# Growth and Mineral Nutrition of *Aquilaria Malaccensis (Karas)* In Two Habitats As Affected By Different Cultural Practices

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## ABSTRACT

*Effects of cultural practice under different habitats, of well-managed monoculture plantation and growing wild under rubber trees, were studied in Aquilaria malaccensis (Karas) leaves. This study was carried out on Karas growing in these two habitats each from Lipis, Pahang and Sepang, Selangor areas in Malaysia; under the control and induced treatments. The parameters studied include wet and dry weight of 50 matured leaves, iron and zinc elemental contents in leaf, iron and zinc uptakes from soil, and leaf and soil moisture contents. Iron and zinc were analysed in Karas leaves and soil by using Instrumental Neutron Activation Analysis (INAA) technique.*

## ABSTRAK

*Kesan dari kaedah penanaman pada habitat berbeza, iaitu ladang monokultur yang ditadbir selia dengan baik dan tumbuh secara meliar di bawah pokok getah, telah dikaji pada daun Aquilaria malaccensis (Karas). Kajian ini telah dilaksanakan terhadap daun pokok Karas yang hidup dalam dua habitat berbeza di Lipis Pahang dan Sepang, Selangor; di bawah perlakuan kawalan dan induksi. Parameter yang dikaji termasuk berat basah dan kering 50 daun matang, kandungan elemen besi dan zink daun, pengambilan besi dan zink dari tanah, dan juga kandungan air daun dan tanah. Kandungan besi dan zink telah dianalisis dengan menggunakan teknik Analisis Pengaktifan Neutron Instrumentasi (APNI).*

**Keywords:** *Aquilaria malaccensis* (*Karas*), growth, mineral nutrition, cultural practices, two habitats

## INTRODUCTION

Trees of *Aquilaria* spp. (Thymelaceae) have been harvested for centuries from the wilderness of countries such as Malaysia, Indonesia, Thailand, the Philippines, Myanmar, Vietnam and Laos for agarwood (*gaharu*), a resinous commercial wood used as incense (Chakrabarty *et al.*, 1994; Jalaluddin, 1977; Ng *et al.*, 1997). A total of 25 species of *Aquilaria* are found in the world. The five species that can be found growing in natural forests of Malaysia include *Aquilaria hirta, A. beccariana, A. rostrata, A. malaccensis* and *A. microcarpa. Aquilaria malaccensis* is common in Peninsular Malaysia whilst *A. microcarpa* is common in Sarawak (Sepiah, 2011). Chakrabarty *et al.* (1994) suggested that the high commercial value of *gaharu* has stimulated its collection to a level that may threaten the survival of a species. In 1994, because of the persistent pressure from international trade on *Gaharu*, *A. malaccensis* was incorporated into Appendix II of CITES (CITES, 1994). The aims of CITES listing are to regulate and monitor international trade to ensure that the trade does not constitute a threat to the survival of the species (Milner-Gulland and Mace, 1998).

*Aquilaria malaccensis* (*Karas*) can be found growing in various habitats, from rocky, sandy or calcareous, well-drained slopes and ridges to swampy lands. They are adapted at altitudes between 300 m and 850 m above sea level until up to 1000 m. They are also well adapted to daily temperature that ranges from 20 °C to 22 °C (Dayana-Aisyah *et al.*, 2009). In Peninsular Malaysia, the tree growth of *Aquilaria malaccensis* is rather low at 0.33 cm per year in native forests. Nevertheless, tree growth can reach up to 0.8 to 1 cm per year (La Frankie, 1994).

Bose (1926; in Gibson, 1977) first initiated the investigation on *gaharu* resin formation. Formation of agarwood (*gaharu*) is often associated with the physiological reactions of these trees (the host) against pathogenic infections from parasitic fungi. Sepiah (2011) studied and identified these fungi, including the saprophytic and the endophytic fungi, which inhabited the fresh stem tissues as well as the *Gaharu* chip of both *A. malaccensis* and *A. microcarpa*. There was however, no clear indication to associate specific fungus isolated to the *gaharu* resin formation and *gaharu* grade.

The inducement process is still mysterious with some quarters claiming that it was by enzyme and chemical compounds rather than through fungal infection. Nevertheless, the xylem structures of *A. malaccensis* plant are probably responsible for producing the *gaharu* resin through the compartmentalization process that was triggered by physical or mechanical injury or due to invasion by microbes (Chong *et al*,.(2012).

