

**THE EFFECT OF TEA RESIDUE IN PROMOTING PHYTOREMEDIATION OF
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ABSTRACT: Numerous studies on soils have shown that toxic heavy metal, particularly Lead and Cadmium concentration is increasing in the soil around the world. Phytoremediation as a friendly and low cost environmentally method is a suitable remediation strategy. Due to vast medicinal benefits and the easy conditions for growing lavender plant we chose it for cleaning –up the soil. In order to find out the effect of residue of black and green tea in the soil for phytoremediation potential of *L.angustifolia* remaining infusion of green and black tea leaves were added to the composite soil. Mixed Composite soil samples were studied by two different conditions: *Lavandula angustifolia* grown and not grown in it by different pHs after every 10 days. Lead and Cadmium in old and young leaves, roots and soil samples were studied by Flame Atomic Absorption every ten days during two months. Results revealed that young leaves have more potential to absorb lead and cadmium during first days of growing ($p \leq 0.004$). Translocation factor in all conditions were higher than one which indicates that metal concentrations in shoots were higher than roots and the plant is suitable for phytoremediation. The heavy metals uptake rate by this plant is significantly affected by the age of plant cultivated as for lead uptake ($p < 0.003$), while for Cd the p -value was 0.01. *L. angustifolia* plants in infusion green and black tea leaves in soils samples had more potential (25.3%) to uptake and concentrate heavy metals than conventional soil. A positive correlation was found between Pb and Cd levels in root of plants and in the tea added soil in all days.

Key Words: *Lavandula angustifolia*, Phytoremediation, Lead, Cadmium, soil, Tea residue.

INTRODUCTION

Numerous studies on soils have shown that heavy metal, particularly Lead and Cadmium concentration is increasing. According to a report from Department of the Environment Tehran, I.R. Iran in July 2005, some significant sources of Lead and cadmium are: Nickel-Cadmium Batteries, Cadmium pigments, Cadmium stabilizers, Cadmium Coating, fossil fuels, cement, phosphorous fertilizers, Lead batteries, Glasses & ceramic industries, paint manufacturers, Cadmium electronic compounds, Metal plating, Factories with the process of extraction, production and concentration of Lead ore, Industrial wastewater, solid waste and Municipal waste waters [1]. Therefore, the vast industrial waste materials and sewages from a lot factories and different chemical fertilizers and pesticides in Tehran have caused contamination of soils [2]. High persistence and the dynamics of heavy metals such as Cadmium, contribute to hazardous factors both in the solution phase and available soil phase, as observed in plants. Their introduction to plants and the food chain endangers human health and animal life. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological features. For example, Cadmium is considered nonessential for living organisms [3]. The information presented in some research studies has described plants not only as source of food, fuel, and fiber, but also as environmental counterbalances to industrial pollution [4].

