Lead accumulation by jabon seedling (*Anthocephalus cadamba*) on tailing media with application of compost and arbuscular mycorrhizal fungi

L Setyaningsih[1*], Y Setiadi[2], S W Budi[2], Hamim[3] and D Sopandie[4]

[1]Department of Forestry, Nusa Bangsa University, Jl. Kh. Sholeh Iskandar Km 4, Bogor, Indonesia. 16166
[2]Department of Silviculture, Faculty of Forestry, Bogor Agricultural University (IPB), Jl. Bundaran Akademik. Campus IPB Darmaga, Bogor, Indonesia. 16680
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB), Jl. Meranti, Campus IPB Darmaga, Bogor, Indonesia. 16680
[4]Department of Agronomy, Faculty of Agriculture, Bogor Agricultural University (IPB), Jl. Meranti. Campus IPB Darmaga, Bogor, Indonesia. 16680

Email: luluk.setya@gmail.com

Abstract. Lead (Pb) is one of the dangerous heavy metal contained in tailing that needs remediation activity. This study aimed to investigate the potency of jabon to take up and accumulate lead in its tissue by the application of compost and arbuscular mycorrhiza fungus (AMF) on pot observation. In Pb-containing tailing media, the average levels of Pb in roots seedling was 50% greater as compared to the levels of Pb in the stem and leaves of seedlings. Application of compost in tailings media significantly increased ($p \leq 0.5$) the average levels of Pb in the roots and stems, but decreased Pb levels in leaves. Applications AMF significantly decreased ($p \leq 0.5$) the average levels of Pb in the roots, stem and leaves of seedlings by approximately 18-33%. The combination applications of compost and AMF significantly ($p \leq 0.5$) increased the level of Pb in the roots, stems and leaves of seedlings at 6, 16 and 27 fold respectively than that in control plant (without compost and AMF). After 12 weeks exposure, lead bioconcentration factor varied from 0.1-1.6 in seedling tissue with transport factor varied from 0.1-1.0. The application of active compost and AMF increased 1-15 fold lead accumulation from control, and the biggest accumulation was 452.9 $x10^{-2}$ mg/plant with Pb concentration of 1.5 mM. Active compost and AMF application supported jabon seedling to act as lead phytostabilizer and to remove lead from the tailing to the above part of the plant.

1. Introduction

Tailing produced from mining activities has extreme physical and chemical properties, which in addition to lower carbon and macro nutrients, it also contains heavy metals, such as lead (Pb) that can be produced naturally or triggered by mining activity [1,2]. Some reports observed that lead content in the tailings was approximately 4.80 ppm with total dissolved Pb was 172 ppm [2], while from another observation [3] it was 110 mg Pb/kg, and it was already at the critical threshold according to Alloway (year of publication) [4]. Lead has been categorized as a second dangerous element after arsenic [5] and at a certain concentration can be very harmful to living things, if deposited in the food chain, so that the effort to remove this particular element from the environment is a main priority to do.

As other heavy metals, lead is not easy to degrade so that the cleaning efforts (remediation) can be done by stabilizing the soil that reduce the mobility and bioavaibility of contaminants from leaching by wind or water erosion, and or transfer from extracted soil. This activity can be carried out by employing a certain plants by a process known as phytoremediation [6,7]. Phytoremediation is based on the fact that the plant life activities as a pumping using solar energy to extract and concentrate certain heavy metals from the environment [8]. Phyto-stabilization and phyto-extraction are two among several mechanisms of phyto-remediation. Phyto-stabilization uses plants to reduce the availability (bioavailability) of contaminants in the environment [9]. It is mainly due to strong roots arrangement that resists the leaching and erosion of contaminated soil by root absorption of pollutants or precipitate them to the rhizosphere, so that it will be stable [10]. On the other hand, phyto-extraction uses plants that have the ability to accumulate pollutants and further remove them from soil while concentrate them in the plant tissue, for later harvested of its biomass [9]. Dry biomass of plant shoots is expected containing contaminants in higher concentrations than those found in the soil. Thus, the potential remediation of a plant can be determined by measuring the bioconcentration factor (BCF), the value of the transport factor (TF) and the value bioaccumulation [7,10,11,12].

Phytoremediation of lead, however, is often inhibited by the lower solubility of Pb in the soil, or lower Pb translocation to the plant that can be harvested, or Pb toxicity for the plants [7,13]. The selection of resistant plants to Pb and improvement of physical and chemical properties of the tailings using compost and mycorrhizal applications are expected to be suitable to overcome these obstacles. Compost application is important and strongly recommended in agriculture practices [14], and it becomes an important component in tailing reclamation to support phytostabilization [15,16]. In addition to compost, arbuscular mycorrhizal fungi (AMF) has also been recognized to be able to filter heavy metals such as aluminum, arsenic, boron, cadmium, copper, iron, lead, nickel, selenium and zinc to the tolerant concentration for plant growth, so it is less toxic to the plants [17]. On the other hand, AMF is also able to increase accumulation of heavy metals such as Cu, Ni, Pb, Zn, Fe and Co on root and shoot tissues of grass *Ehranta calycina* Sm [18,19,20], as well as accumulation arsenic on Brachiaria Grass *(Brachiaria decumbens)* [21]. An attractive system to advance plant based environmental clean-up is provided by AMF. Its associations are integral functioning parts of plant roots and are widely recognized to enhance plant growth on severely disturbed sites, including those contaminated with heavy metals [22].