Apart from *gaharu* production in the trunks and roots, *A. malaccensis* is also valued for its tea that is produced from its leaf (Sepiah, 2011). This study is thus aimed at comparing growth effects of two different cultural practices in *Karas* leaves, in terms of well-managed monoculture plantation and growing wildly in old rubber plantations, at two sites, under both the control and induced treatments. The growth parameter was measured as wet and dry weights of 50 randomly sampled matured *Karas* leaves. The iron and zinc contents in these leaves, their contents in soils, and moisture contents of leaves and soils were also analysed to compare the ability of this plant to take up these nutrients from different soil types at the two habitats.

## MATERIALS AND METHODS

### *Plant sampling*

*Aquilaria malaccensis* leaves used were sampled from the Sepang and Lipis areas of Malaysia. *Karas* trees were growing in the wild under old rubber trees in Sepang, Selangor whilst trees in Lipis Pahang were growing in a well-managed monoculture plantation (Khairuddin, 2003). At both habitats, 50 matured leaves from control trees as well as from trees that have undergone inducement for gaharu resin production by injection (Khairuddin, 2003), were randomly sampled from each tree, put in sealed plastic bags before being transported back to the Malaysian Nuclear Agency (Nuclear Malaysia) laboratory in ice boxes. It was kept in refrigerator at 4 ºC prior to moisture and mineral content analysis.

### *Soil sampling*

Soils under *Karas* trees were obtained from Sepang and Lipis areas, during each field samplings of leaves. The soil was sampled from 0-20 cm and 20-50 cm depths (20-40 cm in Lipis because of unavoidable stones) by using soil auger, put into separate plastic bags and brought back to the Nuclear Malaysia laboratory for storage under shade prior to moisture and mineral content analysis.

### *Preparation of plant and soil samples*

A total of 50 matured leaves which were randomly sampled were washed clean under running water in the laboratory, each leaf was later carefully dabbed by using clean and moist tissue paper. These leaves were put into paper envelope and weighed for growth determination. This was followed with oven drying at 65 ºC until constant weight, before taking the dry weight and moisture content measurement. These leaves were later ground into powder form with a grinder prior to mineral content analysis by using Instrumental Neutron Activation Analysis (INAA) technique (Nashriyah *et al.,* 1996).

Soil sample of known weight was oven dried at 65 ºC until constant weight, before taking the dry weight. Prior to mineral content analysis by INAA, it was pounded into powder form by using pestle and mortar. Solid soil powder was sieved by using 20 mm sieve followed by 212 nm sieve. The moisture content was subsequently measured and calculated (Nashriyah *et al.,* 1996).

### *Instrumentation Neutron Activation Analysis (INAA)*

Mineral contents for iron (Fe) and zinc (Zn) in leaf and soil were measured by using the multi-elemental Instrumentation Neutron Activation Analysis (INAA) method (Nashriyah *et al.,* 1996). Mineral uptake was calculated as soil-plant transfer coefficient ratio (Nashriyah *et al.,* 2006).

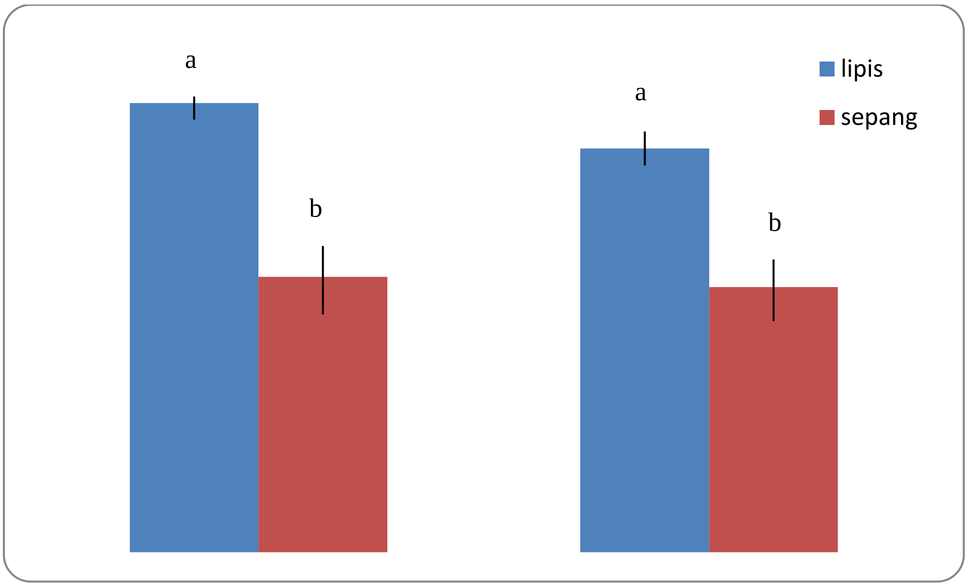
### *Statistical Analysis*

Mean values was statistically compared (Analysis of Variance (ANOVA)) by using the SAS software. Significance of differences of means was accorded to P≤0.01 (Duncan’s).

