

## A Lysimeter Study on Growth and Physiological Characteristics of *Melaleuca cajuputi* Planted In Contaminated Bris Soil

Noor-Liyana-Athirah Muhamad<sup>1</sup>, Hazandy Abdul-Hamid<sup>1,2</sup>, Arifin Abdu<sup>1,2</sup>,  
Nik Mohd Majid<sup>1,2</sup> and Mohd-Kamil Ismail<sup>1</sup>

<sup>1</sup> Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>2</sup> Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

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**Abstract:** Heavy metal pollution in soil and groundwater is currently one of the worst environmental problems the world over because it is significantly toxic to all living things. In this study, a simple lysimeter method was used to determine the heavy metal uptake of *Melaleuca cajuputi* via transport and leaching losses of solutes and to investigate the best Cu and Zn concentrations for the growth and physiological development of this species. *M. cajuputi* seeds were planted in simple lysimeter pots containing beach ridges interspersed with swales (BRIS) soil according to a completely randomized design (CRD). Four different Cu and Zn concentrations were applied—0 (control), 100, 300 and 500 ppm—with nine replications for each treatment. Observations of the growth and physiological performance of the plants at the end of the study showed that 100 ppm of each Cu and Zn yielded the best results for all the parameters measured, whereas 500 ppm of each Cu and Zn yielded the lowest. This finding facilitates better understanding of the growth and physiological attributes of *M. cajuputi*, which shows potential for remediating contaminated sites. Despite the simplicity of the method used in this work, it provides sufficient insight into *M. cajuputi*. It also serves as basis for testing new and alternative methods of designing experiments that effectively measure the heavy metal uptake of plants via leaching losses. Tested plant can be used for phytoremediation.

**Keywords:** Lysimeter, Heavy metal, *M. cajuputi*, BRIS soil, Phytoremediation.

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### I. Introduction

The deposition of atmospheric and industrial waste, mining waste, agricultural chemicals, waste from human activities and incidental accumulations, are only some of the sources of heavy metal contamination (Zubillaga et al., 2008). Heavy metals represent one of the most pressing threats to water and soil resources, as well as to the health of humans and other living things. The recent widespread use of fertilisers in agricultural activities may increase the contamination of groundwater by heavy metals. Inorganic or chemical fertilisers, which contain metal contaminants such as copper (Cu), zinc (Zn), arsenic (As) lead (Pb) and many more, may present numerous advantages because of their availability and easy and fast absorption by plants. However, leaching, wherein soil is depleted of its natural nutrients, is considerably more prevalent with fertiliser use.

Even though Zn and Cu are essential micronutrients for plant growth and physiological performance, high concentrations of these heavy metals can be toxic to plants and soils. The ingestion of such contaminants causes serious health problems to living things, especially human beings. The threat posed by heavy metals to living things is exacerbated by their long-term persistence in the environment (Yoon et al., 2006). In agricultural soil, high Cu contents usually result from the long-term use of Cu-containing fungicides and animal manure; Zn is present in extreme concentrations in the majority of industrial waste (Rossi et al., 2004). Several technologies for remediating soils from heavy metal contaminants have been reported. Nevertheless, as Yoon stated (as cited in Cao et al., 2002 and Mulligan et al., 2001) many of these technologies (e.g. excavation of contaminated material either chemical or physical treatment) are very costly and do not attain long-term or aesthetic result. One of the easiest and most inexpensive ways to remove contaminants from the earth is the phytoremediation method. This method involves the engineered use of green plants to remedy, remove or render environmental contaminants harmless; it is a cost-effective, long-lasting and aesthetic approach to remediating contaminated sites (Yoon et al., 2006).

Given the importance of removing heavy metals from contaminated land (Wong, 2003), an understanding of reactive plant transport in porous media is necessary to predict the fate of pollutants in soils and aquifers (Hu et al., 2007). An alternative technique is to measure the heavy metal uptake of plants via leaching losses. Outdoor leaching or percolation experiments, which are carried out under natural field conditions, generally refer to lysimeter experiments. The original application of lysimeter has elicited increasing attention in the last decade because of the recent rise in groundwater pollution and contamination. Lysimeters are essential tools for monitoring soil, plant and atmospheric conditions. According to Lazarovith (2006, as cited

in Hillel et al., 1969 and Van Barel, 1961), a lysimeter can directly measure actual evapotranspiration rates and facilitate water, fertiliser and solute balance studies. As part of natural physiological processes, plants normally pump water, nutrients, solutes and organic matter from surrounding media. This potential can be used to remove, break down or stabilise contaminants in soil (Robinson et al., 2003).

The selected plant species in this study was *Melaleuca cajuputi*. It is locally known as Gelam or Kayu Putih and belongs to the Myrtaceae family. This species can produce essential oils that are suitable for medicinal purposes; the cajuput oil from *M. cajuputi* has been used as external treatment for headache, toothache, ear-ache, rheumatic cramps and fresh wounds (Lim et al., 2001). Ko Ko (2009, as cited in Doran & Gunn, 1994) indicated that the leaves are also used as flavouring in cooking and as a fragrance and freshening agent in soaps, cosmetics, detergents and perfumes. *M. cajuputi* naturally occurs in swamp forests between old raised sea beaches and mangroves (Lim et al., 2001). It has a potential to survive in sea beaches soil which both waterlogged and well-drained soils. Therefore, this study was conducted using beach ridges interspersed with swales (BRIS) soil as a planting medium to maintain natural occurrence of this species.

Numerous studies indicated that many plant species have been tested because of their ability to accumulate toxic elements (Tlustos et al., 2006), but little research has been directed towards determining the physiological performance and growth responses of *M. cajuputi* by a simple lysimeter method. The capability of this species to survive in water-logging conditions enable the isolation of liquid-form Cu and Zn that leach to groundwater systems—a task that can also be performed using a simple lysimeter method. Different heavy metal concentrations may cause different growth responses in *M. cajuputi*. Madyar (2008) stated that Zn and Cu are considered dangerous for organisms at concentrations of 5 and 1 mg/L, respectively. Hence, the objectives of this study were to determine the heavy metal uptake of *M. cajuputi* via transport and leaching losses of solutes and to investigate the best Cu and Zn concentrations for the growth and physiological performance of the selected species.

## II. Experiment

**2.1 Plant materials and study site:** This study was conducted in a nursery at Faculty of Forestry, Universiti Putra Malaysia (UPM) Serdang, Selangor starting on 1<sup>st</sup> May 2011 until 30<sup>th</sup> September 2011. Seventy two seedlings aged three-month old of *Melaleuca cajuputi* as well as BRIS soil were taken from Setiu, Terengganu. All the seedlings were planted in polybag and then were transferred to simple lysimeter pots which consist of a filter, and piping system.

