

# Phytoremediation of Cd and Pb by Four Tropical Timber Species Grown on an Ex-tin Mine in Peninsular Malaysia

Lai Hoe Ang, Lai Kuen Tang, Wai Mun Ho, Ting Fui Hui, and Gary W. Theseira

**Abstract**—Contamination of heavy metals in tin tailings has caused an interest in the scientific approach of their remediation. One of the approaches is through phytoremediation, which is using tree species to extract the heavy metals from the contaminated soils. Tin tailings comprise of slime and sand tailings. This paper reports only on the finding of the four timber species namely *Acacia mangium*, *Hopea odorata*, *Intsia palembanica* and *Swietenia macrophylla* on the removal of cadmium (Cd) and lead (Pb) from the slime tailings. The methods employed for sampling and soil analysis are established methods. Six trees of each species were randomly selected from a 0.25 ha plot for extraction and determination of their heavy metals. The soil samples were systematically collected according to 5 x 5 m grid from each plot. Results showed that the concentration of heavy metals in soils and trees varied according to species. Higher concentration of heavy metals was found in the stem than the primary roots of all the species. *A. Mangium* accumulated the highest total amount of Pb per hectare basis.

**Keywords**—Cd, Pb, Phytoremediation of slime tailings, timber species.

## I. INTRODUCTION

TIN tailings are part of a landscape in Peninsular Malaysia, major towns along the west-coast of the peninsula came to existence as a result of the mining industries in the 1960's, the tailings cover about 113,750 ha of land surface and about 40% had been used for cultivation of fruit crop, aquaculture, recreation grounds and housing estates [1]. Tin tailings comprising slime, sand and sandy slime tailings [2]. Recent studies show that the ex-mines contain four potentially toxic elements (PTEs) of heavy metals, and they are lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg). The four PTEs were found in fruit crops and fishes produced from ex-mining sites [1]-[3]. The uptake of the heavy metals was found in the

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fruit crops grown from the tailings and their concentrations were higher than the permissible levels of the national food safety standard [2]-[3]. Ingestion of PTEs contaminated food exceeds human tolerance limit may cause severe health problems [3]-[5]. This has drawn interest to develop a scientifically cost-effective remedial measure to remove heavy metals from the contaminated soils. One of the established approaches is through phytoremediation [6]. Phytoremediation is using tree to extract the heavy metals from the contaminated ground, and accumulate them in root, stem and branches. Logically, repeating cycles of planting and harvesting of trees accumulated with heavy metals will eventually reduce the concentration of PTEs in soils to an acceptable level for other uses. A green house study shown that *Acacia mangium* and *A. auriculiformis* accumulated considerable amount of Pb, Cd, As and Hg from sludge treated sand tailings [7]. Phytoremediation is one of the cost-effective approaches to remove the heavy metals from the contaminated ex-mine [6]. Using trees to remove heavy metals from the tin tailings brings other benefits such as improving its site quality. A 10-year old tree stand was found to have ameliorated the harsh microclimate of an ex-tin mine to that of a secondary forest and also have improved its soil properties [1],[3]. Using timber species for phyto-remediation approach has additional benefit as it also yields timber at the end of its rotation [1],[3]. All tree and shrub species have the capacity for uptake of heavy metals but each species may differ in its bioaccumulation potential. It also depends on the species' preference and availability of heavy metals in ionic forms. Marcus [8] reported that *Rinorea bengalensis*, which is a shrub, is a potential bio-accumulator for Ni. Many studies in the temperate show that tree species have been used as bioaccumulators to clean heavy metals such as Ni, Pb, Zn, Cu and Cd [6],[9]. This implies that tree species have a great potential to remove heavy metals for the contaminated mines. However, this ideal cost-effective approach has many limitations, one of them is heavy metal contaminated sites are normally adverse to establishment and growth of plants, e.g. tin tailings. Greening tin tailings with tree species is well documented, but no documentation is available on the phytoremediation potential of these tree species. Hence, this study was embarked to determine the bioaccumulation of Cd and Pb by four selected timber species namely *Acacia*

*mangium*, *Hopea odorata*, *Intsia palembanica* and *Swietenia macrophylla* that are successfully grown on sand tailings.

