

## Accumulation and distribution of heavy metal cadmium in sweet sorghum

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### ABSTRACT

Many species of plants have been studied, as well as applied for cleansing the environment. Previous research has concluded that sorghum plants are highly tolerant to metal pollution and capable of reaching high biomass values in the presence of metals. However, the distribution of heavy metals in plant's parts has not been adequately studied. In this study, two varieties of sweet sorghum (Keller and E-Tian) were grown with 5 levels (0, 5, 10, 25 and 50 ppm) of cadmium (Cd) in order to investigate the accumulation of Cd in plant parts at the hard dough stage. The results clearly showed the absence of Cd in the seeds of the above plants. There was the presence of Cd at the second and fifth leaf when the level of Cd reached 25 - 50 ppm. There was a great correlation coefficient between Cd and the position of the internodes, namely 0.86, 0.96, 0.99, 0.98 with KE, and 0.86, 0.92, 0.94, 0.94 with ET at 5, 10, 25 and 50 ppm Cd ( $P < 0.01$ ), respectively. The greater the internodes, the lower the accumulation of Cd. The aforementioned plants recorded the high accumulation of Cd in their roots, peaking at 23.27  $\mu\text{g/g}$  (dried weight, dw) in Keller and 21.69  $\mu\text{g/g}$  in E-Tian. Based on these results, it is concluded that the distribution of Cd in the studied sweet sorghum can be arranged in the following order: > stem > old leaves > young leaves.

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### 1. Introduction

Heavy metal contamination in soil has become a public concern due to industrial development and human activities, such as mining and smelting of metalliferous ores, electroplating, fertilizer and pesticide application, and fuel production (Garbisu & Alkorta, 2003). Excessive heavy metals, for example, cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), zinc (Zn), and nickel (Ni), in agricultural areas seriously threaten food safety and public health (Järup, 2003). Cadmium (Cd) has been placed at seventh rank among the top toxins, although Cd is a non-essential element for crop plants, it is easily taken up by plants growing on Cd-supplemented or Cd-contaminated soils, entering the food chain and causing damage to plant and human health (Ra-

hat et al., 2012). Elimination or remediation of heavy metal contamination in soil is urgently needed to prevent humans and animals from toxicity.

Sorghum (*Sorghum bicolor* L.) is a pro-poor multipurpose crop providing food, feed, fiber, and fuel across a range of agro-ecosystems (Zheng et al., 2011). Sweet sorghum consists of natural variant cultivars of sorghum with abundant sucrose storage in culm and great biomass and is thereby considered an ideal feedstock for biofuel production (Kokyo et al., 2015). Sweet sorghum will be a competitive candidate species for soil remediation due to its great biomass and strong resistance to adverse environmental conditions.

To preliminarily evaluate its potential for phytoremediation, several morphological and physiological characteristics of sorghum were investi-

gated under heavy metal stresses (Cd, Pb, Zn, Cu) in previous studies (Zhuang et al., 2009; Liu et al., 2011; Soudek et al., 2013). There were several pieces of research which focus on the improvement ability of absorption heavy metal from the contaminated soil (Zhuang et al., 2009; Soudek et al., 2014; Ziarati et al., 2015). The aim of this study was to determine the absorption and distribution of Cd in sweet sorghum plant organs and its distribution in different organs of sweet sorghum.

## 2. Materials and Methods

### 2.1. Plant material and experimental design

The elite line of sweet sorghum Keller (KE) and E-Tian (ET) were chosen as plant materials. Keller (GRIN access code PI 653617) is an elite sweet sorghum line developed by DM Broadhead at US Sugar Crops Field Station at Meridan, Mississippi in 1982. E-Tian (literally meaning Russian Sweet in Chinese) was introduced into China in the early 1970s and known for having high Brix content in its stem (Zheng et al., 2011).

