

# The effects of pre-aluminum treatment on morphology and physiology of potential acidic slope plants

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## Abstract

High temperature and rainfall contribute in turning most of the slope soils acidic in countries like Malaysia. Acidic slope again contributes in a low plant coverage, high eroding potential and instability of slopes. In this context, this study aims at investigating the effects of pre-Aluminum treatment on the growth and development of selected plants on acidic slope. The acidic-tolerant characteristics of *Acacia mangium*, *Leucaena leucocephala* and *Melastoma malabathricum* were determined by subjecting them into pre-Aluminum treatment at germination phase. The results showed that *M. malabathricum* exhibited the highest morphological parameters; root length and dry weight partitioning and physiological performance including photosynthetic rate, stomatal conductance, transpiration rate, Leaf Area Index (LAI), and leaf Aluminum analyses. Within ten weeks of observation, the Al-pretreated *M. malabathricum* showed the highest photosynthetic and transpiration rates as compared to *L. leucocephala* and *A. mangium*. Simultaneously, stomatal conductance was found to be the highest in *M. malabathricum*. Moreover, within ten weeks, soil pH planted with *M. malabathricum* increased by 23.8% and was found to be the highest among the species studied, implying the rehabilitation capacity of this species. The Al-pretreated *M. malabathricum* also displayed the longest root length in acidic soil exhibiting a tolerance mechanism towards soil acidity. Amongst the species studied, *M. malabathricum*, either treated or non-treated, exhibited the best morphological and physiological performances on acidic conditions.

**Keywords:** Acidic slopes; acidic-tolerant plants; aluminium treatment; soil rehabilitation; slope stability.

## 1. Introduction

The intensive development of infrastructures such as housing, agriculture, and highways on hill slopes has become a serious concern for the policymakers due to

the occurrence of landslide hazards at regular intervals and frequent erosion (Mafian *et al.*, 2009; Huat & Kazemian, 2010). When bare and steep surfaces are created as a result of massive human miscalculation and mismanagement to nature, especially on slopes, it demands the need of vegetation to provide stabilization (Komoo *et al.*, 2011; Mugagga *et al.*, 2012; Song *et al.*, 2012). Although the process and system of vegetation is important, the criterion to fix the need is limited due to adverse conditions, mainly climate and soil properties of the slope (Bochet & García-Fayos, 2004; Normaniza & Barakbah, 2006).

In Malaysia, the annual average rainfall of nearly 2,500 mm can cause great acceleration of erosion, often leading to landslide (Shafie, 2009). Besides, deforestation in the country has adverse effects on the hydrological cycle, particularly relating to increase in runoff and erosion (Pradhan *et al.*, 2012). In exposed conditions, almost the entire volume of rain hits the surface and flows as runoff in a short period of time, leading to structural weaknesses in the soil as well as destruction of the topsoil (Mafian *et al.*, 2009; Huat & Kazemian, 2010). To add to this circumstance, lack of vegetation coverage may result in surface runoff that consequently increases the landslide risk. Apart from that, most of the soil in the tropical region becomes arid and barren due to lack of buffer capacity and low clay activities, which result in soil acidity (Koutika *et al.*, 2002). The rain water percolation, which leaches away the basic elements of the soil such as calcium, magnesium, potassium, and sodium from the soil profile is another factor that develops the soil acidity. The main trait of soil acidity is low pH value, which is mainly attributed by the high concentration of aluminum (Al), as well as other acid-caused elements like manganese (Mn), hydrogen (H) and iron (Fe) (Kochian *et al.*, 2004). Aluminum toxicity is the primary stress factor limiting the growth of plants in acid soils (Zsoldos *et al.*, 2003).

Soil acidity has a negative impact on the fertility, biological activities and plant productivity. Acidic land is known as infertile, barren and not suitable for the purpose of agriculture due to the occurrence of toxic elements like aluminum and manganese. These elements create problem in soils as they are more soluble at low pH (Copeland *et al.*, 2012; Wang *et al.*, 2006). Moreover, at soil pH values at or below 5, dissolution of Al-bearing minerals result in toxic Aluminium (Al) forms, inhibit root growth and function, and thus, reduce crop yields (Kochian *et al.*, 2005). In other words, more of the solid form of these elements will dissolve in water when the pH is acidic. There is always a lot of Al present in soils, because it is a part of most clay particles. In addition, soil acidity also affects the effectiveness of soil microorganism activities (Fuentes *et al.*, 2006), affecting the level of releasing nutrients by soil for plant growth. Although these organisms function best at soil pH levels of 8.0, their effectiveness does not drop rapidly until pH levels drop below 6.0. Decomposition of organic matter contributes to aggregation of soil particles, which provides good aeration and drainage (Kidd &

Proctor, 2001). In order to enhance the slope stability, the acidity is one of the major factors that should be reduced by eliminating the acidic elements, which is mainly caused by Al.

Liming, one of the conventional methods, is widely used to correct acidity. However, this method is not cost effective and it contributes soil pollution (Normaniza *et al.*, 2014). Therefore, it is essential to identify the potential acidic slope plants. The potential acidic slope plant must be an Al accumulator as this type of plant has the potential to accumulate high concentration of Al from the soil into its different parts like root and shoot (Misawa *et al.*, 2005). This is how the Al concentration of the acidic slope could be reduced. A number of studies have already assessed the success of the common species for slope stabilization (Kochian *et al.*, 2004; Rohailah, 2011). Soil acidity inhibits the elongation and interconnects the root system, which reduces the nutrient and water uptake, resulting consequently in poor plant growth and slope coverage. Vegetation contributes to slope mass stability by increasing the soil shear strength *via* root reinforcement (Normaniza & Barakbah, 2011). The stunted root profile in acidic slope causes difficulty for the root to bind soil and prevent soil losses especially due to the rainfall (Harter, 2002). Thus, poor root profiles of the plants can be one of the major causes of previous erosion and landslide disasters (Cruden & Varnes, 1996; Saifuddin & Normaniza, 2014a).

The native species are aesthetically harmonious with natural vegetation and thus, suggested for plantation on slope (Normaniza *et al.*, 2014; Normaniza *et al.*, 2008). As most of the plants could not survive on acidic slope, all the three species studied namely, *Acacia mangium*, *Leucaena leucocephala* and *Melastoma malabathricum* were exposed to the high Al concentration (pre-acidic treatment) before transferring them into the real acidic condition of the slope. *A. mangium* has high growth rates on bare soils. It has great ability to buffer temperature and improve soil organic matters (Yang *et al.*, 2009). *L. leucocephala* is one of the nitrogen fixing and fast growing tropical plants (Saifuddin & Normaniza, 2014b; Saifuddin *et al.*, 2015). An aggressive root system of *L. leucocephala* helps to break up compacted subsoil layers, improving the penetration of moisture into the soil and decreasing surface runoff. *M. malabathricum* is a shrub species and can usually be found in abandoned areas. It has the potential to remove Al ion from the soil and its flowering feature can help to enhance the flora-fauna interactions on slopes (Rohailah, 2011). It is anticipated that the Al pre-existence in the Al-pretreated plants would be more adapted to acidic condition of the slope. Hence, the aims of this study are to assess the morphology and physiology of the species studied, either control or Al-treated. The morphological and physiological adaptation and the rehabilitation of soil in terms of soil pH were also monitored.

## 2. Materials and methods

### 2.1. Plant materials: laboratory and glasshouse experiments

The seeds of all the species studied were germinated on moistened cotton in petri dish at the temperature of 25°C for 7 days in the laboratory. The seedlings labeled as Aluminium pretreated (Al-pretreated) were pre-cultured for 2 weeks on the treatment solutions as follows: 200 µM K<sub>2</sub>SO<sub>4</sub>, 200 µM CaCl<sub>2</sub>, 100 µM MgSO<sub>4</sub>, 200 µM Ca(NO<sub>3</sub>)<sub>3</sub>, 300 µM NH<sub>4</sub>NO<sub>3</sub>, 5 µM NaH<sub>2</sub>PO<sub>4</sub>, 10 µM Fe-EDTA, 5 µM MnSO<sub>4</sub>, 0.38 µM ZnSO<sub>4</sub>, 0.16 µM CuSO<sub>4</sub>, 8 µM H<sub>3</sub>BO<sub>3</sub>, 0.06 µM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 50 µM Al (Tolrá *et al.*, 2005). The same treatment solutions were applied for the control species without the element of Aluminum (Al).