## RESULTS AND DISCUSSION

### *Growth*

Growth of *Aquilaria malaccensis* trees in both Sepang and Lipis areas was measured as dry and wet weight (biomass) of 50 matured leaves. Regardless of treatment given (control or induced), trees from Lipis area showed higher biomass value as compared to leaves from Sepang area (Figures 1 and 2).



*****Means followed by the same letter are not significantly different (P≤0.01) (Duncan's).

Figure 1: Dry weight (g) of 50 matured leaves from *Aquilaria malaccensis* trees growing in Lipis and Sepang areas

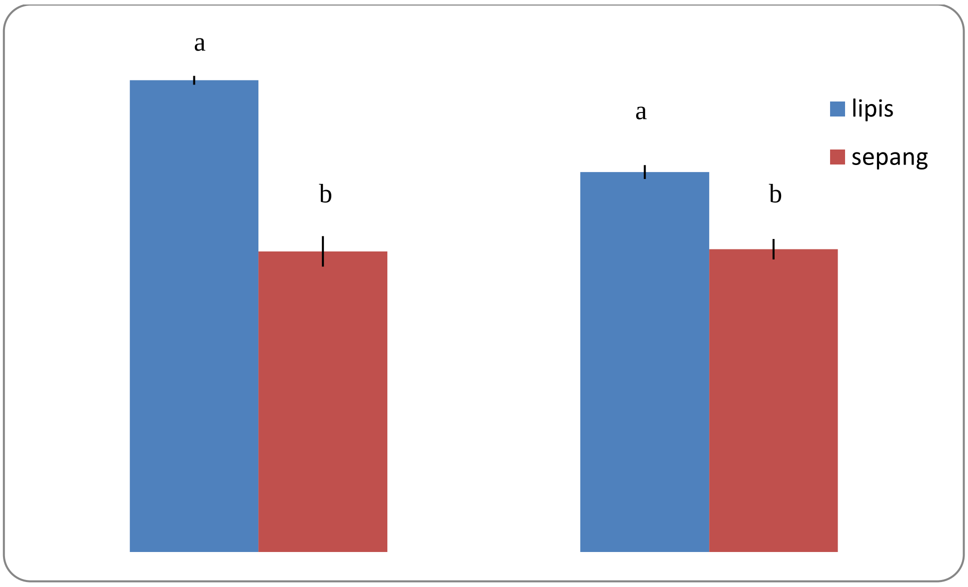


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Figure 2: Wet Weight (g) of 50 matured leaves from *Aquilaria malaccensis* trees growing in Lipis and Sepang areas

The leathery, shiny, simple and alternately arranged leaves were supported by 4-6 cm long stalk or petiole. Dry and wet weights of 50 matured leaves from the control and induced *Aquilaria malaccensis* trees growing in Sepang area were lower compared to control and induced trees growing in Lipis area (Figures 1 and 2). The dry weight mean values of the control samples at Sepang were 7.4 ± 1.0 g, whereas in Lipis area was 12.1 ± 0.7 g. The mean values of leaf dry weight which were measured at 10.9 ± 0.6 g and 7.17 ± 1.0 g, respectively, was higher in the Lipis area than in the Sepang area.

The data of wet weight of leaves at Lipis also showed the higher mean values for both control 31.87 ± 1.11 g and induced 25.67 ± 0.43g trees compared to trees at Sepang at 20.31 ± 1.03 g and 20.46 ± 0.69 g, respectively. These results were expected because trees were well fertilized in Lipis plantation but found growing wild in abandoned rubber plantation in Sepang.

In a physiological study carried out in plantations at Kluang, Johor and Bukit Katil, Melaka between July 2007 and January 2009; Dayana-Aisyah *et al.* (2009) found that the mean values for minimal fluorescence ($F_o$), maximal fluorescence ($F_m$) and variable fluorescence ($F_v = F_m - F_o$) for seedlings planted at Kluang plantation were higher than those at Bukit Katil plantation. Thus it was possible that trees in Lipis were also exposed to direct sunlight that can ensure optimum photosynthesis, whereas trees in Sepang which were growing under the shade of old rubber trees experienced limited photosynthesis.

