (HCl). Plants were harvested after five months for determination of plant growth, biomass and heavy metal analysis. *Aqua regia* method was used as described by Ahmadpouret al. (2010). Cd concentration in plant parts and soil samples was determined using atomic absorption spectrometry (AAS). Total C and N were determined by dry combustion using CNS 2000 analyzer. Three indicators were used to determine the potential of four plant species for phytoremediation of Cd-contaminated soil including translocation factor (TF), bioconcentration factor (BCF) and removal efficiency (RE).

$$BCF = \left[ \frac{\text{Metal Concentration in Roots (mg kg}^{-1}\text{)}}{\text{Metal Concentration in Soil (mg kg}^{-1}\text{)}} \right]$$

$$TF = \left[ \frac{\text{Metal Concentration in Shoot (mg kg}^{-1}\text{)}}{\text{Metal Concentration in Root (mg kg}^{-1}\text{)}} \right]$$

$$RE (\%) = \left[ \frac{\text{Metal in Shoot (mg kg}^{-1}\text{)} \cdot \text{Shoot biomass (kg)} + \text{Metal in Root (mg kg}^{-1}\text{)} \cdot \text{Root biomass}}{\text{Total added Metal per pot (mg)}} \right] \times 100$$

Analysis of variance (one way ANOVA) for growth, heavy metals in soils and plant parts were implemented. Duncan Multiple Range Test (DMRT) was employed to detect any significant differences ( $p \leq 0.05$ ) among and between the treatments of growth media, growth parameters and biomass. Correlation analysis was also performed to relate total Cd concentrations in growth media with dry biomass production and total Cd concentration in plant species. All data obtained in terms of growth, biomass and heavy metals in soil and plants were analyzed using the SAS (Define SAS) program (Release 9.2).

## RESULTS AND DISCUSSION

### Physico-chemical properties of the control media

The physico-chemical properties of the control media are shown in Table 1. The soil used in this study was sandy clay of Munchong series with content, 57.88 ± 1.97% sand, 5.25 ± 0.42% silt and 36.87 ± 1.86% clay. Total N, C, P and K were 0.03 ± 0.03, 0.74 ± 0.05, 0.03 ± 0.002 and 0.1 ± 0.003%, respectively. The soil was acidic with pH 4.62 ± 0.16. This media contain 9.17 ± 1.12 mg kg<sup>-1</sup> available phosphorous with 14.03 ± 1.77 cmol<sub>c</sub>kg<sup>-1</sup> CEC and 0.3 dS m<sup>-1</sup> EC. The concentration of exchangeable cations for K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> were 0.005 ± 0.001, 0.004 ± 0.001 and 0.046 ± 0.005 cmol<sub>c</sub>kg<sup>-1</sup>, respectively. The values of exchangeable Al and H were 0.75 ± 0.13 and

**Table 1.** Selected physico-chemical properties of the control soil.

Soil Property	Value
Texture	Sandy clay
Sand (%)	57.88
Silt (%)	5.25
Clay (%)	36.87
Field capacity (%)	28.92
Total N (%)	0.03
Total C (%)	0.74
Total P (%)	0.03
Total K (%)	0.1
pH (1:2.5 soil to water)	4.62
Available P (mg kg <sup>-1</sup> )	9.17
CEC cmol <sub>c</sub> kg <sup>-1</sup>	14.03
EC dS m <sup>-1</sup>	0.3
<b>Exchangeable cations (cmol<sub>c</sub>kg<sup>-1</sup>)</b>	
K cmol <sub>c</sub> kg <sup>-1</sup>	0.005
Mg cmol <sub>c</sub> kg <sup>-1</sup>	0.004
Ca cmol <sub>c</sub> kg <sup>-1</sup>	0.046
Al cmol <sub>c</sub> kg <sup>-1</sup>	0.75
H cmol <sub>c</sub> kg <sup>-1</sup>	0.13
<b>Total heavy metal (mg kg<sup>-1</sup>)</b>	
Cd	2.6
Cu	9.93
Zn	46.75
Fe	479.4
Mn	30.6

0.13 ± 0.06 cmol<sub>c</sub>kg<sup>-1</sup>, respectively. High concentrations of exchangeable Al and H were the main source of soil acidity. Judging the content of total C and N, CEC, and exchangeable bases, the plant nutrient status in this growth media was relatively low due to the acidic nature of the soil. The concentrations of Cd, Cu, Zn, Fe and Mn were 2.6 ± 0.21, 9.93 ± 0.31, 46.75 ± 4.55, 479.4 ± 22.87 and 30.6 ± 3 mg kg<sup>-1</sup>, respectively.

