Phytoremediation of Cadmium-contaminated agricultural land using indigenous plants

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Abstract— *The content of the heavy metal cadmium (Cd) which is excessive in the soil could affects on the soil and plants health. The aim of this descriptive study was to investigate the ability of selected indigenous plants in stabilizing Cd. The study was conducted at an agricultural production center in Batu City, East Java, Indonesia. There were two stages of this study, namely: (1) analysis of nutrient and heavy metal contamination, and (2) phytoremediation experiment by using five types of indigenous plants. The experiment was arranged in completely randomized design (CRD) with three replications. Once the plants were harvested, the plant materials then were analyzed the heavy metal content remaining in the soil and absorbed by the plants. The heavy metal content analysis used AAS (Atomic Absorption Spectrometry). Subsequently, the analysis result data were calculated for the bio-concentration factor (BCF) and heavy metal reduction. The initial content of heavy metal Cd in the soil prior phytoremediation had passed the threshold value (2.26 mg kg-1). The five indigenous plants tested on the contaminated soil showed a good growth pattern, especially in the fourth week after planting. The average ability of this selected plant to reduce heavy metals Cd was up to 71.2%. The reductions of heavy metals Cd obtained by each plant were Vetiveria zizanioides (71.2%), Eleusine indica, L. (58.9%), Ageratum conyzoides L. (52.2%), Euphorbia hirta (51.8%) and Chromolaena odorata (22.1%).*

***Keywords*— Phytoremediation, contaminated land, heave metal Cd, indigenous plants**

I. INTRODUCTION

Cadmium (Cd) pollution in the soil environment has been rapidly increasing in recent years, as a result of the use of agrochemicals such as fertilizers and herbicide. Phosphatic fertilizers are widely regarded as being the major sources of Cd contamination to the agricultural soil (Gray et al., 1999). Phosphate fertilizer contains a relatively high amount of Cd (up to 500 mg kg^{-1}) as a trace element from its manufacturing process (Loganathan et al., 2003). Hence, the intensive usage of phosphate fertilizer could result in the high Cd contamination in the soil (Tijani et al., 2008; Zeng et al., 2007). The use of organic fertilizer, such as farmyard manure also considered as the source of Cd contamination to the soil. Alloway (1995) reported that farmyard manure typically contains 0.3 - 1.8 mg kg^{-1} of Cd. Large annual application of farmyard manure (35 Mg of fresh manure ha^{-1}) were significant as Cd source to contaminate the soils (Alloway, 1995).

The intensification of agricultural activities in the city of Batu – Indonesia has led to the high usage of agrochemical products to increase the plant yields. However, the extensive usage of agrochemical is likely to pollute the soil by trace elements, and heavy metals originated from the agrochemical (Alloway, 1995). The usage of pesticides and phosphate fertilizers is a common agricultural method by farmers in the city of Batu. Recent soil analysis from the initial study of this experiment in Batu showed that the soil Cd concentration has passed the threshold value of 2.26 mg kg^{-1}. The high concentration of Cd in soils not only toxic to plants but also could generate secondary problems of acidifying the soil (Loganathan, 2003).

Looking at the potential soil contamination of Cd, it is important that farmers are starting to apply the appropriate soil remediation techniques to minimize the damage to plant and soil. One of the proposed soil remediation techniques is phytoremediation of Cd by using the indigenous hyperaccumulator plants. The phytoremediation technique by using indigenous hyperaccumulator plants is considered as the most inexpensive technology, compared to the other physical or chemical soil remediation techniques (McMohan, 2000). The indigenous hyperaccumulator plants could reduce the migration of Cd through the soil medium, also known as the phytostabilisation mechanism (Ogundiran and Osibanjo, 2008; Robinson et al., 2009). A study by Hamzah et al. (2012) also reported the potential of using indigenous hyperaccumulator plants to stabilize heavy metals and remediate contaminated soil to be used as agricultural soil again. The objective of this study is to describe the potential ability of selected indigenous plants in stabilizing Cd from contaminated agricultural soils in the city of Batu - Indonesia.