### *Moisture and Mineral Contents of Leaf*

Moisture and mineral contents were measured in the matured leaves of *Aquilaria malaccensis* trees growing in Sepang and Lipis areas (Figures 3 and 4), to ascertain whether plants were experiencing water stress under open plantation in Lipis. Moisture contents for the control trees were almost similar at both areas. Nevertheless, moisture contents of leaves from the induced trees in Sepang and Lipis areas were significantly differences. The moisture content of leaves for induced trees in Sepang was higher at 65.86 ± 0.01%, as compared to those from Lipis, which was 56.87 ± 0.22 %. Since water content in the induced *A. malaccensis* leaves growing in the Lipis plantation was reduced to less than 60%, this treatment had exposed these trees to water stress. These results were expected because under shade, as in the Sepang samples, leaves would transpire less as compared to when exposed to direct sunlight in an open area plantation, as in Lipis (Whitecross and Amstrong, 1972; Baker, 1974).

Iron content in matured leaves at Sepang was higher than leaves at Lipis area. Iron content in control leaves at Sepang (149.6 ± 0.7 ppm) showed significantly different concentration as compared to leaves at Lipis area (57 ± 0.6 ppm). However, the iron content in leaves of induced trees at both places was nearly similar. On the other hand, zinc contents in leaves of both control and induced trees at both places showed no significant difference, which were 59.6 ± 1.0 ppm and 67.6 ± 0.8 ppm, respectively.

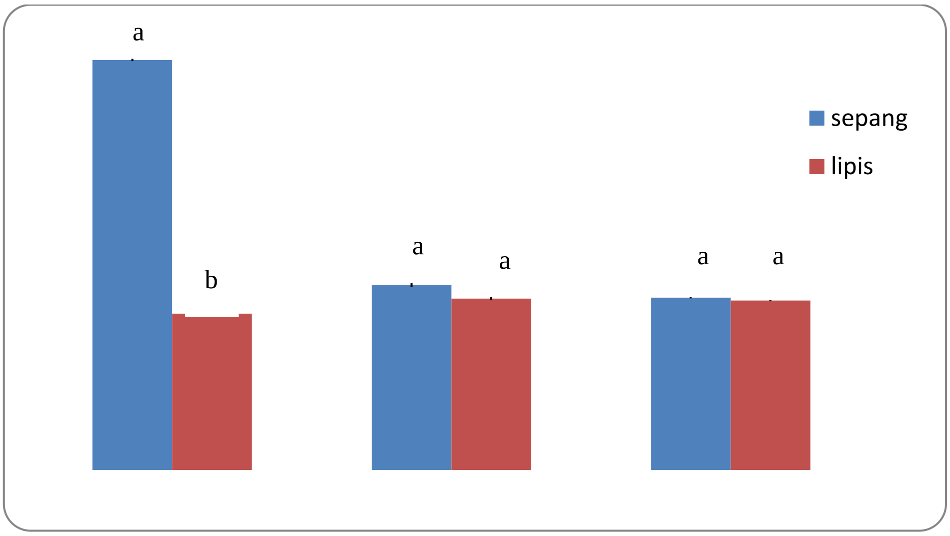

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Figure 3: Mineral (ppm) and moisture contents (%) in leaves from the control *Aquilaria malaccensis* trees growing in Sepang and Lipis areas

### *Soil Moisture Content*

In Sepang soil, moisture content was expected to be optimum because *Aquilaria malaccensis* trees were growing wildly in an old untapped rubber agroecosystem (Khairuddin, 2003). At 0-20 cm soil depth (Figure 5), moisture content at Sepang was measured at 23.1 ± 1.45% and 23.8 ± 1.6% under the control and induced trees, respectively. Similar results were obtained for soil moisture at Lipis, the moisture contents were measured at 22.5 ± 0.8% under the control trees and 22.8 ± 0.9% under the induced trees, respectively. The results showed no significant difference between induced and control trees soils at both places (P>0.01).

Moisture contents of Lipis plantation soil at 0-20 cm and 20-40 cm soil depths were measured to ascertain whether plants were exposed to water stress under open plantation. A lower minimum value at 0-20 cm depth indicated that direct sunlight has reduced the surface soil moisture content slightly.

The results showed that lower moisture content at 20-40 cm soil depth at both areas (Figure 6), was possibly due to higher water uptake by tree roots at the lower soil depth. However, the moisture content in soil under the control trees at both places showed significantly different values. Trees and shrubs, including the weeds, were found to co-exist densely in this hilly agroecosystem (Khairuddin, 2003). Dayana-Aisyah *et al.* (2009) also showed that moisture contents of soils were not significantly different between two plantations, under *Aquilaria malaccensis* seedlings.