Soil was amended with CdCl<sub>2</sub> at final concentrations of 0, 5, 10, 25, 50 mg/kg. The group not treated with CdCl<sub>2</sub> was the control group. The soil was fertilized with base fertilizers (urea, diammonium phosphate, and potassium sulfate), following the technical process for high-yield land application.

Seeds were soaked in warm water at 28°C, then placed on a moist filter paper tray in a warm place for germination. After 3 days, the seedlings were subsequently transplanted into plastic pots (diameter: 30 cm; height 25 cm) with peat soil (2 kg soil for 2 seedlings per pot) and cultivated under glasshouse conditions (28 - 32°C with 14 - 16 h light/22 - 26°C with 8 - 10 h dark). The same care conditions and procedures were used for all experimental and control plants. Each experiment formula and control formula consisted of 12 plants with 3 replications. Leaves and internodes were numbered from the top to the bottom of the plant. The plant materials (root, internodes, leaves, and seed) were harvested when the oldest plants were in the hard dough stage.

### 2.2. Cd concentration assay

The plant samples were dried in a ventilated oven at 105°C for 30 min and 70°C for 48 h and

subsequently ground into powders. 0.1 g of the ground sample was soaked in a mixture of HNO<sub>3</sub> and HClO<sub>4</sub> (3:1; v/v) according to Sun et al. (2008). Cd concentration was determined using a flame atomic absorption spectrometry Hitachi Z5000 (Tokyo, Japan).

### 2.3. Data analysis

The data were calculated using Statistix (version 10.0). Significant differences were determined by the least significant differences (LSD) at a 5% level of probability.

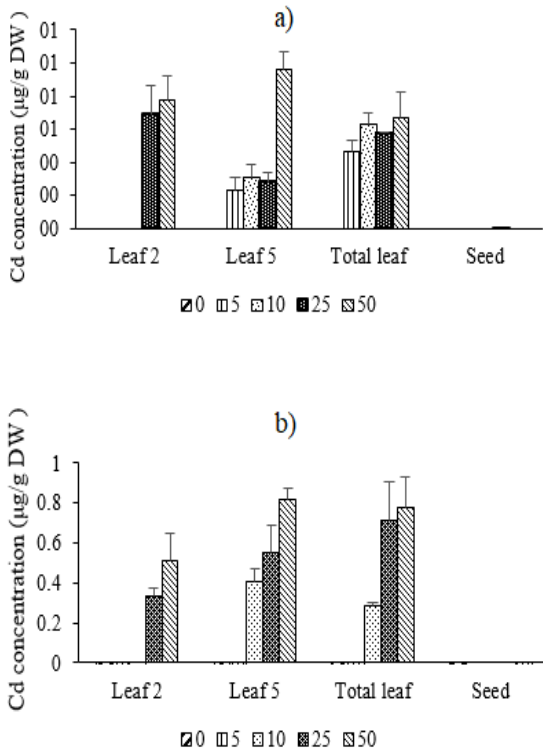
## 3. Results

### 3.1. Cd concentrations in leaves and seeds of sorghum

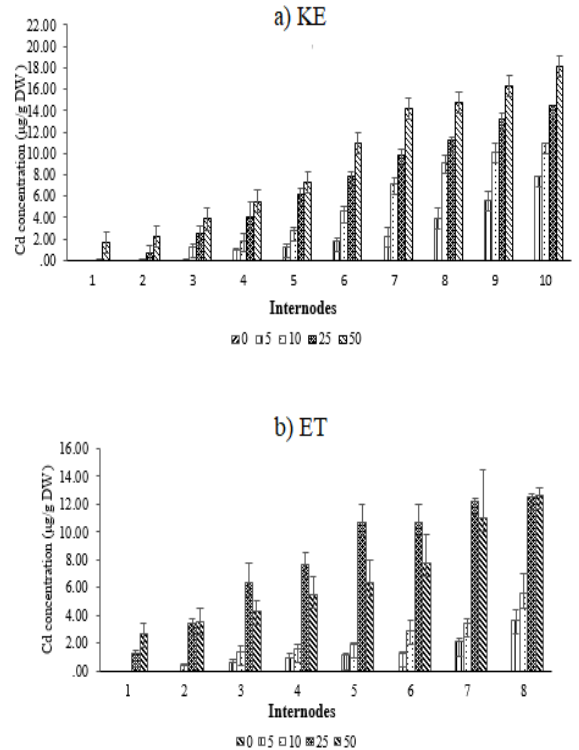
In the control treatment, the concentrations of Cd were not found in any organs of the plant such as the leaf, stem, root, or seed (Figure 1, 2, & 4; Table 1). For the treated plant, there was a significant difference in Cd accumulation in leaf among different Cd treatment levels. In the KE plant, Cd was absent in the second leaf at the lower concentration (5 and 10 ppm), and present when concentration was higher (25 and 50 ppm). The fifth leaf was observed with a presence of Cd at 5 ppm treatment. The highest Cd accumulation was recorded by treated 50 ppm Cd (0.9633 µg/g DW).