II. Material and Method

2.1 Soil sampling and initial chemical analysis

The descriptive study was conducted in the field at Bumiaji Village of Batu City, East Java, Indonesia. Prior to the planting of selected indigenous plant, the soil was analysed for chemical properties. The soil analysis include: pH (H_2O), soil organic carbon (C) content using Walkley and Black method; total nitrogen (N) by Kjeldahl method, total phosphorus (P) by Olsen method, total potassium (K), and total soil Cation Exchange Capacity (soil CEC) using 1 M ammonium acetate (NH_4OAc) at solution pH of 7.0. The soil total Cd was analysed using AAS *(Atomic Absorption Spectrometry).*

The soil samples were taken randomly from 3 farms at each different location in the village of Bumiaji, Batu, Indonesia. The farms were used mainly for shallot and vegetables such as carrots and potato. The soil sampling was collected from the top 10 cm soil depth and then mixed with the other soil samples from different locations to create a composite soil medium. This composite soil medium used for investigating the ability of selected indigenous plants in stabilizing Cd.

2.2 Descriptive experiment: investigating the ability of selected indigenous plants in stabilizing Cd from contaminated soil

There are five plants selected in this descriptive study; three indigenous plants and two hyperaccumulator plants that are commonly found in the village of Bumiaji, Batu, Indonesia. The three indigenous plants selected were *Eleusine indica, L.* (KB-1), *Ageratum conyzoides, L*. (KB-2), and *Euphorbia hirta* (KB-3). The hyperaccumulator plants found to be commonly found in the Bumiaji village are *Vetiveria zizanioides L*. (KB-4), and *Chromolaena odorata* (KB-5). All plants were planted in individual pots contain 10 kg of composite soil medium.

Plant observations were done every week until the plants aged three-months-old. The parameters observed were plants growth and the Cd concentration in roots, stems and leaves. After the plants reach three months old, the roots, stems, and leaves were collected separately. The plant samples then washed with distilled water to remove any excess soil and oven dried at 600 C for 72 hours. The dried plant samples were analysed for the Cd metal content by using AAS (Atomic Absorption Spectrophotometer).

2.3 Cadmium concentration analysis

The total Cd concentration in the soil and plant samples were analysed according to the method described by AOAC (1990). Dry soil and plant samples (1.00 g for each sample) added to a digestion tube with 1 ml of concentrated nitric acid (HNO_3) and 5 ml of 70% perchlorate acid ($HClO_4$) and leave for overnight. The samples then heated at 100°C for 1 hour 30 minutes and after that increased to 130°C for 1 hour. The temperature for second digestion was increased to 150°C for 2 hours 30 minutes (or until all of the yellow steam is exhausted). After all of the yellow steam exhausted, the temperature then was raised again to 170°C for 1 hour. The final temperature for the sample digestion was 200°C for 1 hour (white vapour is formed). Samples digestion was complete when the white precipitate has formed and a 1 ml of clear solution. After the digestion, samples were filled up with distilled water up to 10 ml and then filtered through MM 640 W Whatman filter paper. Analysis for the total Cd concentration from each extract was done by AAS (atomic absorption spectrophotometry) with various Cd standard solution as a comparison.

2.4 Transfer factor (FT) and Bio-concentration Factor (BCF)

The Transfer factor (FT) was calculated to evaluate the rate of Cd transfer between plant roots and shoot. The bio-concentration factor (BCF) indicates the ability of selected indigenous plant in absorbing Cd from the contaminated soil.

$$\text{FT} = \frac{\text{Total Cd concentration in above ground biomass (shoot + leaves)}}{\text{Total Cd concentration in roots}}$$

$$\text{BCF} = \frac{\text{Total Cd concentration in plants}}{\text{Total Cd concentration in soil}}$$

III. RESULTS AND DISCUSSION

3.1 Soil characteristics and heavy metal contamination

The initial soil analysis results used for this descriptive experiment are reported in Table 1. The soil collected from Bumiaji Village of Batu, Indonesia, has a sandy loam texture with the distribution of 56% sand, 8% dust, and 35% clay. The soil has a neutral pH of 6.22, and relatively low nutrient content (0.13% N; 0.64 cmol kg^{-1} available-P; and 0.03 cmol kg^{-1} K). The soil also characterised by the low soil organic matter content (1.73% organic carbon) and low soil Cation Exchange Capacity (CEC) of 6 cmol kg^{-1}. The soil total Cd concentration was 2.26 mg kg^{-1}. Tresnawati et al. (2014) also found that in the several farms of Bumiaji Village contain 4.22 mg Cd kg^{-1} soil, which much higher than the threshold value for agriculture soil (1 mg Cd kg^{-1} soil) (Alloway, 1995).