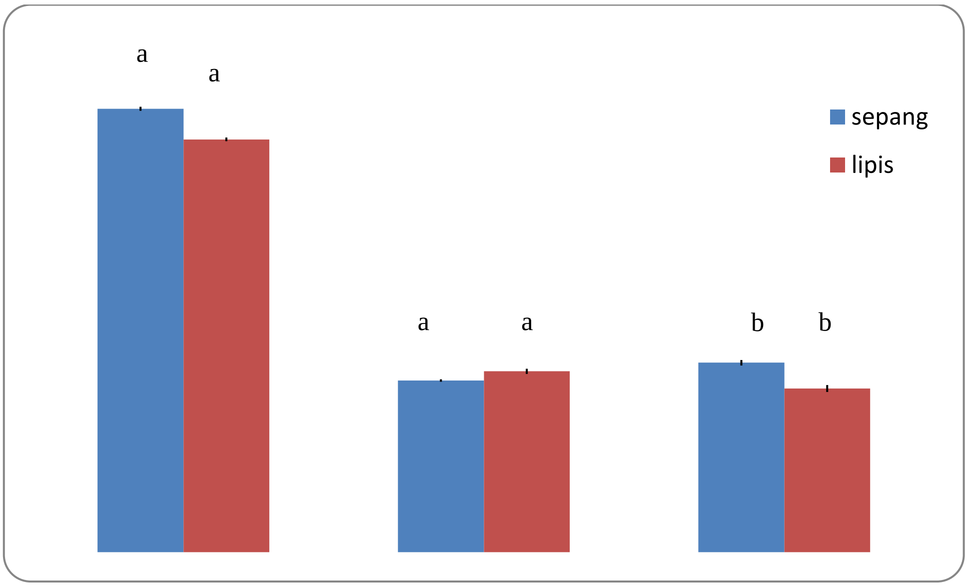

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Figure 4: Mineral (ppm) and moisture (%) contents in leaves from the induced *Aquilaria malaccensis* trees growing in Sepang and Lipis areas

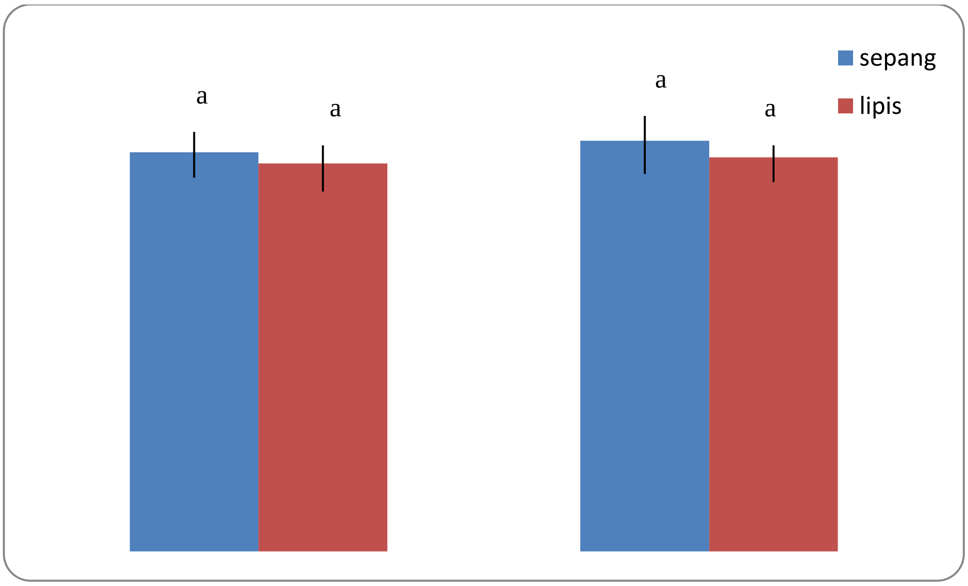

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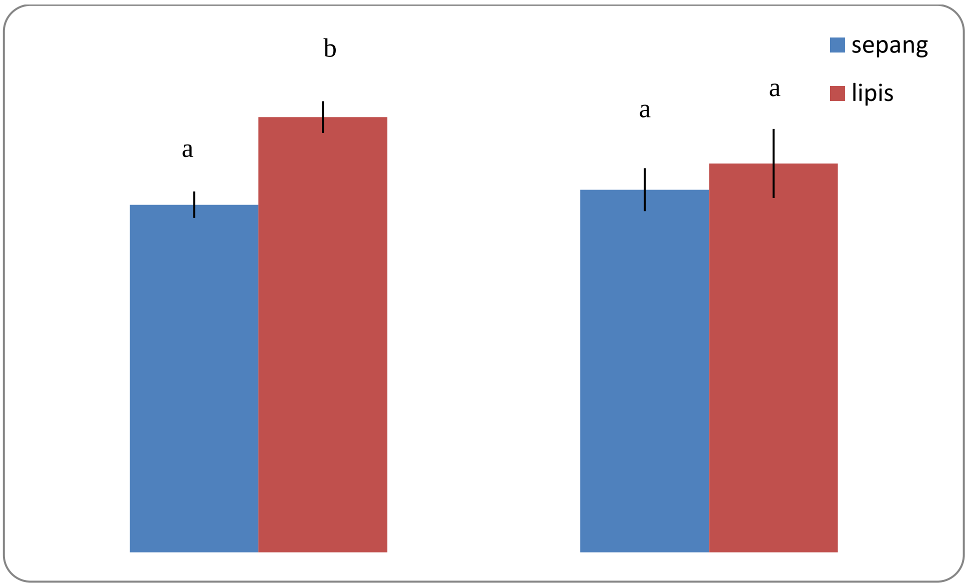
Figure 5: Moisture contents (%) in Sepang and Lipis soils (0-20 cm depth)