The results displayed the absence of Cd in the seed of a plant in both cultivars, even though Cd concentration was increased from 5 ppm to 50 ppm (Figure 1; Table 1). This result indicated that the transport of Cd from the root to the shoots and then to the seed was strongly inhibited. It also suggests that sweet sorghum can be used in safety for providing food, feed, and phytoremediation.

ET plants had a similar trend with KE plant for the accumulation of Cd in organs. By the lower Cd concentration treatments (5 and 10 ppm), Cd was completely absent in leaves and seeds. By the higher Cd treatments (25 and 50 ppm), the presence of Cd in the second leaf and fifth leaf was observed. The fifth leaf had a higher Cd concentration than the second leaf. The higher the concentration Cd treatment, the higher the concentration Cd accumulated in the leaf. There was no presence of Cd in the seed even though Cd concentration was increased from 5 to



**Figure 1.** Cadmium concentration in leaves and seeds of a) sweet sorghum KE and b) ET. (DW: dried weight).



**Figure 2.** Cd concentration in internodes of sweet sorghum. The internodes were numbered according to the proximity to panicles. (DW: dry weight).

50 ppm, similar to the KE seed (Figure 1b, Table 1).

**3.2. Cd concentrations in stems of sweet sorghum**

Compared to the control, more Cd was significantly enriched in the stem of both sweet sorghum cultivars under excessive Cd condition (Figure 2). The accumulation and distribution of Cd in the internodes of sorghum stem were very different. There was a significant difference in Cd concentration between internodes in stem and between Cd treatment levels. This displayed the difference in the ability of absorption and accumulation Cd of sweet sorghum. The Cd concentration in the stem displayed more fold higher than Cd in leaf in both cultivars.

For the control plants, Cd was completely absent in the internodes of the stems of both cultivars. In KE treated Cd plants, under the lower 5 ppm Cd, Cd was not detected in the internodes 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>. Cd was detected from the 4<sup>th</sup> internodes to the 10<sup>th</sup> internodes. The lower internode had higher Cd concentration (ranged

from 0.92 µg/g DW to 7.81 µg/g DW at the 4<sup>th</sup> to 10<sup>th</sup> internode respectively) (Figure 2a). At the 10 ppm of Cd treatment, Cd was absent in the 1<sup>st</sup>, 2<sup>nd</sup> internode, and was detected from the 3<sup>rd</sup> to the 10<sup>th</sup> internodes. The highest Cd concentration was observed at the bottom internode of the stem (10<sup>th</sup> internode, Cd reached up to 10.96 µg/g DW). Cd was recorded at the 2<sup>nd</sup> internode with 25 ppm Cd, Cd concentration in internodes was increased along the stem. The highest Cd at the 10<sup>th</sup> internode was 14.51 µg/g DW by 50 ppm Cd. By the highest 50 ppm Cd treatment, Cd was present at the 1<sup>st</sup> internode (Figure 2a; Table 1) and ranged from 1.65 to 18.13 µg/g DW at 1<sup>st</sup> to 10<sup>th</sup> internode respectively.