TABLE 1
SOIL CHEMICAL PROPERTIES

Element	Chemical Properties
pH (H_2O)	6.22
Organik C (%)	1.73
Total N (%)	0.13
C/N	13.31
P-olsen (cmol kg^{-1})	0.64
K (cmol kg^{-1})	0.03
CEC (cmol kg^{-1})	6.00
Cd (mg kg^{-1})	2.26

The high concentration of total soil Cd in the soil used for this experiment could be a result from the intensive use of agrochemical compounds, including fungicides and P fertilizer. Cadmium compound is often found as an active ingredient in fungicides (Environmental Agency, 2009) and pesticides (Lahuddin, 2007). The rigorous fungicides and pesticides application could increase the soil Cd concentration as expected. An interview was conducted with the farmers in the Bumiaji Village shows that almost 80% of the farmers in the area use fungicides almost constantly every year, thus may add a substantial amount of Cd residue in the soil.

Phosphatic fertilizers are widely regarded as being the major sources of Cd contamination to the agricultural soil (Gray et al., 1999). Relatively high concentrations of Cd (<500 mg/kg) are found in the phosphosites (rock phosphate) used for the manufacture of P fertilizers. The farmers in the Bumiaji Village are regularly applied P fertilizers, commonly with SP-36 (36% P) and NPK (10% P) fertilizer. Due to the high content of trace metal (in this case Cd) in the P fertilizers, it is likely that the level of total soil Cd concentration will increase along with the intensive application of P fertilizers. Moreover, Cd is more available for plants absorption compared to other metal in soil (Widaningrum et al., 2007; Subowo et al., 1999). In order to clarify the prediction of anthropogenic Cd source in the Bumiaji Village, further analysis of the active ingredients of P fertilizers and fungicides used by the farmers are needed to confirm the Cd content added to the soil.

3.2 Descriptive experiment: investigating the ability of selected indigenous plants in stabilizing Cd from contaminated soil

a) Plants Growth

All five indigenous plants used in this descriptive experiment shows a relatively good growth during the eight weeks of the experiment (Figure 1). Of the three indigenous plant tested in this experiment, both of Eleusine indica, L. (KB-1) and Ageratum conyzoides, L. (KB-2) show a better growth compared to Euphorbia hirta (KB-3) (Figure 1a). At the end of the experiment, both Eleusine indica, L. (KB-1) and Ageratum conyzoides, L. (KB-2) had the highest plant height compares to Euphorbia hirta (KB-3), Vetiveria zizanioides L. (KB-4), and Chromolaena odorata (KB-5).

Similar to plant height measurement, Eleusine indica, L. (KB-1) and Ageratum conyzoides, L. (KB-2) also had the highest number of leaves compare to the other plants (Figure 1b). The higher plant growth and leaves production shows that Eleusine indica, L. (KB-1) and Ageratum conyzoides, L. (KB-2) are tolerant to the high soil total Cd content. Moreover, both plants show a better growth than the known hyperaccumulator plants used in the experiment (KB-4 and KB-5). The plants height and leaves production are correlated to the ability plants in absorbing nutrient from the soil (Sastroutomo, 1990). Hence, these results may indicates that Eleusine indica, L. (KB-1) and Ageratum conyzoides, L. (KB-2) not only tolerant of high soil Cd content, but potentially to be hyperaccumulator plant in stabilizing Cd in a contaminated soil.

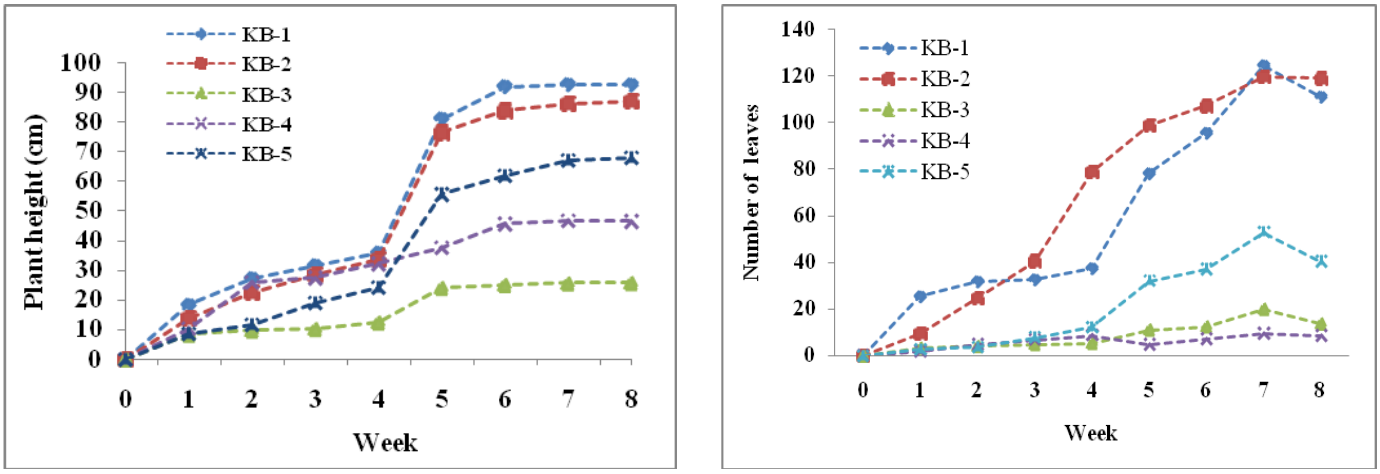

FIGURE 1. A) PLANT HEIGHT AND B) NUMBER OF LEAVES DURING THE 8 WEEKS OF EXPERIMENT
KB-1= *Eleusine indica*   KB-2 = *Ageratum conyzoides*   KB-3 = *Euphorbia hirta*   KM-4 = *Vetiveria zizanioides*
KB-5 = *Chromolaena odorata*

Plants root traits (root length and root dry matter) are presented in the Figure 2. Of the three indigenous plants selected, *Eleusine indica L.* (KB-1) and KB-3 *Chromolaena odorata* (KB-3) showed higher root length (63.0 – 65.1 cm cm^{-3}) compared to *Ageratum conyzoides, L.* (KB-2) (38 cm cm^{-3}). Similar to the root length measurement, the root dry matter of KB-1 and KB-3 also higher than KB-2. The known hyperaccumulator plants tested in this experiment showed a different root length measurement, where *Vetiveria zizanioides L.* (KB-4) had a lower root length compare to *Chromolaena odorata* (KB-5) (Figure 2a). However, lower root length recorded in KB-4 did not resulted in lower root dry matter compared to KB-5. Root dry matter measurement showed that KB-4 had the highest biomass (90 g pot^{-1}) compare to the other plants (Figure 2b).

Hyperaccumulator plants are characterised by: (i) tolerant to high concentration of metal in soil, (ii) accumulate/absorb metal from the soil, (iii) rapid growth rate, (iv) producing high biomass, and (v) has a robust root system (Garbisu et al. 2002). The profuse root system of hyperaccumulator plants is closely linked to the existence of various types of microbial important role in the contaminant degradation in rhizosphere (Alkorta et al., 2004; Krämer *et al.*, 2010). This microbial will help to breakdown heavy metal compounds to be available for roots absorption, thus increase the extraction of metals from soil by accumulator plants.