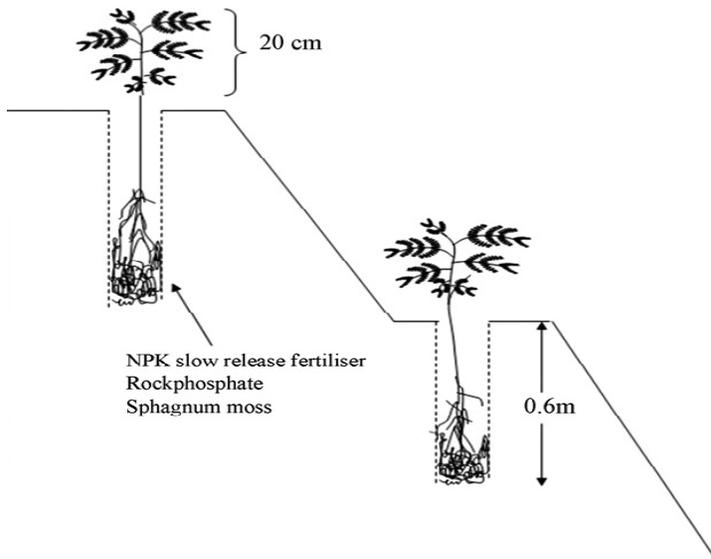
This solution was renewed twice per week to prevent fungal activities. The seeds were grown in a growth chamber under the light condition of 330 µE m<sup>-2</sup> s<sup>-1</sup>, photoperiod of 16 h light/8 h darkness, day/night relative humidity of 50%/80%, day/night temperature of 24 °C/18 °C (Tolrá *et al.*, 2005). After 2 weeks, the seedlings with uniform height of 10 cm were transferred into polythene bag in the glasshouse (temperature 25-32°C, maximum PAR 2000 µE m<sup>-2</sup> s<sup>-1</sup> and relative humidity of 60-90%) located at the Rimba Ilmu, University of Malaya.

### 2.2. Experimental Design

Six plots (each plot of 6 m x 2 m = 12 m<sup>2</sup>) in a completely randomized design (CRD) were set up on the experimental slope in a total area of 102 m<sup>2</sup>, including 6 m<sup>2</sup> of buffer zone between the plots, and 60° of slope angle. The plots had the same soil type, acidic soil (Table 1) with pH ranging from 4.70 to 4.90 (Al concentration = 2757 ppm, relative humidity of 70-90%, temperature 32-38°C, maximum PAR 2025 µE m<sup>-2</sup> s<sup>-1</sup>), at Section 16 (longitude E 101° 39' 0", latitude N 03° 07' 45"), University of Malaya. After 5<sup>th</sup> week observation, the Al-pretreated and controlled plants in the glasshouse, which reached 50 cm high were transferred to the slope. The transplanting of both Al-pretreated and control species was conducted using a modified microclimate plant propagation Technique (Normaniza & Barakbah, 2011). Each seedling was then transplanted into a hole at 0.6 m of soil depth on the slope (Figure 1) with additions of plant supplements such as NPK fertilizer, *sphagnum* moss (15 g/hole) and rock-phosphate, which were only applied with soil in the beginning of planting in order to allow the establishment of the roots and other physiological processes of the plants (Dashti *et al.*, 2014). The information of the plots was described and tabulated in Table 2 and Figure 2. In slope condition, the species studied were grown for another 10 weeks.

**Table 1.** Physical properties of the soil used in this experiment

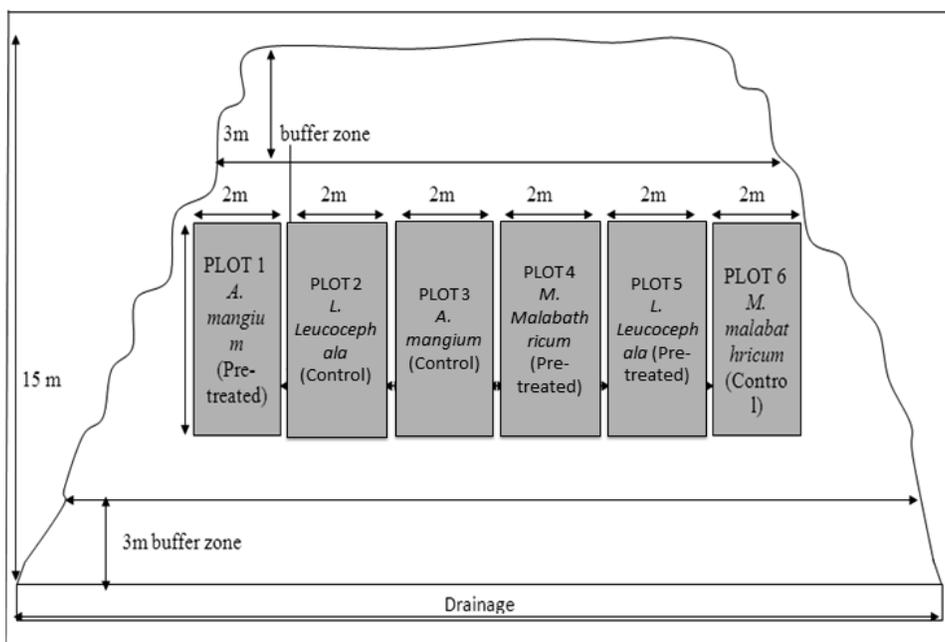
Soil properties	Slope soil
Specific gravity	2.62
Liquid limit	26.9%
Plastic limit	14.5%
Dry unit weight (kN/m <sup>3</sup> )	13.1
Soil Field Capacity	20.3 %
pH	4.7-4.9
Color	6/8/Hue 10 [Bright yellowish brown]
Type	Size distribution
500 to 1.0 mm	12.165 %
250 to 500 mic	29.45 %
100 to 250 mic	38.58 %
50 to 100 mic	13.14 %
<2 to 50 mic	6.64 %



**Fig. 1.** Plant propagation technique on slope

**Table 2.** Description of the experimental plots

Plot	Species	Treatment	Plant Spacing	Rows	No. Of Plant
PLOT 1	<i>A. mangium</i>	Al-pretreated	50 cm	5	10
PLOT 2	<i>L. leucocephala</i>	Control	50 cm	5	10
PLOT 3	<i>A. mangium</i>	Control	50 cm	5	10
PLOT 4	<i>M. malabathricum</i>	Al-pretreated	50 cm	5	10
PLOT 5	<i>L. leucocephala</i>	Al-pretreated	50 cm	5	10
PLOT 6	<i>M. malabathricum</i>	Control	50 cm	5	10


**Fig. 2.** Experimental design on slope

## 2.3. Measurements

### 2.3.1. Morphological parameters: root length and dry weight partitioning

Root length and dry weight partitioning of stem, leaf and root were obtained at the end of each treatment on tenth week (Al-Bader *et al.*, 2014). All parts were oven-dried (80°C) until they reached the constant weight.

### 2.3.2. Physiological parameters: photosynthetic, transpiration rates, and stomatal conductance

Both photosynthesis and transpiration rates were measured using portable photosynthesis system (LICOR, Li-6400XT, USA), and portable porometer (SC-1, Decagon, USA) was used to measure stomatal conductance. Five young expanded leaves of each species were measured randomly. The measurements were taken between 12 p.m. – 2 p.m. with a range of PAR 1800-2100  $\mu\text{E m}^{-2} \text{s}^{-1}$  (normal PAR of 1500  $\mu\text{E m}^{-2} \text{s}^{-1}$ ).