The similar trend was observed in ET, there was a significant difference in accumulation and distribution of Cd in stem among Cd treatment levels. At the lowest Cd treated plant (5 ppm), Cd in 1<sup>st</sup> and 2<sup>nd</sup> internode could not be detected. An increase in Cd was recorded from 3<sup>rd</sup> to the 8<sup>th</sup> internode (0.598 to 3.617 µg/g DW). At the Cd 10 ppm, Cd was absent in the 1<sup>st</sup> internode and present from 2<sup>nd</sup> to 8<sup>th</sup> internode (0.432 to 5.563 µg/g DW). At 25 and 50 ppm Cd, Cd accu-

**Table 1.** Cd concentration in organs of sweet sorghum at the hard dough stage (µg/g DW)

SS Cd	Leaf 2	Leaf 5	Total leaf	Seed	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	Root
0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	0.23	0.47	0.47	ND	ND	ND	ND	0.92	1.29	1.77	2.27	3.98	5.63	7.81	2.85
	±	±	±					±	±	±	±	±	±	±	±
KF 5	ND	0.07 <sup>b</sup>	0.07 <sup>b</sup>	ND	ND	ND	ND	0.29 <sup>d</sup>	0.18 <sup>c</sup>	0.29 <sup>d</sup>	0.34 <sup>d</sup>	0.76 <sup>d</sup>	0.92 <sup>d</sup>	0.86 <sup>d</sup>	0.6 <sup>d</sup>
	0.31	0.63	0.63	ND	ND	ND	1.23	1.86	2.83	4.56	7.16	9.19	10.11	10.96	6.83
	±	±	±				±	±	±	±	±	±	±	±	±
10	ND	0.08 <sup>b</sup>	0.07 <sup>ab</sup>	ND	ND	ND	0.37 <sup>c</sup>	0.35 <sup>c</sup>	0.69 <sup>b</sup>	0.28 <sup>c</sup>	0.49 <sup>c</sup>	0.59 <sup>c</sup>	0.69 <sup>c</sup>	0.86 <sup>c</sup>	0.2 <sup>c</sup>
	0.69	0.28	0.58	ND	ND	0.68	2.48	4.01	6.15	7.86	9.80	11.17	13.22	14.51	13.93
	±	±	±			±	±	±	±	±	±	±	±	±	±
25	0.18 <sup>a</sup>	0.06 <sup>b</sup>	0.01 <sup>ab</sup>	ND	ND	0.2 <sup>b</sup>	0.67 <sup>b</sup>	0.71 <sup>b</sup>	1.40 <sup>a</sup>	0.53 <sup>b</sup>	0.46 <sup>b</sup>	0.59 <sup>b</sup>	0.37 <sup>b</sup>	0.55 <sup>b</sup>	1.19 <sup>b</sup>
	0.77	0.96	0.67	ND	1.65	2.24	3.88	5.54	7.25	10.97	14.14	14.76	16.26	18.13	23.27
	±	±	±		±	±	±	±	±	±	±	±	±	±	±
50	0.15 <sup>a</sup>	0.11 <sup>a</sup>	0.15 <sup>a</sup>	ND	0.24	0.65 <sup>a</sup>	0.96 <sup>a</sup>	0.67 <sup>a</sup>	1.11 <sup>a</sup>	0.54 <sup>a</sup>	0.72 <sup>a</sup>	0.75 <sup>a</sup>	1.06 <sup>a</sup>	0.96 <sup>a</sup>	0.13 <sup>a</sup>
	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ET 5	ND	ND	ND	ND	ND	ND	0.59	0.94	1.10	1.29	2.08	3.62	NA	NA	3.52
	0.41	0.28	0.28	ND	ND	0.43	1.40	1.56	1.91	2.89	3.44	5.56	NA	NA	7.97
	±	±	±			±	±	±	±	±	±	±			±
10	ND	0.07 <sup>c</sup>	0.02 <sup>b</sup>	ND	ND	0.08 <sup>b</sup>	0.36 <sup>c</sup>	0.35 <sup>c</sup>	0.13 <sup>c</sup>	0.80 <sup>c</sup>	0.27 <sup>b</sup>	1.46 <sup>b</sup>	NA	NA	0.49 <sup>c</sup>
	0.33	0.55	0.71	ND	1.29	3.47	6.36	7.64	10.69	10.67	12.19	12.56	NA	NA	14.46
	±	±	±		±	±	±	±	±	±	±	±			±
25	0.05 <sup>a</sup>	0.14 <sup>b</sup>	0.19 <sup>a</sup>	ND	0.16 <sup>b</sup>	0.30 <sup>a</sup>	1.44 <sup>a</sup>	0.92 <sup>a</sup>	1.33 <sup>a</sup>	1.30 <sup>a</sup>	0.18 <sup>a</sup>	0.19 <sup>a</sup>	NA	NA	0.42 <sup>b</sup>
	0.51	0.82	0.77	ND	2.64	3.54	4.28	5.52	6.36	7.80	11.00	12.65	NA	NA	21.69
	±	±	±		±	±	±	±	±	±	±	±			±
50	0.14 <sup>a</sup>	0.06 <sup>a</sup>	0.15 <sup>a</sup>	ND	0.81 <sup>a</sup>	0.96 <sup>a</sup>	0.75 <sup>b</sup>	1.26 <sup>b</sup>	1.56 <sup>b</sup>	1.97 <sup>b</sup>	3.45 <sup>a</sup>	0.56 <sup>a</sup>	NA	NA	0.59 <sup>a</sup>
	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