## II. MATERIALS AND METHODS

### A. Study Site

The study was conducted in a substation of Forest Research Institute Malaysia in Bidor, which is situated 138 km from Kuala Lumpur. Bidor is located at 4° 6' N latitude and 101°16' E longitude and was a famous mining town in the 1940's. The substation covers about 120 ha of ex-mine and it has been successfully greened with some timber tree species.

### B. Soil Sampling

Soil samples of slime tailings were collected from two 0.25 ha plots at grid sampling of 5 x 5 m. Each composite sample comprising soil samples collected from a 25 x 25 m subplot, hence, a 0.25 ha slime tailings had four composite samples. A total of 8 composite samples were prepared from the soil samples collected from 2 x 0.25 ha plots, for determination of pH, total Pb and Cd. The wet pH of the slime tailings was determined using a pH meter, pH 211 (Hanna Instrument).

### C. Tree Sampling and Oven-Dry Weight

Four timber species mentioned earlier are grown on slime and san tailings at eight years after planting. Six trees of each species were randomly harvested from slime tailings. Sample tree was labeled, and its diameter at breast height (dbh), total length, total leaf number, root diameter and leaf area were determined. The total weight of the tree comprising root, stem, and branch was determined using two balances, with maximum ranges of  $2.5 \pm 0.01$  kg and  $150 \pm 0.5$  kg, respectively. Root samples only comprising extractable primary roots and not the secondary roots. For secondary roots that are difficult to separate from the soils during the root extraction were left out. Moisture content of primary root, stem and branch were determined at  $105 \pm 0.1^\circ$  C. Bark was removed from the wood samples of root, stem and branch. The samples were dried until they reached constant weight.

### D. Chemical Analysis

The root and stem samples were also prepared for determination of the two potentially toxic elements namely Pb and Cd using AOAC (Association of Analytical Communities) method (1980) in an ISO commercial laboratory, Chemical Laboratory Sdn Bhd. Heavy metals concentrations of soils and plants were analyzed using Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICPS 7510, Shimadzu, Japan).

### E. Total Photosynthetically Active Leaf Area and Phyto-Extraction Factor

The leaf number of each tree was counted manually, and 50 fully mature leaves were randomly selected for determination of leaf area using a leaf area meter (AMS, Delta-T Devices LTD). Phytoextraction ratio (PER) or bioconcentration factor as defined in the following equation:

$$PER = \frac{\text{Concentration of metal in plants}}{\text{Concentration of metal in soils}}$$

All the data obtained from this study were compared using means and their standard errors (SEM).

## III. RESULTS AND DISCUSSION

### A. Occurrence of Pb and Cd of Slime Tailings

Fig. 1 shows that the occurrence of the four heavy metals in slime tailing varies according to species and soil depths. Total Cd and Pb in slime of the study site is similar to that reported [2], its distribution is evenly between the two depths. Total Pb and Cd were found to be evenly distributed at the two depths of slime tailings. The results show that total concentration of Pb and Cd in slime tailings under the stands of the four species are within the range reported by others studies [2]-[4].

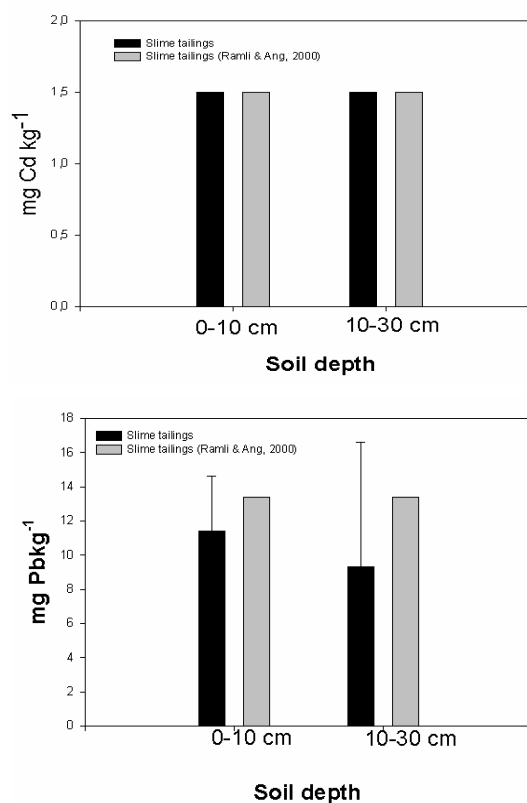


Fig. 1 Total Cd and Pb distributed at 0-30 cm depth of slime tailings compared to the finding of Ramli and Ang [2]

### B. Vegetative Growth Characteristics

Fig. 2 shows that four timber species significantly different total photosynthetically active leaf area. This explains why *Acacia mangium* had significantly the largest oven-dry weight, and followed by *Hopea odorata*, *Swietenia macrophylla* and *Intsia palembanica*. The results indicate that *A. mangium* grown on slime tailings produces the highest yield of timber and thus oven-dry biomass (Fig. 2).