**2.2 Experimental design:** A completely randomized design (CRD) was used in this study. Different Zn and Cu concentrations, i.e. 0 (control), 100, 300 and 500 ppm, were applied. The heavy metals were mixed with water at certain concentrations to create the levels required for the treatments. Each concentration was replicated nine times. After the seedlings acclimatised to the soil medium and the environment, the treatments were applied to each plant. These seedlings were then covered with plastic bags in order to control the transpiration via soil medium and also to avoid the biasness of the data.

### 2.3 Data collection:

**2.3.1 Gas exchange:** Three fully expanded leaves were selected for each plant. Five of gas exchange parameters, namely, net photosynthesis rate ( $A_{net}$ ), stomatal conductance ( $G_s$ ), transpiration rate ( $E$ ), leaf-to-air vapour pressure deficit ( $V_{pdL}$ ) and intercellular  $CO_2$  ( $C_i$ ) were measured using LiCor 6400, Portable Photosynthesis System. This open-type photosynthesis system was equipped with a standard 3cm x 2cm broadleaf cuvette. Calibrations for flow meter and  $CO_2$  zero values had been made before the gas exchange measurements. The  $CO_2$  concentration then was set at  $360 \text{ molm}^{-2}\text{s}^{-1}$  in order to avoid the effect of environmental fluctuating conditions. The cuvette irradiance, temperature, and relative humidity also were set at  $650 \text{ } \mu\text{mol photons m}^{-2}\text{s}^{-1}$  (saturating irradiance),  $25^\circ\text{C}$ , and 40% respectively. Measurements were taken from 7:30 a.m. to 11:30 a.m. to avoid the reduction in photosynthesis at midday.

**2.3.2 Growth performances:** Growth performances of all the seedlings were assessed on the basis of quantitative characteristics such as total height, collar diameter and survival rate. The actual growth performance of *M. cajuputi* in terms of height and diameter was expressed in absolute growth rate (AGR) and relative growth rate (RGR) according to the following equations:

$$AGR = \frac{W_2 - W_1}{\Delta T}$$

Where,

AGR : Absolute Growth Rate

W1 : Height/Diameter at first reading

W2 : Height/Diameter at second reading  
 $\Delta T$  : Different in time per weeks

$$RGR = \frac{\ln(W2) - \ln(W1)}{\Delta T}$$

Where,

RGR : Relative Growth Rate  
 Ln : Longitude  
 W1 : Height/Diameter first reading  
 W2 : Height/Diameter second reading  
 $\Delta T$  : Different in time per weeks

**2.3.3 Analysis of heavy metal content and measurement of pH:** Leachates were collected fortnightly by using a cylindrical tube. On the basis of seedling observations, all the seedlings needed approximately 2 to 3 days to absorb all the treatments applied to the pots. Thus, leachate samples were taken every 2 weeks to avoid data bias and to ensure that the treatments were absorbed by the plants or that the elements leached to the drainage system. The pH of the leachates before and after precipitation was measured using a HI-9828 Multiparameter Hanna instrument. Furthermore, the leachates were subjected to chemical analysis by atomic absorption spectrophotometry (AAS).

**2.3.4 Data analysis:** All the data were analyzed by using one way analysis of variance (ANOVA) to detect the significance of variation among different level of treatment applications. SPSS version 16.0 was used for analyses, and Duncan's new multiple range test was adopted in evaluating multiple comparisons or mean separation. Significant different of means comparisons was set at 0.05. The AGR and RGR were analyzed using mean separation test. Additionally, SigmaPlot version 10.0 was used to derive and plot graphs for all data collected.

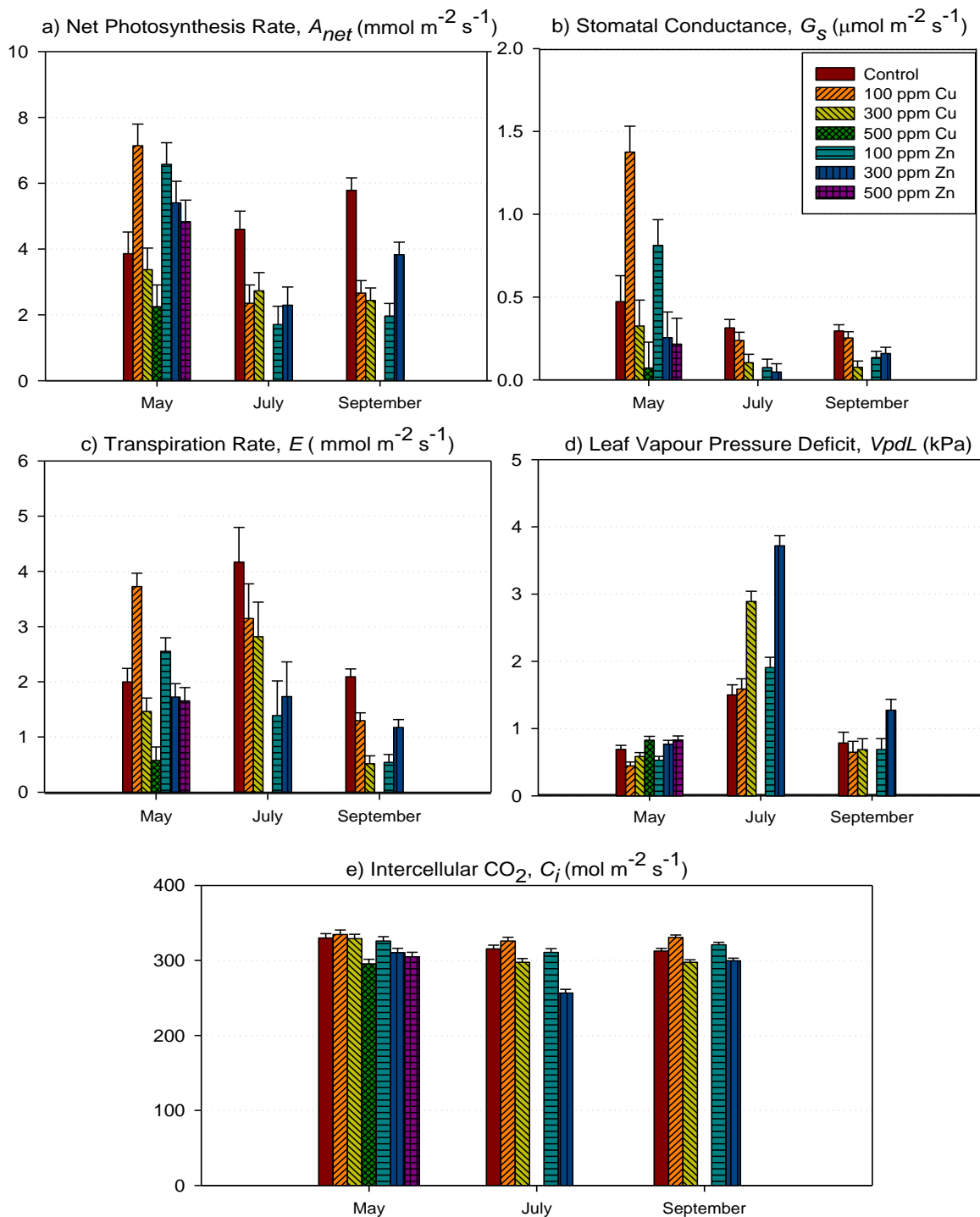
### III. Results

**3.1 Gas Exchange:** The summary of ANOVA for net photosynthesis (Anet), stomatal conductance (Gs), internal intercellular CO<sub>2</sub> (Ci), transpiration rate (E<sub>L</sub>) and leaf to air vapour pressure deficit (VpdL) between level of treatments applications was recorded in Table 1. Highly significant differences at p≤0.05 were found for all the gas exchange parameters between level of applications except in May for Anet. This shows that different level of treatment applications (0, 100, 300, and 500 ppm) gave significant effect and stresses to all the seedlings growth and survivality. The mean values of physiological parameters for all treatments for three selected study period May, July, and September were presented in Fig. 1(a-e). From the graph patterns, each 100 ppm of Cu and Zn were the highest mean values for all gas exchange parameters compared to control treatments except for leaf vapour pressure deficit, (VpdL) on May. The lowest means value for all parameters except VpdL on May were both 500 ppm of Cu and Zn respectively. Overall observation shows that all plant physiological responses on September were decreased dramatically.

**Table 1. Summary of ANOVA for net photosynthesis (Anet), stomatal conductance (Gs), internal intercellular CO<sub>2</sub> (Ci), transpiration rate (E<sub>L</sub>) and leaf to air vapour pressure deficit (VpdL) between level of treatment applications.**

Months	Parameters	Cu		Zn	
		MS	F	MS	F
May	Anet	40.769	10.500*	8.732	2.245 <sup>ns</sup>
	Gs	3.118	13.777*	0.822	3.864*
	Ci	2723.361	5.909*	1739.213	10.146*
	E	15.833	23.193*	1.508	3.919*
	VpdL	0.441	11.277*	0.3	14.048*
July	Anet	22.977	11.044*	45.823	13.156*
	Gs	0.153	6.704*	0.21	9.384*
	Ci	225297.7	1337.630*	196822.5	690.118*
	E	17.939	14.601*	66.571	11.347*
	VpdL	13.363	67.837*	20.84	95.080*
September	Anet	62.88	79.114*	45.415	24.759*
	Gs	0.232	15.719*	0.089	8.351*
	Ci	222694.7	5037.392*	218310.3	1271.145*
	E	13.839	60.012*	3.392	25.109*
	VpdL	1.382	118.105*	2.439	5.354*

Note: \* Significant different at p≤0.05, <sup>ns</sup> Not significant



**Fig. 1(a-e).** The mean values of physiological parameters for all treatments for May, July, and September. a) Net photosynthesis rate,  $A_{net}$ , b) Stomatal conductance,  $G_s$ , c) Transpiration rate,  $E$ , d) Leaf Vapour Pressure Deficit,  $V_{pdL}$ , and e) Intercellular  $\text{CO}_2$ ,  $C_i$ .

### 3.2 Absolute and Relative Growth Rate:

**3.2.1 Plant Diameter:** The graph pattern of absolute diameter growth rate (ADGR) and relative diameter growth rate (RDGR) for each treatments Cu and Zn between different level concentrations were presented in Fig 2(a-b). According to Fig 2(a), it shows that 100 ppm of Cu was the highest for both ADGR and RDGR compared to control treatments only in the first period of the study. The results however were different starting from the second period of the study until the end where 300 ppm Cu was found to be the highest compared to the others. Other than that, starting from the second period until the end, the slowest diameter increment for both

ADGR and RDGR was 500 ppm Cu respectively. Figure 2(b) subsequently shows that the control treatments were the highest starting from the first period of the study until the end for both results ADGR and RDGR. These treatments recorded the best diameter increments as there was no stress conditions occurred. As expected, 500 ppm of Zn was the lowest diameter increment for ADGR and RDGR at the end of study period. The summary of ANOVA for ADGR and RDGR for each Cu and Zn was shown in Table 2. There were no significant differences were found for Cu for first period of study (ADGR1 and RDGR1). However, the rest result shows significant differences at  $p \leq 0.05$  for both Cu and Zn.

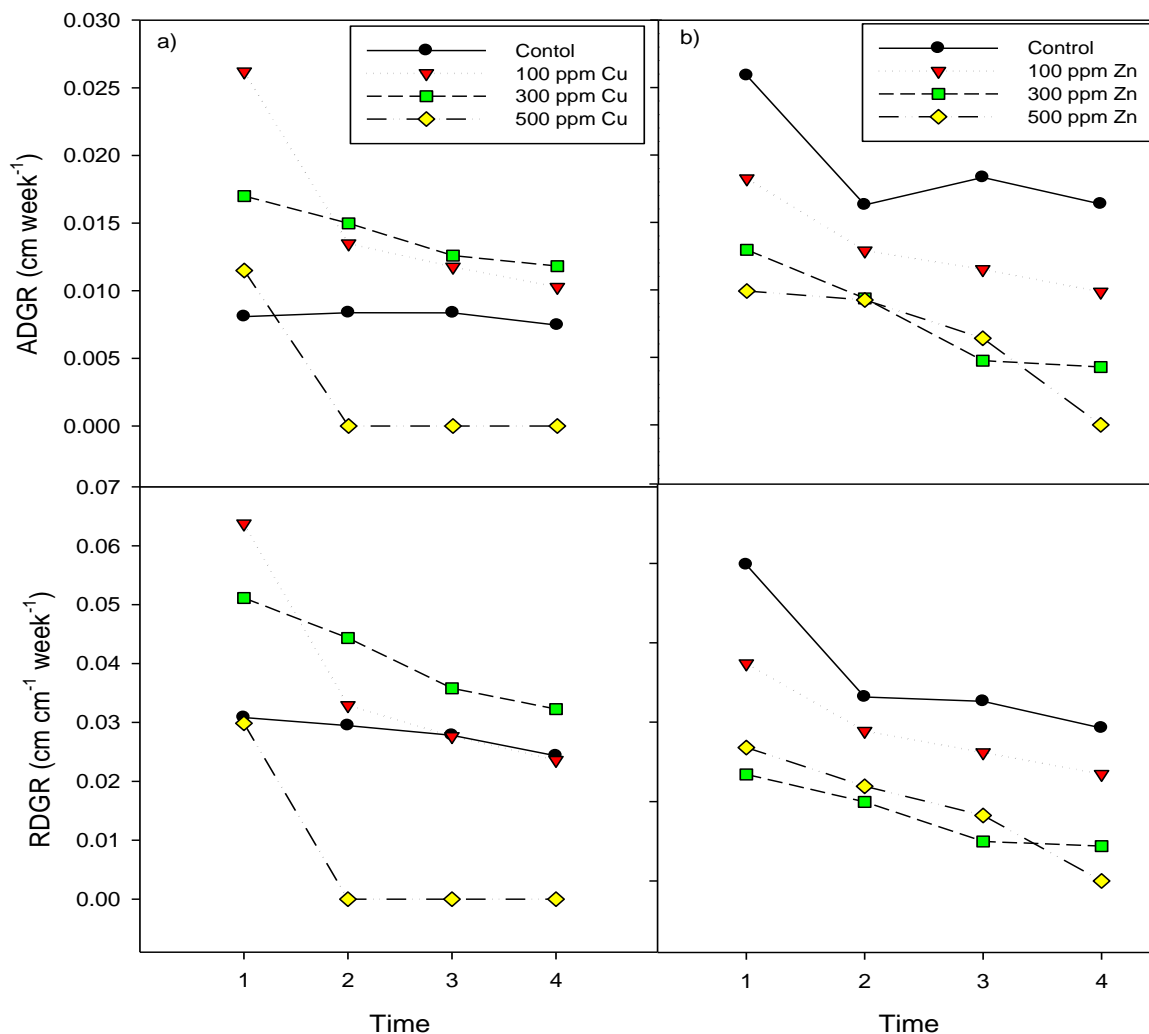


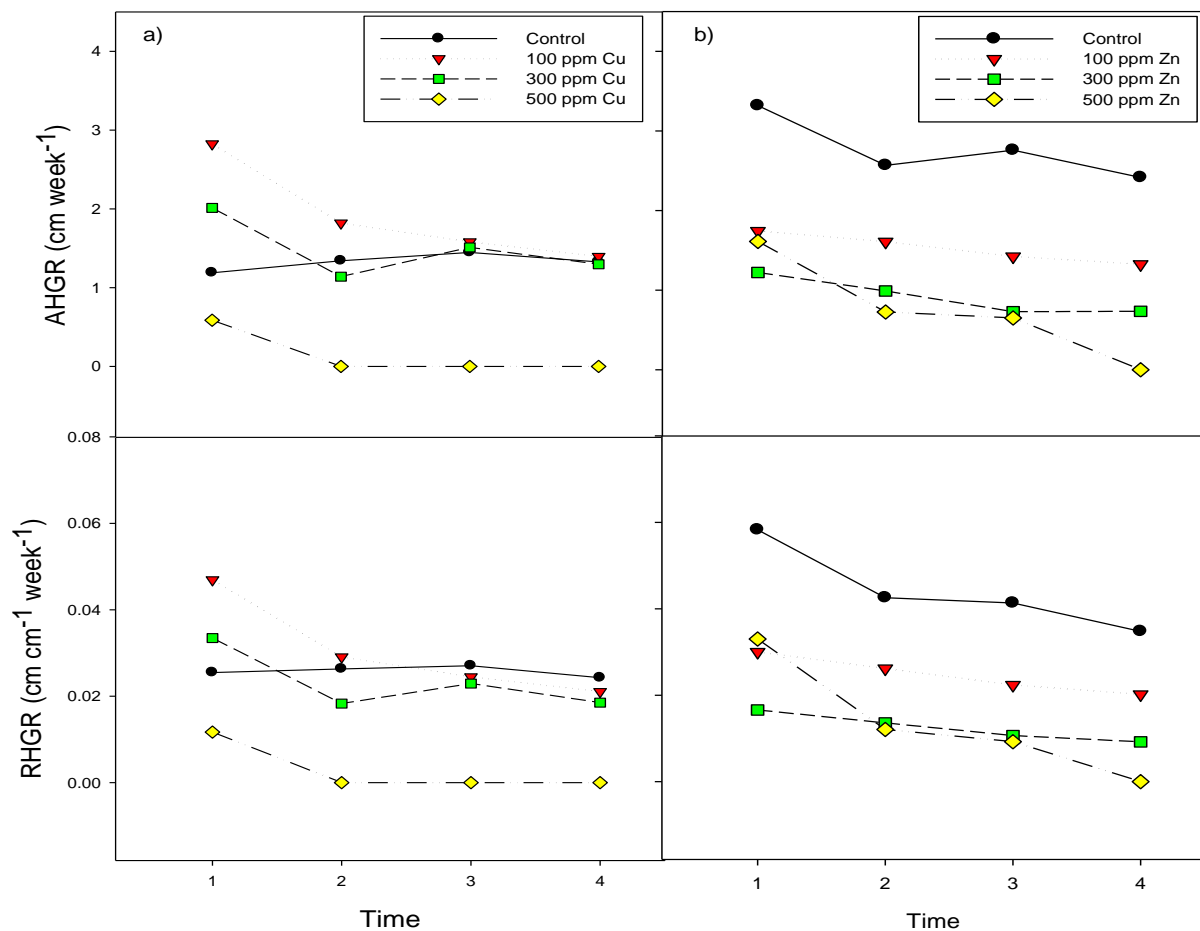
Fig. 2(a-b). The ADGR and RDGR for each treatments Cu (a) and Zn (b) between different level concentrations.

Table 2. The ANOVA for ADGR and RDGR for both Cu and Zn between level of treatments application.

Dependent Variable	Cu		Zn	
	MS	F	MS	F
ADGR1	0.00056	2.5729 <sup>ns</sup>	0.00044	3.7243*
ADGR2	0.00025	3.3451*	0.00040	17.0841*
ADGR3	0.00019	4.9467*	0.00062	33.1752*
ADGR4	0.00014	4.9888*	0.00051	33.3867*
RDGR1	0.00244	1.3444 <sup>ns</sup>	0.00510	4.2378*
RDGR2	0.00183	2.9882*	0.00362	16.6043*
RDGR3	0.00134	4.8395*	0.00408	39.4606*
RDGR4	0.00094	4.9597*	0.00306	46.6807*

Note: \* Significant different at  $p \leq 0.05$ , <sup>ns</sup> Not significant

**3.2.2 Plant height:** The graph pattern of absolute height growth rate (AHGR) and relative height growth rate (RHGR) for treatment Cu and Zn between different levels of concentration were presented in Figure 3(a-b). The AHGR and RHGR of 100 ppm of Cu was the highest recorded in the first and second period of the study compared to control treatments. However, the result for RHGR was found different starting from the third period until the end of the study. Meanwhile, both results of AHGR and RHGR for Zn showed that the control treatment recorded the highest starting from the first period of the study until the end. Then, as expected, the 500 ppm Cu and Zn was found to be the lowest growth increments starting from the second period of the study until the end. The summary of ANOVA for AHGR and RHGR for both Cu and Zn was recorded in Table 3. Highly significant at  $p \leq 0.05$  were found for both Cu and Zn between level of applications along the study period. This is because, certain trees in this study became unhealthy and weak as the time goes by since the stress condition occurred between different levels of heavy metals concentration resulted in decreasing the rate of heavy metal uptake dramatically.



**Fig. 3(a-b).** The AHGR and RHGR for each treatments Cu (a) and Zn (b) between different level concentrations.

**Table 3. Summary of one way ANOVA for AHGR and RHGR for both Cu and Zn between level of treatments application.**

Dependent Variable	Cu		Zn	
	MS	F	MS	F
AHGR1	8.5664	4.0285*	7.6608	3.4763*
AHGR2	4.2911	5.9042*	10.3902	13.2044*
AHGR3	3.6661	5.9973*	13.5163	21.0528*
AHGR4	2.9522	7.0355*	10.6896	23.1791*
RHGR1	0.0020	3.1061*	0.0027	3.4121*
RHGR2	0.0013	6.5686*	0.0030	14.5766*
RHGR3	0.0011	7.1645*	0.0031	23.4826*
RHGR4	0.0008	8.8591*	0.0023	28.2689*

Note: \* Significant different at  $p \leq 0.05$

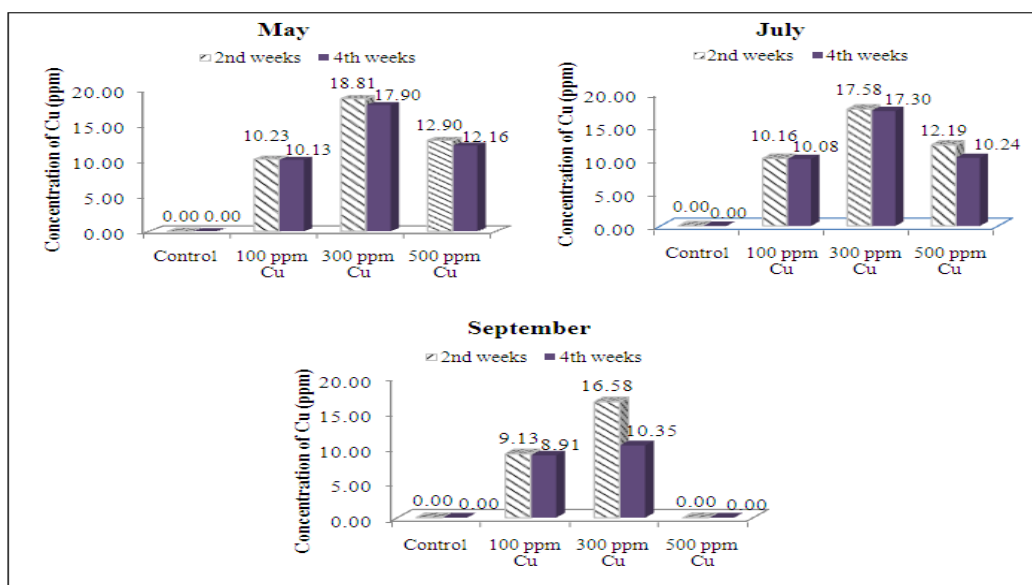
**3.3 Survival Rate:** The survival rate of this species which were recorded every month starting on 1<sup>st</sup> May 2011 until 30<sup>th</sup> September 2011 was shown in Table 4. During 1<sup>st</sup> May 2011 until 31<sup>st</sup> July 2011, all the plants were successfully survive. However, rely on the observations made, the results and graph patterns for all treatments obviously decrease starting on 17<sup>th</sup> July 2011 until the end of study, 30<sup>th</sup> September 2011. Starting on this 17<sup>th</sup> July 2011 until the end of July, each control treatments and 100 ppm of Cu were both decrease to 94.44% respectively meanwhile each control treatments and 100 ppm of Zn were still 100% survive. The 300 ppm Cu was decreased to 72.22% and 500 ppm Cu was decrease to 50%. The 300 ppm Zn was decreased to 83.33% and 500 ppm Zn was decreased to 55.56%. The results for Cu treatments were the same from 1<sup>st</sup> August 2011 until the end of study, which were control and 100 ppm were 88.89%, 300 ppm Cu was 44.44%, and 0% for 500 ppm Cu respectively. However, the control for Zn was still 100% survive until the end of study. The result of 100 ppm Zn was decreased from 91.67% until 88.89%. The 300 ppm Zn and 500 ppm Zn were decreased to 44.44% and 0% respectively until the end.

**Table 4. The Survival Rate for The Plant Species from 1<sup>st</sup> May 2011 until 30<sup>th</sup> September 2011.**

May-September 2011	Treatment Survival Rate (%)							
	Copper				Zinc			
	Control	100 ppm	300 ppm	500 ppm	Control	100 ppm	300 ppm	500 ppm
May	100	100	100	100	100	100	100	100
June	100	100	100	100	100	100	100	100
July	94.44	94.44	72.22	50	100	100	83.33	55.56
August	88.89	88.89	44.44	0	100	91.67	44.44	0
September	88.89	88.89	44.44	0	100	88.89	44.44	0

**3.4 Heavy Metal Content Analyzing:**

**3.4.1 Leachate analysis:** Figure 4 and 5 show leachate analyzing result using the Atomic Absorption Spectrophotometry (AAS) method for Cu and Zn for second and fourth weeks of three selected study period. It shows that in May, the 300 ppm of Cu was the highest concentration of Cu for its leachate sample for both second and fourth weeks of study period which were 18.81 and 17.90 ppm respectively. The same results also was found on July and September for the 300 ppm, however the concentration value were slightly different from May which were 17.58 and 17.30 ppm respectively for July, then 16.58 and 10.35 ppm respectively for September. Otherwise, the second weeks of study period for 300 ppm of Cu was the highest during September which was 16.58 ppm. Nevertheless, the result for 500 ppm of Cu however cannot be recorded because of all tree replications were died already due to the severe stress condition. According to Figure 5, the 500 ppm of Zn was the highest concentration in May for both second and fourth weeks which were 11.56 and 11.53 ppm respectively. The graph patterns however were slightly different in July when the concentration for the fourth weeks of 300 ppm of Zn was decrease to 9.20 ppm. Then, all of the concentration results in September were obviously decrease until 7.02 ppm for the fourth weeks of 300 ppm of Zn whereas the result for 500 ppm of Zn also cannot be recorded similar to the result of Cu above.



**Figure 4.** Concentration of Cu (ppm) after chemical analyzing between different treatments applications.

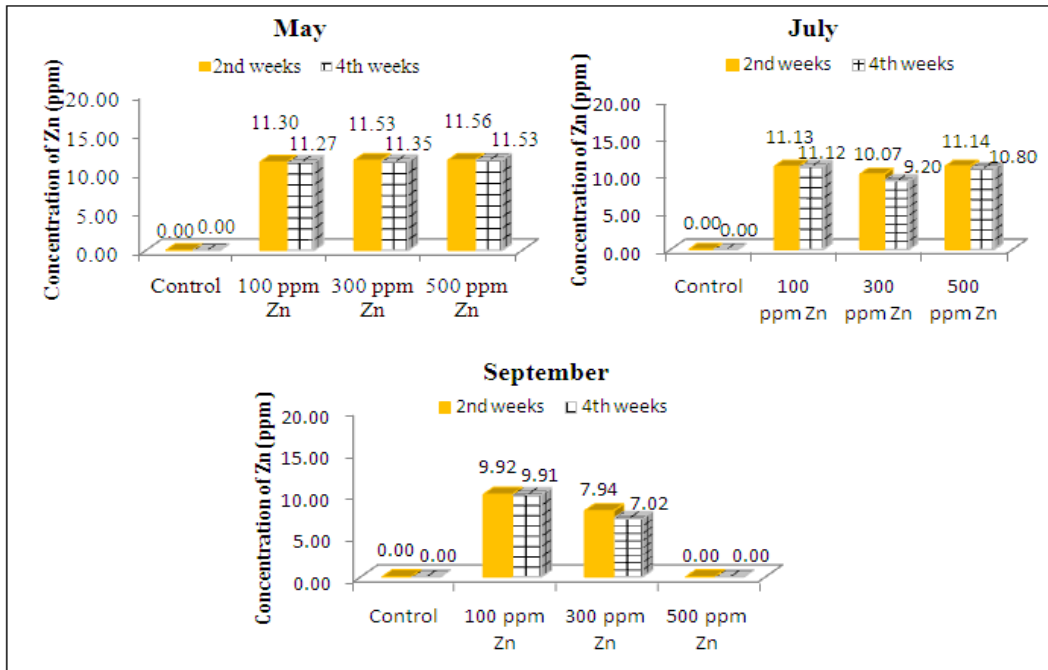


Figure 5. Concentration of Zn (ppm) after chemical analyzing between different treatments applications.

**3.4.2 pH Measurement:** The pH reading for leachate collection of each treatments, Cu and Zn before and after precipitation between different concentration levels of treatments application were shown in Figure 6 and 7. The pH values varied amongst different concentrations, in which the highest concentration (i.e. 500 ppm) generated the lowest pH. This result indicates that high elemental concentrations are more acidic than low ones. Figure 12 and 13 also indicate that all the pH concentrations after precipitation slightly decreased compared with the level generated before precipitation.

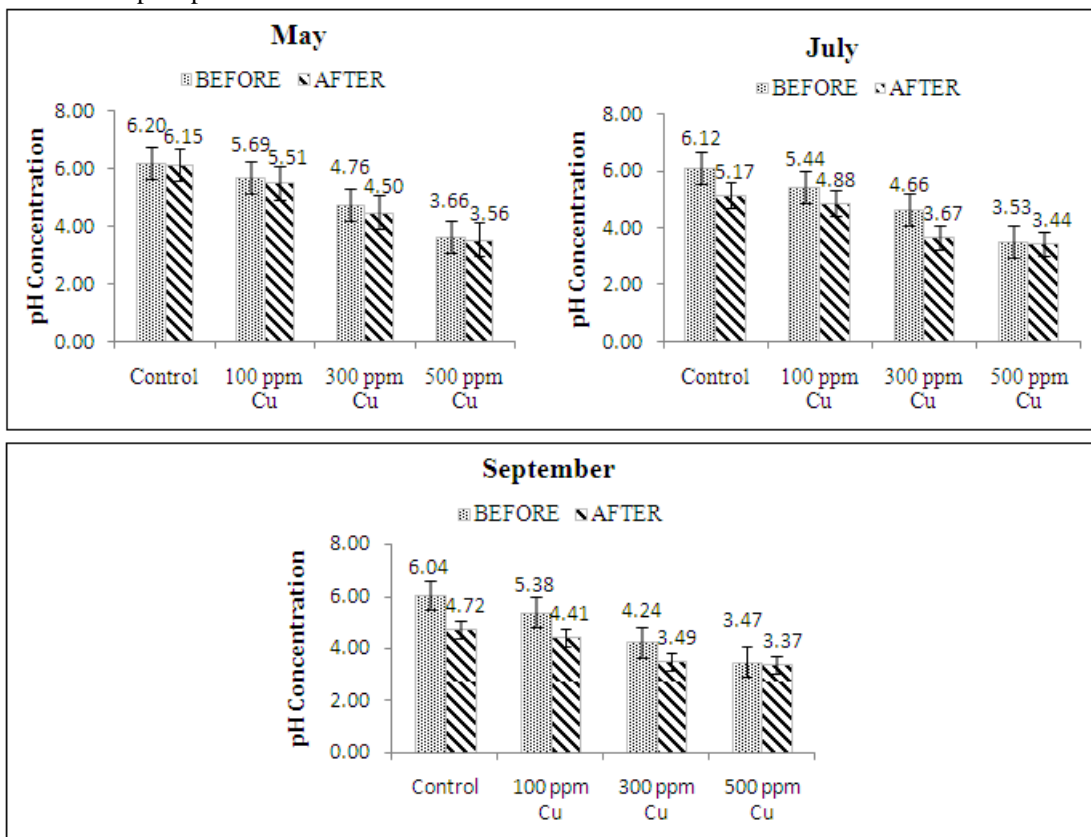


Figure 6. pH measurements before and after precipitation of Cu between different concentration levels of treatments application.



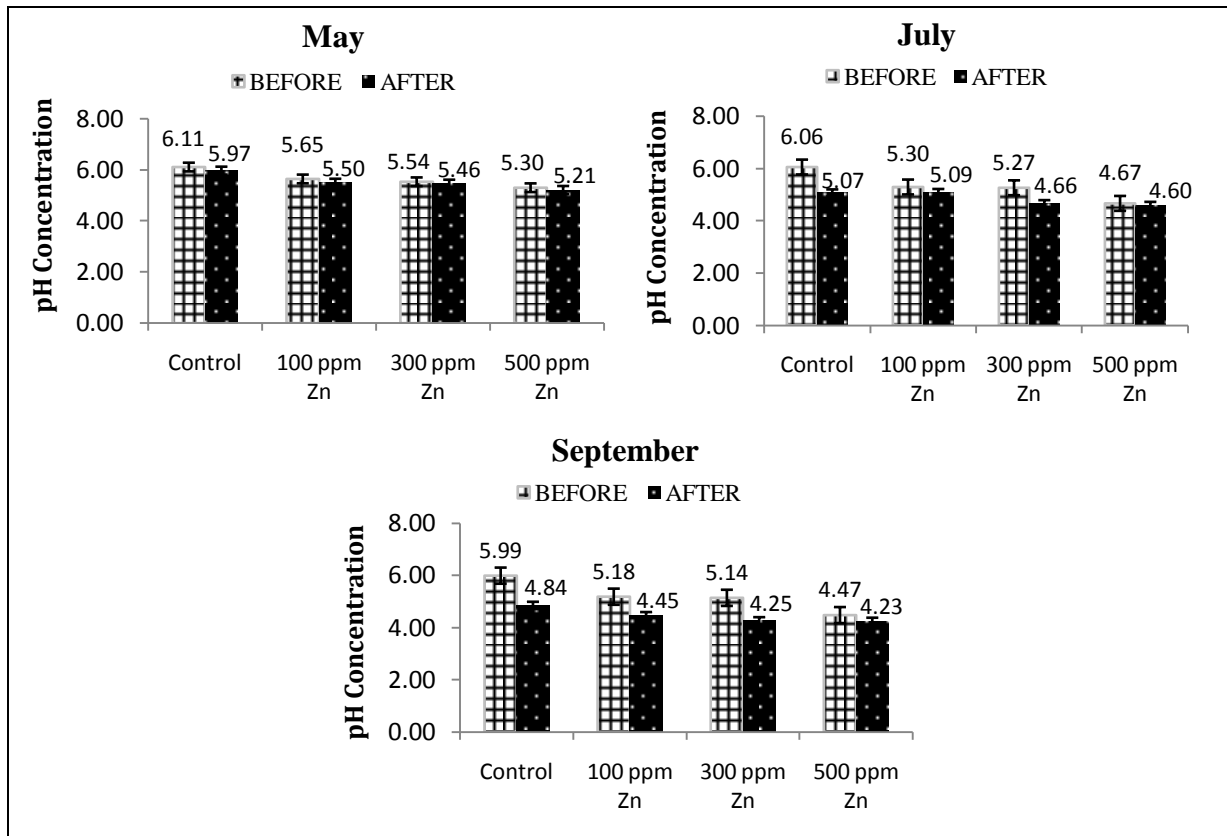


Figure 7. pH measurements before and after precipitation of Zn between different concentration levels of treatments application.

#### IV. Discussions

**4.1 Physiological performance of *M. cajuputi* under different heavy metal concentrations:** To accurately determine the viability of a plant species exposed to certain environmental conditions, a study should go beyond measuring physical development or growth and investigate the species' physiological behaviours. The accomplishment of this goal necessitates effective tools for evaluating the response of plants to different environmental factors; such tools are necessary because many plant traits evolve at the biochemical and cellular levels (Hazandy et al., 2009). A suitable tool would be complex physiological equipment, and the specific instrument used in this study is a portable photosynthesis system (LiCor 6400). In the early stages of the study period, the graph patterns for the mean values of the gas exchange parameters showed that 100 and 500 ppm of Cu and Zn exhibited the highest and lowest values, respectively, for all the gas exchange parameters, except VpdL. However, all the mean values dramatically decreased by the end of the study period. The mean Anet value would have been higher had the Gs value also been higher. According to Andrew et al. (1998), increases in the Anet and Gs of leaves may occur simultaneously because the gas exchange between leaves and the atmosphere is primarily controlled by stomatal conductance. The relationship between stomatal conductance and net photosynthesis is typically positively linear if other factors such as physical environment and leaf characteristics are constant. If leaves are hairy and excessively waxy, mesophyll conductance in the leaves can play a major role in determining photosynthesis rate. This phenomenon highlights intercellular CO<sub>2</sub> as a principal factor.

**4.2 Growth performances of *M. cajuputi* under different heavy metal concentrations:** The physical or growth performance of *M. cajuputi* was determined on the basis of its survival rate, height and diameter after exposure to the selected conditions. According to Hazandy et al. (2009), the traits observed in organisms and the changes that such traits undergo in response to the natural environment can elucidate the means by which specific organisms survive in particular environments. The survival rate in the current work showed that *M. cajuputi* successfully adapted to its environment within the early stages of the study period, that is, between May and June. This adaptation occurs when individual plants reasonably respond to the environment where they are exposed (Hazandy et al., 2009). However, rely on the observations made, the results for all treatments obviously decrease starting on 17<sup>th</sup> July 2011 until the end of study, 30<sup>th</sup> September 2011. Certain treatments were harmful

and caused plant death given the increase in exposure (duration), especially under the highest concentrations (i.e. 300 and 500 ppm of Cu and Zn, respectively). These adverse effects may be due to the toxicity that occurred during the application of Cu and Zn; these elements become toxic when they exceed a maximum soil concentration of 125 and 400 mg/kg, respectively (Rossi et al., 2004).

In addition, the plant height and diameter derived in this study were calculated on the basis of the AGR and RGR. The AGR data can show all the measurements and comparisons of total growth per unit of time, whereas the RGR data can indicate the speed of plant growth (Anon., 2012). The findings indicated that the best growth increment of the plants in the first and second study periods was achieved under exposure to 100 ppm of Cu. This result is attributed to the fact that the AHGR and RHGR values were the highest at every week of a given month. However, the results for the third period to the end of the study slightly differed, with the control treatments of Cu generating the highest AHGR and RHGR values. This result is due to the fact that many of the plants exposed to 100 ppm of Cu became unhealthy and died by the end of the study period. Furthermore, 500 ppm of Cu generated the slowest growth pattern until the end of the study because the plants were exposed to the highest level of stress from the highest Cu concentration.

Meanwhile, the highest AHGR and RHGR of Zn were generated under the control treatments. Under such treatments, the plants' physical characteristics, including the size of the stems and number of leaves, exhibited values that were superior to those from the control Cu treatments. Thus, the successful growth and survival of the plants are attributed to the high AHGR and RHGR values, as well as to the fact that no stress resulted from the Zn concentration. Moreover, 500 ppm of Zn generated the slowest growth patterns, similar to that achieved with 500 ppm of Cu. All the plants under these treatments suffered from stress and became too weak to sufficiently absorb nutrients because they were the most strongly contaminated amongst all the plants. This concentration also caused stunted growth, low leaf production and decreased stem diameter.

The highest diameter growth increment of the plants for both ADGR and RDGR was achieved under 100 ppm of Cu but only in the first study period. From the second period up to the end of the study, the highest increment was accomplished at 300 ppm of Cu. The observations suggest that this phenomenon occurred because of the low leaf production of the control trees at the start of the study. Leaves are very important components of a tree because photosynthesis occurs in these parts. A leaf is regarded as a solar collector full of photosynthetic cells. The raw materials of photosynthesis, water and CO<sub>2</sub>, enter the cells of the leaf and produce sugar and oxygen, which exit the leaf through the opening stomata. Under the control Zn treatments, the plants visibly grew healthily and generated the best diameter increments for ADGR and RDGR across the study period. As expected, 500 ppm of Cu and Zn produced the lowest diameter increments for both ADGR and RDGR at the end of the study.

**4.3 Evaluation of Melaleuca cajuputi as Phytoremediator Species:** The AAS results showed that all the treatments exhibited different concentrations for each Cu and Zn (ppm) after second and fourth weeks of study period, indicating the potential of *M. cajuputi* to rapidly absorb Cu and Zn. This species has been confirmed to be a phytoremediator but not a hyperaccumulator species (Sibli et al., 2013). The average accumulation level for Zn and Cu in the shoots for 12 months field study were 160 and 30 mgkg<sup>-1</sup> respectively whereas the amounts of Zn and Cu projected to be accumulated in single plant were 0.25 and 0.05 g respectively (Sibli et al. 2013). In this study, even though a proportion of the Zn and Cu leached into the piping system because of the BRIS soil characteristics, almost all of them were absorbed and used by the plants. The analysis results for the selected heavy metals correspond with the pH reading for the leachate samples, which exhibited a significant difference before and after precipitation. In addition, the time estimated to extract the metals (i.e Cu and Zn) however were slightly longer compared to others species (i. e. *Acacia Mangium*), about 6 years for Zn and 18 years for Cu, but if good silvicultural practices were applied such as fertilizers may resulted in higher biomass production thus could possibly reduce the time for heavy metal removal (Sibli et al., 2013).

## V. Conclusions

The results of this study provide useful information that can facilitate a good understanding of the growth and physiological attributes of *M. cajuputi*, a potential remediator of contaminated sites (Sibli et al., 2013). The overall results for physiological and growth performance showed that 100 ppm of both Cu and Zn yielded the highest and best results for all the measurement parameters, whereas 500 ppm of both Cu and Zn generated the opposite results. In addition, the physical appearance of the three-month-old plant species (e.g. stem diameter, numbers of leaves, etc.) at the beginning of the study period completely differed from that observed at the end of study. Such differences affected the physiological attributes, plant response and uptake of the heavy metals. Even though only a simple lysimeter method was used in this work, the approach sufficiently serves as basis for developing a new method of plant design that can measure the heavy metal uptake of plants via leaching losses. Newly tested plants can then serve phytoremediation functions. The use of a lysimeter in this study as a preventive device can be an alternative method for protecting the environment, especially

groundwater resources. The method is not only easy to apply, but also an inexpensive and effective tool for detecting potential contaminants before they penetrate into precious natural resources. Potentially harmful crop inclusions, such as pesticide residues or leached heavy metals, are successfully monitored and measured by lysimeter in a uniform manner as these substances move and are modified across the soil profile. With the use of a lysimeter, therefore, we can control, develop and understand excellent management practices, thereby enabling us to achieve optimum performance, quantify potential pollutant sources, incur minimum loss or runoff at minimum costs and gain a good understanding of the infiltration properties of particular soil types.

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### References

- [1] Zubillaga, M. S., Bressan, E. & Lavado, R. S. (2008). Heavy metal mobility in polluted soils: effect of different treatments. *American Journal of Environmental Sciences*, 4(6), 620-624.
- [2] Yoon, J., Cao, X., Zhou, Q. & Ma, L. Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, 368, 456-464.
- [3] Rossi, G., Figliolia, A. & Socciarelli, S. (2004). Zinc and copper bioaccumulation in *Brassica napus* at flowering and maturation. *Engineering in Life Sciences*, 4(3), 271-275.
- [4] Cao, X., Ma, L. Q., Chen, M., Singh, S. P., and Harris, W. G. (2002). Impacts of phosphate amendments on lead biogeochemistry in a contaminated site. *Environmental Science and Technology*, 36 (5), 296-304.
- [5] Mulligan, C. N., Yong, R. N., and Gibbs, B. F. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology*. 60. 193-207.
- [6] Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50, 775-780.
- [7] Hu, N., Luo, Y., Wu, L. & Song, J. (2007). A field lysimeter study of heavy metal movement down the profile of soils with multiple metal pollution during chelate-enhanced phytoremediation. *International Journal of Phytoremediation*, 9, 257-268.
- [8] Lazarovitch, N., Ben-Gal, A. & Shani, U. (2006). An automated rotating lysimeter system for greenhouse evapotranspiration studies. *Vadose Zone Journal*, 5, 801-804.
- [9] Robinson, B., Green, S., Mills, T., Clothier, B., Velde, M.V.D., Laphane, R., ...Dijssel, C. V. D. (2003). Phytoremediation: Using plants as biopumps to improve degraded environments. *Australian Journal of Soil Research*, 41, 599-611.
- [10] Lim, S. C. & Mohd Shukari, M. (2001). *Timber of Gelam (Melaleuca cajuputi Powell)*. Kepong, Kuala Lumpur: Timber Technology Centre (TTC), Forest Research Institute Malaysia (FRIM).
- [11] Ko Ko Juntarajumnong, W. & Chandrapatya, A. (2009). Repellency, fumigant and contact toxicities of *Melaleuca cajuputi* Powell against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst. *Thai Journal of Agricultural Science*, 42(1), 27-33.
- [12] Doran, J. C. & Gunn, B. V. (1994). Exploring the genetic resources of tropical *Melaleucas*. *Forest Genetic Resources Bulletin*, 22, 78.
- [13] Tlustos, P., Szakova, J., Hruby, J., Hartman, I., Najmanova, J., ...Batysta, M. (2006). Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. *Plant Soil Environment*, 52(9), 413-423.
- [14] Madzyar, Y. (2008). Tolerance of *Trichoderma atroviride* (karst.) isolated from freshwater ecosystems towards copper and zinc (Unpublished doctoral dissertation). Universiti Putra Malaysia, Malaysia.
- [15] Hazandy, A. H., Sharifah-Amira, S., Mohd-Hazimy, Y. & Mohd-Kamil, I. (2009). Chlorophyll fluorescence of three dipterocarp species in a reciprocal planting. *Malaysian Forester*, 72(2), 165-173.
- [16] Andrew, J. J. & William, J. D. (1998). The coupled response of stomatal conductance to photosynthesis and transpiration. *Journal of Experimental Botany*, 49, 399-406.
- [17] Anonymous, 2012. Retrieved September 10, 2012 from WikiAnswers: [http://wiki.answers.com/Q/Comparison\\_between\\_Absolute\\_Growth\\_rate\\_with\\_Relative\\_Growth\\_Rate#ixzz1ypLRf31p](http://wiki.answers.com/Q/Comparison_between_Absolute_Growth_rate_with_Relative_Growth_Rate#ixzz1ypLRf31p).
- [18] Sibli, N. M., Majid, N. M., Shazili, N. A. M. & Abdu, A. (2013). Assessment of *Melaleuca cajuputi* as heavy metals phytoremediator for sewage sludge contaminated soil. *American Journal of Applied Sciences*, 10(9), 1087-1092.
- [19] Sibli, N. M., Majid, N. M., Shazili, N. A. M. & Abdu, A. (2013). Growth performance, biomass and phytoextraction efficiency of *Acacia mangium* and *Melaleuca cajuputi* in remediating heavy metal contaminated soil. *American Journal of Environmental Sciences*, 9(4), 310-316.