*Means followed by the same letter are not significantly different (P≤0.01) (Duncan's).
Figure 6: Moisture contents (%) in Sepang and Lipis soils (20-40 cm depth)

### *Mineral Content of Soil*

The content of zinc in soil under the control trees (0-20 cm and 20-40 cm depth) showed significantly different values at Sepang and Lipis areas (Figures 7 and 8). However, there was no significant difference in zinc content of soil under the induced trees at both places (Figures 7 and 8). The results obtained showed that the mean values of zinc under the control trees at Sepang were 24.23 ± 1.6 ppm which was lower compared to soil under the control trees at Lipis (38.8 ± 1.8 ppm) at 0-20 cm (Figure 7). A similar pattern of results was recognized with the content of zinc in soil under the control trees at 20-40 cm soil depth; 25.78 ± 0.72 ppm and 42.5 ± 0.95 ppm at Sepang and Lipis, respectively (Figure 8). These results were in contrast with lower content of zinc in matured leaves as measured from the control trees in the Lipis area.

The content of iron in 0-20 cm and 20-40 cm soil depths under the control and induced trees at both places showed significantly different values. The amount of iron in soil at Lipis was higher than in Sepang area. The results obtained showed that the mean values of iron in soil under the control trees (0-20 cm depth) at Sepang were 17721 ± 1.24 ppm which was lower compared to the mean values of iron in soil under the control trees at Lipis (33578 ± 0.65 ppm). The similar pattern of results obtained was recognized with the mean values of iron in soil under control trees in 20-40 cm soil depth; 20866 ± 0.92 ppm and 36163 ± 0.43 ppm at Sepang and Lipis, respectively (Figure 9). The amount of iron in 0-20 cm soil depth under the induced trees at Sepang (14611 ppm) was lower than at Lipis (33047 ppm). On the other hand, the amount of iron detected in 20-40 cm soil depth under the control trees at Sepang (18465 ppm) was lower than at Lipis (40343 ppm) (Figure 10).

### *Bioconcentration or Soil Plant Transfer Coefficient of Mineral*

It is important to know the correlation between plant and site conditions in order to achieve the best growth performance and productivity of *Aquilaria malaccensis* seedlings (Dayana-Aisyah *et al.*, 2009). Mineral elemental bioconcentration or soil plant transfer coefficient (Nashriyah *et al.*, 2006) was measured in this study as a parameter for zinc and iron uptakes in *A. malaccensis*.

The bioconcentration of iron at 0-20 cm and 20-40 cm was significantly higher in *A. malaccensis* in both control and induced treatments in Sepang habitat (Figures 11 and 12). Since the uptake was very low from soil at the ratio of less than 0.014 (Figures 11 and 12), it was possibly negligible in term of toxicity.

Iron was a much less important mineral nutrient for *karas* as reflected by the trace amount accumulated. Even though both soils contain much higher amount of iron than zinc, this plant did not take up iron in high amount because it will become toxic if bioconcentrated.

The bioconcentration of zinc was also higher in Sepang habitat as compared to Lipis habitat at 0-20 cm and 20-40 cm soil depths, but it was observed only from the control treatment (Figures 13 and 14). Inducement caused the bioconcentration in Lipis plantation to increase, however the difference between both habitats were not significant.