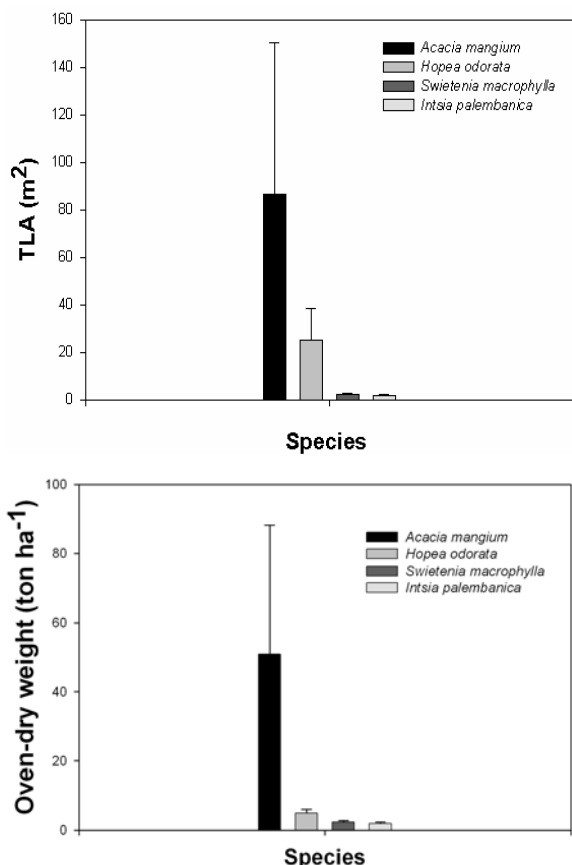


Fig. 2 The total photosynthetically active leaf area (TLA) per tree and over-dry weight per ha of the four timber species. The estimation of over-dry weight per hectare basis was based on the mean and standard deviation of the oven-dry weight of the sample trees with sample size n= 6, and planting density of 500 trees/ha

### C. Total Pb and Cd

The wet soil pH of slime tailings was determined and ranged from pH 4.11 to 4.37 (n=8). The acidic soil environment can be as low as pH2.5 at the root zones of mango trees. Exudates of root are responsible for lowering the rhizosphere pH of the feeder roots [11]. This explains the availability of ionic forms of Pb and other heavy metals to the plants.

Lead and Cd are non-essential but toxic to plants, especially in adversely affecting photosynthetic activities [12]. Fig. 3 shows that Pb was accumulated by the four species and partitioned in the stem except for *S. macrophylla* only accumulated Pb in the root. The total amount of Pb accumulated by the stem and root of the timber species covering  $0.2 \pm 0.1$  to  $6.4 \pm 4.7$  gPb ha<sup>-1</sup>. Stem and root concentrations of Pb did not differ significantly by their standard deviations for the four species. *Acacia mangium* has accumulated the biggest amount of Pb, and followed by *H. odorata* and *I. palembanica*. High amount of total Pb sequestered by *A. mangium* is mainly due to its relatively high uptake ability and fast growth rate (Fig. 3). The result indicates that *A. mangium* and *H. odorata* sequestered the heavy metals in stem via root-uptake.

*Acacia mangium* and *H. odorata* did not significantly accumulate Pb in their stems and primary roots (Fig. 3). Interestingly, Pb was found only in the primary roots of *S. macrophylla*. Most of the studies prove that root normally sequestered higher concentration of Pb than shoot tissues and some trees partitioned Pb only in roots [13]-[14]. Root to shoot translocation of ionic Pb is well established. Low solubility of Pb in soil does stop phytoextraction, as plants grown in low total soil Pb of  $< 4$  mg l<sup>-1</sup> was found to have deposited ionic Pb from soils through precipitation to form complexes with anions including sulphate or carbonate in the intracellular level in higher pH in root, but releases it when pH dropped in the xylem, and transport it to shoots [14]. Higher concentration of Pb in root and shoot tissues of plants will not cause adverse effects to their essential physiological activities. The effects of Pb toxicity in the plants were not observed as other findings show that Pb ions were sequestered and safely fixed into part of cell wall or vacuole through thiol peptides, and the sequestration does not disturb other essential physiological activities [15]-[17].

