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LEAD ACCUMULATION IN DIFFERENT PARTS OF OKRA PLANT (*Abelmoschus esculentus*)

Nguyen Minh Hung, Nguyen Viet Hiep, Bui Ngoc Dung and Nguyen Xuan Hai
VNU University of Sciences, Nguyen Trai, Hanoi, Vietnam
E-Mail: nguyenxuanhai@hus.edu.vn

ABSTRACT

This paper presents experimental result of lead (Pb) affect on okra growth, yield and accumulation in different parts of plant. Results show that Pb concentrations in soil in the range of 16.13 ppm - 350 ppm positively affect okra plant height, yield constituents and yield. Concentration of Pb in roots leaves and fresh fruit of okra plant was in proportion to the concentration of Pb in soil. Lead was accumulated mainly in the roots of the okra plant, then in leaves and fruits. Especially, the concentrations of Pb in fresh okra fruits (eatable part) were lower than allowable limits. The okra plants can be used as multi-purpose trees for Gray Soil with Pb concentrations below 210 ppm.

Keywords: lead accumulation, okra, multi-purpose plant.

INTRODUCTION

Lead and compounds of Pb is very harmful to the human body and animals. Lead enters the human body through drinking water, air and especially Pb contaminated food. WHO statistics show that approximately 600, 000 cases annually retardation in children due to lead poisoning. It is worth noting that 99% of children have been infected by lead in low and moderate-income countries.

Although Pb is not an essential element for plants but easily up-taken and accumulated in different parts of the plant (Pallavi Sharma *et al*., 2005) [1]. Pb uptake by plants depends on many factors such as pH, soil characteristics, cation exchange capacity of the soil, as well as other physico-chemical parameters and plant varieties.

In recent years, the lead content of vegetable soil in Southeast region of Vietnam tends to gradually increase, especially in areas near the industrial parks. Some monitoring sites showed that Binh Chanh district soil reached 74.98 ppm, District 12 - 64.44 ppm, Thu Duc - 58.77 ppm, Hooc Mon - 49.25 ppm. while the study results of Bui Cach Tuyen *et al.* (1998) pointed out that the tight correlation between Cu, Zn, Cr, Pb and Cd contents in soil and heavy metal content in crops [2].

In Vietnam hardly apply the model to treat contaminated soil with heavy metals as developed countries in the world. The use of plants to treat contaminated soil gives better results (Vo Van Minh, Vo Chau Tan, 2005) [3]. However, using plant to treat with heavy metals are not acceptable by farmers because of using a wild species in the field usually takes a long time and farmers can not get benefit from their soil at that time.

The studies of the world have confirmed that lead in particular and heavy metals in general, accumulated in plant biomass in the different parts differently. They tend to be high or very high accumulation in root sections, lowered in stems, branches, leaves, flowers and fruit... Thus, if we can choose the vegetable, flower or fruit accumulates lead high in unused parts, and low in edible parts will have practical significance, that plant so-called multi-purpose. Following this research, entitled theme "Application of biological methods solves heavy metal pollution in soil and water for vegetable growing areas in eastern and southern Mekong Delta" was discovered some species are capable of use as one multi-purpose tree, they were: *Limnocharis flava*, Okra (*Abelmoschus esculentus)*, *Psophocarpus tetragonolobus* and *Colocasia gigantean*.

Okra *(Abelmoschus esculentus)* is one of vegetable to be grown much in the Southeast region and the Mekong Delta with growth duration of 100 days or less, productivity gains 22-25 tons/ha, effective high economic (net interest on 60 million/ha), easy to grow, large biomass (130-180 ton/ha), easy to harvest biomass for burning or processing. Most of the area cultivated okra (*Abelmoschus esculentus)* in particular and vegetables in general are on Gray Soils of Southeast and Central Highlands regions (Nguyen Manh Chinh, Pham Anh Cuong, 2007) [4].

This experiment was conducted for assessing the level of lead accumulation in different parts of the okra at various assumed concentration of lead and proposing okra as a multi-purpose vegetable on lead contaminated soils.