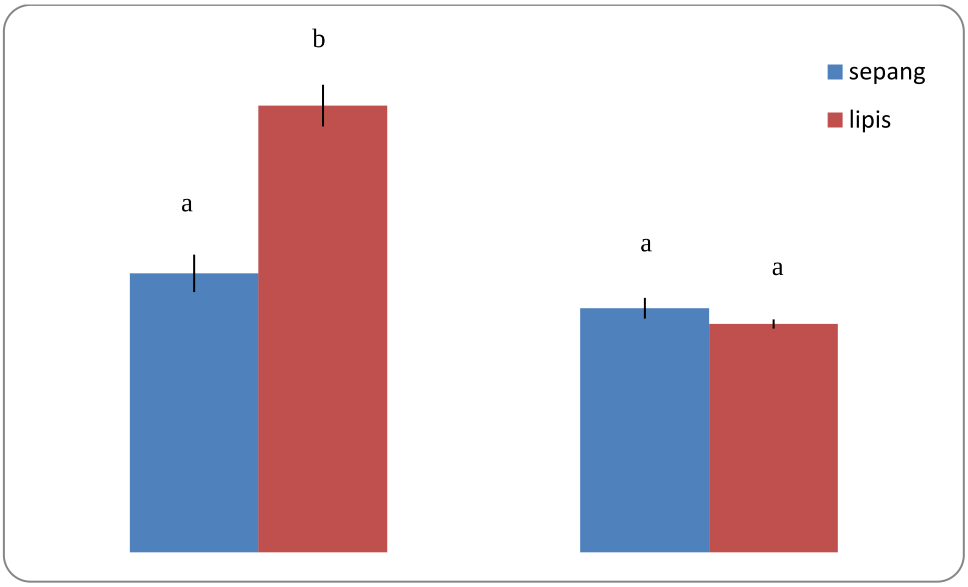


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Figure 7: Zinc content (ppm) in Sepang and Lipis soils (0-20 cm depth)

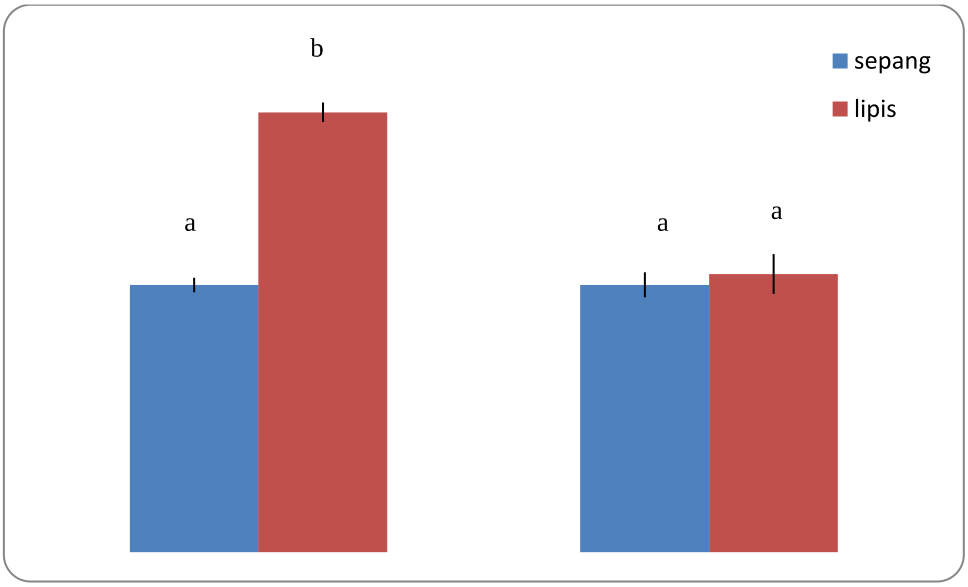

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Figure 8: Zinc contents (ppm) in Sepang and Lipis soils (20-40 cm depth)