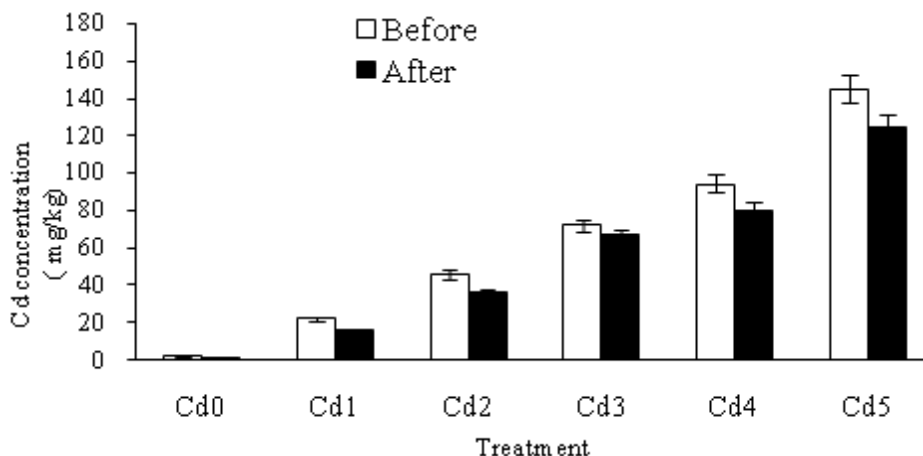
#### Cadmium concentrations in the growth media before planting and after harvest

Cadmium concentration in the growth media before planting ranged from 2.13 to 145.25 mg kg<sup>-1</sup> having the highest concentration (145.25 ± 5.36) in Cd<sub>5</sub> as compared to other treatment levels and the lowest (2.13 ± 0.22) was recorded in the control (Cd<sub>0</sub>). There was a significant difference ( $p \leq 0.05$ ) among treatments in Cd concentration in the growth media before planting (Figure 1). Cd concentration in the growth media after planting was also varied under different Cd added to the growth

media. It ranged from 1.47 to 124.92 mg kg<sup>-1</sup> having the highest (124.92 ± 5.17 mg kg<sup>-1</sup>) in Cd<sub>5</sub> and the lowest (1.47 ± 0.09 mg kg<sup>-1</sup>) in control media. Cd concentration in the growth media decreased at harvest as compared to Cd concentration in the growth media before planting having the highest reduction (31.06%) under control media and the lowest (7.13%) in Cd<sub>3</sub>. Generally, the Cd concentration in both media before and after planting, increased with increase in the Cd concentration applied to the growth media (Figure 1). The concentration of Cd in normal soil ranged from 0.01 to 2.0 mg kg<sup>-1</sup> (Alloway, 1995). However, Kabata-Pendias and Pendias (1984) reported that the critical Cd level in soil is between 3 to 5 mg kg<sup>-1</sup>. The growth performance of *Hopea odorata* in terms of basal stem diameter, height and number of leaves under various Cd concentrations are shown in (Figure 2 a, b, c). Significant difference ( $p \leq 0.05$ ) was observed among different Cd concentrations in basal stem diameter, plant height and number of leaves at harvest. The highest basal stem diameter (11.53 ± 0.22 mm) was recorded in control media followed by Cd<sub>2</sub> (10.05 ± 0.17 mm) and Cd<sub>1</sub> (9.67 ± 0.13 mm) as compared to other Cd concentrations and the lowest basal stem diameter (8.45 ± 0.29 mm) was found in Cd<sub>4</sub>. However, there was no significant difference ( $p \leq 0.05$ ) between Cd<sub>4</sub> and Cd<sub>5</sub> in basal stem diameter. Control media exhibited the maximum height (91.25 ± 1.44 cm) followed by Cd<sub>2</sub> (83.18 ± 1.56 cm) and Cd<sub>1</sub> (80.75 ± 1.38 cm) while the lowest (62.9 ± 0.79 cm) was recorded in Cd<sub>5</sub>. The number of leaves ranged from 40 to 77 with the highest (77 ± 1.55) in control media followed by Cd<sub>2</sub> (69 ± 3.52) and Cd<sub>1</sub> (65 ± 4.5) while the lowest (40 ± 0.95) was observed in Cd<sub>5</sub>. The basal stem diameter and number of leaves increased from 9.67 to 10.05 mm and 65 to 69 when soil treated with Cd<sub>1</sub> and Cd<sub>2</sub>, respectively. However, a reduction was observed at higher Cd concentrations. Generally, the higher concentration of Cd reduced the growth parameters while an increase was observed within each Cd levels during the growth period. As described for the previous mentioned species, the growth of *H. odorata* was also reduced with increasing Cd concentration added to the growth media. These results reveal that *H. odorata* may tolerate soils contaminated with Cd since the growth parameters increased every month indicating the normal growth, but higher levels of this non-essential element have an adverse effect on growth parameters. This result was in line with the findings of a study by Wu et al. (2009). Similar result was also obtained by Unterbrunner et al. (2007).

#### Dry biomass of leaves, stems and roots

Leaves, stems and roots dry biomass are presented in Table 2. Leaves dry biomass was significantly different ( $p \leq 0.05$ ) among Cd concentrations. The dry biomass of leaves increased from control media (20.88 ± 0.28 g) up



**Figure 1.** Change in cadmium concentrations in the growth media after cultivation of *H. odorata* as influenced by different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Means ± standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).