### 2.3.3. Leaf area index (LAI) and leaf aluminum concentration

At 7-day interval, Leaf Area Index of the species was measured using leaf area instrument (AccuPAR-LP80, UK). At the end of experiment, the plants were harvested and oven-dried at 80°C to a constant dry weight. The leaves in triplicates of the species studied were thoroughly washed and rinsed with distilled water. The samples were dried in oven at 40°C, ground into fine powder and stored in fresh plastic polythene bags. The powdered samples of the leaves were digested by wet digestion method and the Aluminum concentration of leaf was determined by using atomic absorption spectrophotometer (Dhiman *et al.*, 2011).

### 2.3.4. Soil analysis: Soil pH

In order to determine the rehabilitation process of the acidic slope, the soil was analyzed. Both parameters were measured diagonally across the plot with three replications. The measurement was taken at 7 days interval for ten weeks using a pH meter (pH meter BASIC 20, CRISON, Barcelona) at 0.5 m of soil depth and 0.5 m around the plant root.

### 2.3.5. Statistical analysis

Statistical analysis was performed using SIGMAPLOT 2000. The one way ANOVA was applied to evaluate the significant differences among the means. The significant ( $p < 0.05$ ) difference among the means was compared using the Fisher's Least Significant Difference (LSD). Microsoft Excel was used for regression analysis and graphical presentation.

## 3. Results

### 3.1. Morphological traits

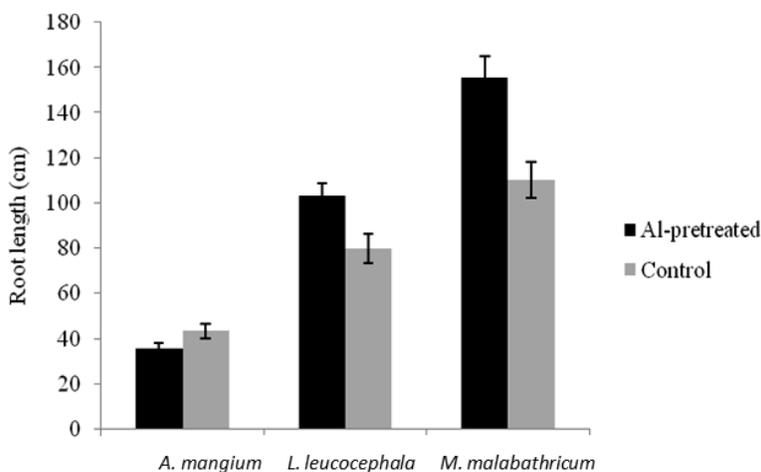
#### 3.1.1. Root length

The root length of both Al-pretreated *M. malabathricum* and *L. leucocephala* reached more than one meter of soil depth after ten weeks of experiment (Figure 3). In fact, the

root length of Al-pretreated *M. malabathricum* and *L. leucocephala* was almost four and three fold of the root length of Al-pretreated *A. mangium*, respectively. However, the root length of Al-pretreated *A. mangium* was significantly lower than the control treatment.

### 3.1.2. Dry weight partitioning

All species showed higher leaf dry weight in Al-pretreated than in control plants (Figure 4). *M. malabathricum* exhibited highest among the test species. In contrast, the shoot dry weight for all Al-pretreated species was significantly lower than the control. Similar result was observed for root dry weight of Al-pretreated *A. mangium*, but not in the other two species. Interestingly, in all Al-pretreated species, *M. malabathricum* exhibited the highest total biomass, followed by *L. leucocephala* and *A. mangium*. The same trend was found in all control species. In general, the total biomass for every species is higher in Al-pretreated plants than in control plants except for *A. mangium*.



**Fig. 3.** Root length of the species studied after ten weeks of treatment. Vertical bars represent standard deviation and vertical lines represents  $LSD_{p<0.05}$ .

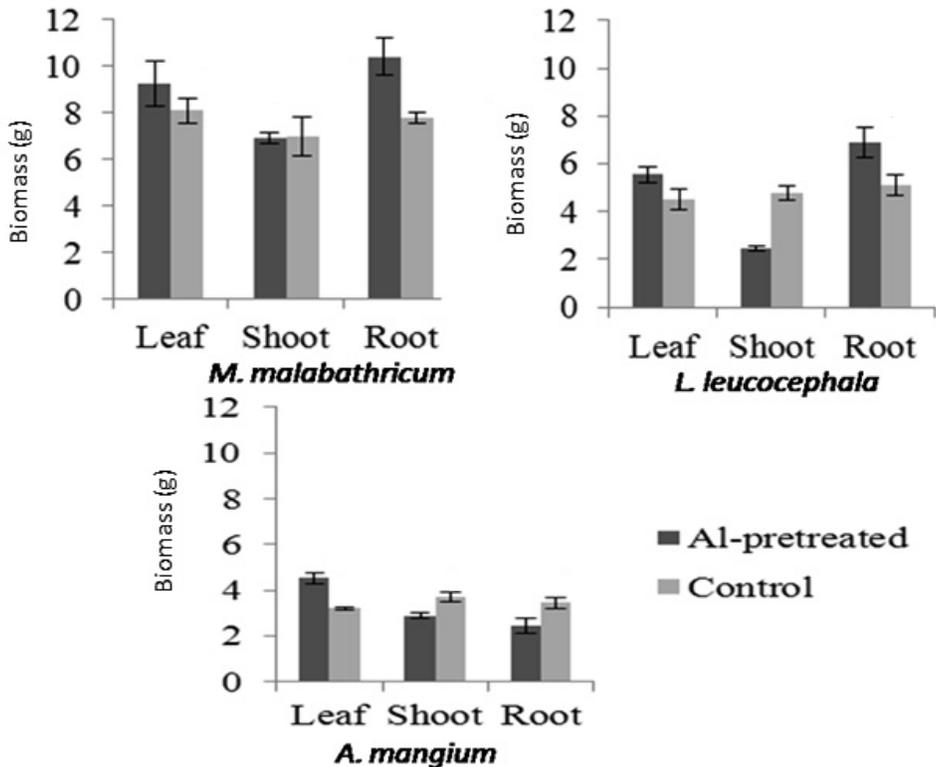
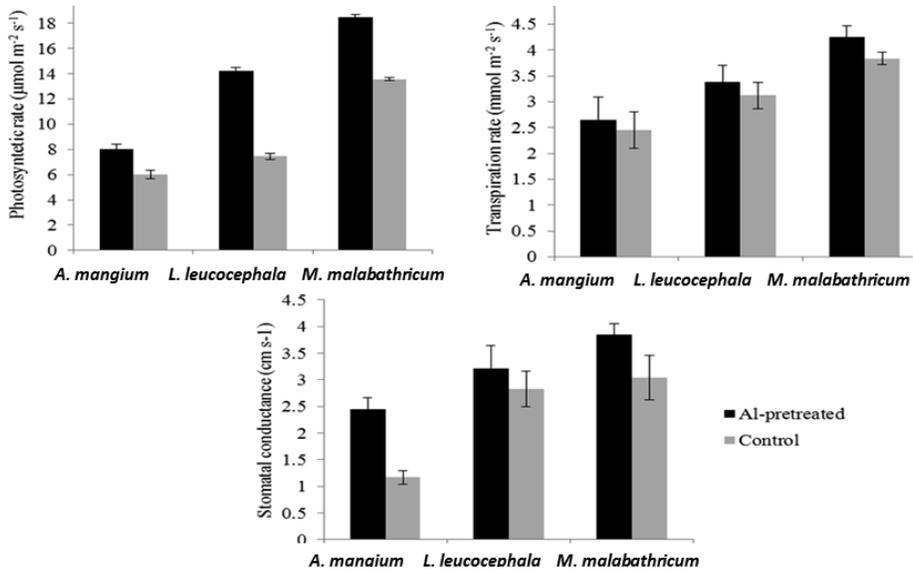


Fig. 4. Comparison of dry weight partitioning of three species studied in controlled and pre-aluminum treatment. Each value represents the mean of 5 replications. Vertical bars represent standard deviation and vertical lines represents LSD<sub>p<0.05</sub>