*H. odorata* and *I. palembanica* had accumulated Cd of the ex-tin mine (Fig. 3). However, the amount of Cd extracted by the two species was small and ranged only from 0.1 to 0.4 g Cd ha<sup>-1</sup> the root system of these two species were deeper than 0.3 m which Cd was not detected, and able to tap into the leached Cd at the lower depth.

Cadmium is not an essential element to plants but it is toxic and reduces vegetative growth of plants [18]. Cadmium was undetected in the primary roots of all four tree species but was found in stem *H. odorata* and *I. palembanica*. This could be due to several Cd translocation pathways in plants, the two probable pathways include first, through expulsion mechanism where Cd is translocated from root to shoot through xylem, and precipitated and accumulated in leaf cells or outer stem cells, and later removed from the plant through abscission of leaf or stored in the bark. Some plants translocate and expel Cd to the surface of the roots, and later trapped in the root-bark. Cadmium treated plants had significant amount of Cd detected at the root cap and not found in the shoot supported the expulsion mechanism, e.g. *Zea mays* [19],[20].

Second, through detoxification mechanism of ionic heavy metals from xylem and sequestered them into plant tissues. Cadmium is assimilated in the stem through phytochelatin PCs-complex that alleviate the phyto-toxicity of Cd trapped ionic Cd into vacuole. Silicon complexes reduce cell damages caused by Cd toxicity in plants and protect membrane integrity of the root caps of *Z. mays*, *Oryza sativa*, *Brassica chinensis* and strawberry [19][20][21]. Hence, the presence of total Cd in both the timber species but did not cause any visual symptoms of Cd toxicity could probably be due to effectiveness of Si-complexes in alleviating Cd-toxicity. *Hopea odorata* and *I. palembanica* had significantly higher total Cd concentration in the stem, and implies that they have the potential to be used as phytoremediators of Cd contaminated sites.

Both expulsion and assimilation mechanism in plant have only one main purpose that is to protect the feeder roots from damages caused by Cd toxicity, as the roots have direct contact to ionic Cd in the soils. Hence, it is anticipated that primary roots without bark with a diameter size range of 3 to 10 cm did not have any trace of Cd. The finding implies *H. Odorata* and *I. Palembangica* efficiently translocate ionic Cd through xylems of the primary root and only sequestered Cd in their stem in favorable conditions through PCs complex and suitable pH. The accumulation of Cd is explained plainly through asymlastic and apoplastic regulations [18],[22].

Total Cd concentration of the secondary and feeder roots were not determined in this study, as these roots normally are left in the soils after timber extraction. However, high Cd was detected in root system of Cd spiked plants in other studies [18]-[22].

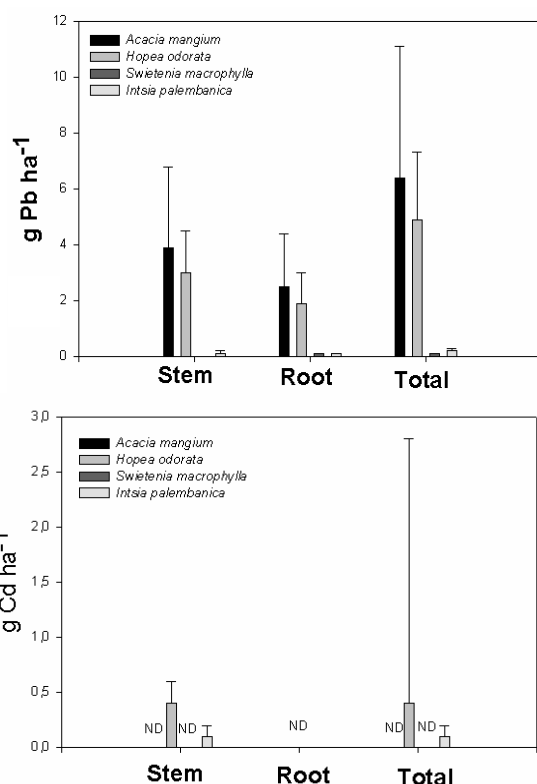


Fig. 3 Total accumulated lead (Pb) and cadmium (Cd) by four timber species grown on the ex-tin mine. The estimation of total Pb and Cd per hectare basis was computed from the mean biomass of six sample trees x mean concentration of metal of six sample trees x 500 trees per ha. ND denotes below detection limit

To examine the species preference over species of heavy metals, a phyto-extraction ratio is used (Fig. 4). Phytoextraction ratio (PER) which is an adaptation from bioconcentration factor (BCF)[23]. This study only quantified the primary roots and excluded the secondary, tertiary and feeder roots as their removal from soils can be an uneconomical efforts. However, heavy metals enriched primary roots can be easily removed during logging activities.