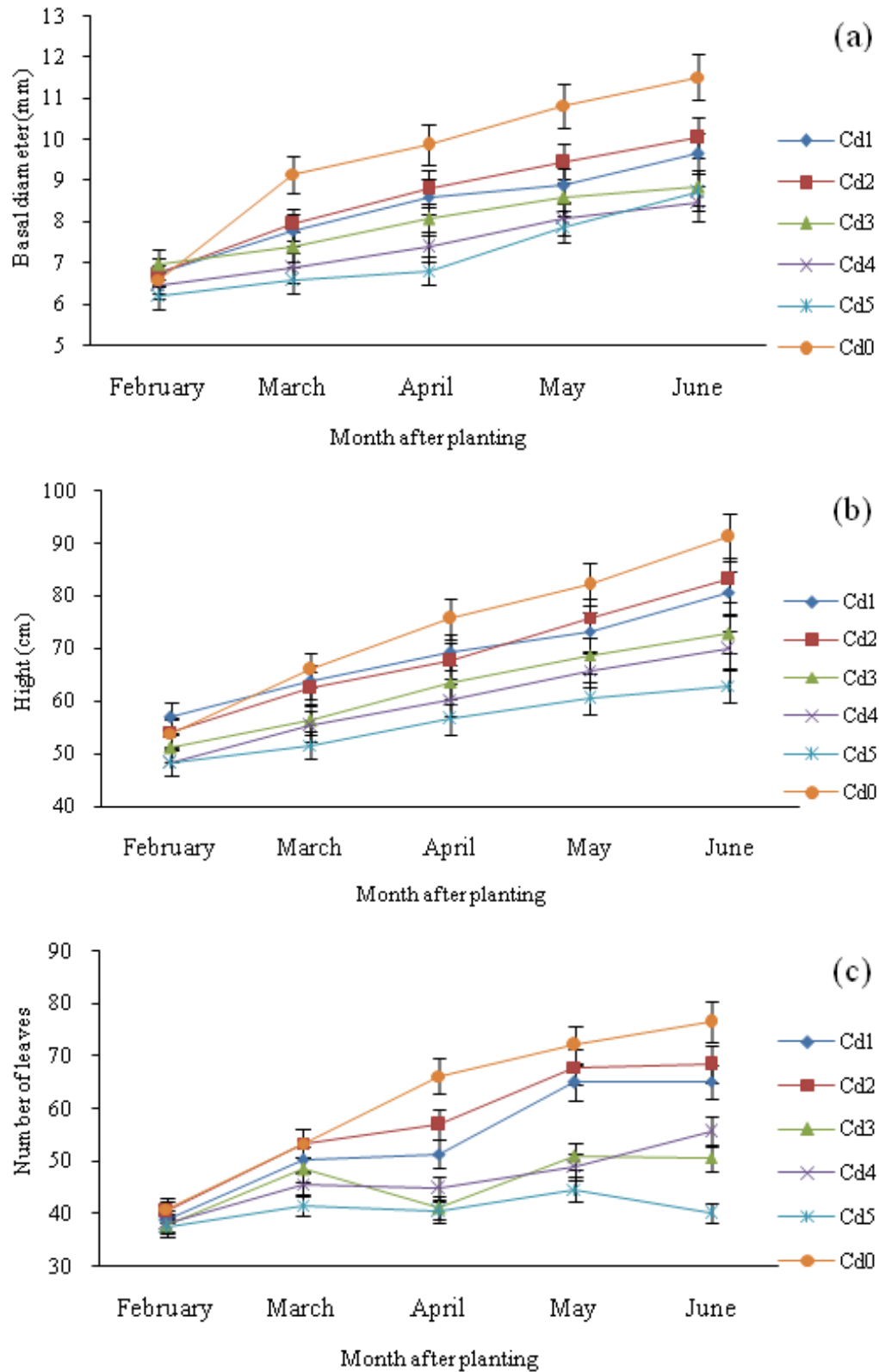
to Cd<sub>2</sub> (23.8 ± 1.03 g). However, the leaves dry biomass decreased at higher Cd concentrations added to the growth media where the lowest dry biomass (17.68 ± 0.93 g) was found in plants treated with Cd<sub>5</sub>. The effect of Cd concentrations on stem dry biomass was not steady. There was a significant difference ( $p \leq 0.05$ ) among Cd concentration in stems dry biomass. The highest stem dry biomass (22.03 ± 0.59 g) was observed in seedlings grown in the growth media treated with Cd<sub>2</sub> compared to the lowest stem dry biomass (16.35 ± 0.46 g) in seedlings treated with Cd<sub>5</sub>.

As shown in Table 2, the roots dry biomass was significantly different ( $p \leq 0.05$ ) among Cd concentrations. The dry biomass of root showed the similar trend with the stem dry biomass. The roots dry biomass increased from control media (13.48 ± 0.27 g) up to Cd<sub>2</sub> (15.05 ± 0.61 g). However, there was no significant difference ( $p \leq 0.05$ ) among seedlings grown in control media with plants treated with Cd<sub>1</sub> and Cd<sub>2</sub>. The roots dry biomass then decreased with increasing the Cd applied to the growth media where the lowest dry biomass (11.1 ± 0.21 g) was found in plants grown in the growth media treated with Cd<sub>5</sub>. The dry biomass among different plant parts was in the following order: leaves > stems > roots. It was observed that the dry biomass of leaves, stems and roots decreased at higher cadmium concentration in the growth media. This probably occurred due to the adverse effect of Cd on cell expansion or division, and may be via its influence on DNA, RNA or protein metabolism (Auda and Ali, 2010). Jadia and Fulekar (2008b) reported same result on alfalfa where the biomass of this species decreases the Cd concentration increased to (40 to 50 mg kg<sup>-1</sup>). However, the result obtained by Liu et al. (2010) on 40 cabbage cultivars showed that the biomass of Liaodaquikang, Suancaiawang and Beijingxiaozha 56