Phytoremediation is the most emerging field of environmental biotechnology and is an environment friendly technique to remove heavy metals from contaminated soil or water, which involves the extraction of metals by plant roots and then translocation to the shoots [5,6]. The most of plant roots have natural ability to absorb the heavy metals of the soil, behaving as natural phytoextractors [7,8]. Phytoextraction is the use of plants to remove contaminants from soil by accumulation in plant tissue and this is a promising clean-up technology for a variety of metal containing soil [9-11]. The choice of a suitable remediation strategy depends on many factors, one of which is the degree of the risk presented by metal-polluted soils. The commonly used physicochemical analyses of soil metal content are not representative enough for risk evaluation, as they do not directly address biological availability and metal toxicity. This prompted the development of biological evaluation tests, including microbial tests for the rapid detection of heavy metal bioavailability [12], which are complementary to the former chemical analysis. In addition, several plant-based tests have been developed for monitoring soil metal phytotoxicity [13,14] but further optimization has also been suggested [15]. In general, a metal phytoextraction protocol consists of the following elements: (1) cultivation of the appropriate plant/crop species on the contaminated site, (2) removal of harvestable metal-enriched biomass from the site [16], and postharvest treatments (i.e., composting, compacting, thermal treatments) to reduce volume and/or weight of biomass for disposal as a hazardous waste, or for its recycling to reclaim the metals that may have an economic value [17]. In Iran many vegetables and crops contaminated by lead and cadmium [18-23] therefore finding friendly and low cost environmentally method seems essential. Phytoextraction is a potentially effective remediation strategy for chemicals and heavy metals at contaminated sites where removal or treatment is not practical in other ways due to costs and time needed. For phytoextraction to be effective, we need vigorously growing plants (>3 tons dry matter/acre-yr), an easily harvestable aboveground portion, and a plant that accumulates large amounts of metals (~ 1000 mg/kg) in aboveground biomass. To achieve clean-up within three to five years, the plant must accumulate about ten times the level in soil (for example, if the level in soil is 500 mg/kg, then the concentration in the plant must be almost 5000 mg/kg to clean-up the soil in a few years). Many members of the botanical family Lamiaceae is grown in Iran not only for medicinal benefits and applications but also as an ornamental plant for aromatic parks and garden design as well as the wide application of lavender essential oil in pharmaceutical and fragrance industries [24]. Lavender produces well on soils that are nutrient deficient for most other crops. Lavender is a perennial, bushy shrub growing 0,3 to 1,2 m high. True lavender has a compact and rounded growth form. There are 48 species of lavender with hundreds of various genotypes differentiated by variations ranging from growth form to chemical composition of essential oil [25]. There are three main species within the genus producing lavender essential oil: *L. angustifolia* (true lavender, English), *L. latifolia* (spika, broad leaves), *L. angustifolia* x *L. latifolia* (lavandin) [25]. Lavender is native to the Mediterranean region south to tropical Africa and east to India. Nowadays the cultivated forms are planted in gardens world-wide and so they are occasionally found growing wild, as garden escapees, well beyond their natural range. Around fifty distinct species of lavender grow throughout the world, including *lavandula vera* and *lavandula officinalis*. Within this family the genus *Lavandula* comprises 30 known species among which three are economically important: *Lavandula angustifolia*, *Lavandula latifolia* and the hybrid lavandin *L. angustifolia* x *L. latifolia* [26,27]. Pharmaceutically, this plant and its preparations have long been used for carminative, antispasmodic, antidepressant, expectorant, anti-rheumatic, relaxant, sedative, anti-inflammatory and tonic properties [28-32]. Due to vast medicinal benefits and the easy conditions for growing lavender plant we chose it for cleaning –up the soil. The aims of this research were to

- 1- Cleaning Up Contaminated Soil by *Lavandula angustifolia* Mill and their potential ability of to phytoextract Heavy metals (Lead and Cadmium).
- 2-Compare of the soil pH and the phytoextraction rate based on different growth stages of the plant.
- 3- Determine metal transfer factors from soil (TFS) of *Lavandula angustifolia* Mill.
- 4- Study the effect of adding black and green tea leaves in soil and the metal transfer factors.

MATERIAL & METHODS

Soil sampling

A composite soil sample (5000 gr) was collected from depth of 0-35 cm from a yard in the center of Tehran in order to simulate the conditions of soils in the contaminated lands with industrial sewages. 30, 10, 5, 30 and 30 mM/L of $Pb(NO_3)_2$, $Cd(NO_3)_2$, $CaHPO_4$ and K_2SO_4 respectively. In order to find out the effect of residue of black and green tea in the soil for phytoremediation potential of *L. angustifolia* 1000 gram of remaining infusion of green and black tea leaves were added to the composite soil. Mixed Composite soil samples were studied by two different conditions: *Lavandula angustifolia* grown and not grown in it by different pHs after every 10 days. Metal contents were detected by Atomic Absorption Spectrophotometer by wet digestion method in Research Laboratory in Pharmaceutical Sciences Branch University. At the beginning of study, soil profile characteristics were observed and recorded by a packet penetrometer (CI-700A, soil Test Inc., USA).

Soil samples were mixed, homogenized and separated into three parts, 1/3 of each samples was air-dried and pass through a 2 mm sieve in order to determine p and k content, pH and electrical conductivity and particle-size distribution. The other 2/3 was passed through a 2 mm sieve without drying and 1/3 of it used to determine heavy metals concentration by Atomic Absorption Spectroscopy (AAS) after digestion with aqua-regia. The samples were analyzed by an Atomic Absorption Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals: Pb and Cd using at least three standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines [33].

