Accumulation and distribution of lead (Pb) in different tissues of Lucky bamboo plants (*Dracaena sanderiana*)

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ABSTRACT

Lucky bamboo plants (*Dracaena sanderiana*) were used to study the accumulation and distribution of lead (Pb) in tissues of root, stem and leaf, as well as the impact of lead accumulation on the anatomical structure of these tissues. *Dracaena sanderiana* plants were exposed to $Pb(NO_3)_2$ solution at the Pb concentrations of 0; 200; 400; 600; 800; 1,000; 2,000; 3,000 and 4,000 mg/L for 60 days. The results showed that the more the Pb concentration was used, the more the amount of lead was accumulated and deposited. The tolerance limit of *Dracaena sanderiana* was 800 mg/L of Pb in water. The lethal concentration for plants was 4,000 mg/L Pb. When the concentrations of Pb in the solution were higher than the tolerance limit of the plant, the growth of *Dracaena sanderiana* could be inhibited. *Dracaena sanderiana* could accumulate up to 39,235 mg/kg Pb in the presence of Pb at 800 mg/L. Lead was accumulated mainly in roots (97.5%) and deposited mainly in the cell walls and the spaces between cells in tissues of roots. In the stems and leaves of *Dracaena sanderiana*, lead accumulation was limited and distributed mainly around vascular bundles. Lead accumulation caused changes in the anatomical structure of root, stem and leaf tissues. The accumulation and distribution of Pb is mainly in the cell walls and the space of cells; it could be a detoxification mechanism for Pb of *Dracaena sanderiana*.

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1. Introduction

Today, the continuous development of science and technology has brought many economic benefits to humans. However, this also makes the environment more seriously polluted. In the world, the pollution of heavy metals, especially lead (Pb) pollution is becoming increasingly popular due to the massive development of human, agricultural and industrial activities (ATSDR, 1993). Lead is the most dangerous substance in the environment, and has caused deleterious effects not only to the environment, but also to the public's health (EPA, 2000). Thus, the search for Pb treatment methods has to be taken in priority in many countries.

Different methods of lead decontamination are available, the most commonly is physical chemistry. They can process the contaminated soil in-situ or ex-situ by using techniques involving chemicals, such as chelating and physical methods such as pumping or heating. Recently, a technique based on the use of plants has been developed for remediation of soil and water. This technique is well known "phytoremediation" that is less invasive and less expensive than the physical

and chemical techniques (EPA, 2000), but it involves the identification of plant species with particular capabilities. To be used in lead decontamination, the plants should tolerate the presence of high concentrations. In addition, they must be capable of accumulating the contaminant into their roots or in their shoots and have detoxifying mechanisms. However, these plant species were rather limited. Finding out plant species that represent its lead removal ability to reduce environmental pollution is one of the most interesting subjects towards scientists.

Recently, the research of Hao (2011) indicated a plant's ability in absorbing Cu, Ni, Hg, Cd and Cr for the process of phytoremediation, namely, Lucky bamboo plants (*Dracaena sanderiana*). Sereshi et al. (2014) had also shown that *Dracaena sanderiana* has the ability to grow well in garbage wastewater. However, the accumulation and distribution of lead by *Dracaena sanderiana* has not been researched yet.

Consequently, this study has been conducted to determine the accumulation and distribution of lead in *Dracaena sanderiana* plants as well as the impact of Pb on changes in the anatomy structure of those tissues.

2. Material and Methods

2.1. Plant materials and experiment

Lucky bamboo plants (*Dracaena sanderiana*) were collected, cut into sections about 45 cm, and were cultivated in greenhouse at Thu Dau Mot University, Binh Duong, Vietnam. Plants were cultivated in distilled water for 60 days in order to obtain homogeneous plants. Eighty one cloned plants selected by tree height, root length, number of leaves, and healthy condition, were transplanted to plastic pots containing 15 L $Pb(NO_3)_2$ solution (Merck), pH level of 4.5 with the following Pb concentrations: 0; 200; 400; 600; 800; 1,000; 2,000; 3,000 and 4,000 mg/L. The experiment was conducted for 60 days, after this period, plants were harvested and subsequently divided into roots, stems and leaves. The experimental design was completely randomized with three replicates. Every 10 days, experimental plants were evaluated for the total content of Pb in plant parts and after 60 days experimental plants were evaluated for Pb distribution and anatomy structure. Experimental solution didn't change for 60 days.

2.2. Determination total content of Pb in parts of *Dracaena sanderiana*

Plant materials including roots, stems and leaves were collected at 7 time periods (0, 10, 20, 30, 40, 50 and 60 days) and washed 3 times with distilled water, oven dried at 70°C for 24 h, then milled. Approximately 1 g of milled plants was placed in 100 mL digest tubes, added with 10 ml HNO_3, 2 mL $HCLO_4$ and 2 mL H_2O_2 then digested at 180°C until the samples were completely digested. The concentration of Pb in digestates was determined by AAS (Atomic Absorption Spectrometer) (Shimadzu, Japan).

2.3. Determination of Pb distribution in roots, stems and leaves tissues of *Dracaena sanderiana*

The observations of lead on plant tissues were made using histochemical methods which are considered to be fast, simple and accurate (Glater & Hernandez, 1972; Tung & Temple, 1996). Sodium rhodizonate is a specific good chromophoric reagent which gives red to blackish brown color with a buffer solution at pH of 2.8. Lucky bamboo plant parts (roots, stems and leaves) of each treatment Pb level were fixed by formaldehyde acid acetic for 24 h. The aim of the fixation was to obtain the same tissue as the original one. Then, samples were cut thin with a hand microtome. Thin sections are then soaked in a solution of sodium rhodizonate 0.2% (added one drop of acetic acid buffer solution of pH 2.8) for 30 min. Tissue samples were then rinsed with distilled water and placed in an object glass and observed on a microscope.