### 3.2. Physiological traits

#### 3.2.1. Photosynthetic rate, stomatal conductance and transpiration rate

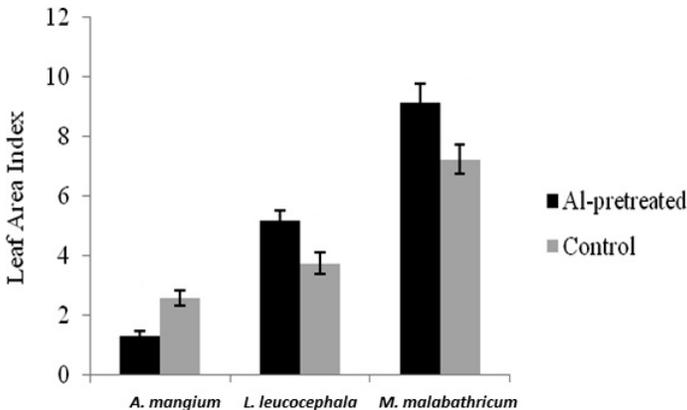
The entire Al-pretreated species showed significantly higher photosynthetic rate compared to the control. Photosynthetic rate of Al-pretreated *L. leucocephala* was almost twice than that of control (Figure 5). Likewise the transpiration rates of all Al-pretreated species studied (except *A. mangium*) were significantly higher than the control. The highest transpiration rate was shown by *M. malabathricum*, followed by *L. leucocephala* and *A. mangium* in both the treatments. In the case of *L. leucocephala*, there was no positive effect of pre-aluminum treatment on stomatal conductance. The stomatal conductance of Al-pretreated *M. malabathricum* and *A. mangium* were significantly higher than the control.



**Fig. 5.** Comparison of physiological performance of the three species studied in controlled and pre-aluminum treatment. Each value represents the mean of 5-10 replications. Vertical bars represent standard deviation and vertical lines represents LSD<sub>p<0.05</sub>

### 3.2.2. Leaf area index (LAI) and leaf aluminum analyses

Leaf Area Index (LAI) of Al-pretreated *A. mangium* was significantly lower compared to the control (Figure 6). Unlike *A. mangium*, the LAI of *L. leucocephala* and *M. malabathricum* were significantly higher in Al-pretreated compared to control. However, the Al-pretreated *M. malabathricum* performs well in the parameter studied, almost double than the Al-pretreated *L. leucocephala*. The results showed that at the beginning of the experiment, the Al concentration in the leaves of all Al-pretreated species was two to four folds higher than the control (Figure 7).



**Fig. 6.** Leaf Area Index (LAI) for Al-pretreated and control plants on experimental slope. Vertical bars represent standard deviation and vertical lines represents LSD<sub>p<0.05</sub>

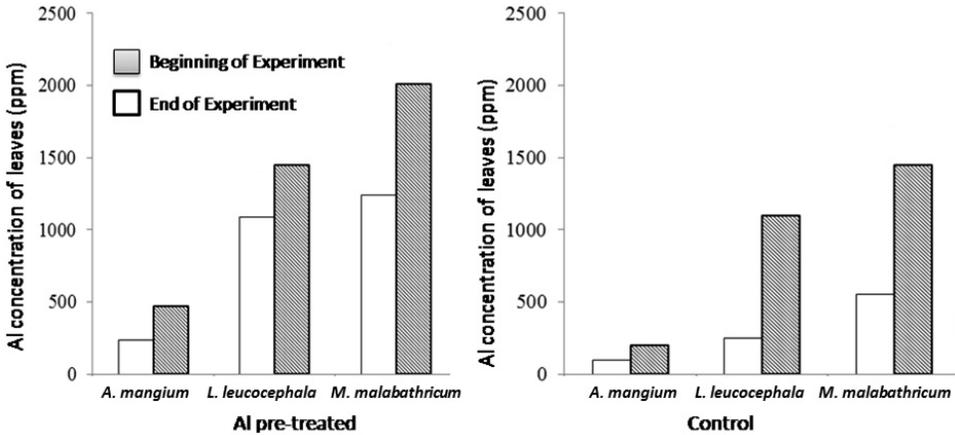


Fig. 7. Al concentration of leaves of three species studied in the beginning of experiment and at the end of experiment. Vertical lines represents  $LSD_{p<0.05}$

### 3.2.3. Soil pH

Soil pH of the slope was measured for ten weeks of experiment for plot grown by Al-pretreated and control species (Figure 8). In the beginning of experiment, soil pH at the slope was ranging from 4.70 to 4.90 for every plot before those species were transferred to the slope. After transplanting, there was a significant increment in the soil pH. At the end of experiment, plot grown with Al-pretreated *M. malabathricum* (Plot 6) exhibited the highest increment in soil pH with 23.8%, followed by *L. leucocephala* (16.0%, Plot 5) and *A. mangium* (7.5%, Plot 1). The same trend was found in the plot grown by control species, though the results for control plots were more consistent than those of Al-pretreated species. This was due to the soil pH that had dropped rapidly at week three and four respectively, before increasing the pH value in the following week. However, at the end of experiment, the soil pH of the plots grown by Al-pretreated *M. malabathricum*, *L. leucocephala* and *A. mangium* was 5.99, 5.59 and 5.32, respectively. Meanwhile for the control treatment, the value of soil pH grown with *M. malabathricum*, *L. leucocephala* and *A. mangium* was 5.60, 5.42 and 5.12, respectively.

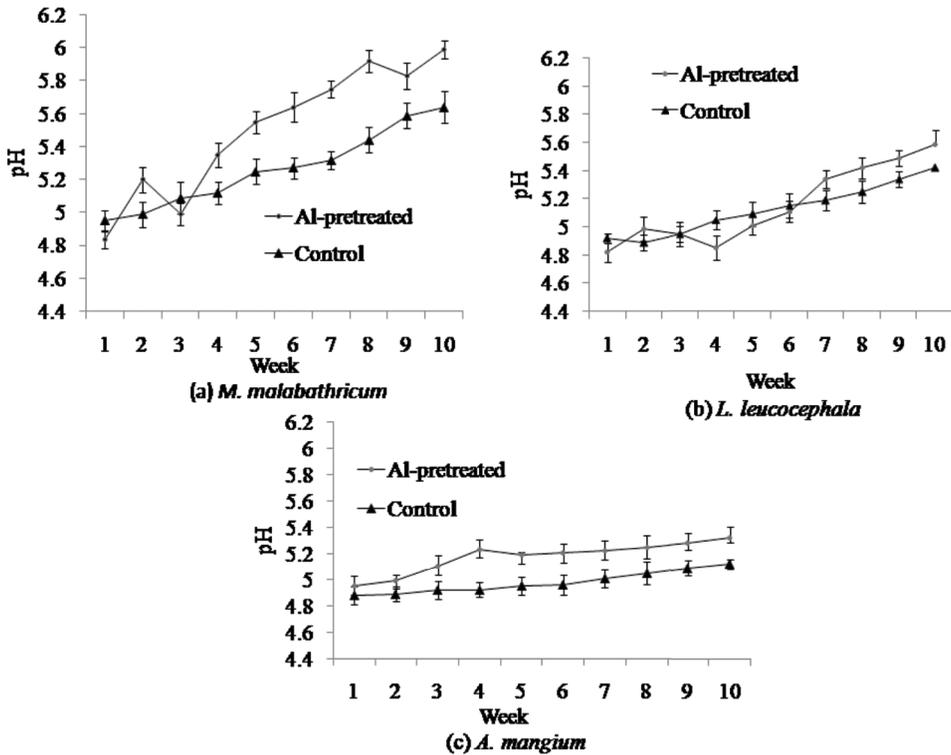


Fig. 8. Soil pH changes grown by Al-pretreated and control plants of the three species studied. Vertical bars represent LSD<sub>p<0.05</sub>

## 4. Discussion

### 4.1. Morphological and physiological characteristics

The root length of Al-pretreated *A. mangium* was found significantly lower than the control treatment. The root of Al-pretreated *A. mangium* possibly needs more time to release organic acid anions such as citrate, malate or oxalate from the root apices in response to Al-stress and thus, reduce the root elongation (Prasetyo, 2007). The significant effect of pre-Aluminum treatment had forced *M. malabathricum* and *L. leucocephala* (except *A. mangium*) in establishing longer root length that helps in nutrient and water absorptions, thus improving the plant growth as well as better dry weight performance. The deep rooting system enhanced soil reinforcement, prevented soil erosion and increased the water uptake (Abraham *et al.*, 2009; Preti & Giadrossich, 2009).