Sampling method

Plants of 'true lavender' (*L. angustifolia*) were purchased at a local market in Tehran and grown indoors. The plant identified by Dr. Gholamreza Amin, (Department of Pharmacognosy) as *Lavandula angustifolia*. Lavender. Standard growing conditions were maintained at 23-25 °C and the light from OSRAM FLUORA® fluorescent lamps L 30 W/77. Different parts: shoots and roots of 50 greenhouse –grown *Lavandula angustifolia* in the same situation were studied in summer, autumn and winter 2013. Samples were watered each day by tap water (Tehran tap water). The studied samples were managed by the same light situation and some circumstances in order to be compared with each other due to determine the ability of *Lavandula* in phytoextraction of Lead and Cadmium from soil. The soil by different pH (5.9 up to 6.9) put into 50 vases (65 cm x 45 cm) and samples were grown in eight examined soils (by tea residue and without tea residue) and no plants were grown in two others as they have been considered as control group in soils, as the same procedure in the other reports of scientists who have investigated the effects of soil acidification on Cadmium phytoextraction [7,8,34]. As soil acidification might cause some negative side effects such as increasing solubility of some toxic metals and leaching them into the groundwater and creating another environmental risk. Therefore, at the beginning of study, we tried to control pH at the range of 5.9 up to 6.9 in samples of soils. Different parts of *L. angustifolia* Plant samples (shoots, roots and leaves) were separated and washed and digested by wet method according the standard protocol for measuring Cadmium and Lead. Mean values were calculated, and analysis of variance (ANOVA) and Student's t-test were performed. Bioaccumulation factors (BAF-s) were calculated for heavy metal contents of plant parts (mg/kg) / heavy metal content of soil (mg/kg), for each metal. The uptake rate is given by the following equation [35, 36, 37].

$$U = (\text{TSCF}) (T) (C) (1)$$

Where U = uptake rate of contaminant, mg/day

TSCF = transpiration stream concentration factor, dimensionless

T = transpiration rate of vegetation, L/day

C = aqueous phase concentration in soil water or groundwater, mg/L.

The last port used to determine nitrate and ammonium 2M KCl extraction followed by determination using flow injection method. All the soil data are expressed on a dry basis. The soil by different pH put into eight vases and samples were grown in six examined soils and no plants were grown in two others as they have been considered as control group in soils, as the same procedure in the other reports of scientists who have investigated the effects of soil acidification on Pb and Cd phytoextraction [34, 38]. As soil acidification might cause some negative side effects such as increasing solubility of some toxic metals and leaching them into the groundwater and creating another environmental risk. Therefore, at the beginning of study, we tried to control pH at the range of 5.9 up to 6.9 in samples of soils. Physical and chemical properties and concentrations of heavy metals in soils before and after adding Cadmium and Lead and also without tea residue after the growth period measured. In order to assess amount of heavy metals transfer from soil to plant (shoot and root), translocation factor was determined by dividing metal concentration at shoot by its concentration at root. The ratios were higher than one it was considered as suitability of plant at that condition for use in phytoremediation.

RESULTS

Chemical extraction of the soil profile before adding specified amounts of heavy metals is shown in the table 1 and electrical conductivity and nitrate content in different layers is indicated in table 2. Data is averages of the profiles.

Table 1- Physical and Chemical properties of studied soil before adding Lead and Cadmium and cultivated *L. angustifoli*

Characteristic	Quantity	Characteristic	Quantity
Soil Texture	Loam	Pb (mg/kg DW)	1.9805
Clay (%)	24.53	Cd (mg/kg DW)	0.0967
Silt	37.11	Zn (mg/kg DW)	1.0065
Sand (%)	38.36	Cu (mg/kg DW)	6.3210

Table 2- The characteristics of soil samples comparing with their depth and pH

Layer (depth cm)	pH (H ₂ O)	Electrical conductivity dS/cm 1:1	NO ₃ -N mg/kg DW	NH ₄ -N mg/kg DW
1 (0-15)	6.6	0.47	65.2	9.84
2 (15-35)	6.7	0.27	26.7	8.83

Plant availability of Lead and Cadmium depends on soil properties such as soil pH and contains exchange capacity and on the distribution of metals among several soil fractions. Results indicated that the rate of heavy metals uptake by this plant is significantly affected by pH ($p < 0.003$). Translocation factor in all conditions were higher than one which indicates that metal concentrations in shoots were higher than roots and the plant is suitable for phytoremediation. The maximum Cadmium uptake rate was in pH = 6.3 and by 30 day *L. angustifolia* while for lead the best situation is pH = 6.5 and after 20 days of growing. Figure 1, 2, 3 and 4 show the lead and cadmium content in soil, old and young leaves. Young leaves have more potential to absorb lead and cadmium during first days of growing ($p < 0.004$). The heavy metals uptake rate by this plant is significantly affected by the age of plant cultivated as for lead uptake ($p < 0.003$), while for Cd the p -value was 0.01.