<sup>a-d</sup>Data with different letters in the same column of 1 cultivar means significant difference at 0.05 level. SS: sweet sorghum; I: internode; ND: not detected; NA: not applicable.

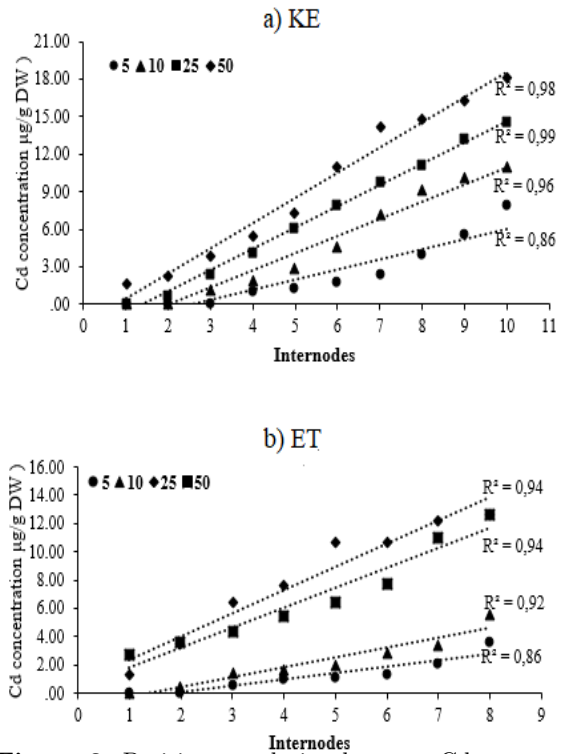
mulation was strongly increased along the stem. Cd accumulation in the 8<sup>th</sup> internodes was nearly 6-fold higher than that in the 1<sup>st</sup> internodes (Figure 2b; Table 1). Comparisons with the seedling stage showed Cd accumulation in the stem at the hard dough stage was observed 4 fold higher. This result indicates that the accumulation of Cd was increased more during the longtime of growth.

Under Cd exposure, the enriched Cd inhibited differential distribution within the stem of both KE and ET cultivars, which positively correlates with the position of internodes numbered according to the proximity to panicles. Increases in Cd concentration along the stem from the top internode to the lower internodes could be easily observed. There was a strong positive correlation between Cd concentration and internode positions along the stem.

The correlation coefficient of KE plant (0.86, 0.96, 0.99, 0.98 for KE and 0.86, 0.92, 0.94, 0.94 for ET by the treated 5, 10, 25 and 50 ppm Cd treatment respectively,  $P < 0.01$ ). Cd preferentially accumulated in the lower internodes, while accumulating less in the upper ones (Figure 3). This indicates that the transport process of Cd from the root up to the tops was strongly inhibited. Hence, Cd concentration in the top internodes was very low, as in the leaf, and completely absent in the seed.

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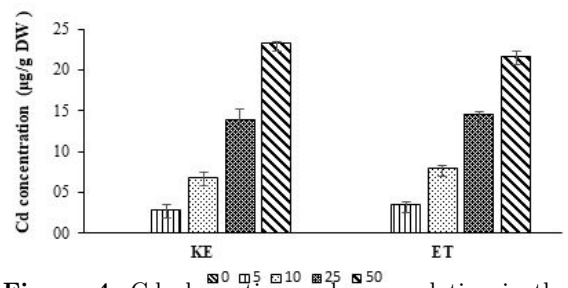
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**Figure 3.** Positive correlation between Cd concentration and internode position along the stem. The internodes were numbered according to the proximity to panicles. R indicates the Pearson correlation coefficient.

### 3.3. Cd concentration in the root of sweet sorghum

KE and ET plants could accumulate a high concentration of Cd in the root. There was a significant difference among Cd exposed levels, which displayed differences in absorption and accumulation capacities of Cd in the plant (Figure 4).



**Figure 4.** Cd absorption and accumulation in the root of sweet sorghum (DW: dry weight).

#### 4. Discussion

The partitioning of Cd to different plant organs plays important role in the toxicity of Cd to plants. At the seedling and the hard dough stage, the distribution of Cd was different among organs of sweet sorghum. Results were consistent with previous studies, which showed was Cd in order root > stem > leaf (Barros et al., 2009; Soudek et al., 2013; Ziarati et al., 2015). Tuerxun et al. (2013) found that the Cd concentration in leaves, root, and stem of two sweet sorghum varieties increased as to the increased of added Cd content and to the elongation of exposure time. For both varieties of sweet sorghum, roots contained the highest Cd content, followed by stem and leaf (Tuerxun et al., 2013). However, Izadiyar & Yargholi (2010) studied on Cadmium absorption and accumulation in sorghum found that the maximum concentration can be observed in Sorghum root and the minimum concentration in sorghum stem. Cadmium concentration in different parts of the tested plant species is the following order of ranking: root > leaf > stem (Izadiyar & Yargholi, 2010). Probably, the response of sweet sorghum to Cd toxicity is not the same as other sorghums.

The results also displayed that the old leaf (the fifth leaf) can accumulate higher Cd than the young leaf (the second leaf) (Figure 1). Maria et al. (2013) indicated that roots and old leaves are the main metal sinks suggesting a defense or tolerance mechanism of the plants to avoid toxic levels in physiologically most active apical tissues (Maria et al., 2013). Moreover, the position of the fifth leaf was lower than the second leaf along the stem. Combined with the results about distribution Cd in the internodes of the stem (Figure 2), it could be concluded that the process of Cd transport in stem decided the distribution of Cd in aerial parts such as leaf, stem, and seed. Several studies determined the accumulation of Cd in the grain of sorghum (Zhuang et al., 2009; Angelova et al., 2011). Angelova et al. (2011) studied heavy metals accumulated in different sorghums, included grain sorghum, technical sorghum, sugar sorghum, and Sudan grass grown on the soils contaminated with heavy metals (Pb, Cu, Zn, Cd). Their results showed that heavy metal content in the grains of Sudan grass, technical, and sugar sorghum were in the normal range (below the maximum permissible concentrations) and did not reach the phytotoxic levels

(Angelova et al., 2011). In our result, although Cd treatment was increased from 5 ppm to 50 ppm, there was completely absent of Cd in seed in both cultivars of sweet sorghum (Figure 1; Table 1). Hence, in the present research, the accumulation of Cd was in the following order: roots > stems > old leaf > young leaf > seed. The accumulation of Cd in the stem of sweet sorghum was studied, but all previous studies have no attention to the distribution of Cd in each internode along the stem. This is also one of the new observations of our study.