Al-tolerant plants exhibited better dry weight performance than Al-sensitive plants (Ma *et al.*, 1997). Therefore, *M. malabathricum* can be regarded as more tolerant towards acidic condition of the slope compared to *L. leucocephala* and *A. mangium*

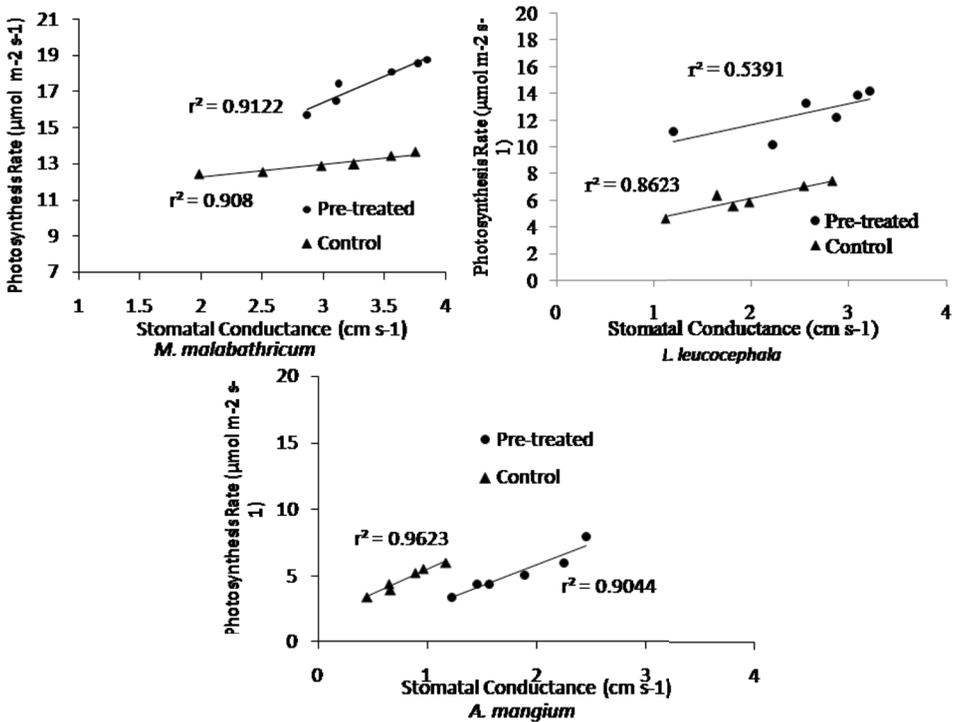
as it performed the highest values. The contrary result shown by shoot dry weight of Al-pretreated plants of all species may be due to the early Al-stressed condition which contributed these species to accumulate the Al in their shoot. As a result, the toxic Al in the shoot might slow down the growth of shoot. In addition, the result behind the low performance of *A. mangium* was perhaps related to the inhibition of the root elongation by Al-stress (Kikui *et al.*, 2005). This result showed good performance of the two species (*M. malabathricum* and *L. leucocephala*) on acidic slope condition, especially on pre-Al treatment plants.

The good performance in photosynthetic rate was shown by Al-pretreated as compared to the control species. The high photosynthetic rate of plants may be attributed to the huge amount of chlorophyll pigment in leaf, which was in agreement with the findings of other researchers (Hamlyn, 1998; Saifuddin & Normaniza, 2012). Thus, this finding implied that Al-pretreated or acidic stressed species have high acid tolerance mechanisms and potentiality. Furthermore, among the Al-pretreated species, *M. malabathricum* exhibited the highest photosynthetic rate, followed by *L. leucocephala* and *A. mangium*. The results indicated that the Al-pretreated *M. malabathricum* increased the photosynthetic rate as it has tolerance mechanism towards acidity. This promising characteristic had set *M. malabathricum* apart from the other two species as a potential acidic slope plant. In addition, a deeper root length in Al-pretreated *M. malabathricum* might influence in higher potassium uptake which was essential in chlorophyll synthesis of leaves. Hence, this will give rise to the chlorophyll content which led to high photosynthetic rate (Ainsworth & Rogers, 2007). The species with higher photosynthetic rates indicated that they are more efficient in utilizing light for enhancing growth and are more likely to grow faster, an essential characteristic for a slope colonizer (Saifuddin *et al.*, 2013).

In addition, the highest transpiration rate shown by *M. malabathricum*, followed by *L. leucocephala* and *A. mangium* in both treatments could be attributed to the maximal root system. This enhanced the water absorption capacity of the Al-pretreated species as an adaptation towards acidity (Eleftheriou *et al.*, 1993). Moreover, it has been shown that 99% of water is lost to transpiration and only 1% will be evaporated. Therefore, apart from Aluminum tolerant, higher transpiration rate can also be regarded as another essential criterion for a potential acidic slope plant. As a result, the slope would be drier and stable. This water cycle system is imperative to the slope condition in order to avoid the soil from being saturated. As *M. malabathricum* was noted to have the highest transpiration rate compared to the other species in both treatments, this species could be considered to fulfill the acidic slope plant standards. In the case of Al-pretreated *M. malabathricum* and *L. leucocephala*, high leaf area as well as stomatal opening may contribute to high transpiration rate, hence in favor to reduce the water saturation level of the slope.

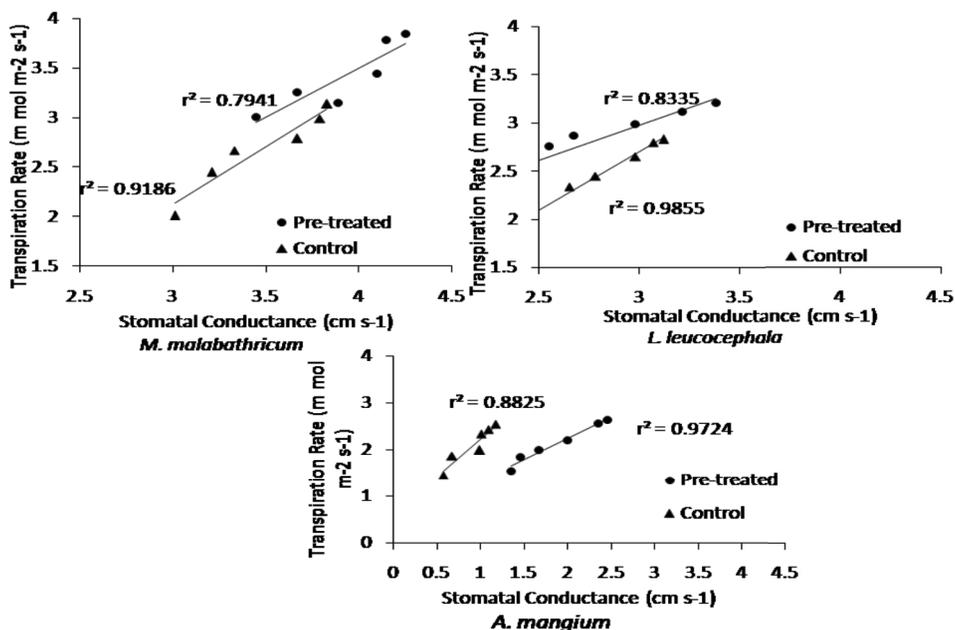
The stomatal conductance operates in apparent contradictory way due to the minimized water loss relative to the amount of CO<sub>2</sub> uptake. The results also indicate that the low rate of stomatal conductance can be one of the mechanisms to conserve water, another significant criterion of a slope plant. However, the tolerance mechanisms in stomatal conductance of different species are likely to depend on the particular morphological and physiological characteristics (Heterington & Woodward, 2003). For example, longer root lengths of *M. malabathricum* in both treatments imply the ability of the species to absorb water from the soil. As a mechanism to maintain turgor potential to a substantial level through osmotic adjustment, *M. malabathricum* increases the stomatal conductance to release the excessive water from the plant to the air. This criterion is also regarded as a tolerance mechanism of this particular species to maintain itself as pioneer vegetation on the acidic slope.

Stomatal conductance and photosynthetic rate of all species appeared to be highly correlated in both treatments but with varying degrees (Figure 9). The stomatal opening in both *L. leucocephala* and *M. malabathricum* is at a similar range in which the Al-pretreated plants showed a higher photosynthetic rate, implying that the pre-Aluminum treatment give more positive effect on the growth of the species studied. In addition, the Al-pretreated plants tend to use internal carbon in order to increase the photosynthetic rate at the lower range of stomatal conductance. The increase in net photosynthesis is positively correlated to the increment in stomatal conductance (Jarvis & Davies, 1997). However, both treatments of *A. mangium* showed contrary results, indicating that the Al-treatment did not give any significant effect on photosynthetic rate. This may be due to the fact that the control plants tend to close stomata completely to reduce the water loss and it is a fact that the closure of stomata is a very effective protection for plants exposed to severe stress levels. The Al-pretreated *M. malabathricum* showed the highest stomatal conductance and photosynthetic rate in both treatments.



**Fig. 9.** Relationship between stomatal conductance and photosynthetic rate in Al-pretreated and control species. The data point represents 5-10 replications. The regression has been analyzed separately due to the prominent effect of the pre-Al treatment on photosynthesis rate subjected to varying degree of stomatal opening

Similar trend was observed in the correlation between stomatal conductance and transpiration rate, both parameters were directly related (Figure 10). At the same range of stomatal conductance, the transpiration rate of Al-pretreated *M. malabathricum* and *L. leucocephala* exhibited higher values, indicating that the pre-aluminum treatment would enhance the water absorption capacity. Therefore, the stomatal conductance of both species was increased in order to release abundant water, thus increasing the transpiration rate as well. While, both the treatments of *A. mangium* had lower transpiration rates regardless of the stomatal conductance values, low transpiration rate was observed in control due to the adaptation mechanism in order to conserve more water.



**Fig. 10.** Relationship between stomatal conductance and transpiration rate in Al-pretreated and control species. The data point represents 5-10 replications. The regression has been analyzed separately due to the prominent effect of the pre-Al treatment on transpiration rate subjected to varying degree of stomatal opening

#### 4.2. Aluminum accumulation characteristics

The photosynthetic rates of all species were increased with increasing soil pH (Figure 11). Perhaps this soil pH increment was caused due to the acidic elements uptake (Aluminum) by the plants. Thus it has improved the soil organic matter as well as increased the nutrients, e.g. P and K. In addition, to undergo an acid soil rehabilitation process, all species had to increase their soil pH by setting up several tolerance mechanisms, for example by increasing the soil pH around the root apices (Kochian *et al.*, 2004). Watanabe *et al.* (2008) found that the roots of *M. malabathricum* exude large amount of mucilage and the root mucilage is generally known to immobilize metal cation such as Al in the rhizosphere. However, their studies indicated that the unique root mucilage (higher charge density) of *M. malabathricum* facilitates Al uptake in this specie by absorbing more negatively charged anion than positively charged cation to maintain the root's surface pH of above 5.0. This root-mediated mechanism was prominent in increasing the soil pH around the rhizosphere. Besides, lowering the soil pH was responsible to inhibit the root growth (Watanabe *et al.*, 2000). However, some species will protect themselves from excess Al toxicity (main cause of acidity) by increasing the pH around the root apices (Vitorello *et al.*, 2005). The root length increases in order to get some nutrients for their growth as the soil pH increase. As the

availability of essential nutrients for the process become higher, the photosynthetic rate will also be increased. However, similar photosynthetic rate obtained in *A. mangium*, indicated no significant effects of the pre-Aluminum treatment on the relationship of both the parameters studied. Among the treatment studied, Al-pretreated *M. malabathricum* exhibited the highest increment in soil pH and photosynthetic rate.

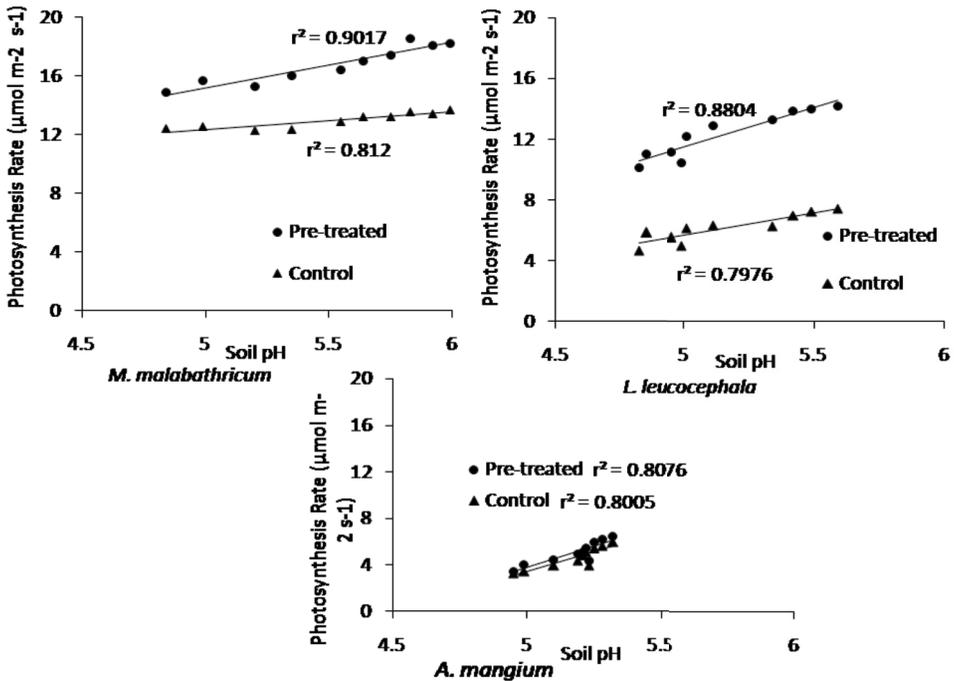


Fig. 11. Relationship between soil pH and photosynthetic rate in Al-pretreated and control. The data point represents 5-10 replications. The regression has been analyzed separately due to the prominent effect of the pre-Al treatment on photosynthesis rate subjected to varying soil pH

Leaf area index (LAI) of the Al-pretreated plants, except *A. mangium*, was significantly higher compared to control. It may be due to the excessive Al that disturbed the root mineral uptake of the species and cause the plant's growth inhibition. The Al-pretreated *M. malabathricum* showed the best performance than Al-pretreated *L. leucocephala* and these results obtained in the current experiment were depending on the level of the Aluminum accumulation characteristics of both species. This causes *M. malabathricum* to create tolerance mechanism by increasing leaf area to accumulate more Al in its leaves and then being removed from the plant, when the leaves drop (Watanabe *et al.*, 2001).

Al concentration in the leaves of all Al-pretreated species was higher than the control at the beginning of the experiment due to the pre-existence of Al during germination (pre-aluminum treatment). The early exposure also could be an advantage

to the Al-pretreated species to easily adapt on the harsh condition of the slope compared to the controlled species. The control species began to accumulate Al in their leaves as the Al concentration increased rapidly at the end of the experiment. According to the previous experiment that was performed by Jansen *et al.* (2002), the mean content of Al in the leaves of plants was 200 ppm. In the current experiment, at the end of observation, the leaves of both Al-pretreated and control species absorb two to ten times higher than that of Jansen's findings. In addition, Al accumulation in the leaves was one of the mechanisms to resist Al stress (Jansen *et al.*, 2003). Thus, the results indicated that all species studied have accumulated the Al in their leaves as a tolerance mechanism towards high Al stress on acidic slope.

The plants which exhibit Al concentration more than 1000 ppm in their leaves are reportedly classified as an Al accumulator (Jansen *et al.*, 2002). Hence, as the leaves of control *L. leucocephala* and *M. malabathricum* accumulate more than 1000 ppm of Al, the species could be classified as Al accumulators. On the other hand, *A. mangium* has only accumulated about 400 ppm, indicating that this species is a non-accumulator. In contrast, Ma *et al.* (1997) found that *A. mangium* had high efficiency to accumulate Al. The different result obtained may be due to the different condition of exposing the *A. mangium* to the high Al concentration. One explanation could be that the experiment done by Ma *et al.* (2002) was in the laboratory, whereby in this experiment, *A. mangium* was planted on the real condition of acidic slope, which possibly has influenced the results. Therefore, *A. mangium* has not shown a prominent criterion as a potential slope plant.

The decrease in the soil pH on both control and Al-pretreated (Figure 8) plots probably due to the low progress of both Al-pretreated species in accumulating Al in their leaves during adaptation phase. The soil pH was higher in all plots grown by Al-pretreated species implying that Al pre-existence had forced the species to change their physiological traits; for example, by producing higher root length that helps in improving soil organic matters and later rehabilitate the acidic slope. If the soil was too acidic, the availability of N, P and K decreased and affected the plant growth (Dobermann & Fairhurst, 2000). Although the acidic slope was not rehabilitating completely in this experiment, it was hypothesized that the soil pH will increase in time and ultimately would reach to the optimum soil pH for plant growth.

## 5. Conclusion

From this study it was revealed that the Al-pretreated species especially *M. malabathricum* and *L. leucocephala* exhibited better physiological performance. The treated plants exhibited several tolerance mechanisms including the root length enhancement, partial stomatal closure, and high LAI. The interaction and compilation of all tolerance mechanisms contribute to the rehabilitation of the acidic soil. In

addition, amongst the species studied, *M. malabathricum* exhibits the best potential slope species, and can be regarded as a strong Al-accumulator with high tolerance mechanisms.

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## References

- Abraham, J.M., Richard, B.P., Pamela, H.T., Sarah, R. & Sharda, M. (2009)** Long-term relationship among atmospheric CO<sub>2</sub>, stomata, and intrinsic water use efficiency in individual trees. *American Journal of Botany*, **96**:1779-1786.
- Ainsworth, E.A. & Rogers, A. (2007)** The response of photosynthesis and stomatal conductance to rising (CO<sub>2</sub>): Mechanisms and environmental interactions. *Plant, Cell and Environment*, **30**:258-270.
- Al-Bader, D.A., Shuail, D.A., Al-Hasan, R. & Suleman, P. (2014)** Intertidal seagrass *Halodule uninervis*: Factors controlling its density, biomass and shoot length. *Kuwait Journal of Science*, **41**(1):171-192.
- Bochet, E. & García-Fayos, P. (2004)** Factors controlling vegetation establishment and water erosion on motorway slopes in Valencia, Spain. *Restoration Ecology*, **12**:166-174.
- Copeland, S.M., Bruna, E.M. Barbosa Silva, L.V. Mack, M.C. & Vasconcelos, H.L. (2012)** Short-term effects of elevated precipitation and nitrogen on soil fertility and plant growth in a Neotropical savanna. *Ecosphere*, **3**(4):31.
- Cruden, D.M. & Varnes, D. (1996)** Landslide types and processes. In: Turner A.K.; Shuster R.L. (eds) *Landslides: Investigation and Mitigation*. National Research Council, Transportation Research Board, Special Report, **247**:36–75.
- Dashti, N.H., Montasser, M.S., Ali, N.Y. & Cherian, V.M. (2014)** Influence of plant growth promoting rhizobacteria on fruit yield, pomological characteristics and chemical contents in cucumber mosaic virus-infected tomato plants. *Kuwait Journal of Science*, **41**(2):205-220.
- Dhiman, A., Nanda, A. & Ahmad, S. (2011)** Metal analysis in *Citrus sinensis* fruit peel and *Psidium guajava* leaf. *Toxicology International*, **18**(2):163-167.
- Dobermann, A. & Fairhurst, T. (2000)** Nutrient disorder and nutrient management. Handbook Series. Norcross Georgia, Potash and Phosphate Institute (PPI). Potash and Phosphate Institute Canada (PPIC) and International Rice Research Institute, USA.
- Eleftheriou, E.P., Moustakas, M. & Fragiskos, N. (1993)** Aluminate-induced changes in morphology and ultrastructure of *Thinopyrum* roots. *Journal of Experimental Botany*, **44**(2):427-436.
- Fuentes, J.P., Bezdicsek, D.F., Flury, M., Albrecht, S. & Smith, J.L. (2006)** Microbial activity affected by lime in a long-term no-till soil. *Soil and Tillage Research*, **88**(1–2):123-131.
- Hamlyn, G.J. (1998)** Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany*, **49**:387-398.
- Harter, R.D. (2002)** Acid soils of the tropics. ECHO technical note. University of New Hampshire. pp. **8**.
- Hetherington, A.M. & Woodward, F.I. (2003)** The role of stomata in sensing and driving environmental change. *Nature*, **424**:901-908.
- Huat, B. B.K. & Kazemian, S. (2010)** Study of root theories in green tropical slope stability. *Electronic Journal of Geotechnical Engineering*, **15**:825-1834.

- Jansen, S., Broadley, M.R. Robbrecht E. & Smets, E. (2002)** Aluminium hyperaccumulation in Angiosperms: A review of its phylogenetic significance. *The Botanical Review*, **68**:235-269.
- Jansen, S., Watanabe, T., Dessein, S., Smets, E. & Robbrecht, A. (2003)** A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. *Annals of Botany*, **91**:657-663.
- Jarvis, A.J. & Davies, W.J. (1997)** The Coupled Response of Stomatal Conductance to Photosynthesis and Transpiration. *Journal of Experimental Botany*, **49**:399-406.
- Kidd, P.S. & Proctor, J. (2001)** Why plants grow poorly on very acid soils: are ecologists missing the obvious? *Journal of Experimental Botany*, **52**:791-799.
- Kikui, S., Sasaki, T., Mackawa, A., Miyao, M. & Hirochika, H. et al. (2005)** Physiological and genetic analyses of aluminium tolerance in rice, focusing on root growth during germination. *Journal of Inorganic Biochemistry*, **99**(9):1837-1844.
- Kochian, L.V., Pineros, M.A. & Hoekenga, O.A. (2005)** The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant and Soil*, **274**:175-195.
- Kochian, L.V., Hoekenga, O.A. & Pineros, M.A. (2004)** How do crop plants tolerate acid soils? Mechanism of aluminum tolerance and phosphorus efficiency. *Annual Review of Plant Physiology and Plant Molecular Biology*, **55**:459-493.
- Komoo, I., Aziz, S. & Sian, L.C. (2011)** Incorporating the Hyogo Framework for Action into landslide disaster risk reduction in Malaysia. *Bulletin of the Geological Society of Malaysia*, **57**:7-11.
- Koutika, L.S., Sanginga, N., Vanlauwe, B. & Weise, S. (2002)** Chemical properties and soil organic matter assessment in fallow systems in the forest margins benchmark. *Soil Biology and Biochemistry*, **34**(6):757-765.
- Ma, J., Hiradate, S., Nomoto, K., Iwashita, T. & Matsumoto, H. (1997)** Internal detoxification mechanism of Al in hydrangea: identification of Al form in leaves. *Plant Physiology*, **113**:1033-1039.
- Ma, J., Hiradate, S. & Matsumoto, H. (2002)** High aluminium resistance in buckwheat II. Oxalic acid detoxifies aluminium internally. *Plant Physiology*, **117**:753-759.
- Mafian, S., Huat, B.B.K. Rahman, N.A. & Sing, H. (2009)** Potential plant species for live pole application in tropical environment. *American Journal of Environmental Sciences*, **5**:759-764.
- Misawa, S., Osaki, M. & Watanabe, T. (2005)** Aluminium accumulation in the roots of *Melastoma malabathricum*, an accumulating plant. *Canadian Journal of Botany*, **83**(11):1518-1522.
- Mugagga, F., Kakembo, V. & Buyinza, M. (2012)** A characterisation of the physical properties of soil and the implications for landslide occurrence on the slopes of Mount Elgon, Eastern Uganda. *Natural Hazards*, **60**:1113-1131.
- Normaniza, O. & Barakbah, S.S. (2006)** Parameters to predict slope stability—Soil water and root profiles. *Ecological Engineering*, **28**:90-95.
- Normaniza, O. & Barakbah, S.S. (2011)** The effect of plant succession on slope stability. *Ecological Engineering*, **37**:139-147.
- Normaniza, O., Faisal H.A. & Barakbah, S.S. (2008)** Engineering properties of *Leucaena leucocephala* for prevention of slope failure. *Ecological Engineering*, **32**:215-221.
- Normaniza, O., Saifuddin, M. & Aimee, H. (2014)** Contribution of vegetation to alleviate slope's erosion and acidity. In Maria C. Hernandez-Soriano (Eds.), *Environmental risk assessment of soil contamination* (pp. 519-543). Rijeka, Croatia, INTECH. ISBN 978-953-51-1235-8. doi: 10.5772/57228.
- Pradhan, B., Chaudhari, A., Adinarayana, J. & Buchroithner, M. (2012)** Soil erosion assessment and its correlation with landslide events using remote sensing data and GIS: a case study at Penang Island, Malaysia. *Environmental Monitoring and Assessment*, **184**(2):715-727.
- Prasetyo, T. (2007)** Effects of Al on root morphology and physiology of two maize hybrids. Faculty of Agriculture. M.Sc. Thesis. Unpublished.

- Preti, F. & Giadrossich, F. (2009)** Root reinforcement and slope bioengineering stabilization by Spanish Broom (*Spartium junceum* L.). *Hydrology and Earth System Sciences*, **13**:1713-1726.
- Rohailah, M.I. (2011)** Rehabilitation of acidic soil using potential slope plants. M.Sc. Thesis. University of Malaya.
- Saifuddin, M., Normaniza, O. Rahman, M.M. & Amru, N.B. (2015)** Soil reinforcement capability of two legume species from plant morphological traits and mechanical properties. *Current Science*, **108**(7):1340-1347.
- Saifuddin, M., Normaniza, O. & Rahman, M.M. (2013)** Influence of different cutting positions and rooting hormones on root initiation and root-soil matrix of two tree species. *International Journal of Agriculture and Biology*, **15**:427-434.
- Saifuddin, M. & Normaniza, O. (2014a)** Evaluation of hydro-mechanical properties and root architecture of plants for soil reinforcement. *Current Science*. **107**:845-852.
- Saifuddin, M. & Normaniza, O. (2014b)** Hydrological and mechanical properties of plants to predict suitable legume species for reinforcing soil. *Chinese Science Bulletin*, **59**:5123-5128.
- Saifuddin, M. & Normaniza, O. (2012)** Physiological and root profile studies of four legume tree species. *Life Science Journal*, **9**(4):1509-1518.
- Shafie, A. (2009)** A Case study on floods of 2006 and 2007 in Johor, Malaysia. Colorado State University
- Song, Y.S., Hong, W.P. & Woo, K.S. (2012)** Behavior and analysis of stabilizing piles installed in a cut slope during heavy rainfall. *Engineering Geology*, **129–130**:56-67.
- Tolrà, R.P., Poschenrieder, C., Luppi, B. & Barceló, J. (2005)** Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. *Environmental and Experimental Botany*, **54**:231-238.
- Vitorello, V.A., Capaldi, F.R. & Stefanuto, V.A. (2005)** Recent advances in aluminium toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology*, **17**:129-143.
- Wang, J., Raman, H., Zhang, G., Mendham, N. & Zou, M. (2006)** Aluminum tolerance in barely (*Horidium vulgare* L.): Physiological mechanisms, genetics and screening methods. *Journal of Zhejiang University Science*, **7**:769-787.
- Watanabe, T., Osaki, M. & Tadano, T. (2000)** Effect of aluminium on growth of melastoma (*Melastoma malabathricum* L). Proceedings of the international symposium on impact of potential tolerance of plants on the increased productivity under Aluminium stress. Karuizawa, Japan. 15-16 September 2000. Pp. 45-50.
- Watanabe, T., Osaki, M. & Tadano, T. (2001)** Al uptake kinetics in roots of *melastoma malabathricum* L. – an Al accumulator plant. *Plant and Soil*, **231**:283-291.
- Watanabe, T., Misawa, S. Hiradate, S. & Osaki, M. (2008)** Root mucilage enhances Aluminium accumulation in *Melastoma malabathricum*, an Aluminium accumulator. *Plant Signaling and Behavior*, **3**(8):603-605.
- Yang, L., Liu, N., Ren, H. & Wang, J. (2009)** Facilitation by two exotic Acacia: *Acacia auriculiformis* and *Acacia mangium* as nurse plants in South China. *Forest Ecology and Management*, **257**(8):1786-1793.
- Zsoldos, F., Vashengyi, A. Pecsvaradi, A. & Bona, L. (2003)** Influence of silicon on aluminum toxicity in common and durum wheat. *Agronomy*, **23**:349-354.

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## آثار المعالجة بالألومنيوم على شكل وفسولوجيا نباتات المنحدرات الحمضية المحتملة

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### خلاصة

ارتفاع درجة الحرارة وهطول الأمطار يساهم في تحويل معظم أنواع تربة المنحدرات إلى تربة حمضية في بلدان مثل ماليزيا. المنحدر الحمضية تسهم مرة أخرى في انخفاض الغطاء النباتي، واحتمالية تآكل عالية وعدم استقرار المنحدرات. في هذا السياق، تهدف هذه الدراسة في التحقيق في آثار المعالجة بالألومنيوم على نمو و تطور مجموعة مختارة من النباتات على المنحدرات الحمضية. خصائص تحمل الحمضية لكل من (*Acacia mangium*, *Leucaena*) معالجات بالألومنيوم في مرحلة الإنبات. أظهرت النتائج أن *Melastoma malabathricum* أبدى أعلى المعايير الشكلية: تقسيم طول الجذر والوزن الجاف، والأداء الفسيولوجي بما في ذلك معدل التمثيل الضوئي، تصريف خلايا الثغور، ومعدل النتح، ومؤشر مساحة الورقة (LAI)، وتحليل الألومنيوم في الأوراق. في غضون عشرة أسابيع من المراقبة، أظهرت *Melastoma malabathricum* المعالجة أعلى معدلات البناء الضوئي والنتح بالمقارنة مع *Acacia mangium*, *Leucaena leucocephala*، على نفس النحو، كان معدل تصريف خلايا الثغور هو الأعلى في (*Melastoma malabathricum*). وعلاوة على ذلك، في غضون عشرة أسابيع، ارتفعت حموضة التربة المزروعة (*Melastoma malabathricum*) بنسبة ٨، ٢٣٪، ووجد أنها الأعلى بين تلك الأنواع، مما يعني قدرة هذا النوع على إعادة التأهيل. *Melastoma malabathricum* المعالج بالألومنيوم أيضاً أظهر أطول الجذور في التربة الحمضية مستعرضاً آلية التحمل تجاه حموضة التربة. بين تلك الأنواع التي تمت دراستها، أظهر نبات *Melastoma malabathricum*، سواء المعالج أو غير المعالج، أفضل أداء مورفولوجي وفسولوجي في الظروف الحمضية.