2.4. Determination of changes in anatomical structure of roots, stems and leaves tissues of *Dracaena sanderiana*

Experimental plants were taken from each treatment Pb level and then separated according to the roots, stems and leaves, which were then cleaned with distilled water and soaked in a solution of formaldehyde acetic acid for 24 h. Plant parts that have been fixed in formaldehyde acetic acid solution were then cut as thin as possible using a hand microtome, then stained in double with methylene blue and carmine red. Protocol for implementing a temporary microscope template was made according to the method of Hoang

& Nguyen (2004). The samples were observed by using a light microscope. Parameters measured were profile anatomical structure of roots, stems and leaves. Measurements were conducted on the thickness of tissues. These observations were conducted using Optika B - 383PL microscope (linked with a computer).

2.5. Data analysis and statistical analysis

Anatomical tissues size was measured by Optika Vision Pro software. Values are calculated as the mean of 3 repetitions. All data were averaged and statistically processed using Statgraphics centurion XVI software. Least significant difference (LSD) test was used to compare Pb contents in roots, stems and leaves of plants on different treatments.

3. Results and Discussion

3.1. Accumulation of Pb in *Dracaena sanderiana* plants

Results obtained from Figure 1, 2, 3 showed that levels of Pb in solution affected accumulated Pb ability in parts of *Dracaena sanderiana* plants. Pb content accumulated in roots, stems and leaves at different Pb levels tended to increase along with increasing Pb concentration and indicated a significant difference at 5% level according to the LSD test. The more Pb concentration increases, the more content of Pb accumulated in plants. However, contents of Pb in roots weren't significant difference in longer Pb exposure time (Pb contents was accumulated in the roots at concentrations 200; 400; 600; 800; 1,000 and 2,000 mg/L had significant difference at 5% level ($P < 0.05$) at 0 to 40 days, but wasn't significant difference at 50 days and 60 days) (Figure 1). This showed that *Dracaena sanderiana* may have a certain accumulation threshold. If accumulated content of Pb in plants surpasses the threshold, plants will halt absorption Pb from outside.

The tolerance limit of Pb in solution of *Dracaena sanderiana* was 800 mg/L (Ho et al., 2019). When the concentrations of Pb in the medium were higher than the tolerance limit of the plant, the growth of *Dracaena sanderiana* could be inhibited. The lethal concentration of Pb was 4,000 mg/L (All of the plants died at this level at 30 days). The highest Lead accumulation capacity in roots of *Dracaena sanderiana* was 38,518 mg/kg dw for the solution with Pb concentrations < 1,000 mg/L and 60,570 mg/kg dw for the solution with Pb concentrations > 1,000 mg/L (at 60 days of the experiment). Compared with Pteris vittata with accumulated Pb content in the roots 3,157 mg/kg dw at 3,000 mg/kg level (Tran et al., 2011), *Dracaena sanderiana* has a higher ability for accumulation Pb in the roots. Lead concentrations 2,000 mg/kg, 3,000 mg/kg and 4,000 mg/kg also significantly inhibited the growth of Pteris vittata. *Lantana camara* L. could tolerate Pb at a pollution level of 4,000 mg/L. After 1 day contacting with this Pb level, *Lantana camara* L. could accumulate 5,252 mg/kg dw Pb in the roots (Diep & Garnier-Zarli, 2007) and thus *Dracaena sanderiana*'s accumulation ability is lower than it (1,009 mg/kg dw in 1 day). Lead accumulation capacity in stems and leaves of *Dracaena sanderiana* was also shown to be higher than in some plant species such as *Chrysopogon zizanioides*, *Ricinus communis*, *Conyza canadensis*, *Oryza sativa*, *Pfaffia glomerata*, *Elsholtzia splendens* (Kumar & Prasad, 2018), with the highest Pb content in stem was 2,263 mg/kg dw (at 4,000 mg/L) and in leaves was 389.52 mg/kg dw (at 2,000 mg/L).

Pb content in parts of *Dracaena sanderiana* at all concentrations of Pb arranged in the order of roots > stems > leaves. At Pb levels from 200 to 4,000 mg/L, accumulated Pb content in roots, stems and leaves was 97.50%, 1.95% and 0.55%. Similar results were also reported in many plants such as *Lantana camara* L. (Diep & Garnier-Zarli, 2007), *Armeria maritima*, *Agrostis tenuis* and *Cardaminopsis halleri* (Dahmani et al., 2000). This result showed that the Pb transportation capacity of *Dracaena sanderiana* was limited only with upper organs as stems and leaves. Thus, *Dracaena sanderiana* could use phytostabilization or phytofiltration mechanism for absorptionPb. According to these two mechanisms, absorbed Pb was accumulated mainly in the roots.

3.2. Distribution of Pb in *Dracaena sanderiana* plants

3.2.1. Distribution of lead in roots tissues

Staining sodium rhodizonate gives red to blackish brown color in tissues where lead has accumulated. The appearance of red stains found in two forms as diffusive or granular deposits (Glater &

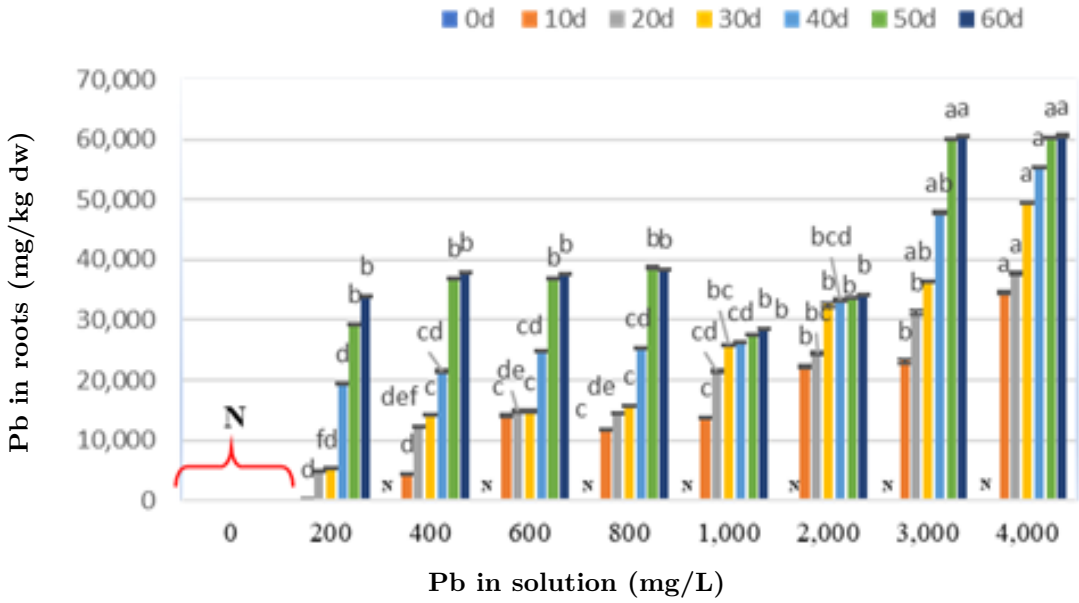


Figure 1. Accumulation of Pb in roots (mg/kg dry weight) of *Dracaena sanderiana*.
Note: Data in chart columns with different letters indicate a significant difference at 5% level according to LSD test; N: negative; dw: dry weight, analysis threshold = 0.006 mg/L); d: day.

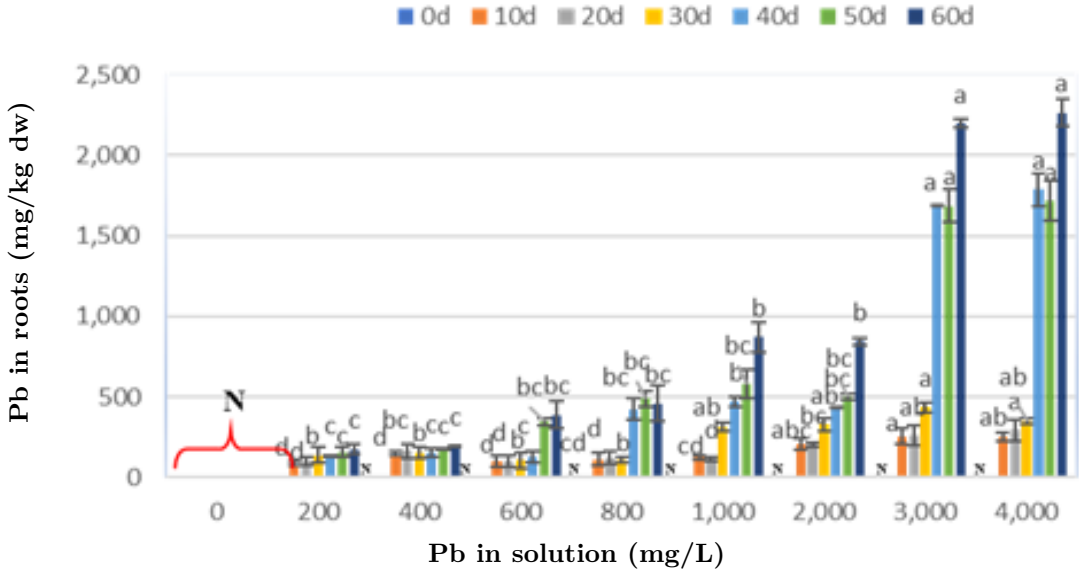


Figure 2. Accumulation of Pb in stems (mg/kg dry weight) of *Dracaena sanderiana*.
Note: Data in chart columns with different letters indicate a significant difference at 5% level according to LSD test; N: negative; dw: dry weight, analysis threshold = 0.006 mg/L); d: day.

Hernandez, 1972). At the root tissues could be seen that Pb deposited mainly in granular form (Fig 4A).

In the root tissues of *Dracaena sanderiana*, Pb was distributed mainly in the spaces between cells and linked to the cell walls (Figure 4B). This distribution could be mainly way to distribute Pb in the root tissues and be a tolerance and limitation strategy for Pb toxicity (Al-Saadi et al., 2013). Pb binds to the cell walls because of high affinity with components such as lignin, pectin, polysaccharide, cellulose (Krzesłowska et al., 2009). More

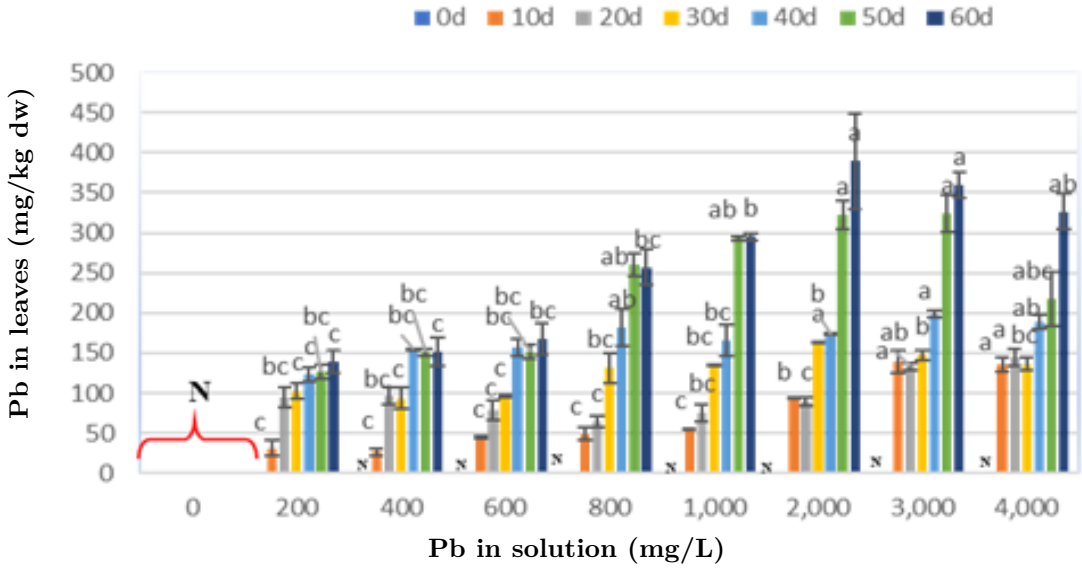
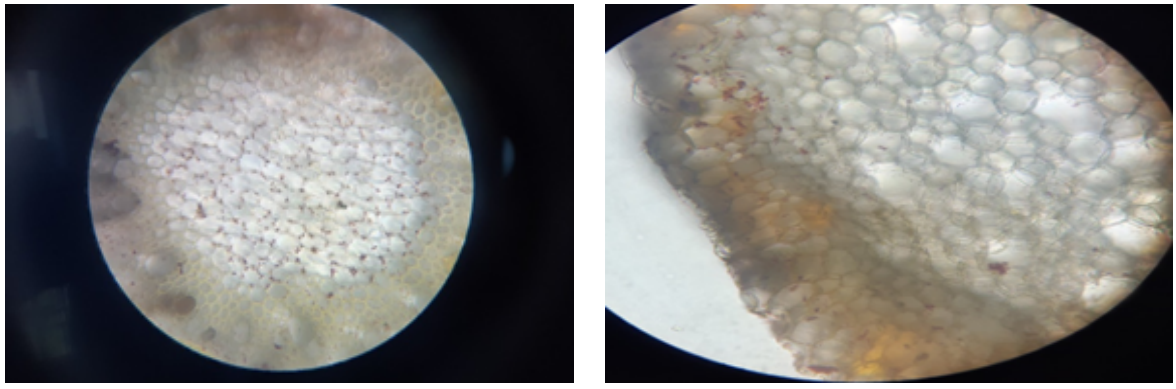


Figure 3. Accumulation of Pb in leaves (mg/kg dry weight) of *Dracaena sanderiana*.
Data in chart columns with different letters indicate a significant difference at 5% level according to LSD test; N: negative; dw: dry weight, analysis threshold = 0.006 mg/L); d: day.



(A) (B)

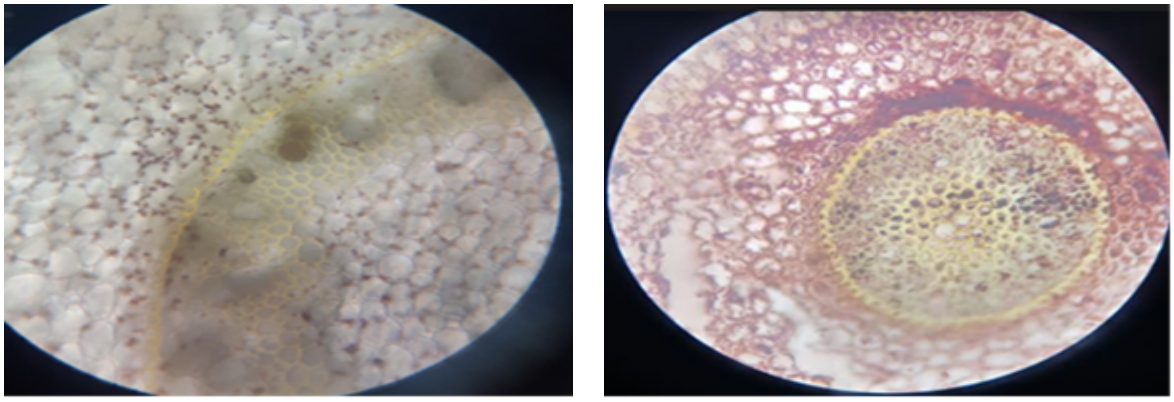
Figure 4. Distribution of Pb in root tissues *Dracaena sanderiana* plants: (A) Pb granular form (10X); (B) Distribution of Pb in epidermis and parenchyma at 800 mg/L Pb (40X); Bar: 1.1mm and 0,4mm; E: epidermis; P: parenchyma.

than 90% of Pb accumulated in the roots was found insoluble in the spaces between cells and tightly bound to the cell walls (Jiang & Liu, 2010).

In the roots, Pb tended to move to the xylem tissue to be transported to stems and leaves. However, Pb mobility is limited by the endodermis barrier. So lead accumulation increases in the endodermis, especially on the casparian strip with dark red color (Figure 5A). At lethal concentration (4000 mg/L), endodermis barrier is broken and Pb infiltrates through casparian into tissues (Figure 5B) The endodermis acts as a barrier to the migration of Pb from roots to stems and leaves (Azmat et al., 2006). This may partly explain for the result that the content of Pb accumulates in roots higher than in stems and leaves.

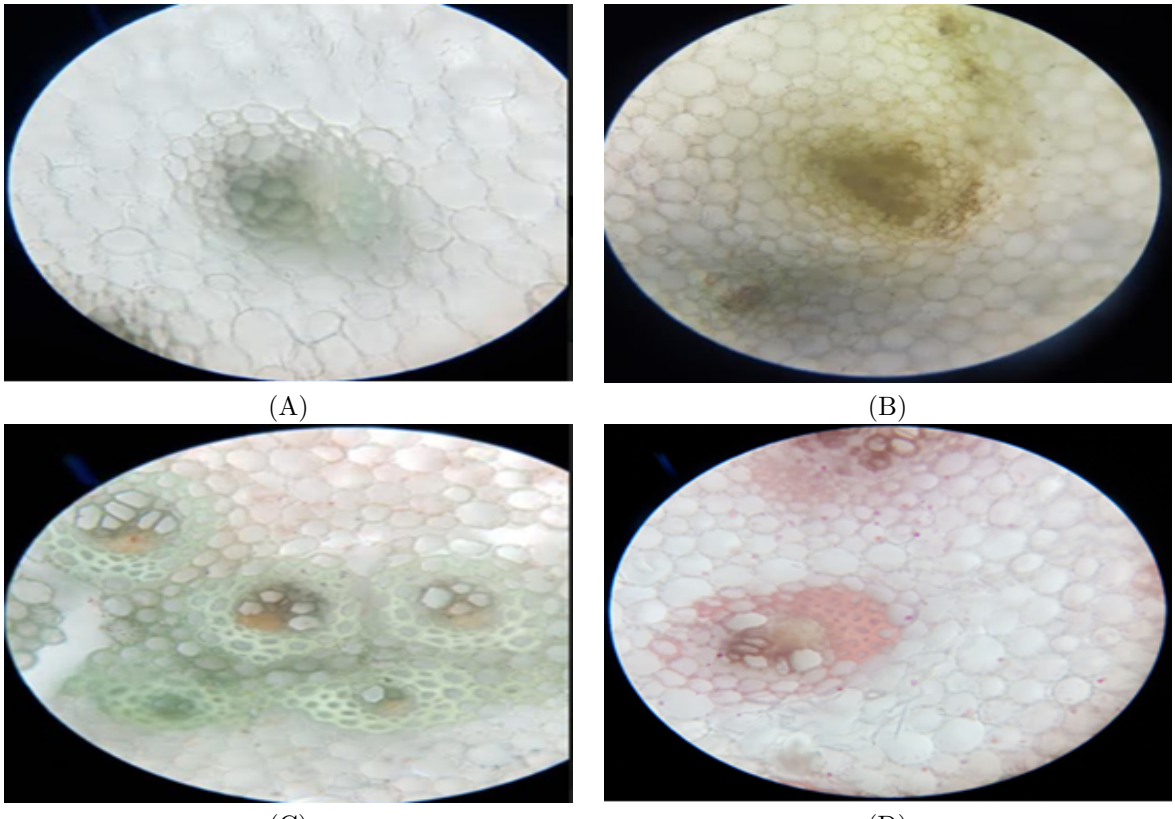
3.2.2. Distribution of Pb in stems tissues

The Pb form deposited mainly in the stem as a diffuse form and a small part showed in granular form. In the stem, Pb content accumulated and deposited the most around the vascular bun-



(A) (B)

Figure 5. Distribution of Pb in root tissues of *Dracaena sanderiana* plants. (A) Distribution of Pb at endodermis at 1000 mg/L Pb (40X); (B) Distribution of Pb at lethal concentration (4000 mg/L) (40X); Bar: 0,4mm; En: Endodermis; C: Casparian strip.



(A) (B)

(C) (D)

Figure 6. Distribution of Pb in stems tissues of *Dracaena sanderiana* plants. (A, B, C and D: control, 400 mg/L, 800 mg/L and 2000 mg/L; VB: vascular bundles; Bar: 0.4 mm; (40X).

dles and tended to diffuse to nearby tissues (Figure 6B, 6C, 6D). Lead content accumulated also increased with the concentration of lead treated (Figure 6). High concentration of lead deposited on the vascular bundles could exceed the tolerance of plants and reduce vascular size and expand the xylem space. This phenomenon has also been identified in the study of Al-Saadi et al.

(2013).

3.2.3. Distribution of Pb in leaves tissues

According to the observation of Pb distribution in leaf tissues (Figure 7), red marks didn't detected in leaf tissues of *Dracaena sanderiana* that exposed lead at a concentration of 200 mg/L to 2000 mg/L. Red marks only detected at concentrations of 3000 mg/L and 4000 mg/L. The results showed that the color in leaves tissues is not different between control, concentration of 200 mg/L and concentration of 2000 mg/L (Figure 7A, 7B, 7C). In contrast, red marks were detected at concentration of 3000 mg/L Pb and observed in vascular bundles (Figure 7d). It is possible that the concentration of lead accumulated very low in leaves at treatment concentrations is less than 3000 mg/L so the color has not been seen.

3.3. Response of plant tissues to lead accumulation

3.3.1. Response of root tissues

This study examined the effect of toxic Pb on *Dracaena sanderiana*. The results showed that Pb markedly affect the treatmented *Dracaena sanderiana* plants as compared to the control. A pronounced effect of Pb on plant roots tissues structure of *Dracaena sanderiana* showed the rapid respond to accumulated Pb, through a increasing in size of tissues. Epidermis, parenchyma, xylem and pith tissues were increased 1.36 - 2.48; 1.01 – 1.55; 1.01 – 122 and 1.01 – 1.55 times, respectively when were observed at 200 – 400 mg/L Pb (Figure 8). These tissues tended to increase when Pb levels in solution increase, especially to the epidermis and parenchyma tissues. These results could be explained that lead accumulation in root tissues accelerates maturation of cells as well as the formation of secondary cell walls. When plant cells are exposed to Pb, the synthesis polysaccharides in cell walls increase as resulting for significant thickening of the cell walls (Raven et al., 1999).

Thicker root tissues could also be the detoxification way for Pb of *Dracaena sanderiana* in order to create the physical barrier and reduce migration of Pb to the upper parts of the plants. Similar results were also reported by Tupan & Azrianingsih (2016) who said that the size of parenchyma and endodermis tissues of Thalassia hemprichii roots have increased in Pb poisoning condition. In 250 mg/L Pb, the number of xylem tissue layers in Lens culinaris increased more than three times compared to the control (Azmat et al., 2006). In addition, the thickening of the cell walls also creates more sites for Pb binding and thus increases extracellular sequestration (Gomes et al., 2011). Thicker cell walls have also been detected in *F. hygrometrica protonemata* (Krzesłowska et al., 2009).

3.3.2. Response of stem tissues

Epidermis tissue and vascular bundles in the stems at Pb levels of 200, 400, 600 and 800 mg/L were thicker than 1.05 - 1.18 times and 1.02 - 1.10 times as compared to the control plant (Figure 9). The size of the epidermis tissue and vascular bundle were decreased when Pb concentrations above 800 mg/L. In contrast, xylem tube diameter of stems in all Pb levels increased 1.08 - 1.36 times as compared to the control treatment.

At lower concentrations (200, 400, 600, 800 mg/L), the thicker epidermis tissue could be the result of stimulation when the presence of lead in low concentrations was diffused from vascular tissue leading to more maturation of the cell walls. Krzesłowska et al. (2010) suggested that, when a small amount of lead penetrates in the cell membranes, it interacts with cellular components and increases the thickness of the cell walls. He also reported that the presence of low levels of Pb will stimulate the growth of plants. However, at higher lead levels (1000, 2000, 3000 and 4000 mg/L), the epidermis, cell layer occupying and including vascular size decreased. These changes may be due to the effects of toxic lead thresholds when linked to cell walls that do not make cells thicker which disrupt hormonal balance. The effect on water hydrolysis of cells as causes the cells lose much water (Gomes et al., 2011).

Dracaena sanderiana is a plant that can survive in both soil and water environments. When living in a water environment, the *Dracaena sanderiana* itself, like other aquatic plants, needs a large intercellular space to bring oxygen to the roots. Therefore, *Dracaena sanderiana* has many vascular bundles in the stem anatomy structure. Vascular bundle size tends to increase lead concentration to 800 mg/L. This change may be the way to the tree against the loss of oxygen. Al-Saadi et al. (2013) found changes in intercellular spaces in the stem of a aquatic plant *Potamoge-*

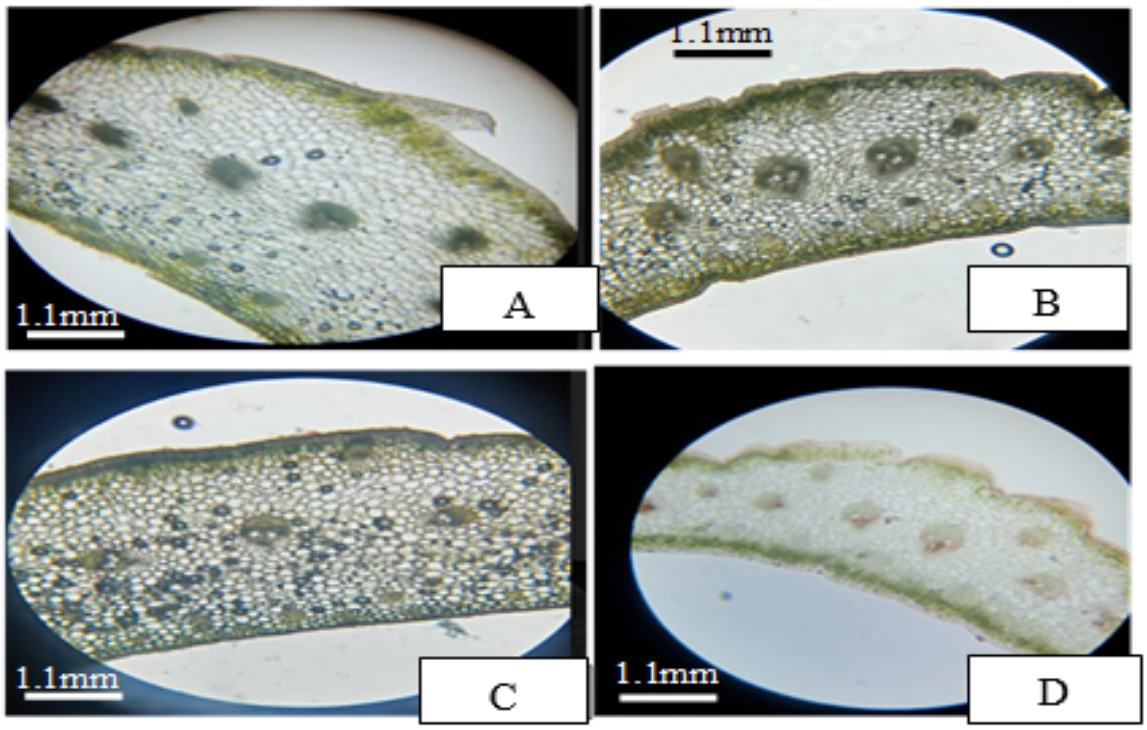


Figure 7. Distribution of Pb in leaves tissues of *Dracaena sanderiana* plants. (A): Control; (B): 200 mg/L; (C): 2000 mg/L; (D): 3000 mg/L; Bar: 1.1mm; (10X).

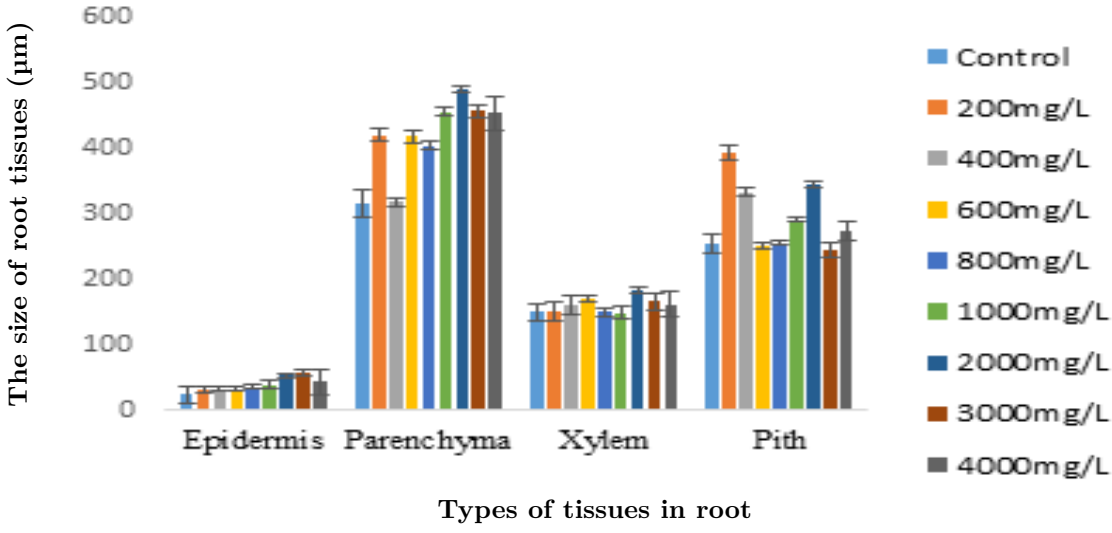


Figure 8. Changes in the anatomical tissues structure of Dracaena sanderiana roots.

ton sp. and concluded that the change is because Pb is transported in the intercellular space so the intercellular space must expand to prevent oxygen loss (Al-Saadi et al., 2013). The increased size of vessels can be an important strategy of live plants in the wetland environment not only facilitates the transport of oxygen in the tree, but also increases the potential for carrying oxygen to the root zone.

3.3.3. Response of leaf tissues

The size of the upper epidermis, parenchyma and vascular bundle was greater than the con-

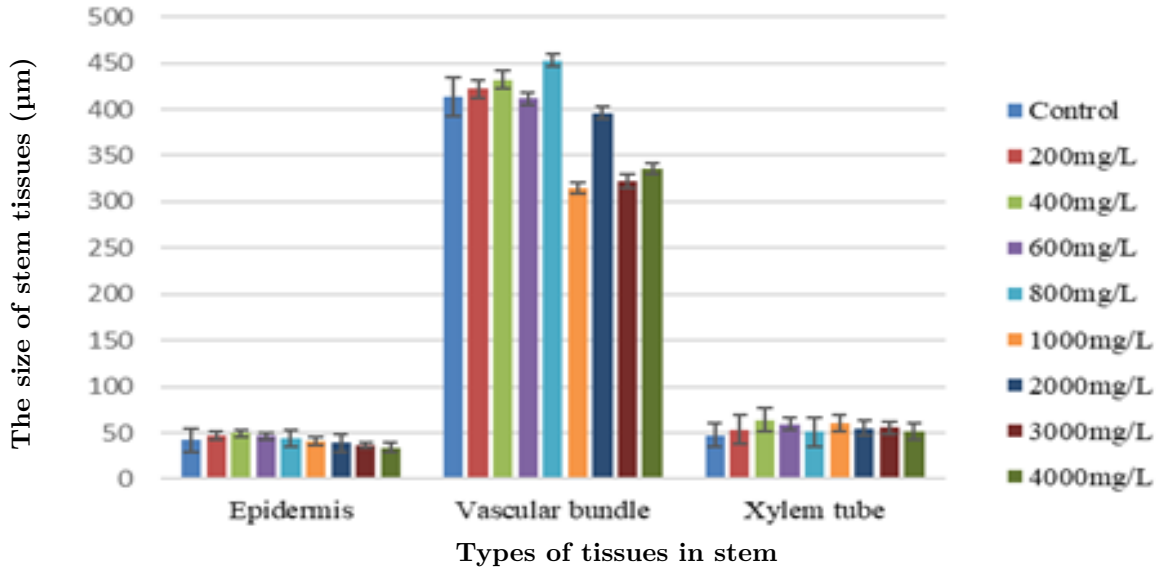


Figure 9. Changes in the anatomical tissues structure of *Dracaena sanderiana* stem.

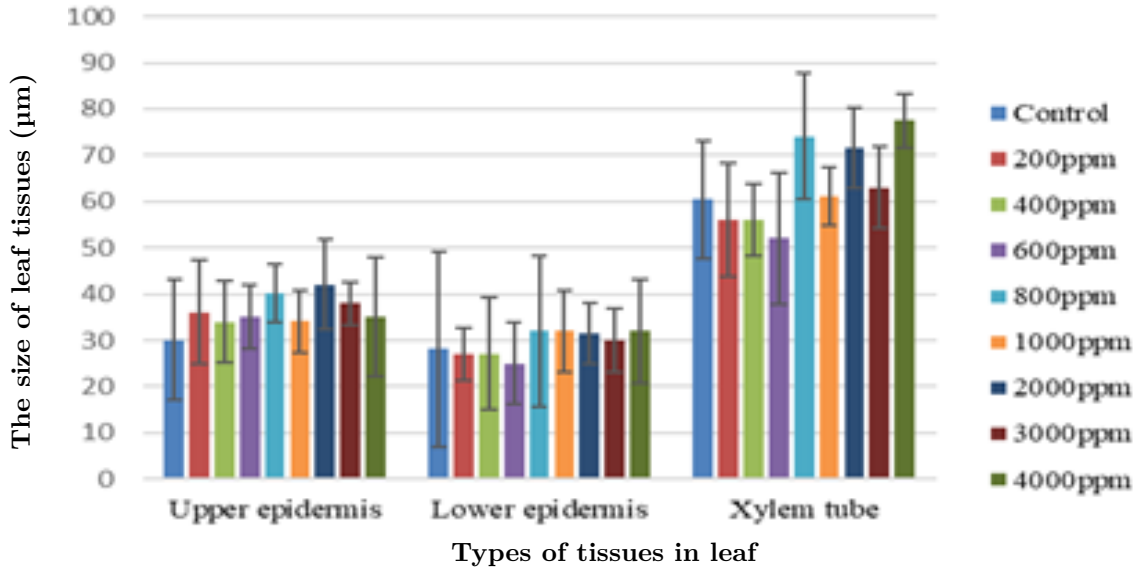


Figure 10. Changes in the upper epidermis, lower epidermis and xylem tissues structure of *Dracaena sanderiana* leaf.

trol 1.13 - 1.32 times; 1.37 - 3.95 times and 1.06 - 1.55 times, respectively (Figure 10, 11). However, the increase in size in these tissues varies with lead concentration and depends on the type of tissue. The structure of other types of tissue such as the lower epidermis, vascular bundle also changes with the concentration of lead. The size of the lower epidermis and xylem at 200, 400 and 600 mg/L Pb decreased greatly compared to the control. Otherwise at 800, 1000, 2000, 3000 and 4000 mg/L Pb, they increase greatly compared to the control (1.13 - 1.14 times) (Figure 10). This proves that the leaves of *Dracaena sanderiana* trees have a reaction to lead. When plants are contaminated with lead, plants have some lead-resistant mechanisms, isolating lead in vacuole is one of those mechanisms. To accumulate lead in vacuoles, the vacuole size must stretch and change the leaf anatomical structure. The change in leaf anatomical structures may be related to

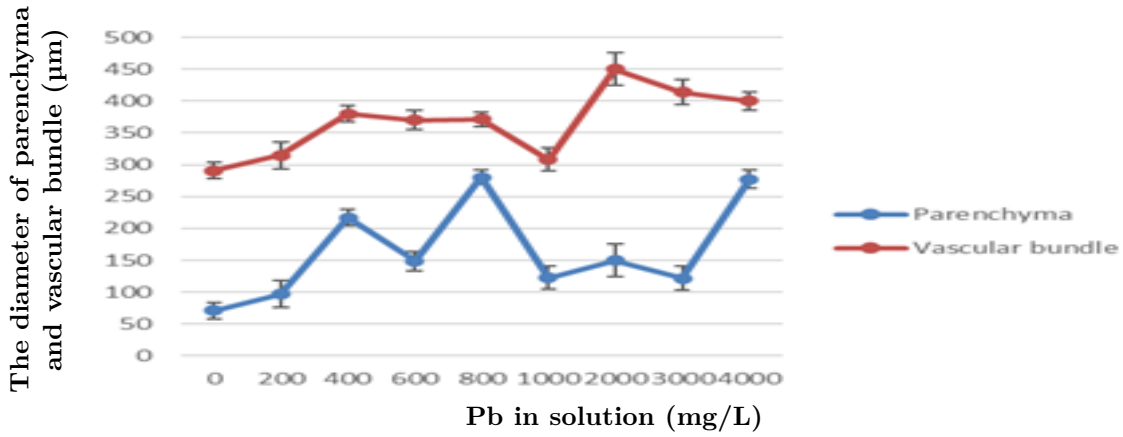


Figure 11. Changes in the parenchyma and vascular tissues structure of *Dracaena sanderiana* leaf.

lead content in the leaves. In this study, the results changed significantly in epidermal size and leaf tissue may be related to this problem. The change in leaf anatomical structure is significant at lead concentrations of 2000, 3000 and 4000 mg/L and this is also the concentration at which leaves accumulate the most lead content.

With the results of the surgery leaves obtained showed that the leaf has many characteristics adapted to high lead environment. Leaf epidermis, parenchyma and vascular bundle increased more than to help plants accumulate lead, and limit evapotranspiration, increasing the ability to accumulate water to detoxify the tree, on the other hand, the increased vascular bundle diameter helps facilitate transport oxygen to the roots zone (compared to the control treatment).

4. Conclusions

Lucky bamboo plants (*Dracaena sanderiana*) have the ability to accumulate and distribute lead in roots, stems and leaves tissues. Pb content is accumulated mainly in the roots and increases with the increase in concentration of Pb. In the roots, Pb is deposited mainly on cell walls and intercellular. In stems and leaves, Pb deposited mainly around the vascular bundles. The distribution of Pb made the anatomical structure of the roots, stems and leaves change markedly. With the capacity for accumulation and distribution of Pb, *Dracaena sanderiana* can become a well bio-accumulated plant as well as a biofilter plant for lead heavy metal pollution.

Conflict of interest

The authors declare no conflict of interest.

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