Hence, concentrations of primary root with stem alone were used in the calculation of PER.

The phyto-extraction ratio shows that *H. odorata* and *I. palembanica* are better bioaccumulators of Cd than the other two species, but none of the timber species accumulated Pb in the tissues more than the soil Pb concentration.

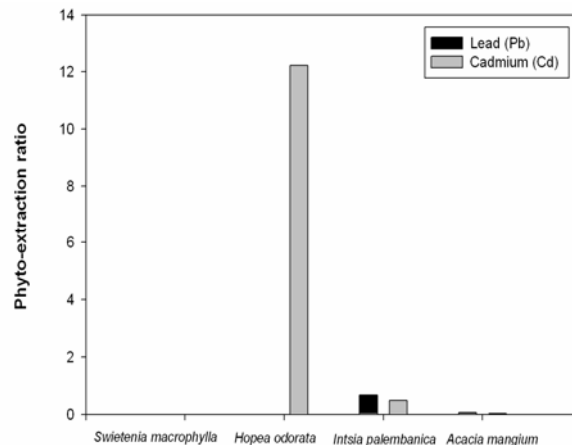


Fig. 4 Phyto-extraction ratio of lead (Pb) and cadmium (Cd) of the four timber species

#### IV. CONCLUSION

Based on the recent finding, phytoremediation may not be economically feasible to be used to clean a contaminated site as tree species used in the cleansing processes accumulate less than 1% Pb in its above-ground woody tissues [13]. None of the four timber species is having economic potential for remediation of soil Pb and Cd if based on the present understanding.

Four timber species have demonstrated their phytoextraction potential in sequestration of Pb and Cd from the ex-tin mine. The amount of heavy metals accumulated by the four timber species is relatively small. Four timber species were found to accumulate Pb and Cd from the contaminated site but only *H. odorata* and *I. palembanica* had greater concentration of Cd in the woody tissues than the soils.

The finding of this study shows that *A. mangium* and *H. odorata* had significantly accumulated greater total amount of Pb than *S. macrophylla* and *I. palembanica* because of their relatively faster growth and thus having greater oven-dry biomass. Hence, it is interesting to note that phytoextraction factor alone may not be sufficient to be used for assessing the potential of tree species for removing heavy metals from the contaminated sites. A more realistic estimate of heavy metal removal capacity of plants should also take into consideration of their biomass growths.