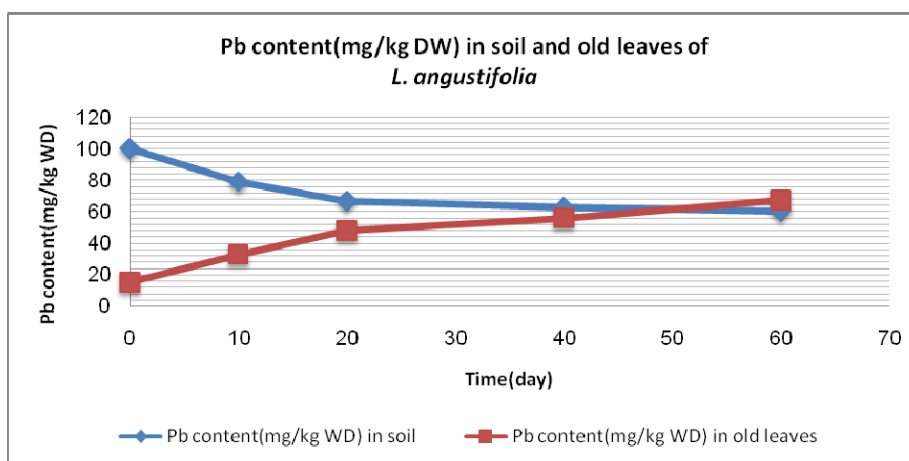


Figure 1-Lead Content (mg/kg DW) in soil and old Leaves of *L. angustifolia* during 60 days of growing in tea residue added in contaminated soil

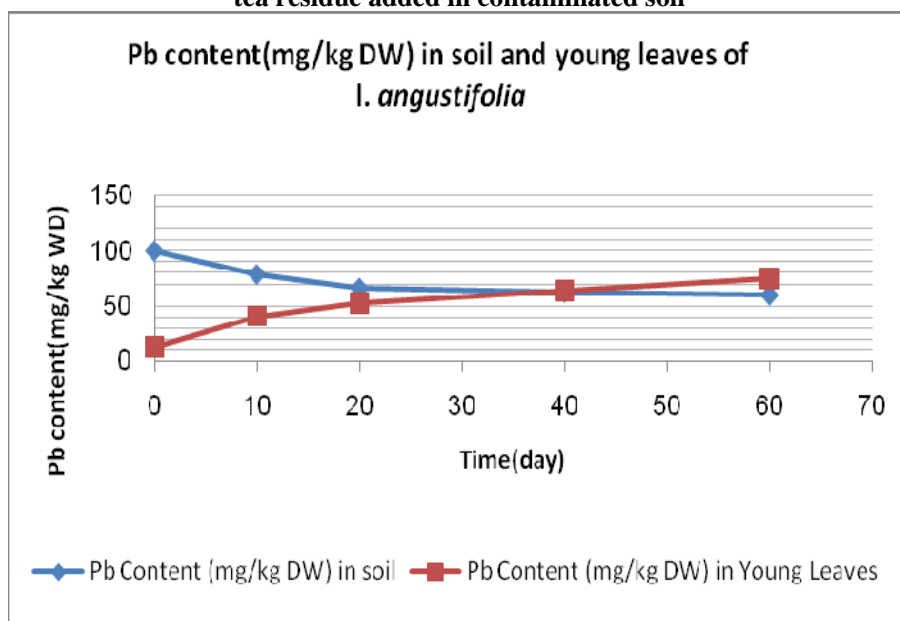


Figure 2-Lead Content (mg/kg DW) in soil and young Leaves of *L. angustifolia* during 60 days of growing in tea residue added in contaminated soil