increased under Cd concentrations (1.0, 2.5 and 5.0 mg kg<sup>-1</sup>) indicating the high tolerance of these cultivars to Cd toxicity. Anget al.(2010) described that one of the way for Cd translocation in plant is detoxification mechanism of Cd from xylem and sequestration of this metal into plant tissue. Cd can be assimilated in the stem via phytochelatins PCs-complex (phytochelatin-cadmium) resulting in the reduction of the toxicity of Cd trapped into vacuole. However, Kuzovkina et al. (2004) described that Cd as a non-essential element is known as a strong phytotoxic elements by interfering with enzymes activates, restricting the DNA-mediated transformation in microorganisms as well as to inhibit in symbiosis between microbes and plants.

#### Plant total dry biomass in response to cadmium treatments

Total dry biomass was varied under different Cd concentration in the growth media ranging from 45.13 to 60.88 g. A significant difference ( $p \leq 0.05$ ) was observed among soil Cd treatments in total dry biomass production. The highest total dry biomass (60.88 ± 1.78 g) was recorded in Cd<sub>2</sub> followed by Cd<sub>1</sub> (55 ± 0.85 g) as compared to Cd<sub>5</sub> which gave the lowest total dry biomass (45.13 ± 0.51 g) (Figure 3). The production of total dry biomass decreased with increase in the Cd concentration in the growth media, indicating the adverse effect of Cd on dry biomass production specifically at higher Cd concentrations (Chiang et al., 2006). Heavy metals such as Cd can create indirect toxicity by replacing essential elements at cation exchange areas in plant species (Jadia and Fulekar, 2009; Taiz and Zeiger, 2002). Cd in plants also can inhibit the transportation of Ca<sup>2+</sup> and K<sup>+</sup> and abscisic acid in

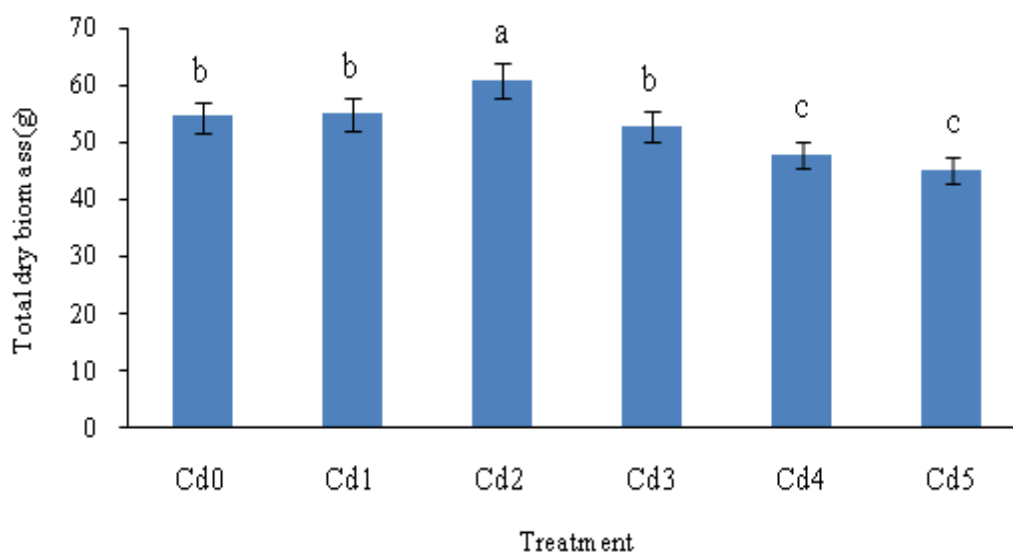


**Figure 2.** Plant basal diameter (a), height (b) and number of leaves (c) of *H. odorata* at different months after planting as influenced by different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Means ± standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).

**Table 2.** Leaves, stems and roots dry biomass (g) of *H. odorata* after 5 months growth at different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>).

Treatment	Plant part			Total
	Leaf	Stem	Root	
Cd <sub>0</sub>	20.88±0.28 <sup>b</sup>	20.18±0.05 <sup>ab</sup>	13.48±0.27 <sup>ab</sup>	80.61
Cd <sub>1</sub>	21.15±0.36 <sup>b</sup>	20.28±0.2 <sup>ab</sup>	13.58±0.68 <sup>ab</sup>	55.01
Cd <sub>2</sub>	23.8±1.03 <sup>a</sup>	22.03±0.59 <sup>a</sup>	15.05±0.61 <sup>a</sup>	60.88
Cd <sub>3</sub>	20.33±1.14 <sup>b</sup>	19.28±1.58 <sup>bc</sup>	13.13±0.45 <sup>bc</sup>	52.74
Cd <sub>4</sub>	19.08±0.82 <sup>bc</sup>	17.23±0.72 <sup>cd</sup>	11.6±0.97 <sup>cd</sup>	47.91
Cd <sub>5</sub>	17.68±0.93 <sup>b</sup>	16.35±0.46 <sup>d</sup>	11.1±0.21 <sup>d</sup>	45.13

Different letters within a column represent significant difference among means at a 5% level following Duncan Multiple Range Test ( $p \leq 0.05$ ). Data with  $\pm$  is mean standard error (S.E.).



**Figure 3.** Total dry biomass of *H. odorata* as influenced by different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Different letters indicate significant difference among means at a 5% level following Duncan multiple range test ( $p \leq 0.05$ ). Means  $\pm$  standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).

guard cell (Jadia and Fulekar, 2008a).

#### Relationship between total cadmium in growth media and plant dry biomass

Cd concentration in the growth media and total biomass of *H. odorata* was significantly ( $p \leq 0.01$ ) related to each other ( $r = -0.70$ ). The negative correlation between these two parameters revealed that the total dry weight of species decreased with increase in the total Cd concentration in the growth media. Cd inhibits the biosynthesis of chlorophyll and the alleviation of chlorophyll content could result in reduction of shoot biomass (Orcutt and Nilsen, 2000). Metals cannot degrade inside the plant cells and can create toxicity when they accumulate above

the threshold by destroying the cell structure and interfering with some of cytoplasmic enzymes (Assche and Clijsters, 1990).

#### Cadmium concentration in various plant parts (leaves, stems and roots)

The Cd concentration in different plant parts are shown in Table 3. The concentration of Cd in leaves under various Cd concentrations applied to the growth media was significantly different ( $p \leq 0.05$ ). The Cd concentration in leaves showed inconsistent trend under different Cd added to the growth media. The highest Cd concentration in leaves ( $10.31 \pm 0.64$  mg kg<sup>-1</sup>) was recorded in leaves of seedlings grown in the growth media treated with Cd<sub>3</sub>

**Table 3.** Cd concentrations ( $\text{mg kg}^{-1}$ ) in various parts of *H. odorata* in different treatments including 0, 25, 50, 75, 100 and 150  $\text{mg Cd kg}^{-1}$  ( $\text{Cd}_0$ ,  $\text{Cd}_1$ ,  $\text{Cd}_2$ ,  $\text{Cd}_3$ ,  $\text{Cd}_4$  and  $\text{Cd}_5$ ).

Treatment	Plant part			Total
	Leaf	Stem	Root	
$\text{Cd}_0$	$1.51 \pm 0.13^c$	$1.30 \pm 0.08^e$	$1.71 \pm 0.06^c$	4.52
$\text{Cd}_1$	$1.65 \pm 0.06^c$	$12.49 \pm 0.62^d$	$110.15 \pm 7.84^b$	124.29
$\text{Cd}_2$	$3.94 \pm 0.35^b$	$16 \pm 0.79^d$	$116.55 \pm 10.55^b$	136.49
$\text{Cd}_3$	$10.31 \pm 0.64^a$	$33.21 \pm 2.23^c$	$169.16 \pm 8.72^a$	212.68
$\text{Cd}_4$	$3.78 \pm 0.31^b$	$68.11 \pm 1.9^b$	$175.4 \pm 2.03^a$	247.29
$\text{Cd}_5$	$2.31 \pm 0.16^c$	$105.99 \pm 7.83^a$	$181.93 \pm 11.83^a$	290.23

Different letters within a column indicate significant difference among means at a 5% level following Duncan multiple range test ( $p \leq 0.05$ ). Data with  $\pm$  is mean standard error (S.E.).

as compared to the lowest concentration ( $1.51 \pm 0.13 \text{ mg kg}^{-1}$ ) which was observed in leaves of plants grown in control media. The Cd concentration in leaves was then significantly decreased starting from  $\text{Cd}_3$  to  $\text{Cd}_5$  indicating the toxicity effect of Cd at higher concentration. In the case of stem, Cd concentration increased significantly ( $p \leq 0.05$ ) from  $1.3 \text{ mg kg}^{-1}$  to  $105.99 \text{ mg kg}^{-1}$  in stems of seedlings grown in control media and  $\text{Cd}_5$ , respectively. However, there was no significant difference ( $p \leq 0.05$ ) between the Cd concentrations in the stem of seedlings grown in the growth media treated with  $\text{Cd}_1$  and  $\text{Cd}_2$ .