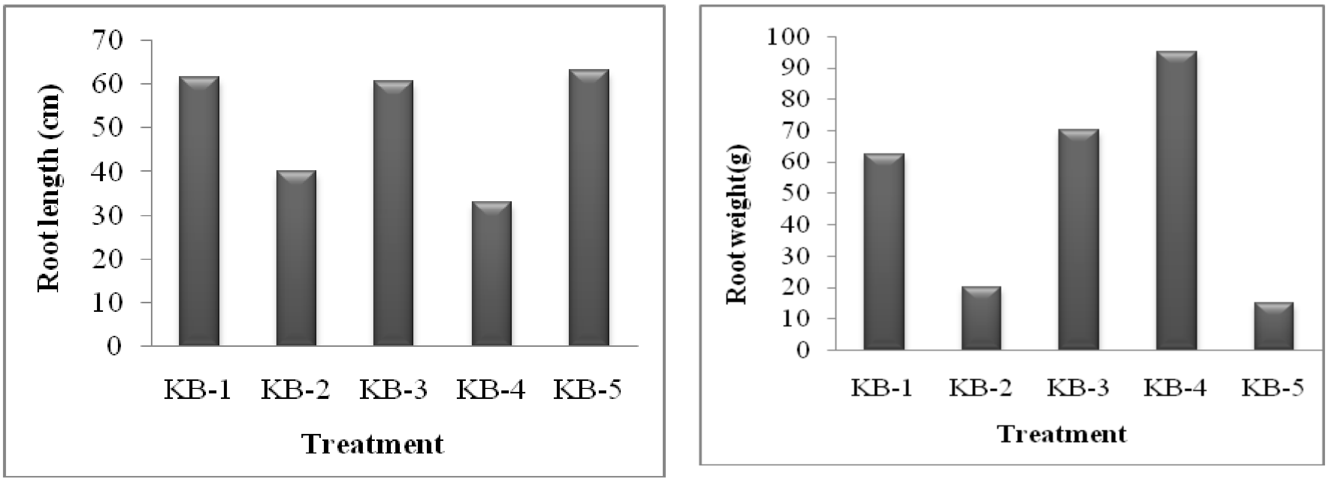

FIGURE 2. A.) PLANTS ROOT LENGTH AND B.) DRY ROOT WEIGHT AFTER 8 WEEKS OF EXPERIMENT
KB-1= *Eleusine indica*   KB-2 = *Ageratum conyzoides* KB-3 = *Euphorbia hirta*
KB-4 = *Vetiveria zizanioides*   KB-5 = *Chromolaena odorata*

b) Cadmium absorption by plants

The most acceptable metals extraction from contaminated soil is by using plant (phyto-extraction), and the remediation method is known as phytoremediation. This process takes advantage of the ability of plants to take up, accumulate, and/or degrade constituents that are present in soil and water environments. Some plants are referred as heavy metals hyperaccumulators plant, as they have the ability to store large amounts of these metals that do not appear to be used in their function. Phytoremediation of elemental pollutants involve plants extracting and translocating a toxic cation or oxyanion to above-ground tissues for later harvest or sequestering the element in roots to prevent leaching from the site.

Some heavy metals accumulator plants are tend to store heavy metals in its roots, while other translocate heavy metals into its shoot and/or leaves. The translocation factor (TF) is commonly used to evaluate the heavy metals storage in plant tissues, where TF > 1 indicates the effective translocation of heavy metals from roots to shoot (Fayiga and Ma, 2006; Rezvani and Zaefarian, 2011). Results from this descriptive experiment showed that most of the indigenous plant are having TF more than 1, with only exception of *Eleusine indica L.* (KB-1) (Table 2). *Vetiveria zizanioides L.* (KB-4) had the lowest TF (Table 2) compare to the other plants tested, indicates the preference of this plant in storing Cd in its roots.

TABLE 2
CADMIUM CONCENTRATION IN PLANT ROOTS, UPPER PART OF PLANTS, TRANSLOCATION FACTOR (TF), AND BIOCONCENTRATION FACTOR (BCF) IN EACH PLANT.

Treatment	Cd concentration in roots (mg kg^{-1})	Cd concentration in upper part of plants (mg kg^{-1})	Total Cd concentration (mg kg^{-1})	TF	BCF
E. indica	0.6	0.3	0.9	0.6	0.4
A. conyzoides	0.5	0.6	1.1	1.2	0.5
E. hirta	0.5	0.6	1.1	1.3	0.5
V. zizanioides	0.4	0.2	0.6	0,5	0.3
C. odorata	0.8	0.9	1.8	1.1	0.8

Bioconcentration factor value (BCF) is used to evaluate the ability of selected plants in extracting heavy metals from the soil (Ma et al., 2001). The BCF for selected indigenous plants in this experiment are presented in Table 2. Plants with BCF value higher than 1 mg kg^{-1} are categorised as hyperaccumulator, while plant with BCF value lower than 1 mg kg^{-1} are considered as accumulator of heavy metals (Mellem et al., 2009; 2012). All of the plant tested in this experiment are having BCF value lower than 1. Hence, these plants are considered accumulators of Cd. The highest Cd accumulation was occurred in *Chromolaena odorata* (KB-5) and the lowest accumulation by *Vetiveria zizanioides L.* (KB-4) (Table 2).

The rate of Cd uptake from soil to plant tissue was influenced by several aspects including: (1) the movement rate of Cd from the soil to the root surface; (2) ion concentration of Cd on the root surface; (3) metal transport from the roots surface into the root; (4) contact time, and (5) translocation from the roots to the plant canopy (Alloway, 1995; Brown et al., 1995).The low value of BCF found in this experiment might attributed to the contact time and the low ion concentration of Cd on the root surface. Further experiments on the Cd concentration in the rhizosphere are needed to confirm these explanations.