In addition to its utilization as alternative industrial-forest tree, Jabon, one of the fast-growing timber species, [23], has been chosen as afforestation and reforestation tree in the post mining area. However, detailed information regarding Jabon seedling ability to perform remediation activities on lead-contaminated tailings has not been revealed. Likewise, activated compost utilization and AMF to support the ability of forest trees seedlings in phytoremediation of tailing media is still very limited.

This study aimed to determine the effect of compost and AMF to support Jabon seedlings ability to accumulate lead in the tailings contained lead.

2. Methods

Research was carried out at Greenhouse Laboratory of Silviculture, Faculty of Forestry and Research Center of Biological Resources and Biotechnology, Bogor Agricultural University (IPB), Bogor, Indonesia.

2.1. Research design

This study was carried out using completely randomized design (CRD) with three factors. The first factor was addition of Pb (NO3)2, consisted of 4 levels: 0, 0.5, and 1.5 mM of Pb (equivalent to 0, 150, 450 mg kg^{-1} of tailing); The second factor was compost, consisted of 2 levels (without and with the addition of compost (1: 20 w / w)); and the third factor was AMF inoculants consisted of 2 levels (with and without AMF Mycofer Inoculant). Mycofer Inoculant consisted mix of 4 species of AMF, i.e: *Glomus manihotis* INDO-1, *Glomus etunicatum* NPI 126, *Gigaspora margarita*, *Acaulospora tuberculata* INDO-2.

2.2. Preparation of Media

Tailing was collected from the tailing dam in the area of national gold mining company, owned by PT Aneka Tambang Tbk. UBPE Pongkor, Bogor, Indonesia. Dried tailing was characterized by the following properties: tend to be alkaline (pH 8) with very low carbon (0.24%) and nitrogen (of 0.02%) content, while the availability of other macro nutrients was (P = 6 ppm and Mg = 0.73 cmol/kg). Total Pb content was 114 ppm and available Pb was 17.3 ppm. The activated compost was made from cow manure that was combined with rock phosphate and rice husk with the ratio of 500:20:50 (weight). One liter of bio-activator solution that had been diluted with 100 liters of water was added and the mixture was incubated for a week. Chemical properties of compost was: having pH slightly acid (6), the carbon content was very high (17.78%), macro nutrients content (N, P, K, Mg, Ca) was very high (1:57%, 2765 ppm, 1768 ppm, 21:01 cmol / kg, 40.61 cmol / kg respectively). Pb content was 22 ppm and only 0.2 ppm was in the form of available. Tailings and compost were mixed with the ratio of 20: 1 (w / w).

2.3. Preparation of seedlings and AMF

Jabon seeds were obtained from the Tissue Culture Laboratory, SEAMEO BIOTROP, Bogor, Indonesia, which was in the form of plantlets that had been acclimatized. Jabon seedling were prepared into small pots for 3 weeks before transferring to the tailings for the experimental treatment. AMF inoculation was carried out while the seedling were prepared and after being transferred to tailing media. A total of 10 grams of Mycofer inoculant containing spores approximately 25-50 AMF was applied in the middle of the hole of seedlings during planting.

2.4. Pb Treatment

The solution of Pb $(NO_3)_2$ was prepared to provide Pb treatment with 3 levels (0, 0.5, and 1.5 mM of Pb). The 50 ml amount of Pb $(NO_3)_2$ was applied to the seedling by pouring the solution to the area around the roots of 1 week seedlings.

2.5. Biomass measurements

Jabon seedlings biomass was measuring the dry weight of shoots and roots of seedlings. Seedlings were harvested and dried in an oven at a temperature of 70°C to obtain a constant dry weight (estimated at between 20-28hours).

2.6. Lead content measurements

Pb content was analyzed using the American Public Health Association method [24] by Atomic Absorption Spectroscopy (AAS) which previously had been calibrated with a standard solution of Pb. Pb accumulation in seedling tissue was calculated by measuring the levels of Pb in the tissue. After the seedlings were harvested and separated into 3 part (roots, stems and leaves), then they were dried in the oven with a temperature of 45 ± 2°C until constant weight. Special for roots seedling, before being dried they were soaked first for 30 minutes in 1.0 mM H-EDTA and rinsed three times in H_2O in order to eliminate attachment Pb on the root surface [25]. The value of Pb then was used as the basis for calculating value of Factor Bioconcentration/BF (Bioconcentration Factor/BCF), Factor Transport/FT (Translocation Factor/TF) and bioaccumulation /B (bioaccumulation/B) [7], by the equation as follows:

$FB = C_{root} / C_{soil}$ ... (C_{root} is the concentration of Pb in roots and C_{soil} is Pb concentration in soil / media)

$FT = C_{aerial} / C_{root}$ .... (C_{aerial} is the concentration of Pb in the shoots)

$B = C_{aerial} \times DW$ ..... (DW is tissue dry weight)

The data of Pb, FB, FT and bioaccumulation of Pb in the plant tissue were analyzed using SPSS data processing version 15 by comparing mean and analysis of variance.

3. Results

3.1. Jabon Seedling biomass

After 12 weeks of growth, biomass of Jabon seedling was measured. Single factor compost (C) influenced significantly ($p \leq 0.05$) to the seedling biomass except the roots biomass. Compost application increased the average of stem, leaf and total biomass of Jabon seedling, respectively 50%, 200% and 110%. Single factor mycorrhiza also significantly influenced ($p \leq 0.05$) the average biomass of Jabon seedlings, except biomass of stem. Applications of AMF increased the biomass of roots, leaves and total biomass for 48%, 20% and 14%, respectively. While the single factor Pb gave significant effect to the leave`s biomass, only. But in general, Pb exposure has reduced seedling biomass, as total plant biomass reached 49% when the seedlings were exposed to 0.5 mM Pb, and decreased 14% when exposed to 1.5 mM Pb. Double and third interaction factor, compost-mycorrhiza-Pb, did not significantly affect the Jabon biomass. However, application of compost and mycorrhizal increased the biomass of Jabon leaf by more than 200%, and 140% . The greatest total biomass of seedling was found in the treatment of compost-AMF-Pb 0, amounting to 228.9 g (table 1).

3.2. Pb levels in the tissue of Jabon seedlings

The measurements of lead content in Jabon tissue seedlings was carried out 12 weeks after planting. The result showed that lead was found in all parts of the seedling tissues, either in the roots, stems and leaves with the value varied between 0.2 - 86 ppm. The average levels of lead in the roots was larger up to 50% (30.2 ppm) than in the stem (16.2 ppm) and leaves (15.4 ppm) of the seedlings. The application of compost on the media tailing significantly ($p \leq 0.05$) increased average level of Pb in the roots and stems, but it was decrease in leaves.

Applications of AMF significantly lowered ($p \leq 0.05$) average levels of Pb in roots, stems and leaves of seedlings, with the decrease of 18-33%. Exposure to $Pb(NO_3)_2$ by 0.5 mM (150 mg kg^{-1} tailings) resulted in an increase of lead content on the whole seedling tissues, but it decreased significantly when the Pb treatment in the tailings reached 1.5 mM (Table 2). The combination of compost together with AMF significantly ($p \leq 0.05$) increased the level of Pb in the roots, stems and leaves of control seedling (without Pb treatment) up to 6, 16 and 27 fold respectively. However, on the seedling exposed to lead tailings by 0.5 mM, the application caused the level of Pb in the seedling was lower (46.8 ppm in the roots, 30.2 ppm in the stem and 16.5 ppm in the leaves. When seedlings were exposed to 1.5 mM of Pb, the effect of compost and AMF application was inconsistent, since Pb content was decreased in the roots and leaves, while it was increased in the Jabon stem (table 2).

3.3. Bioconcentration factor of Pb (BF)

Bioconcentration factor (BF) shows the comparison of lead level which is transported into the seedling tissues to that present in the tailing media. After 12 weeks of lead exposure in the tailing media, lead bioconcentration factors varied widely, from 0.1-1.6. The highest BF presented by roots tissue followed by the stems, and then the leaves. The higher value of FB (exceeded 1) was shown only by the seedlings applied with compost without any exposure to lead (Pb Compost-0). The effect of AMF to BF was inconsistent among all applications so that there was no particular pattern on the value of BF of lead (figure 1).

3.4. Transport Factors of Pb (TF)

Transport Factors (TF) illustrates the comparison of Pb in the shoot (stems and leaves) to that in the roots of seedlings. The TF value was divers in Jabon seedling after 12 week application in media tailing exposed by different lead concentration and compost application in combination with mycorrhizal fungi (Figure 2). TF value of seedling Jabon was ranged between 0.1 - 1.1 for stem and

0.1 - 1.0 for leaves. The application of compost enhanced TF of lead to an average of 0.7 on the stem and 0.4 on the leaves. Applications of AMF did not change the average of TF (0.4 - 0.5). Increased lead exposure lowered TF on the stem to an average of 0.3, but increased TF of leaves up to 0.6. The TF value ≥ 1 was found only in seedlings without compost, without AMF and at the Pb level of 0.5 mMol, with compost, without AMF and Pb 0, and with compost and AMF and Pb 1.5 (Figure 2).

Table 1. Dry biomass of Jabon seedlings grown on tailing media exposed to different Pb concentrations and different applications of compost and AMF at 12 weeks after planting.

Treatment			Root		Stem		Leaves		Total	
			================== gram ====================							
Without Compost	-AMF	Pb 0	24.0	ab	70.0	bc	82.9	bc	176.8	bcd
		Pb 0.5	12.0	ab	2.0	a	15.5	ab	29.2	ab
		Pb 1.5	5.8	a	6.3	a	14.3	ab	26.2	ab
	+AMF	Pb 0	25.0	ab	24.6	ab	54.6	bc	104.0	bcd
		Pb 0.5	18.2	ab	16.3	ab	40.2	bc	74.5	bc
		Pb 1.5	25.3	ab	12.8	ab	50.3	bc	88.2	cd
Compost	+AMF	Pb 0	8.3	a	24.5	ab	111.8	bcd	144.4	bcd
		Pb 0.5	15.4	c	39.2	bc	68.6	bc	122.9	bcd
		Pb 1.5	14.6	bc	40.0	ef	168.9	bcd	223.3	de
	+AMF	Pb 0	20.7	ab	33.5	bc	174.8	bcd	228.9	de
		Pb 0.5	10.8	a	24.2	ab	72.1	bc	106.8	bcd
		Pb 1.5	18.8	ef	42.2	bc	162.5	bcd	223.3	de
F-Level		C	0.09	ns	36.68	s	42.04	s	32.93	s
F- Level		M	22.54	s	2.04	ns	3.09	s	4.95	s
F- Level		P	0.37	ns	0.51	ns	2.19	s	1.44	ns
F- Level		C*M	9.1	s	1.03	ns	0.01	ns	0.41	ns
F- Level		C*P	0.69	ns	0.75	ns	2.21	s	1.67	ns
F- Level		M*P	2.87	s	1.41	ns	1.05	ns	1.2	ns
F- Level		C*M*P	0.29	ns	0.26	ns	0.98	ns	0.48	ns

Note: Average data followed by similar letter in the same column means not significantly different based on 5% of DMRT test.
ns : $p > 0.05$, not significantly different based on 95 % confidence interval of DMRT test
s : $p \leq 0.05$, significantly different based on 95% confidence interval of DMRT test
C : Compost, M : Micorrhiza, P : Pb, AMF : Arbuscular Mycorrhizal Fungi

3.5. Bioaccumulation of lead in tissue seedlings

Bioaccumulation of lead describes the amount of lead absorbed and accumulated in the seedling tissues. The result showed the diversity of bioaccumulation values in the seedling tissue which was influenced by Pb treatment and application of compost in combination with AMF as shown in Table 3. The entirely treatments either single, double and triple interaction of the factors including Pb treatments, compost and AMF significantly influenced bioaccumulation of Pb ($p \leq 0.05$) in the Jabon seedlings.

Part of Jabon shoots (stems and leaves) seem to accumulate more lead than the roots, reaching an average of 137.6×10^{-2} mg/plant at the tip. While in the roots it was only 43.3 x 10-2 mg/plant. The application of compost provided a significant increase ($p \leq 0.05$) on Pb accumulation, and in the shoots it was more than three-fold, but the increase was not significant in the root seedlings. The AMF application also increased Pb accumulation up to 50% in the shoots and 22% in the roots. Pb treatment also increased significantly Pb accumulation in the tissue seedlings, the largest accumulation when exposed to 0.5 mM Pb was in the root (56.4 x 10-2 mg / plants) and stems (45.4 x 10^{-2} mg / plant), and when it was exposed to 1.5 mM of Pb the largest accumulation was in the leaves (152.1 x 10^{-2} mg / plants) and shoots (190.3x10^{-2} mg / plant) (Table 3).

Table 2. The Pb content in the tissue of Jabon seedlings grown on tailing media exposed to different Pb concentrations and different applications of compost and AMF 12 weeks after planting.

Treament			Root		Stem		Leave	
			----------------------- ppm ----------------					
Without Compost	-AMF	Pb 0	3.5	a	1.1	a	0.3	a
		Pb 0.5	60.8	f	60.2	b	60.5	d
		Pb 1.5	43	e	1.9	a	17.7	bc
	+AMF	Pb 0	2.5	a	1.3	a	0.2	a
		Pb 0.5	26.4	c	10.5	ab	12.6	abc
		Pb 1.5	33.3	cd	3.9	ab	12.6	abc
Compost	-AMF	Pb 0	21.4	b	15.7	ab	6.8	ab
		Pb 0.5	42.2	de	20.3	ab	12.7	abc
		Pb 1.5	39.2	de	7.7	ab	10.7	abc
	+AMF	Pb 0	26.4	c	17.1	ab	8.4	ab
		Pb 0.5	46.8	e	30.2	ab	16.5	ab
		Pb 1.5	27.6	c	27.4	ab	20.7	c
F-level		C	6.66	s	1.75	ns	3.14	s
F-level		M	22.57	s	0.58	ns	7.32	s
F-level		P	163.30	s	10.62	s	25.08	s
F-level		C*M	27.23	s	7.68	s	18.05	s
F-level		C*P	20.38	s	3.47	s	15.66	s
F-level		M*P	13.57	s	4.04	s	11.49	s
F-level		C*M*P	16.15	s	3.95	s	8.18	s

Note: Average data followed by similar letter in the same column means not significantly different based on 5% of DMRT test.
ns : $p > 0.05$, not significantly different based on 95 % confidence interval of DMRT test
s : $p \leq 0.05$, significantly different based on 95% confidence interval of DMRT test
C : Compost, M : Micorrhiza, P : Pb, AMF : Arbuscular Mycorrhizal Fungi

4. Discussion

Jabon seedlings (12 weeks after planting) were still capable to grow following exposure to Pb for up to 1.5 mM However, plant biomass was declined for nearly 50%. It indicates that Pb can affect the metabolism of Jabon seedling or even Pb can be toxic at certain concentrations [8, 26]. It was reported that up to 1500 ppm, Pb exposure has caused death of *Paraserianthes falcataria* as indicated by clorosis and necrosis [27]. The existence of Pb ion can take over Mg ions in chlorophyll that may inhibit enzymatic activity of those enzymes responsible in the synthesis of chlorophyll [28,29], thus resulting metabolic constraints that reduces plant growth.

The application of compost and AMF, both in single and double treatments, has increased the total biomass of Jabon seedlings for more than 140% as well as improving seedlings vegetative performance when exposed to lead. The role of compost in improving seedling tolerance to lead exposure presumably due to improvements in the chemical, physical and biological tailing media that support metabolic activities of the plants Production of enough carbohydrates via photosynthesis will provide energy to improve plant growth through the activity of cell division, as well as to produce compounds (i.e organic acids) to chelate and efflux lead toward cells that do not perform active metabolism [30,31,32,33]. This compartment may reduce the toxic effects of lead to plant cells although level of Pb in tissue is increased, as observed in our study (table 2).

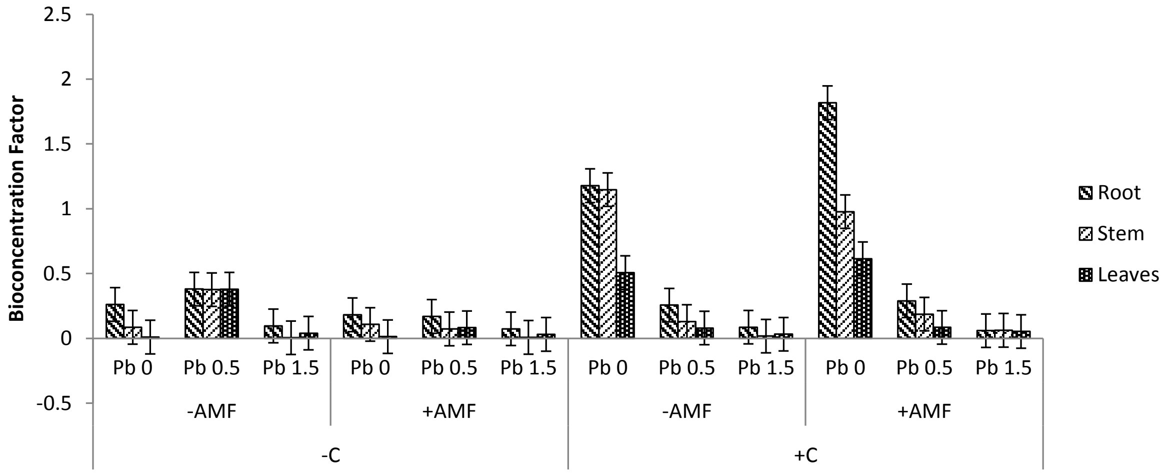

Figure 1. Bioconcentration factor (BF) of Pb in the tissue of Jabon seedlings grown at tailing media exposed to different Pb concentration and different application of compost (C) and AMF, 12 weeks after planting.

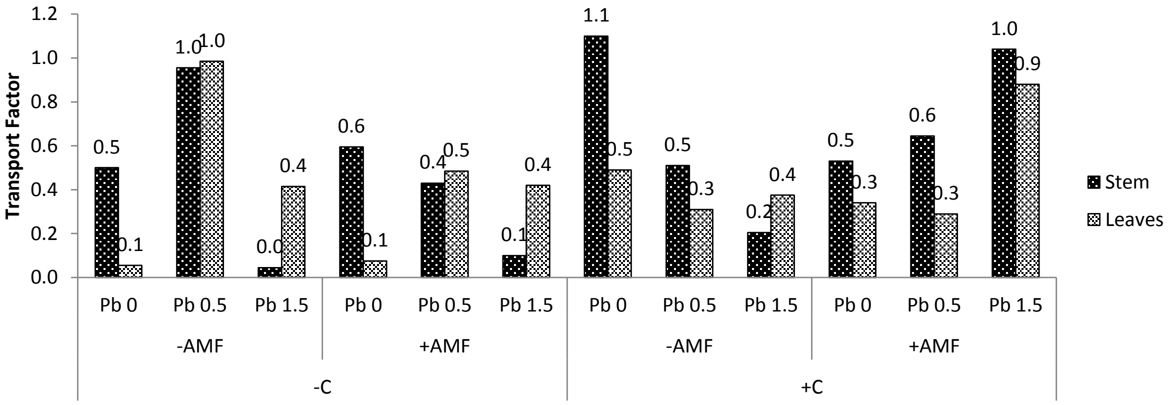

Figure 2. Transport Factor (TF) of Pb in the tissue of Jabon seedlings grown at tailing media exposed to different Pb concentration and different application of compost (C) and AMF, 12 weeks after planting.

AMF may contribute in improving seedling biotmass through direct and indirect ways. AMF may reduce the negative effects of Pb or increase seedlings tolerance by reducing level of Pb in tissue seedlings (table 2). Mycorrhizae may collect the heavy metals in the extrametrical mycelium, thus decreases heavy metals concentration in soil-root area as well as roots and shoots in the network [34, 35]. Mycorrhizae may help plants to escape from the toxic level of specific pollutants through the production of detoxification compounds, i.e such as organic acids or by binding pollutants in the tissues of fungi in the roots and form a physical barrier thaht inhibit the translocation of pollutants into the stem of the host plant [35]. Moreover, mycorrhizae can indirectly improve the ability of plants to survive on contaminated soil by improving plant nutrition status especially in soil with low phosphorus bioavailability, protecting plants from root pathogens, drought stress, improving soil aggregation and increasing the retention against xenobiotics [35].

Table 3. Bioaccumulation of Pb in the tissue of Jabon seedlings grown at tailing media exposed to different Pb concentration and different application of compost and AMF 12 weeks after planting.

Treatment			Root		Stem		Leaves		Shoot	
			------------- x 10^{-2} mg/plant							
Without Compost	-AMF	Pb 0	8.2	a	7.7	ab	2.5	ab	10.2	ab
		Pb 0.5	69.4	bc	11.8	ab	96.0	cde	107.8	bcd
		Pb 1.5	22.3	a	1.2	a	26.1	ab	27.3	ab
	+AMF	Pb 0	6.1	a	3.2	a	1.1	a	4.3	a
		Pb 0.5	46.5	b	17.1	abc	51.1	abc	68.2	abc
		Pb 1.5	82.3	c	5.0	a	63.9	bcd	68.9	abc
Compost	-AMF	Pb 0	16.5	a	38.5	cd	76.3	bcd	114.7	bcd
		Pb 0.5	62.3	bc	79.5	e	87.6	cd	167.1	cd
		Pb 1.5	54.8	b	30.8	bc	181.2	f	212.1	de
	+AMF	Pb 0	53.17	b	57.3	de	147.2	ef	204.5	d
		Pb 0.5	47.53	b	73.0	e	119.6	de	192.6	cd
		Pb 1.5	50.33	b	115.6	g	337.2	g	452.9	e
F-level		C	622.5	tn	30409	n	118427	n		
F-level		M	682.1	tn	2585.7	n	13271	n		
F-level		P	9035.7	n	2137.7	n	51679	n		
F-level		C*M	75.9	tn	2132.9	n	20659	n		
F-level		C*P	853.7	n	605.2	n	29531	n		
F-level		M*P	1791.1	n	1728.1	n	8431.7	n		
F-level		C*M*P	21110	n	1650.9	n	326.1	n		

Note: Average data followed by similar letter in the same column means not significantly different based on 5% of DMRT test.
ns : $p > 0.05$, not significantly different based on 95 % confidence interval of DMRT test
s : $p \leq 0.05$, significantly different based on 95% confidence interval of DMRT test
C : Compost, M : Micorrhiza, P : Pb, AMF : Arbuscular Mycorrhizal Fungi

Jabon seedlings grown on the tailing media for 12 weeks which were exposed with different concentrations of lead were able to absorb and distribute Pb in all parts of their tissues, where the lead was mostly accumulated in root and less accumulated in stem and leaves. Bioconcentration factor values ranged from 0.1 to 1.6, demonstrated that the level of lead in the roots was widely varied, starting from the lowest for only one-tenth to the level of even exceeded from the concentration in the tailings media. The average levels of lead in roots was still 50% greater than in the stems and leaves, suggesting that Pb accumulation pattern was root> stem> leaf. This pattern is similar with previous study in other plants, including *Jatropha* [36] and *Chlorophytum comosum* [28]. The Application of Pb 0.5 mM significantly increased Pb level in the tissues, however when 1.5 mM of Pb was applied, the Pb level in the tissues was decreased (table 2) showing that application of high concentration of may inhibit Pb accumulation in the seedling tissues.

The application of compost markedly increased the levels of Pb in root and stem for 14% and 46%, repspectively, but to decreased by 30% in the leaves. The increased levels of Pb on the tissues in response to compost application was likely triggered by humic acid which is is able to bind metal as well as the normal pH value of compost (pH 6-7) which increased metal absorption by plant roots

[28,37]. It is reported that complex compound of humic acids consisted of carboxyl group and phenolic is able to form a complex binding with metal ions. Indeed, chelating formation is an important aspect of humic acid's role in regulating the availability (bioavaibility) of ion metals [38, 39]. Utilization of humic acid to increase heavy metal availability in the soil such as cadmium and lead has been demonstrated in wheat (*Triticum aestivum*). It is reported that higher concentration of humic acid (0, 140, 280 and 560 mg / kg) in the pot, leads to the increased concentration of heavy metals in plant biomass, while the plant biomass decreased with the increasing concentrations of heavy metals in wheat [40].

The AMF application, on the other hand, generally decreased levels of Pb in the tissues by 15% in the roots, 17% in the stem, and 33% in the leaves. Similar results were exhibited in previous study as lower levels of metals were detected in the tissues of infected Mindi (*Melia azedarach* Linn) seedlings with AMF in the tailing media applied with compost [2] and in maize infected with *Glomus intraradices* [41]. Meanwhile, opposite result was found in *G. intaradices*-inoculated tobacco plant [42] as well as in Brachiaria Grass [16]. It seems likely that AMF exhibits diverse effects on the levels of metal absorption by plants which is highly associated with one of the mechanisms of mycorrhizal fungi to protect the plant from heavy metal toxicity [22,43,44].

Based on the value of transport factor (TF) Pb in stem and leaves which were 0.5 and 0.4, respectively, it is likely that Pb was not transported from the roots to the shoots for more than 50%. The application of compost and FMA, although it increased the value of TF (the range of 0.4-0.7), however, the contribution was not significant because the range was still below one. This condition indicated that Jabon seedling grown in the tailing media applied with compost and AMF still categorized as metal excluder group or the plant prefer to save metal in the root [11]. The types of plants that are tolerant to heavy metals with high value of BF and lower TF has been suggested to be used as phytostabilizer in the contaminated area together with cover crop vegetations [45]. Jabon may have such properties and therefore has potential function as phytostabilizer for tailing area in order to minimize the occurrence of Pb migration into underground water and to reduce potential risk of entering Pb to the food chain.

Bioaccumulation becomes an important indicator to estimate potential crop in remediation activity. The application of compost and AMF provide a significant increase to the accumulation of lead in all parts of the seedling tissues, with the increase of 16-fold by compost, and 4-fold by AMF application. Consequently, a significant increase in biomass by compost and AMF application (Table 1) may cause even higher Pb accumulation. Such result has also been reported in the kenaf seedlings which treated with chicken manure application [7]. This fact indicates that despite the fact that Jabon seedling is categorized as metal excluder (TF<1), however, its capability to accumulate high amount of Pb in the shoots support the application of this plant as phytoextractor [42]. In addition, application of AMF and compost markedly promotes the Jabon seedling to conduct remediation activity.

5. Conclusion

After 12 weeks of lead exposure in the tailing media, the average levels of Pb in the roots of Jabon seedlings was up to 50% higher than that in the stem leaves. Compost increased the level of Pb in the roots and stems. Application of AMF decreased Pb levels for about 18-33%. Application of compost and AMF increased the accumulation of lead with the highest accumulation of lead was detected at 452.9 $x10^{-2}$ mg/plant in the shoot of the seedlings. Compost and AMF play an important role in phytoremediation activity mediated by jabo*n* seedling.

Acknowledgements

Acknowledgements to the Governments of Indonesia C.q. Directorate General of Higher Education for the research fund. Thanks are also conveyed to the Forest Biotechnology Laboratory, Research Center for Biological Resources and Biotechnology, Bogor Agricultural University, and Silviculture Laboratory, Nusa Bangsa University, Bogor, Indonesia.

References

[1] Ang L H and Ang T B 2000 *Proceedings of the Fourth Conference on Forestry and Forest Products Research* (Kuala Lumpur, Malaysian: Forest Research Institute Malaysia) pp 195–205
[2] Setyaningsih L 2007 *Pemanfaatan cendawan mikoriza arbuskula dan kompos untuk meningkatkan pertumbuhan semai Mindi (Melia azedarach Linn) pada media tailing tambang emas Pongkor* (Bogor: Sekolah Pascasarjana, Institut Pertanian Bogor)
[3] Pierzynski G M and Schwab A P 1993 *Journal Environ Qual* **22** 247-254
[4] Alloway B J 1995 *Heavy Metals in Soil. 2nd Edition* (London: Blackie Academic and Profesional)
[5] ATSDR Information Center 2015 *The ATSDR 2015 Substance Priority List*, https://www.atsdr.cdc.gov/spl/, Friday, 21 Oct. 2016, 5.55 am
[6] Clistenes W A and Baoshan X 2006 *Scientia Agricola* **63(3)**
[7] Mun H W, Ang L H and Lee D K 2008 *Journal of Environmental Science* **20** 1341-1347
[8] Kopittke P M, Asher C J, Kopittke R A and Menzies N 2007 *Environmental Pollution* **150** 280-287
[9] Salt D E, Smith R D and Raskin I 1998 *Annu. Rev. Plant Physiol Plant Mol Biol* **49** 643–68
[10] Kramer U 2005 *Biotechnology* **16** 133-141
[11] Baker A J M 1981 *Journal Plant Nutr* **3** 643-654
[12] Kim I S, Kang K H, Jhonson-Green P and Lee E J 2003 *Environmental Pollut* **126** 235-243
[13] Cunningham S D and Berti W R 2000 *Phytoextraction and phytostabilization: Technical, economic and regulatory considerations of the soil lead issue* In phytoremediation of contaminated soil and water. (Terry N, Banuelos G. eds) (Florida Lewis Publication) 359-376
[14] Altuhaish A, Hamim and Tjahjoleksono A 2014 *Emir. J. Food Agric* **26**(8) 716-722
[15] Ernst WHO 2005 *Chemie der Erde*, **65**(S1) 29-42
[16] Adriano D C, Wenzel W W, Vangronsveld J and Bolan N S 2004 *Geoderma* **122** 121-142
[17] Norland M 1993 *Soil Factors Affecting Mycorrhizal Use in Surface Mine Reclamation* (Bureu of Mines Information Circular: United States Departement of Interior)
[18] Killham K and Firestone M K 1983 *Plant Soil* **72** 39-48
[19] Smith S E, Read D J 1997 *Mycorrhizal Symbiosis* (London: Academic Press Limited)
[20] Chen B D, Zhu Y G and Smith F A 2006 *Chemosphere* **62** 1464-1473
[21] Gomes M P, P Silva M and C Nascentes 2015 *Bioremediation Journal* **19(2)** 151-159
[22] Shaker-Koohi and Sajjad 2014 *Int J Adv Biol Biom Res* **2(5)** 1854-1864
[23] Krisnawati H, Maarit K and Markku K 2011 *Anthocephalus cadamba Miq.Ecology, silviculture and productivity* (Bogor: Center for International Forestry Research)
[24] American Public Health Association 1998 *Standar methods for the examination of water and waterwaster* (USA: APHA, AWWA, WEF, Maryland, USA 20 the edition) p 3.56 & 4.178
[25] Jarvis M D and Leung D W M 2002 *Environmental and Experimental Botany* **48** 21-32
[26] Kabir M, Zafar I M and Shafiq M 2009 *Advances in Environmental Biology* **3(2)** 184-190
[27] Setyaningsih L, Y Setiadi, D Sopandie and B S Wilarso 2012 *JMHT* **XVIII (3)** 177-183
[28] Wang Y, Tao J and Dai J 2011 *African Journal of Biotechnology* **10** 14516-14521
[29] Xiao W, Hao H, Liu X Q, Liang C, Chao L, Su M Y and Hong F H 2008 *Biology of Trace Elements Research* **126** 257-68
[30] Taylor G J 1991 *Plant Biochemistry and Physiology* **10** 57-93
[31] Tong Y P, Kneer R and Zhu Y G 2004 *Trends Plants Sci* **9(1)** 7-9
[32] Reichman S M 2002 *The Responses of Plants to Metal Toxicity: A review focusing on Copper, Manganese and Zinc* (Melbourne: Australian Minerals & Energy Environment Foundation)
[33] Yang X, Ying F, Zhenli H and Peter J S 2005 *Journal of Trace Elements in Medicine and Biology* **18** 339–353
[34] Marschner H 1995 *Mineral Nutrition of Higher Plant* (London: Academic Press 2nd Ed)

[35] Vosatka M, Jana R, Sudova R and Martin V 2006 *Soils Phytoremediation Rhizoremediation* **9A** 237-257
[36] Shu, Xiao, Yin L Y, Zhang Q F and Wang W B 2011 *Environ Sci Pollut Res* **(9)** 1-10
[37] Lee S Z, Chang L, Yang H H, Chen C M and Liu M C 1998 *J Hazand Mater* **63** 37-49
[38] Stevenson F J 1994 *Humus Chemistry, Genesis, Composition, Reactions* (New York: Wiley) p 496
[39] Ghabbour E A and Davies, G 2001 *Humic Substances: Structures, Models and Functions* (Cambridge, U.K.: RSC publishing) ISBN 978-0-85404-811-3
[40] Baodong C and Yong-Guan Z 2006 *Journal of Soils and Sediments* **6(4)** 236-242
[41] Malcova R, Vostka M and Gryndler M 2003 *Applied Soil Ecology* **23(1)** 55-67
[42] Sudova R, Daniela P, Tomas M and Miroslav V 2007 *Applied Soil Ecology* **35** 163–173
[43] Leyval C, Turnau K and Haselwander K 1997 *Mycorrhiza* **7** 139-153
[44] Karimi A, Habib K, Mozhgan S and Mirhassan R S 2011 *African Journal of Microbiology research* **5(13)** 1571-1576
[45] Yoon J, Cao X D, Zhou X Q and Ma L Q 2006 *Science Total Environment* **368** 456-464