Based on the data presented in Table 3, there was significant difference ( $p \leq 0.05$ ) among different Cd concentrations applied to the growth media in the concentration of Cd in roots. The concentration of Cd in roots increased significantly with increasing the Cd concentration added to the growth media where the highest Cd concentration ( $181.93 \pm 11.83 \text{ mg kg}^{-1}$ ) was recorded in the roots of seedlings grown in the growth media contaminated with  $\text{Cd}_5$  compared to the lowest Cd concentration ( $1.71 \pm 0.06 \text{ mg kg}^{-1}$ ) in roots of seedlings grown in control media. Although, there was no significant difference ( $p \leq 0.05$ ) in Cd concentration among the roots of seedlings grown in the growth media treated with  $\text{Cd}_1$  and  $\text{Cd}_2$  and media treated with  $\text{Cd}_3$ ,  $\text{Cd}_4$  and  $\text{Cd}_5$ . The Cd concentration in various plant parts was in the following rank; roots > stems > leaves. The concentration of Cd in root increased with an increase of concentration of this metal in the growth media. Similar result was attained by Liu et al. (2006) on the accumulation of Cd by roots and shoots of maize (*Zea mays* L.) where the concentration of Cd in roots and shoot of cultivars of this species increased significantly with increasing Cd levels. The uptake and accumulation of heavy metals by plant depends on the plant genotype and can be affected by physical and chemical properties of soil, and bioavailability of heavy metals in soil (Shuhe et al., 2005). In contrast to the tested plant species in current study, there are some species such as lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* L.) and tobacco (*Nicotiana tabacum* L.) with ability to accumulate high concentrations of Cd

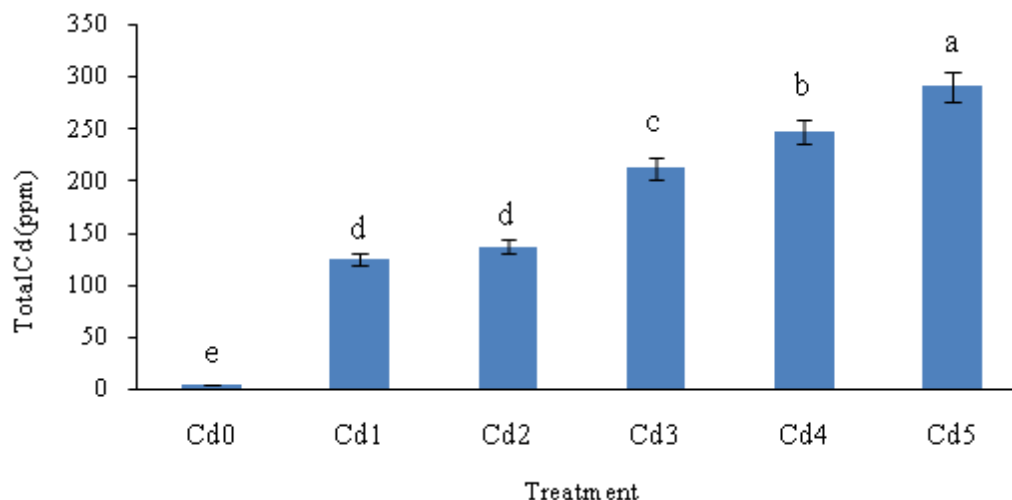
in leaves rather than roots (Jadia and Fulekar, 2008a).

### Total cadmium concentration in the plant

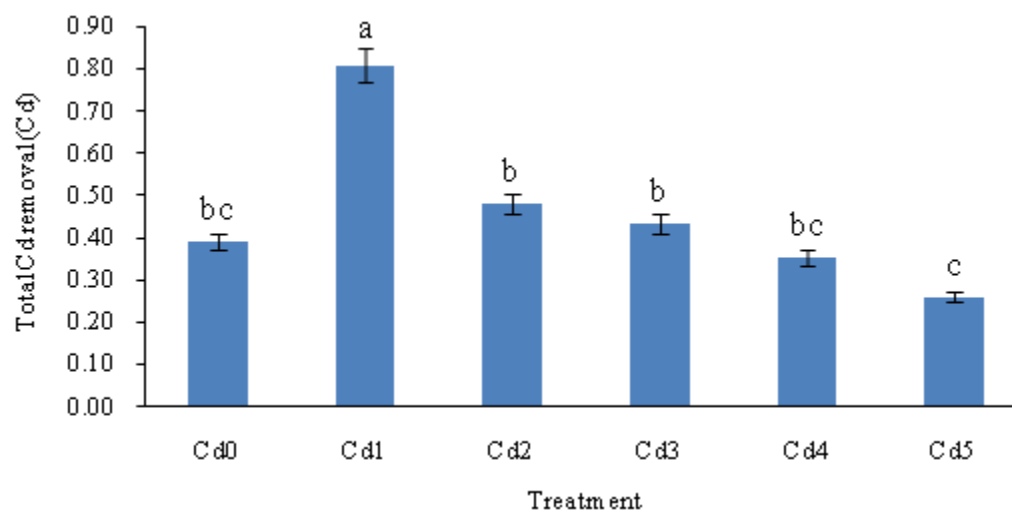
Total plant Cd concentration varied under different treatment levels. It ranged from 4.52 to 290.3  $\text{mg Cd kg}^{-1}$ . There was a significant difference ( $p \leq 0.05$ ) among treatments in total Cd concentrations. The maximum total Cd concentration ( $290.3 \pm 13.38 \text{ mg kg}^{-1}$ ) was found in  $\text{Cd}_5$  followed by  $\text{Cd}_4$  ( $247.28 \pm 2.87 \text{ mg kg}^{-1}$ ) while the minimum ( $4.52 \pm 0.15 \text{ mg kg}^{-1}$ ) recorded in control media (Figure 4). It was observed that the total Cd concentration in *H. odorata* increased with increasing of Cd concentration applied to the growth media increased (Wu et al., 2009). The plant ability to accumulate metals depends on heavy metals availability in soil and the metabolic patterns of plants (Liu et al., 2010). Iannelli et al. (2002) reported the similar result where *Phragmites australis* plants accumulated most of Cd in the roots than leaves when treated with high levels of  $\text{CdSO}_4$  ( $50 \mu\text{M}$ ) in hydroponic culture.