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REFERENCES

- [1] Ang, L.H. and Ang, T.B. 2000. Greening the tin tailings area in Malaysia. In: S. Appanah, M.Y. Yusmah, W.J. Astinah and K.C. Khoo (eds.), *Proceedings of the International Conference on Forestry and Forest Products Research: 195-205. FRIM, Kepong, Malaysia*
- [2] Ramli Mohd Osman & Ang, L.H. 1999. The occurrence of some important potentially toxic trace elements in an ex-mining land located in Bidor, Perak. In P120-127, Proceedings of Malaysian Science and Technology Congress 1999. 8-10<sup>th</sup> November, Hilton Kuching, Sarawak.
- [3] Ang, L.H., TB Ang and LT Ng. 1998. Site toxicity of tin tailings and its implications on land use in Peninsular Malaysia. In P 116-123, Proceedings of MSTC 1998. 23-25 November, 1998. Kota Kinabalu.
- [4] Ang, L.H. & Ng, L.T. 2000. Trace element concentration in mango (*mangifera indica L.*), seedless guava (*Psidium guajava L.*) and papaya (*Carica papaya L.*) grown on agricultural and examining lands of Bidor, Perak. *Journal of Tropical Agricultural Science* 23(1): 15-22.
- [5] Ang, L.H., Ho, W.M., Ramli Mohd Osman, Maimon Abdullah, Chung, P.Y. & Ng, L.T. 2000. The update of potentially toxic elements in some economically important plants and fish produced from ex-mining sites in Bidor, Perak. In Pxxxx, Proceedings of the Malaysian Science Technology Convention 2000, 18-20 September, 2000, Kota Kinabalu, Sabah.
- [6] Pulford, I.D. & Watson, C. 2003. *Environmental International* 29 (2003):529-540
- [7] Ho, W.M. and Ang L. H. 2003. Bioaccumulation of lead, cadmium, arsenic and mercury from domestic sewage sludge in *Acacia mangium* grown on sand tailings. Paper presented in COSTAM 2003, 12-14<sup>th</sup> Kuching, Sarawak. 7p (Unpublished paper).
- [8] Marcus Jopony. 1999. Phytoremediation as an alternative to technologies for remediation of heavy metal contaminated site. In P128-134, COSTAM, proceedings of MSTC 1999, 8-10 Nov, 1999. Kuching, Sarawak.
- [9] Li, M.S. 2006. Ecological restoration of ex-mineland with particular reference to the metalliferous mine wasteland in China: A review of research and practices *Science and Environment* 357 (2006):38-53
- [10] AOAC methods. 1980. Official methods of analysis of the association of official analytical chemists, Washington, D.C. P385-413.
- [11] Ho, WM., Maimon A. & L.H. Ang. The concentrations of potentially toxic elements (PTEs) in vegetables cultivated on sand tailings. In P109-116, Proceedings of Malaysia Science and technology Congress 2000. 18-20, September 2000, USM, Sabah.
- [12] Skorzynska-Polite, E. Basynski, T. 1997. Difference on sensitivity of the photosynthetic apparatus in Cd-stressed runner bean plants in relation to their age. *Plant Science* 128: 11-21.
- [13] Cunningham, S.D. & W. R. Beri. 2000. Phytoremediation and phytostabilization: Technical, economic, and regulatory considerations of the soil-Lead issue, Norman Terry(Ed.). Phytoremediation of contaminated soil and water. CRC Press LLC, Florida.
- [14] Sharma P & R.S. Dubey. 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.* 17(1):35-52
- [15] Mehra, R.K. & Tripathi, R.D. 2000. Phytochelatins and metal tolerance. In Agrawal, S.B., Agrawal, M.(Eds.). *Environmental Pollution and Plant Responses*. Lewis Publisher. Boca Raton, FL, USA 367p.
- [16] Rauser, W.F. 1995. Phytochelatins and related peptides structure, biosynthesis, and functions. *Plan Physio.*109:1141-1149.
- [17] Zenk, M.H. 1996. Heavy metal detoxification in higher plants- a review. *Gene* 179:21-30.
- [18] Larsson, H. Asp H., Bornman J. Influence of prior Cd<sup>2+</sup> exposure on the uptake of Cd<sup>2+</sup> and other elements in the phytocheletion-deficient mutant, cad1-3, of *Arabidopsis thaliana*, *Jour. Experimental Botany*, 2002:53(368):447-453.
- [19] Liang YC. Wong JWC, Wei L. 2005. Silicon-mediated enhancement of Cadmium tolerance in maize (*Zea mays L.*) grown in Cadmium contaminated soil. *Chamosphere* 58:475-483
- [20] Song, A., Li, Z. Liang, Y. 2009. Silicon-mediated alleviation of Cadmium toxicity in roots of *Brassica chinensis* is mainly attributable to Silicon-enhanced antioxidant defense capacity and Silicon suppressed oxidative damages. UC Davis. The proceedings of the International Plant Nutrition Colloquium XVII. Retrieve from: <http://www.escholarship.org/UC/item/94p7w2cn>
- [21] Zhang CC, Wong LJ, Nie Q, Zhang WX, Zhang FS . 2008. Long-term effects of exogenous silicon on Cadmium translocation and toxicity in rice (*Oryza sativa*) *Environ. Exp. Bot.* 62:300-307.
- [22] Redjala, Tanegmart, Streckeman, Thibault & Moral, Jean Louis. 2009. Influence of plant cadmium content on root cadmium uptake. UC Davis. The proceedings of the International Plant Nutrition Colloquium XVI. Retrieve from: <http://www.escholarship.org/UC/item/5xw4q4pm>
- [23] Niu, Z., Sun L., Sun T, Li Y.S. & Wang, H. 2006. Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture.