JABON (*Anthocephalus cadamba* Roxb) POTENCY FOR REMEDIATING LEAD (Pb) TOXICITY UNDER NUTRIENT CULTURE CONDITION

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ABSTRACT

Information on metal adaptation of plants is necessary to understand the mechanism of heavy metal, including lead (Pb), remediation mediated by forest plants in contaminated land. This study aimed to find out the adaptation mechanism of jabon (*Anthocephalus cadamba* Roxb) seedlings to excessive lead level based on the tolerance index of growth performance and lead transport to plant tissue. The seedlings were exposed to lead ($Pb(NO_3)_2$) with the concentrations of 0, 0.5, 1, 1.5, 5 and 10 mM in nutrient culture for 15 days. The result showed that the tolerance index (TI) of the seedlings was significantly decreased by Pb exposure up to 1.5 mM, but the TI values were more than 75%. All seedlings died at Pb concentration of 5 mM and up. Pb accumulated in all parts of the seedlings, with the highest concentration found in the leaves (735.9 ppm) under Pb concentration of 0.5 mM. The Pb was found to be transported to the top portion of the seedlings indicated by Transport Factor (TF) that was more than 1. The results suggest that jabon can adapt to excessive Pb exposure up to 1.5 mM and has the potential as a remediator plant.

Keywords: accumulation, jabon, lead, nutrient culture, tolerance

INTRODUCTION

Lead (Pb) is considered as the second most dangerous heavy metal after arsenic and it is among the biggest pollutants in water and terrestrial ecosystems generated from a variety of activities including household and industrial works (Ebrahimpour & Mushrifah 2008; Tangahu *et al*. 2011; Alaribe & Agamuthu 2015). In a certain concentration, Pb is toxic to plants, animals, and microorganisms. When absorbed by the human body, it will produce various deleterious effects on the hematopoietic, renal, reproductive and central nervous system, mainly through increased oxidative stress (Flora *et al*. 2012).

Heavy metal contamination in the soil can reduce soil fertility for long-term. High Pb levels in the soil need remedial action to enable the soil to regain its normal function (Tangahu *et al*. 2011). Phytoremediation has become an effective, environmentally friendly and affordable technological solution through the extraction or removal of inactive metals and metal pollutants from contaminated soil (Henry *et al*. 2013; Sharma & Pandey 2014). Phytoremediation is based on the fact that plants have membrane active pumps that work through solar energy to extract certain heavy metals from the environment to proceed with the translocation, bioaccumulation, or contaminant degradation depending on the abilities of the entire plant body (Rascioa & Navari-Izzo. 2011; Tangahu *et al*. 2011).

Jabon (*Anthocephalus cadamba*) is known as a tropical forest species with high tolerance to marginal land, and high shoot and roots biomass productions (Krisnawati *et al*. 2011; Setyaningsih *et al*. 2012). Forest plants with deep roots

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character and great biomass have a great potential to be used as remediator plant (Capuana 2010) in contaminated forest land. However, there is still limited information about the tolerance character of tropical forest plants to heavy metals and their potentcy as remediators. Each plant may have a tolerant level and pattern of different adaptations to heavy metal exposure conditions. There is still little information related to tolerant level of Jabon to lead, even though many other species have been extensively evaluated such as sunflower (Niu *et al.* 2010; Kim *et al.* 2009), canola, mustard and vetiver grass (Kim *et al.* 2009), *Lantana camara* (Alaribe & Agamuthu 2015) and *Pinus radiata* (Javris & Leung 2002). Therefore, the effort to learn about the tolerance and remediation capability of Jabon to heavy metal Pb is necessary and important.

This research was conducted in nutrient culture media to determine (1) the tolerant level of Jabon seedlings to Pb by observing root growth and biomass, and (2) the degree by which Jabon seedlings absorb Pb.

MATERIALS AND METHODS

Ninety Jabon seedlings aged one month old with 5 cm height were obtained from the Tissue Culture Laboratory of the SEAMEO Regional Centre for Tropical Biology (BIOTROP) in Bogor, Indonesia, and transferred into culture media in plastic box containers equipped with aerator tubes for lead exposure experiment. These seedlings were grown in aerator tubes containing 1 litre of liquid nutrient culture media (Sopandie 1990) adapted for 4 days and then subjected to a treatment media using Pb for 15 days. Adaptation nutrient was 1/5 dosage of nutrient culture without Pb. Media treatment was 1/3 dosage of nutrient culture treated by Pb (NO3)$_2$ with 6 different Pb concentrations i.e. 0, 0.5, 1, 1.5, 5 and 10 mM, respectively (Setyaningsih *et al.* 2012). During the experiment, the media culture was set to have pH 4.5-5 using NaOH and HCl. Composition of nutrient culture refers to the "Sopandie Nutrient Solution" (Sopandie 1990) consisting of distilled water and nutrient solution which contained 1.5 mM Ca(NO$_3$)$_2$.4H$_2$O; 1.0 mM NH$_4$NO$_3$; 1.0 mM KCl; 0.4 mM MgSO$_4$; 1.0 mM KH$_2$PO$_4$; 0.50 ppm MnSO$_4$; 0.02 ppm CuSO$_4$.5H$_2$O; 0.05 ppm ZnSO$_4$.7H$_2$O; 0.05 ppm H$_3$BO$_3$; 0.01 ppm (NH$_4$)$_6$ Mo$_7$O$_{24}$.4H$_2$O; 68 mM FeEDTA. During the adaptation and treatment, the aeration was supplied using electrical aerator.

Parameters analysed in the experiment were root and shoot growth and plant biomass to verify the response of plants to all Pb treatments. Pb content was analyzed only from samples of control plants (without Pb), the plants with lowest Pb treatment (0.5 mM), and the plant with highest Pb treatment by which the plants were still alive until the end period of the treatment, to compare the accumulation capacity of plants under lower and higher Pb concentration in the media.

Seedling growth was observed by measuring the length of roots and stems, and dry biomass weight when the plant samples were harvested. The number of dead seedlings was also counted. The tolerance index (TI) was calculated at different Pb concentrations using the following equation: TI (%) = 100 x (root length under metal treatment)/(root length in the control solution) (Wang *et al.* 2011). If TI <50 = Not tolerant; 50≤TI <100 = Medium tolerant; TI ≥100 = Tolerant (professional judgment).

Pb accumulation in seedling tissue was calculated by measuring the concentration of Pb in the roots, stems and leaves of control plants, the plants with lowest Pb treatment (0.5 mM), and the highest treatment that were still alive. The seedlings were harvested and dried in the oven with a temperature of 45 ± 2°C until its weight was constant. Special preparation for seedling roots, before being dried, considted of soaking them in 1.0 mM H-EDTA for 30 minutes to remove Pb attached on the root surface and rinsed three times in H$_2$O (Javris & Leung 2002). Pb concentration was determined using Atomic Absorption Spectroscopy (AAS) which previously had been calibrated with a standard solution of Pb. The obtained Pb concentration was then used for calculating the value of Bioconcentration Factor (BCF), Transport Factor (TF) and Bioaccumulation (B) (Mun *et al.* 2008), using the following the equations:

BCF $= C_{root} / C_{soil}$
　　　where C_{root} is the concentration of Pb in roots and C_{soil} is Pb concentration in soil / media

TF $= C_{aerial} / C_{root}$
　　　where C_{aerial} is Pb concentration in the shoots

B $= C_{aerial}$ x DW (g Pb/plant) where DW is tissue dry weight

The study was carried out in completely randomized design for 6 treatments of different Pb concentrations, i.e. P0 = without Pb (0 mM), P1 = 0.5 mM of Pb, P2 = 1.0 mM of Pb, P3 = 1.5 mM of Pb, P4 = 5 mM of Pb and P5 = 10 mM of Pb. Each treatment was repeated 3 times. Three plants were used per unit of treatment, so that the total plants were 54 plants. All the data were analysed using the Microsoft Excel and SPSS 16 to calculate the analysis of variance and to compare the means of the treatments by DMRT analysis. The analysis of regression was also carried out to recognize the typical pattern of the treatment effects (exposure of Pb).

RESULTS AND DISCUSSION

Pb Tolerance of Jabon Seedling

All Jabon seedlings exposed to Pb up to 1.5 mM were able to survive until 15 days. However, seedlings exposed to Pb of 5 mM and 10 mM showed toxicity symptoms during the second day and completely died after 5 days suggesting that 5 mM is a toxic concentration for Jabon seedling. This supports the findings of Hadi and Aziz (2015) and Wang *et al.* (2011). The symptom started with chlorosis and necrosis on the tip of the leaves (the leaves turned to yellow and dried up) on the 2nd day, followed by the appearance of brown spots on the 3rd day. On the 4th day, most of the leaves rolled up and finally dried on 5th day followed by drying of the petiole and branch in the following days. Toxic symptoms also appeared in the roots of the seedlings. By this reason, the measurement of Pb concentration of the highest treatment was carried out from the plants treated by 1.5 mM of Pb. The seedlings grown in the media without Pb had fibrous root with white and transparent colour. The seedlings exposed to 0.5 - 1.5 mM of Pb had white fibrous roots with some dark gray spost (about 1 cm) while the whole roots of seedlings exposed to 5 and 10 mM of Pb had became dark indicating cell death.

Symptoms of toxicity due to Pb began with yellowing leaves, the appearance of brown spots in almost all the leaves, leaf curling and leaf drying as well as stem drying on following days with a grayish black color of roots. These symptoms had also been reported in other plants such as *Chlorophytum comosum* (Wang *et al.* 2011), *Vigna*

unguiculata (Kopitte *et al.* 2007) and *Paraserianthes falcataria* (Setyaningsih *et al.* 2012). Chlorosis and necrosis occurred presumably because photosynthetic pigment was damage as a result of decreased production of chlorophyll a and b when Pb exposure increased up to 1500 ppm (Wang *et al.* 2011). The declined production of chlorophyll occurred as a result of heavy metal disturbance that replaced magnesium (Mg), an important ion in the central of chlorophyll molecules, and inhibit the synthesis of chlorophyll by inhibition of enzyme activity (Wang *et al.* 2011; Xiao *et al.* 2008).

Root damage was very likely caused by the rapid inhibition of root growth. Inhibition of root growth could be caused by inhibition of cell division in root tip due to immobilization of Pb ion in the cell wall (Jiang & Liu 2010). Roots became blackish, short and fat. This phenomenon has also been reported on the roots of *V. unguiculata* which became shorter and had more secondary roots (Hadi & Aziz 2015), and also on the roots of *P. falcataria* in response to Pb exposure (Setyaningsih *et al.* 2012).

Pb treatment for 15 days caused significant decrease ($p < 0.05$) in root length, dry weight and tolerance index (TI) of Jabon seedlings. Root length value ranged from 8.3 to 11 cm which gradually decreased by increasing Pb concentration exposure (Table 1). Dry weight of seedlings had value range of 1.28 - 1.63 g which decreased following the pattern of open polynomial curve of $y = 0.48x^2 - 0.94x + 1.63$ ($R^2 = 1$, $p < 0.05$). The TI of Jabon seedlings ranged from 75% to 100% which decreased significantly with the increase in Pb concentration exposure, and the maximum reduction was 25% (Table 1).

There was no significant change on the growth performance of Jabon seedlings when exposed to Pb concentration up to 1.5 mM, with TI value of 75% or higher. This data showed that Jabon seedlings can be categorized as having intermediate tolerance to Pb. The TI value of Jabon seedling to Pb is apparently smaller than *P. falcataria* seedling with TI value of 90% when exposed to the same level of Pb (Setyaningsih *et al.* 2012), and also smaller than *Chlorophytum comosum* with TI value of 73% even though it was exposed to 1500 mg kg^{-1} (1500 ppm) of Pb for 90 days (Wang *et al.* 2011). Moreover, the TI of Jabon was also even smaller compared to *Acacia farnesiana*, known as Pb bioaccumulator, which

Table 1 Growth of jabon seedling exposed to Pb on nutrient culture during 15 days

Pb (mM)	Root Length (cm)	Dry Biomass (gram)	TI (%)
Pb 0	11.0 ± 1.0 b*	1.63 ± 0.34 b	100 bc
Pb 0.5	8.8 ± 0.2 a	1.28 ± 0.14 a	80 ab
Pb 1	8.3 ± 0.6 a	1.38 ± 0.33 a	75 a
Pb 1.5	8.5 ± 0.5 a	1.30 ± 0.13 a	77 ab

Note: *mean ± standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test

the TI decreased only 8% when exposure to 1000 mg l^{-1} Pb for 60 days (Amalia *et al*. 2011). The TI level of Jabon to Pb is better than cuttings of *Jatropha curcas* which has a TI value less than 80% when exposed to 0.5 mM of Pb and became only 39.2% when it was exposed to 4 mM Pb (Shu *et al*. 2011), or even lower if it was compared to the growth of annual plant such as cowpea (*Vigna unguiculata*) which was recorded to be hampered by only being exposed to 1 µM of Pb (Kopitte *et al*. 2007).

Pb Concentration, Distribution and Bio-accumulation in Jabon Seedlings

Exposure of Jabon seedlings to Pb for 15 days in liquid culture caused an increase of Pb concentration in seedling tissue up to 735 ppm or approximately 12.6 times higher in seedlings under control treatment. The largest average of Pb concentration was found in the leaves (735.93 ppm), then in the roots (459.04 ppm) and the in the stems (438.96 ppm) (Table 2). The pattern of Pb concentration in response to the increasing Pb exposure followed the trend of open downward polynomial curve of $y = -626.79x^2 + 1077.9x + 76.81$ ($R^2 = 1$, $p < 0.05$) (Figure 1).

Comparison of Pb concentration in seedling tissues to that in media indicates the value of Bioconcentration Factor (BCF). BCF value of Jabon seedlings was found to range from 0.5 to 2.9, and the biggest average of BCF was found in the leaves (0.6 - 4.6). The BCF value of Jabon seedlings exposed to 0.5 mM of Pb was the highest as compared to that of Jabon exposed to 1.5 mM of Pb or lower concentration (Figure 2).

Comparison of Pb content in the stems and leaf tissues to that of the root seedlings indicates the value of the transport factor (TF). The TF value of Pb in Jabon seedlings was found to range from 0.4 to 1.63. The TF value of leaves was greater than that of stem. The TF value increased by Pb exposure to 0.5 and 1.5 mM, with the highest TF value observed in seedlings exposed to 0.5 mM of Pb (Figure 3).

Pb bioaccumulation in Jabon seedlings was observed to range from 1.73 to 36.55 x 10^{-2} mg/plant. The highest Pb accumulation was recorded in roots at 3.74 to 36.55 x 10^{-2} mg/plant. The seedlings exposed to 0.5 mM of Pb had the highest bioaccumulation value ranging from 10.41 to 36.55 x 10^{-2} mg/plants, followed by the seedlings exposed 1.5 mM of Pb (Table 3). The highest value of bioaccumulation occurred in the roots treated by 0.5 mM of Pb that was almost 10 times higher than that of the control, i.e. 36.55 x 10^{-2} mg/plant (Table 3).

Table 2 Pb concentrations on parts of jabon seedlings after 15-days exposure in nutrient culture

Pb Exposure (mM)	Pb Concentration per g dry weight (ppm)		
	Roots	**Stem**	**Leaves**
Pb 0	76.81 ± 18.60 b*	27.61 ± 15.98 a	58.38 ± 34.97 a
Pb 0.5	459.04 ± 94.76 d	438.96 ± 206.63 c	735.93 ± 205.78 d
Pb 1.5	283.32 ± 1.06 c	164.34 ± 24.44 ab	360.06 ± 56.35 c