### Cadmium removal by total plant biomass

Removal efficiency based on plant biomass is defined as the total concentrations of metal and dry biomass of plants to total loaded metal in soil (Li et al., 2009). Cd removal was varied among different Cd concentration in soil. A significant difference ( $p \leq 0.05$ ) was observed among treatment levels in Cd removal. It was in the range of 0.26 to 1.17 %. The highest Cd removal ( $1.17 \pm 0.08 \%$ ) was noted in  $\text{Cd}_1$  followed by  $\text{Cd}_2$  ( $0.99 \pm 0.11 \%$ ) as compared to the control media which gave the lowest Cd removal ( $0.19 \pm 0.04 \%$ ) (Figure 5). The Cd removal decreased with increase in the Cd concentration added to the growth media which may be associated with the reduction of plant dry biomass at higher Cd concentration. As a strong phototoxic element to plants; Cd influences the plant growth and development reversely and ceases their life quickly due to its great



**Figure 4.** Total plant Cd concentration as influenced by different treatment levels including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Different letters indicate significant difference among means at a 5% level following Duncan multiple range test ( $p \leq 0.05$ ). Means  $\pm$  standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).



**Figure 5.** Total Cd removals as influenced by different treatment levels including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Different letters indicate significant difference among means at a 5% level following Duncan multiple range test ( $p \leq 0.05$ ). Means  $\pm$  standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).

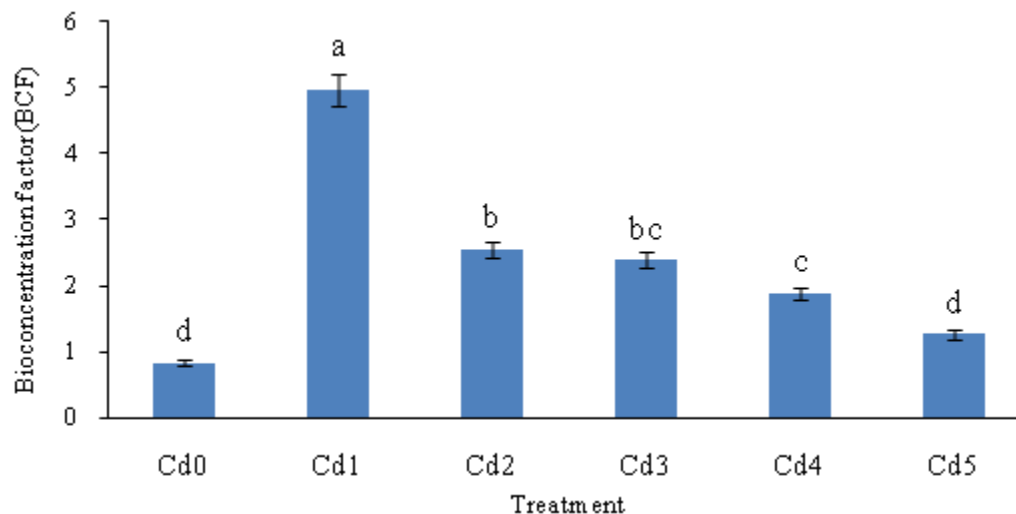
solubility and high toxicity (Das et al., 1997; Kuzovkina et al., 2004).

#### Bioconcentration and translocation factor of cadmium

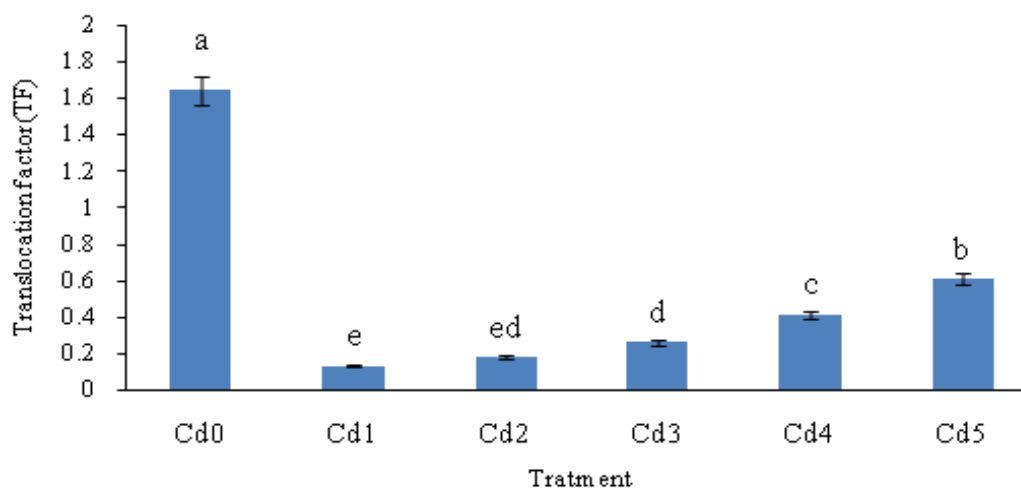
Bioconcentration factor (BCF) index is defined as the ratio of heavy metal concentration in plant roots to that in