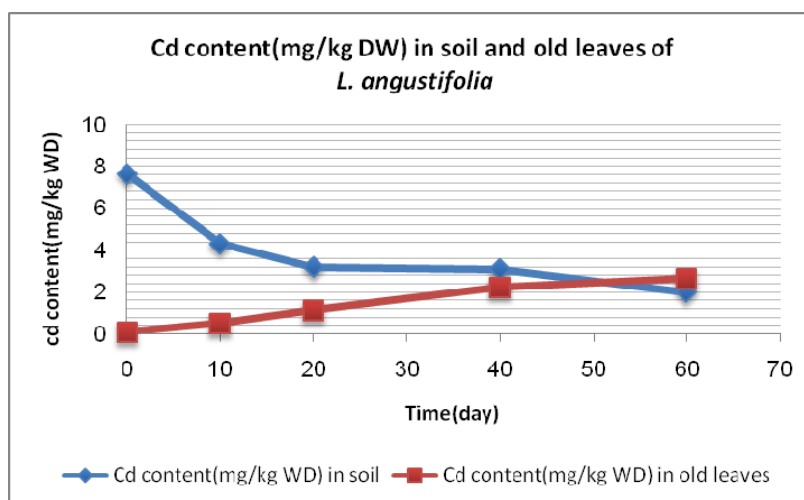


Figure 3-Cadmium Content (mg/kg DW) in soil and old Leaves of *L. angustifolia* during 60 days of growing in tea residue added in contaminated soil

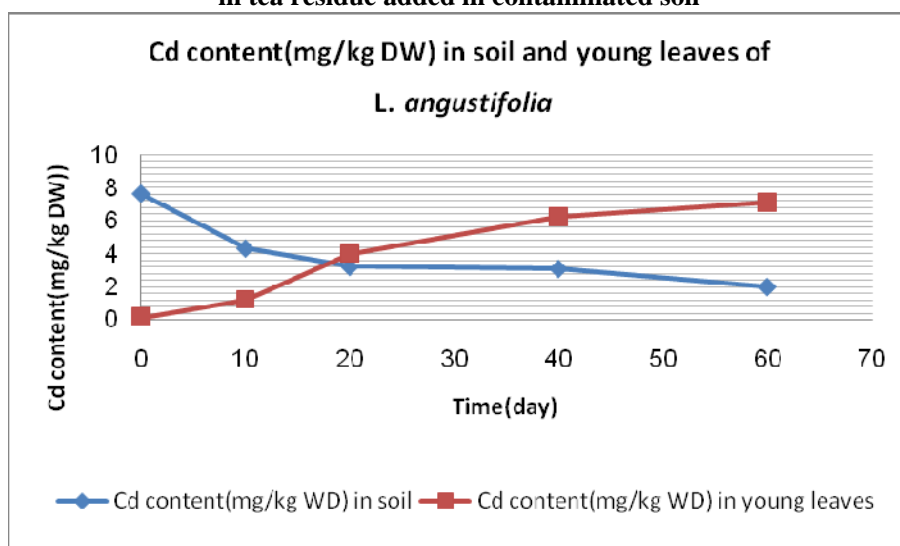


Figure 4-Cadmium Content (mg/kg DW) in soil and young Leaves of *L. angustifolia* during 60 days of growing in tea residue added in contaminated soil

The Cadmium uptake rate by this plant is significantly affected by tea residue added in the contaminated soil ($p < 0.005$), in table 3 the conventional contaminated soil compared by tea residue added soil.

Table 3- Comparing Cadmium content in conventional soil and tea leaves added soil *L. angustifolia* grown

Time (day)	Cd content (mg/kg DW) in conventional soil	Cd Content (mg/kg DW) in tea leaves added soil
0	7.6654	7.3298
20	4.3389	3.9906
30	3.2207	3.112
40	3.1076	2.4537
60	2.0078	1.6301

L. angustifolia plants in infusion green and black tea leaves in soils samples had more potential (25.33%) to uptake and concentrate heavy metals than conventional soil (figure 5). In order to find out in which time of growing, root of plant has more potential to absorb heavy metals, all studied times are compared in figure 6 for up-taking from soil during 60 days of this research. The maximum lead uptake rate was in 40 day *L. angustifolia* growing.