The soil analysis after six weeks of experiment showed that all five plants tested in this descriptive experiment were able to reduce soil Cd concentration (Figure 3). However, it worth noted that *Chromolaena odorata* (KB-5) only extract 22.1% from soil, whereas the other plants were able to extract Cd from soil up to 50% (Figure 3). The low soil Cd reduction observed in the *Chromolaena odorata* (KB-5) indicates that although *Chromolaena odorata* has been proven to be an effective remediator plants (predominantly Hg and Pb) (Hamzah et al., 2012), it did not suitable for Cd remediation. Of the three indigenous plants tested (KB-1, KB-2 and KB-3), *Eleusine indica L.* (KB-1) had the highest soil Cd reduction compare to the two other indigenous species (Figure 3).

Metal accumulator plants extract metal (in this case is Cd) from the contaminated soils and accumulate the metal in its canopy to be reprocessed or disposed when the plants are harvested (Chaney et al., 2008). Based on the traits of metal accumulator plants, *Eleusine indica L.* (KB-1) is the most suitable plants for stabilising Cd in the Bumiaji Village. The *Eleusine indica L.* (KB-1) has shown to be tolerant to high concentration of soil Cd, produce high root biomass, and has shown to the ability to reduce available Cd from a contaminated soil. Further experiment are needed to test the efficiency of *Eleusine indica L.* (KB-1) in extracting Cd from soil.

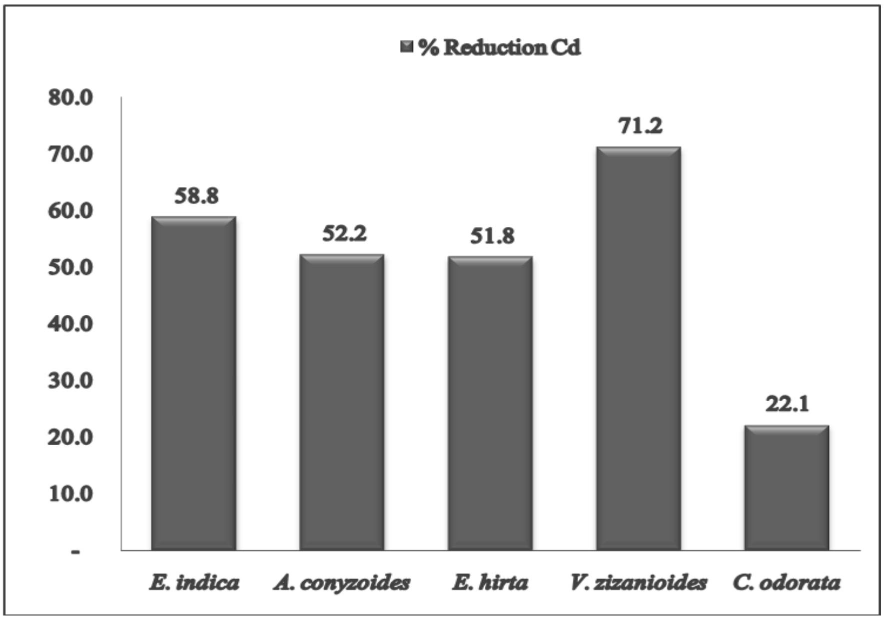

FIGURE 3. PERCENTAGE OF CD REDUCTION ON EACH PLANT

IV. CONCLUSION

Soil in the research site of Bumiaji Vilage of Batu regency, Indonesia had a low fertility levels and the soil available Cd was higher than the permitted concentration for agriculture. Three indigenous plant (*Eleusine indica* L., *Ageratum conyzoides*, L and *Euphorbia hirta,* L.) have been tested for the ability in stabilizing Cd from the contaminated agricultural soil. *Eleusine indica* L. has shown the ability of accumulating Cd in its roots, while both of *Ageratum conyzoides,* L and *Euphorbia hirta,* L. accumulate Cd in its stalks and leaves. Of the three indigenous plants tested in this experiment, *Eleusine indica* L. was the most tolerant plants against Cd contamination, and also proven to be the suitable plants in reducing soil available Cd.

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