The absorption and accumulation of Cd in the root of both sweet sorghum cultivars in this research were consistent with previous studies, root was the highest Cd accumulated part in the plant (Kokyo et al., 2015; Muratova et al., 2015; Nawab et al., 2015). Cadmium was accumulated primarily in the roots of sorghum plants and then transferred to the shoots. Sweet sorghum accumulated high Cd in roots and stems, while the shoots had a very low concentration of Cd. Because of the detoxification mechanism in the plant, the plant can uptake and accumulate Cd without being harmed (Cheng, 2003; Etim, 2012; Laghlimi et al., 2015).

The inhibition of transport of Cd from roots to shoots may reflect a self-defense mechanism. Studies of Pinto et al. (2006) showed that contamination levels of Cd resulted in a corresponding increase in concentrations of phytochelatin, produced by Sorghum. Phytochelatins are an important class of cysteine-rich poly peptides, the production of which was increased in response to excessive absorption of metal ions, such as Hg and Cd by plants (Pinto et al., 2006). Soudek et al. (2013) found that in the time dependence experiment the cadmium concentration in roots become generally greater than in shoots. The roots seem to have a barrier to prevent the transport of cadmium to shoots (Soudek et al., 2013).

Many species, including sweet sorghum, accumulate toxic metals mainly in the roots (Maria et al., 2013; Soudek et al., 2014; Ziarati et al., 2015). For sweet sorghum, increases in the concentrations of Cd in the soil lead to a higher accumulation of this metal in the root. Previous studies demonstrated that sorghum plants were highly tolerant to metal pollution and able to reach high biomass, even in the presence of heavy metals (Marchiol et al., 2007; Epelde et al., 2009; Liu et al., 2011). These results once again confirmed

the ability to clean up contaminated heavy metal Cd soil of sweet sorghum (Figure 4).

The amount of Cd accumulated in the plant is limited by several factors including 1) Cd bioavailability within the rhizosphere; 2) rates of Cd transport into roots via either the apoplastic or symplastic pathways; 3) the proportion of Cd fixed within roots as a Cd- phytochelatin complex and accumulated in the vacuole; and 4) rates of xylem loading and translocation of Cd (Rahat et al., 2012).

## 5. Conclusions

An overall increase of Cd concentration was found in all tissues of the plants (roots, stem, young, mature, and old leaves) by increasing the Cd contamination in the soil. Regardless of treatments, Cd concentration in roots always exceeded those in the aboveground dry matter because of a low translocation from roots to shoots. There were significant differences between the heavy metal contents in root, stem and leaf. The Cd was accumulated in the order that root > stem > old leaves > young leaves. The results clearly showed that the absence of Cd in the seeds of the above plants.

This study detected that sorghum also had considerable accumulation ability to Cd in root and stem. The absence of Cd in seed and inhibition of translocation Cd from root to the shoots may represent the avoid effect on the food chain, which should be suitable for bioremediation.

Furthermore, Cd is accumulated preferentially in the lower internodes while scarcely accumulated in the upper internodes of both sweet sorghum lines KE and ET. These results suggested that excessive Cd accumulation is avoided in leaves, inflorescence, and seeds essential for photosynthate fixation and reproduction. Therefore, Cd accumulation in lower internodes benefits the resistance of sweet sorghum to Cd toxicity.

In conclusion, sweet sorghum should be a competitive candidate species for soil remediation due to its great biomass and strong resistance to adverse environmental conditions.

## Conflicts of interest

The authors declare no conflicts of interest.

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