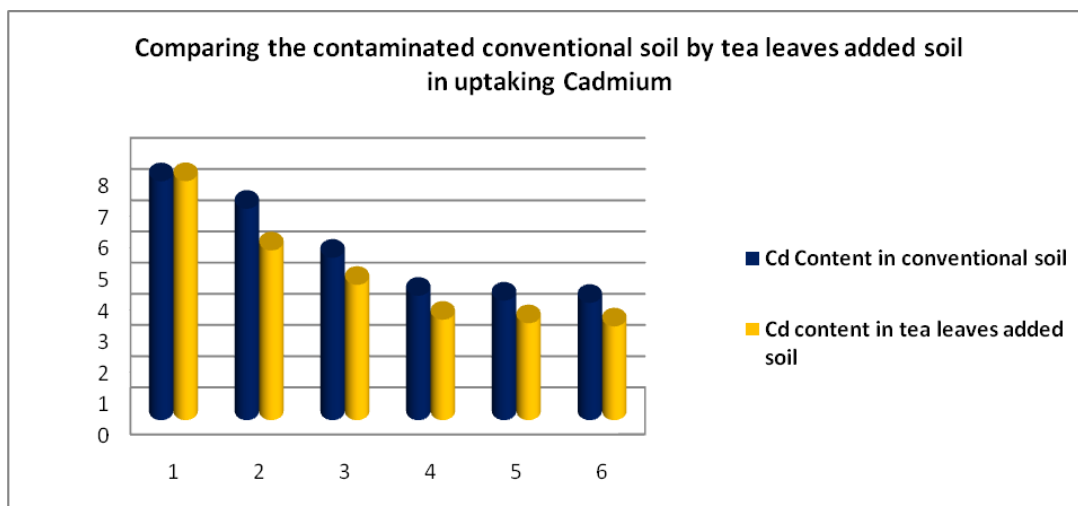


Figure 5- Comparing Cadmium up-taking potential of *L. angustifolia* growing in different condition of soil (Conventional and tea residue added).

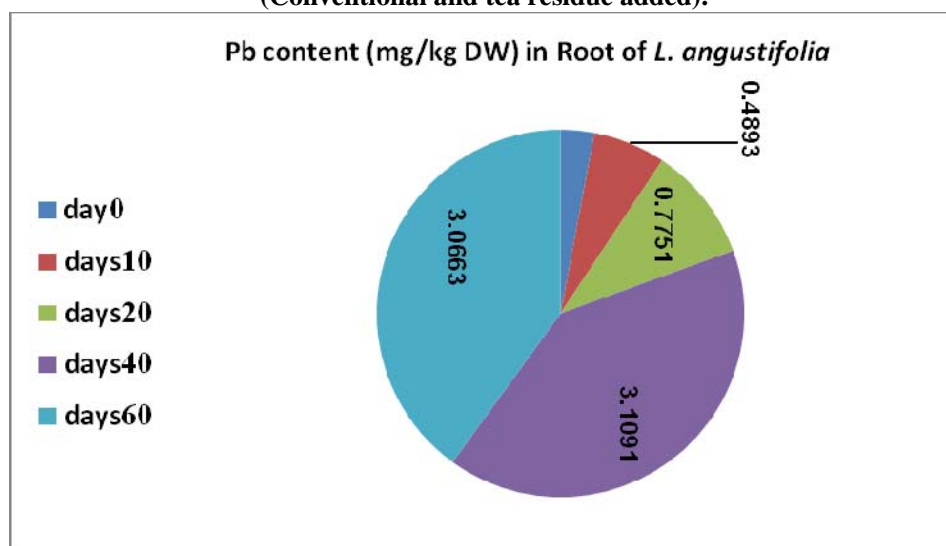


Figure 6- Lead absorbed content (mg/kg DW) by root of plant during 60 days of study.

CONCLUSION

A positive correlation was found between Pb and Cd levels in root of plants and in the tea added soil in all days. Lavender accumulated Pb from the entire soil profile proportionally to its level in the soil and Pb contamination did not reduce the plant biomass. This confirms that by adding some organic matters even those which have no other important applications such as tea residue, the mobility of heavy metals from soil in roots and leaves of plants could be increased.

As heavy-metal uptake by plants is a complex process and in other studies have been mentioned that, influenced by numerous factors that interact with one another and the availability and mobility of metals in the soil [39-41], the addition of tea residue in soil-particle and significantly increase in lead and cadmium contents in acidic pH (6.3-6.5) which are believed that the reason is binding the heavy metals to soil particles. This study revealed that the soil pH and the organic matter content are considerable metal factors in the soil and enhance transition factor. This implies that by controlling soil nutrients and other factors such as pH, metal removal by plants would be easily accomplished and could be utilized for better condition for phytoextraction and survive environment. Although long-term monitoring and evaluation of phytoremediation technology for other species is still needed to demonstrate efficacy, to further define suitable plants and applications, and to gain acceptance from regulatory agencies.

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