Note: *mean ± standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test
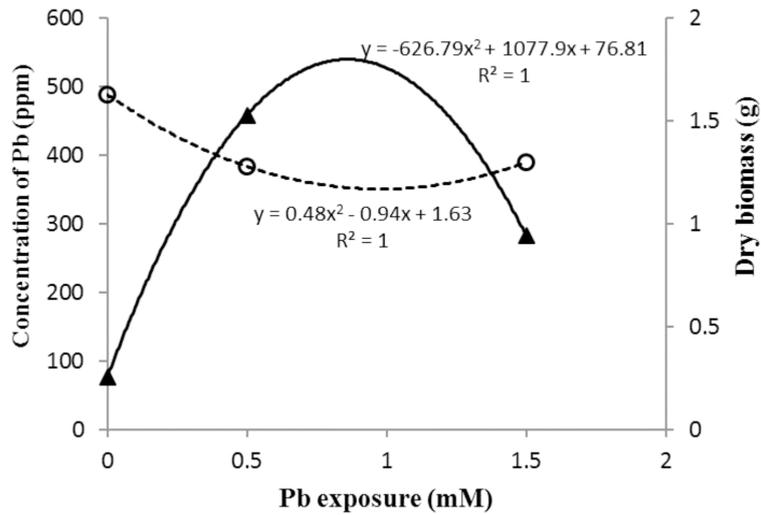
Figure 1 Relation of Pb concentration (▲) in root and dry biomass (o) of jabon seedling on Pb exposure
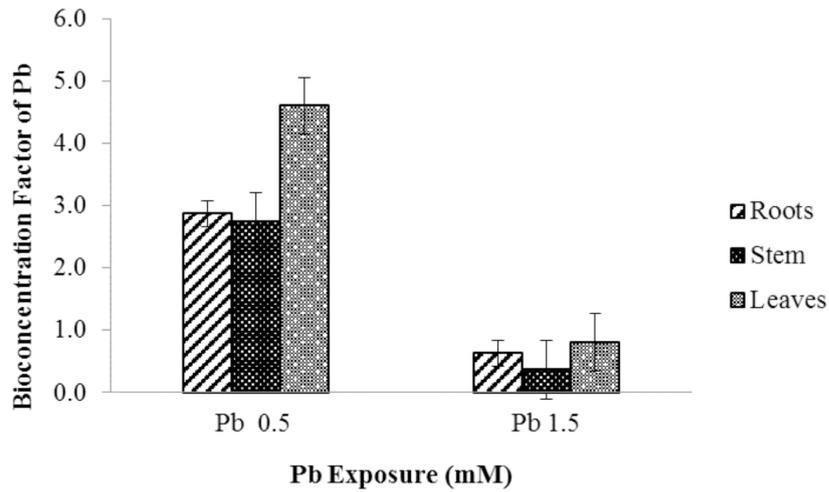
Figure 2 Bioconcentration Factor of Pb on roots, stem and leaves of Jabon seedlings exposed to Pb during 15 days (Note: The dashed line showed the threshold of plant categorized as Pb extractor (Tangahu *et al.* 2011))
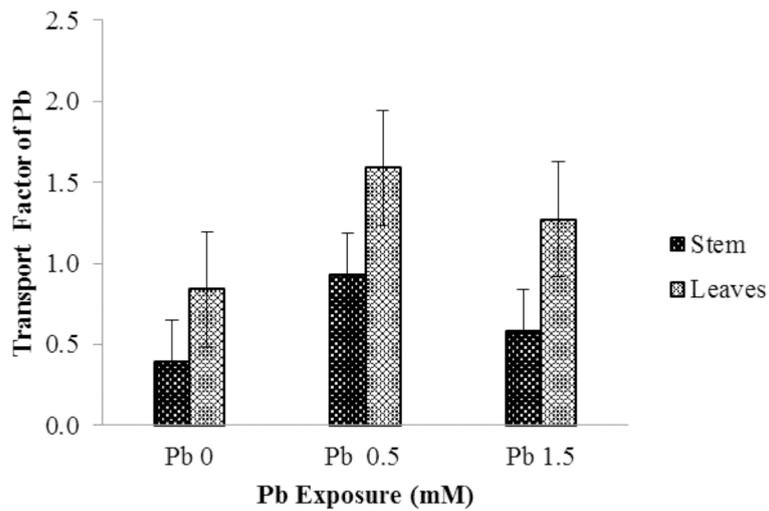
Figure 3 Transport Factor of Pb on stems, and leaves of Jabon seedlings exposed to Pb during 15 days

Table 3 Pb bioaccumulation in jabon seedlings exposed to Pb during 15 days in nutrient culture

Pb (mM)	Bioaccumulation (10^{-2} mg/plant)		
	Roots	Stem	Leaves
Pb 0	3.74 ± 1.06 a[*]	2.82 ± 0.11 a	1.73 ± 0.56 a
Pb 0.5	36.55 ± 8.90 c	10.41 ± 1.15 c	24.58 ± 0.76 c
Pb 1.5	18.12 ± 1.98 b	7.18 ± 1.27 b	8.98 ± 0.82 b

Note: [*]mean $\pm$ standard deviation followed by the same letter in the same column indicates no significant difference in the error rate of 5% by DMRT test

Jabon seedlings exposed to Pb for 15 days in a nutrient culture led to an increase of Pb concentration in the seedling tissues. Based on BCF value which ranged between 0.4 and 4.6, the Pb exposure in the media culture led to the increase of Pb concentration in the tissues of the seedlings with the highest concentration recorded at 735 ppm or 12.6 times higher than that of the control (Table 2). The leaves of the seedlings exposed to 0.5 mM of Pb were found to have the largest Pb concentration followed by the roots and then stems, with the TF values was ranging from 0.9 to 1.59 (Table 2, Fig. 1 and 2). This result showed that Pb concentration in Jabon seedlings was greater as compared to that of *Pinus radiata* (390 ppm) (Javris & Leung 2002), but lower than that in kenaf (4281 ppm) (Mun *et al.* 2008), *P. falcataria* (1773 ppm) (Setyaningsih *et al.* 2012) and *Platanus orientalis* (12.7 ppm) (Monfared *et al.* 2013).

The data also showed that BCF value of seedlings exposed to 1.5 mM of Pb was lower than that exposed to Pb 0.5 mM. The exposure of 1.5 mM of Pb inhibited the plants more and caused more damage than the exposure to 0.5 mM of Pb, which resulted in the reduction of Pb accumulation from media to the seedling tissues. Another possibility was the leakage or efflux of Pb and exit back to the media due to damaging effect of Pb. Pb concentration in seedling roots followed the polimonial pattern of open downwards, while seedling biomass (dry weight) followed the polimonial pattern of open up in response to the increased Pb concentration (Fig. 3). In other words, exposure to 0.5 mM Pb caused the highest Pb concentration in the seedling tissue and resulted in the lowest dry biomass. This suggests that the growth declining of Jabon seedling is highly correlated with the levels of Pb in the tissue, the higher level of Pb

caused decreasing of growth more indicated by lower dry biomass. Such conditions was also reported by other investigator in *C. comosum* (Wang *et al.* 2011).

The higher TF value of seedling due to Pb exposure as compared to control suggested that the transfer of Pb into the shoot of seedling occurred efficiently. TF value of Jabon seedlings was likely to exceed one (TF> 1) indicating that jabon seedling prefered to keep Pb in the shoots which could classify jabon as a metal accumulator (Tangahu *et al.* 2011).

Pb accumulation in Jabon roots was the largest which was almost 3 times of that in the stem (2.82 – 10.41 x 10^{-2} mg/plant) and 2 times of that in the leaves (1.73 - 24.58 x 10^{-2} mg/plant). Therefore, the sequence of Pb accumulation in Jabon seedling was the roots > leaves >stem. This phenomenon has also been reported in some other plants such as Kenaf which has been reported to accumulate Pb up to 97% of the total bioaccumulation (Mun *et al.* 2008) and the Indian Mustard which accumulated Pb more than 95% (Reisinger *et al.* 2008). However, the Pb accumulation capacity of jabon is still lower compared with some other species that have been characterized as Pb accumulators.. These Pb accumulators include *Brassica campestris* L, *Brassica carinata* A. Br.*, Brassica juncea* (L.) *Czern.* and *Brassica nigra* (L.) *Koch* which could accumulate more than 100 mg Pb g^{-1} dry weight (Tangahu *et al.* 2011).

Although the Pb concentration in the tissue was relatively small, because this plant produced high biomass with high BCF and TF values, the total Pb accumulation of Jabon seedling was relatively high. Based on the concept that remediation effectiveness depends on the amount of contaminant accumulated by plant biomass and/or by contaminant level in plant tissues

(Rodriguez *et al.* 2005), we conclude that Jabon may have potential function as Pb remediator.

The efficiency of metal translocation from roots to shoots is one of the key factors to distinguish whether the plant is categorized as metal hyper-accumulator or not. Metal accumulation in the shoot are often limited by the binding of metal cations that is being exchanged in the cell walls of xylem (Kim *et al.* 2009). However, the hyper-accumulator plant may have the ability to overcome this limitation by forming a negative charge or neutral formation of organic-metal complex. Some aspects such as plant species, properties of medium, the root zone, vegetative uptake and addition of chelating agent are several factors which can affect the uptake mechanism of heavy metals (Rodriguez *et al.* 2005; Tangahu *et al.* 2011). Formation of metal-organic acid complex also can be an important mechanism to increase the Pb uptake by plants although free ions of metal are easier to be accessible.

CONCLUSIONS

Jabon seedlings exhibited tolerant characteristic at least up to 1.5 mM (450 ppm) of Pb, based on some indicators of tolerant index. The Pb was distributed to all part of the seedlings, with the highest concentration found in the leaves (735.9 ppm). However, because of higher biomass, the highest bioaccumulation was found in the roots (36.55×10^{-2} mg per plant). Jabon seedling can be classified as metal accumulator and may have great potential as remediator in the lead-contaminated areas.

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