soil (Malik et al., 2010) whereas translocation (TF) is defined as the ratio of heavy metal concentration in aerial parts of plant to that in roots (Karami and Shamsuddin, 2010). The BCFs were varied under different Cd concentrations in the soil and it was in the range of 0.83 to 4.96. There was a significant difference ( $p \leq 0.05$ ) among treatments in BCFs. The highest BCF (that is  $4.96 \pm 0.28$ ) of Cd was found in Cd<sub>1</sub> as compared to other treatment levels, while control media exhibited the lowest BCF





**Figure 6.** Bioconcentration factor as influenced by different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Different letters indicate significant difference among means at a 5% level following Duncan Multiple Range Test ( $p \leq 0.05$ ). Means  $\pm$  standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).



**Figure 7.** Translocation factor as influenced by different Cd concentrations including 0, 25, 50, 75, 100 and 150 mg Cd kg<sup>-1</sup> (Cd<sub>0</sub>, Cd<sub>1</sub>, Cd<sub>2</sub>, Cd<sub>3</sub>, Cd<sub>4</sub> and Cd<sub>5</sub>). Different letters indicate significant difference among means at a 5% level following Duncan Multiple Range Test ( $p \leq 0.05$ ). Means  $\pm$  standard errors (S.E.) are shown in error bars ( $p \leq 0.05$ ).

(0.83  $\pm$  0.08) (Figure 6). The BCFs were >1 under various treatment levels except in control media. The BCF value of Cd usually ranged from 1 to 10 (Li et al., 2006). The BCFs decreased with increase in the Cd concentration in the growth media, which may indicate the restriction in soil-root transfer at higher Cd concentrations in the soil (Justin et al., 2011). Ho et al. (2008) found that the BCFs of Pb in kenaf (*Hibiscus cannabinus* L.) were >1 (1.92 to 3.21) when grown in sand tailings.

TFs were also varied under different Cd concentrations

in the growth media and a significant difference ( $p \leq 0.05$ ) observed among treatments in TFs. This index was in the range of 0.13 to 1.64. Control media showed the highest TF (1.64  $\pm$  0.05) as compared to the other treatment levels while Cd<sub>1</sub> exhibited the lowest TF (0.13  $\pm$  0.01) (Figure 7). It was observed that TFs increased with increase in the applied Cd concentration in the growth media. However, the TFs under different Cd levels were <1 except in control media indicating that *H. odorata* was unable to translocate Cd from the roots to the shoots

efficiently. Baker (1981) reported that plants are classified into accumulator if heavy metal concentration ratio (shoot to root) is more than one and excluder if this ratio is less than one. A pot experiment showed that the translocation factor in five cultivars of cabbage (New Beijing 3, Saixin 5, Fengyuanxin 3, Shuishiyang 91-12 and Liadaqiukang) was  $<1$  under various concentrations of Cd (1.0, 2.5 and 5.0 mg kg<sup>-1</sup>) (Liu et al., 2010). The translocation of Cd is often restricted due to the ability of this element to create Cd-phytochelatin complex by sequestration in the vacuole (Lux et al., 2011). Cd movement from root to shoots probably occurs within the xylem. The levels of free Cd in the symplast can be influenced highly by cellular sequestration of Cd and, therefore, it can affect the movement of Cd throughout the plants (Niu et al., 2007).

### Relationship between total cadmium in the growth media and plant species

Correlation analysis between total Cd concentration in the growth media and in *H. odorata* was significantly different ( $p \leq 0.01$ ). The total Cd concentration in the growth media was significantly related to total Cd concentration in *H. odorata* ( $r = 0.92$ ). This positive correlation indicated that total concentration of Cd in *H. odorata* increased with an increase in total concentration of this metal in the growth media. This result was in line with the result obtained by Wu et al. (2009) on poplar where Cd accumulation increased with increase in the Cd concentration in growth media.

### Conclusion

*H. odorata* planted in control media showed the highest production of basal diameter, plant height and number of leaves. The highest total dry biomass production was recorded in Cd<sub>2</sub> (60.88 ± 1.78 g). The maximum total Cd concentration (290.23 ± 13.38 mg kg<sup>-1</sup>) and total Cd removal based on total dry biomass (0.81 ± 0.06%) were found in Cd<sub>5</sub> and Cd<sub>1</sub>, respectively. Cd was highly concentrated in roots. *H. odorata* exhibited the high BCF and low TF. The BCFs were  $>1$  except in control media, whereas TFs were  $<1$  except in control media. The maximum BCF (4.96) of Cd was found in Cd<sub>1</sub> while the highest TF (1.64) was recorded in control media. Therefore, *H. odorata* can be used as an excluder based on the suggestion of Baker (1981) and remediate Cd-contaminated soil in phytoremediation through phytostabilization method to prevent distribution of Cd in contaminated areas.

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