MATERIALS AND METHODS

Materials

- **Plant:** Okra (*Abelmoschus esculentus)* - VN1 variety, providing by Southern Seed Company (SSC).
- **Soil:** Gray Soil with pH = 4.7. Humus content is 1.4%, Total nitrogen content - 0.13%, total phosphorus - 0.04%. Potassium content - 0.21% and CEC at 3.63 meq/100g soil.
- **Lead:** $Pb_3\ (CH_3COO)_2$ solution was added to the soil according to the experimental treatments.

Methods

The experiment was conducted in greenhouse of Soils and Fertilizers Research Institute (Vietnam), consisting of 4 treatments, each treatment has 5 metal pots, dimensions of 20cm x 20cm x 20cm, each pot containing 7 kg of soil.

Treatment-1 (CT1): The control - the concentration of lead is 14.00 ppm in soil
Treatment-2 (CT2): lead added to 62.11 ppm
Treatment-3 (CT3): lead added to 215.88 ppm
Treatment-4 (CT4): lead added to 371.56 ppm

Fertilizers are used as follows: 0.15 N, 0.15 P_2O_5, 0.08 K_2O per 1 kg dry soil.

Parameters and monitoring methods

Height of okra plant before flowering: Use a ruler to measure from the stump to the top of the longest leaf. Periodically measure the height of trees each 10 days.

Length and diameter of okra fruit: Use Palme clamp meters with accuracy of 0.2 mm.

Fruit weight: Harvest and weight after 9 days flowering

Total lead content in soil: TCVN 6496, ISO 11047:1995.

Lead content in parts of the plant: AAS - Atomic Absorption Spectrophotometric. Destruction of plant sample by wet ash method at 550°C.

Data processing

The data were processed by statistical one -way ANOVA analysis.

3. RESULTS AND DISCUSSIONS

3.1. Effect of different lead concentrations on the growth and development of Okra

3.1.1. Effect of lead content to the height and growth of Okra

The height is one of the important indicators to evaluate the growth of okra plants in Pb different concentration levels in soils. In addition to tree height depends on the genetic characteristics of the variety; plant height was more concerned with environmental conditions such as climate, soil, fertilizer, water.

The study results (Table-1) show that at the time of 10 days and 20 days after germination, the higher lead levels in soil was, the shorter plant height tended. However, at day 30 and later, the concentration of lead in soil at 62.11 ppm (CT2) and 215.88 ppm (CT3) has a stimulating effect on plant height compared to the low concentration of lead (14.00 ppm) as well as much higher (at 371.56 ppm).

Table-1. Effect of Pb different concentrations in soil to okra plant height.

N	Treatments	**Okra plant height** (cm) **at different stage**					
		10 days	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
1	CT 1	$19.90^a \pm 0.54$	$27.64^b \pm 0.62$	$36.58^a \pm 0.92$	$46.87^a \pm 1.11$	$55.33^a \pm 0.81$	$62.7^a \pm 0.96$
2	CT 2	$19.01^a \pm 0.75$	$26.34^{ab} \pm 0.75$	$36.24^a \pm 0.94$	$47.00^a \pm 1.21$	$56.47^a \pm 1.21$	$63.57^a \pm 1.66$
3	CT 3	$18.26^a \pm 0.64$	$25.32^{ab} \pm 0.51$	$38.07^a \pm 1.45$	$51.67^a \pm 2.34$	$62.10^a \pm 1.94$	$68.75^a \pm 2.27$
4	CT 4	$17.44^a \pm 0.58$	$24.10^a \pm 0.67$	$34.84^a \pm 0.37$	$48.50^a \pm 1.00$	$56.76^a \pm 1.82$	$62.6^a \pm 2.29$

Note: The values in the same column have the same letters, the difference was not statistically significant (α = 0.05).

Investigations on growth of okra plants, the results (Table-2) show that they are quite similar trend compared to the height of the tree. After 10 and 20 days of germinating, Pb higher level reduces the growth rate compared to control. To the 30th day, the rate of growth of okra plant change significantly when compared to control. The highest growth rate at CT2 and then at CT3. In 40 days time, the growth rate of plant height reaches a maximum value in all treatments, and reached the highest value was 1.34 cm^3/day (CT3). In particular, according to the One -way ANOVA analysis, growth rate in height after 40 days time difference statistically significant at α = 0.05 in all treatments.

Table-2. Effect of Pb concentration to growth height.

N	Treatments	**Rate of growth height** (cm/day) **at stage**					
		10 days	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
1	CT 1	$0.65^b \pm 0.05$	$0.77^a \pm 0.02$	$0.89^a \pm 0.04$	$0.95^a \pm 0.01$	$0.83^a \pm 0.05$	$0.74^a \pm 0.02$
2	CT 2	$0.56^b \pm 0.02$	$0.73^a \pm 0.05$	$0.99^a \pm 0.04$	$1.07^{ab} \pm 0.07$	$0.87^a \pm 0.09$	$0.71^a \pm 0.05$
3	CT 3	$0.39^a \pm 0.02$	$0.71^a \pm 0.02$	$1.27^b \pm 0.1$	$1.34^{bc} \pm 0.09$	$1.06^a \pm 0.05$	$0.68^a \pm 0.11$
4	CT 4	$0.36^a \pm 0.09$	$0.67^a \pm 0.02$	$1.07^{ab} \pm 0.04$	$1.41^c \pm 0.09$	$0.97^a \pm 0.06$	$0.58^a \pm 0.07$

Note: The values in the same column have the same letters, the difference was not statistically significant (α = 0.05).

As known, lead is not the essential element for plants. Lead affects the activity of many enzymes with different metabolic pathways. At high concentrations, Pb inhibits the activity of the enzyme. Besides, some of the enzymes are more active in the presence of Pb. In addition, Pb promotes the formation of oxidative reactions in plants, causing plant stress, leading to increase activity of antioxidant enzymes. Most of the cases, Pb negatively affect the photosynthesis process of plants (Burzynski, 1987) [5]. However, at low concentration, Pb is considered to promote the synthesis of chlorophyll in the leaves.

These results are also consistent with the announce of Vo Van Minh, Vo Chau Tan (2007) [6], Luong Thi Thuy Van (2012) [7], about Pb absorption of vetiver in artificially contaminated soil (adding $PbCl_2$ into the soil). At a certain level, it will be toxic to plants through inhibition of the physiology of plant growth. The cause of this phenomenon may be due to the addition of Pb salts in the soil, in the early stages of the growth process, the plant was shocked by Pb in soil leads to growth in plant height in treatment added more Pb lower than treatments without additional Pb - CT1. After some time, the okra gradually adapt to the environment, plant growth and gradually rising higher than CT1 (Control). That means, in the scope of this study, Pb capable of promoting okra growth. However, when prolonged period of growth, the rate of plant growth in the CT 3 and CT 4 declines, due to the gradual accumulation of Pb in okra and Pb inhibits its growth process.

3.1.2 Effect of soil lead concentrations in the different constituents of okra yield

Effect of Pb concentrations to fruit size (length and diameter of fresh fruit): The impact of Pb concentrations in soil to the length and diameter of okra fruits (Table-3) show that in the 3 high lead levels (62.11 ppm, 215.88 ppm, 371.56 ppm), the length and diameter of okra fruit is not affected (reduced), but increases compared to control (14.00 ppm). The length and diameter of the largest okra at the concentration of lead in soil is 215.88 ppm (CT3), then the concentration of 371.56 ppm (CT4) and 62.11 ppm (CT2). Thus, the upper limit on the concentration of lead in soil at 371.56 ppm did not affect the length and diameter of okra fruit.

3.1.3 Effect of Pb concentration to the average weight of fresh okra fruits

Weight of fresh fruit has an important role to show the efficiency of plant okra photosynthesis. Fresh weight depends on many factors such as seed, fertilizer, and cultivation conditions, weather etc.

Experimental results indicate that in the experimental conditions, 3 concentration levels of lead (62.11 ppm, 215.88 ppm, 371.56 ppm), in excess of substrate concentration (14.00 ppm), will not reduce the average weight of fresh fruits/okra plant, on the contrary, they stimulate to increase the weight compared to control, respectively 11.48%, 34.90% and 29.26%. At 215.88 ppm lead concentration is most effectively increasing the weight of okra. At 371.56 ppm, average weight of fresh fruit despite reduced but still higher than the control.

So, 3 levels of Pb concentrations in soil - 62.11 ppm, 215.88 ppm and 371.56 ppm significantly influence on the growth and fresh weight of okra plant. The size of okra fruit increased to 7.82%, 17.84% and 13.69%, respectively compared to control. Meanwhile, the weight of fresh fruit increased, respectively, 11.48%, 34.9% and 29.26% compared to control. It indicates, Pb concentrations in soil range from 0 - 350 ppm had a positive impact on productivity of plants with 2 criteria are the length and weight of fresh fruit. However, if the concentration of Pb in soil is higher than 350ppm, the effect is slowing down and lower.

3.2. Effect of different concentrations of lead in soil to lead accumulation in plant parts of okra (A. *esculentus* L.)

3.2.1. Pb accumulation in leaves and roots of the Okra plant

Results analyzed Pb concentrations in leaves and roots of okra tree showed that Pb accumulation in plant parts different varying with the lead levels in soil. Pb concentrations in the study range, levels of Pb accumulation in roots, trunk and leaf seem in proportion to the total Pb content in the soil. The amount of Pb applying to the soil rapidly increased Pb accumulation in plants. Pb content in roots of okra plants increased over 2.7 times, 3 times and 4 times the concentration corresponding to 62.11 ppm (CT2), 215.88 ppm (CT3) and 371.56 ppm (CT4) than at natural soil level in the control - 14.00 ppm (CT1). Pb content in roots the lowest cumulative at 100.3 ppm in control formula (CT1) and the highest value at 411.63 ppm with highest concentration of Pb added to the soil (CT4). Similarly, the relative concentration of Pb in okra leaves increased 3.8 times, 5 times, and nearly 8 times the concentration corresponding to 62.11 ppm (CT2), 215.88 ppm (CT3), and 371.56 ppm (CT4) compared to control. Pb content in leaves accumulates lowest in CT1 (10.35 ppm), while highest - at CT4 (82.02 ppm). Experimental results showed that at concentrations below 62.11 ppm Pb, the ability to uptake and accumulate Pb in the tree increases, but at the concentration higher 62.11 ppm this ability somewhat reduces.

Easy to find, levels of Pb accumulated mainly in roots (from 100.31 to 411.63 ppm), a small fraction is transported to trunk and leaf (from 10.35 to 82.02 ppm) (Table-3). This shows clearly that, although Pb is not an essential element for plants but Pb was easily absorbed and accumulated in the plant parts. But Pb in particular and other heavy metals in general, tend to be higher accumulated in roots than in other parts of the plant. After sucking into the tree roots, Pb will stay in roots than many other parts of the tree. Because Pb has a close association with the carboxyl group of galacturonic carbohydrate and glucuronic acid in cell walls, this has limited the transport of Pb through the cell wall. In addition, the transport of Pb from the roots to the other plant organs is limited by the barrier of endothelial cells of the roots [8].

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Table-3. The accumulation of Pb in the leaves and dried root of the okra plant at different concentrations of Pb.

N	Treatments	In root (ppm)	In leaves (ppm)
1	CT 1	100.31[a] ± 2.29	10.35[a] ± 0.89
2	CT 2	266.84[b] ± 6.54	39.41[b] ± 2.02
3	CT 3	318.57[c] ± 5.27	51.35[b] ± 3.71
4	CT 4	411.63[d] ± 19.65	82.02[c] ± 5.02

Note: The values in the same column have the same letters; the difference was not statistically significant ($\alpha = 0.05$)

3.2.2. Effect of concentration of Pb in soil to Pb accumulation in okra fruit

With the aim of finding the multi-purpose trees, so analysis Pb content in products is indispensable. With the okra plant, eatable part is fruit.

Analyzed results of Pb concentrations in fresh okra fruits (Table-4) show that the concentration of Pb in soil affects the ability of Pb accumulation in okra fresh fruit. Pb accumulation levels in fresh fruits of CT1, 2, 3, 4 in proportion to the concentration of Pb in soil and Pb concentrations accumulate of CT2, 3, 4 increase respectively compared to CT1 (control) 121.7%, 140.2% and 292.9%, respectively. Pb content is the lowest accumulated 0,033 mg/kg fresh material in CT1 (control) - not adding Pb and Pb concentrations are highest accumulation in CT4 (highest pollution levels are 350 ppm) 0.097 mg/kg fresh fruit.

Pb accumulation in green cabbage and salad Pb found in proportion to the concentration of Pb in soil by Nguyen Xuan Cu [9].

Based on national technical regulations to limit pollution of heavy metals in food (QCVN 8-1: 2011/BYT) [10], the maximum limit of Pb allowed in vegetables fruit is 0.1 mg/kg. Can be seen, the average levels of Pb in fresh fruit in CT1, CT2, CT3 are much lower than the maximum limit ($0.033 < 0.040 < 0.046 < 0.1$ mg/kg). Particularly, in CT4 with the highest pollution levels, the concentration of Pb in fresh nearly reaches the maximum limit issued by the Ministry of Health [10].

Result of the Pb accumulation in fresh fruits also showed that some of the sample of CT3, CT4, and the Pb content in fresh fruit exceeds the maximum limit allowed (Table-5).

So, with these areas contaminated soil Pb at severe level (> 210 ppm) should not plant okra for fruit because of the risk for human toxicity is very high.

Table-4. Pb accumulation in some okra fruit sample.

Treatments	Pb content in fresh fruit (mg/kg fresh weight)
CT 3- 1	0.183
CT 3- 2	0.122
CT 3- 3	0.135
CT 4- 2	0.105
CT 4- 4	0.118
CT 4- 4	0.145
CT 4- 2	0.330

3.3. Evaluate the possibility of using Okra (*A. esculentus L.*) as a multi- purpose plant

Multi- purpose plant must be able to grow and develop normally on contaminated soils, accumulate heavy metals from medium to high in parts considered as agricultural by-products, which are discarded after harvesting, but requires a low accumulation of heavy metals (below the permissible level) in the part that humans use as food (edible) or other purposes.

Vo Van Minh *et al.*, 2007 [6] reported that vetiver is capable of absorbing all of the heavy metals such as Cd, Zn, Pb, Cu but at low concentrations. However, due to its high biomass of total heavy metals are absorbed and removed from the environment is very large.

After 3 months of planting, vetiver absorbed from 0.05 to 0.23 mg Cd/10kg soil, from 19.78 to 39.51 mg Zn/10kg soil and from 0.28 to 5.87 mg Pb/10kg soil [6]. The use of vetiver to treat heavy metal contaminated soil solution is feasible but does not bring much economic value to farmers in some areas having Pb pollution levels not too high.

Nguyen Xuan Cu and others conducted the research uptake of Cu, Pb, Zn of green cabbage [9]. The results showed that when the amount of Pb in soil increased, the height and yield were significantly reduced but the Pb content in vegetables has increased. Pb contents in the green cabbage on polluted soil Pb concentrations at 100 ppm, 150 ppm, 200 ppm, respectively were 0.96 ppm, 1.67 ppm, and 1.79 ppm. Pb content in vegetables listed above excesses the prescribed safety limits allowed for Pb contamination in leafy vegetables (< 0.3 ppm; QCVN 8-1: 2011/BYT [10]). Thus, green cabbage only meets the requirements for the absorption of Pb but not satisfied with the Pb content in vegetables.

Meanwhile, through this research, okra plant has good growth on seriously Pb polluted soil (210 ppm and 350 ppm) and Pb concentrations of okra fruit were lower than permissible threshold (< 0.1 ppm), although at 350 ppm, the concentration almost reached permissible level (0,097 ppm). Thus, okra plant meets both 2 criteria of multi-purpose tree (bioremediation and giving edible fruit), can be grown on Pb contaminated soils at limit of Ministry of Natural Resource and Environment for agricultural land (70 ppm) [11] and severity (210 ppm).

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4. CONCLUSIONS

Pb concentrations in the range of 16.13 ppm - 350 ppm positively affect okra plant height, yield constituents and yield.

Increasingly the Pb concentrations from 14 ppm to 350 ppm in soil, concentration of Pb accumulation in roots, leaves and fresh fruit in proportion to the concentration of Pb in soil. Lead was accumulated mainly in the roots of the tree, then in leaves and fruits. Especially, the concentrations of Pb in fresh okra fruits (eatable part) were lower the permissible level (QCVN 8-1: 2011/BYT).

The okra plants can be used as multi-purpose trees for Gray Soil with concentrations below 210 ppm of Pb.

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