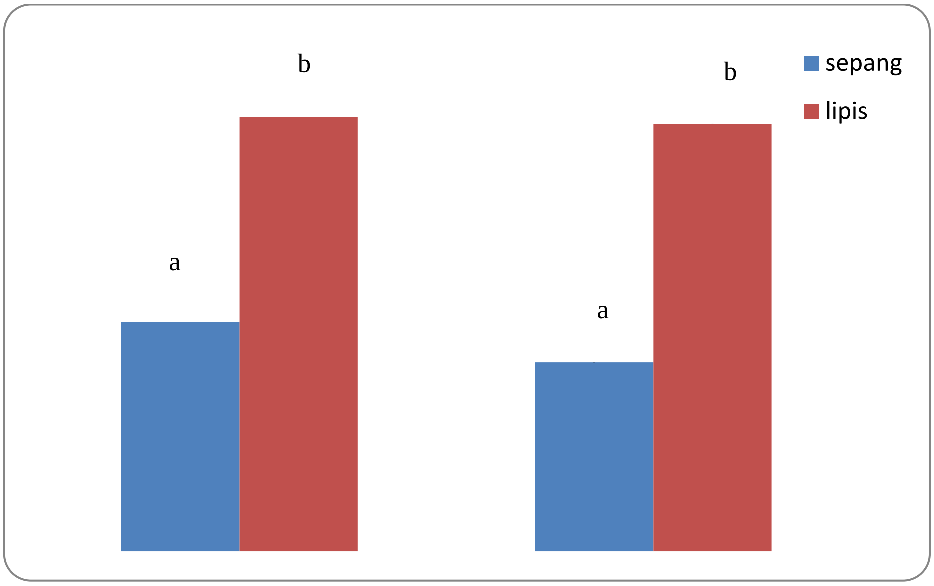

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Figure 9: Iron contents (ppm) in Sepang and Lipis soils (0-20 cm depth)

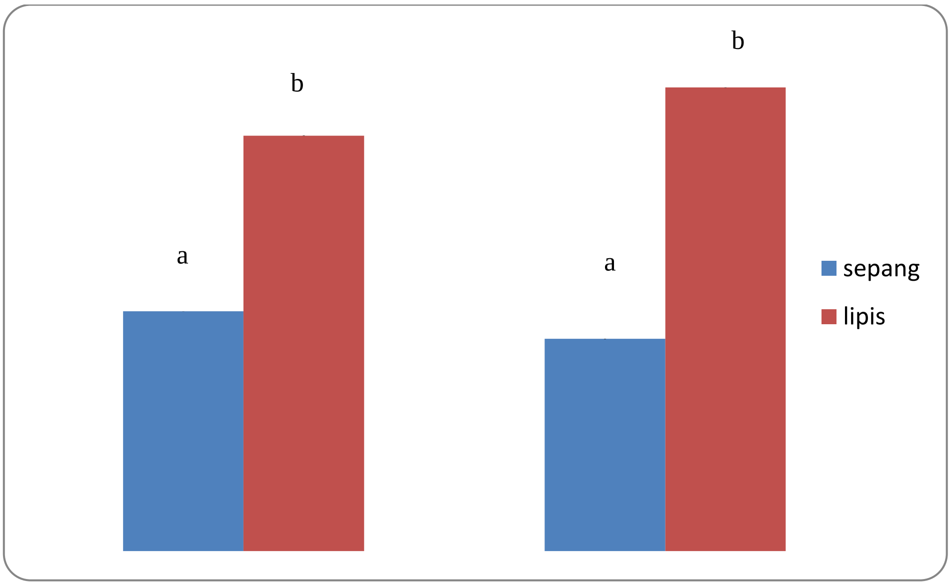

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Figure 10: Iron contents (ppm) in Sepang and Lipis soils (20-40 cm depth)

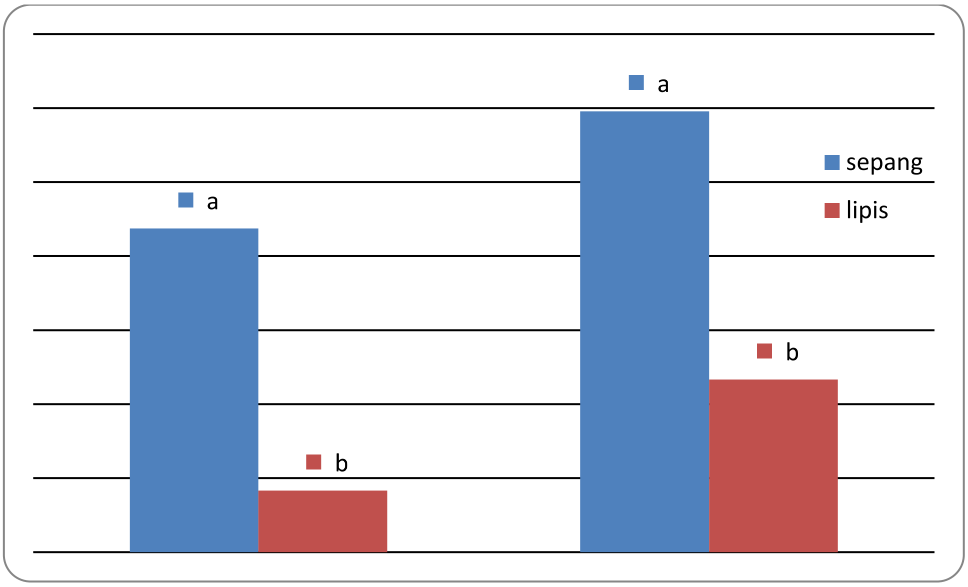

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Figure 11: Bioconcentration of iron in Sepang and Lipis soils at 0-20 cm soil depth

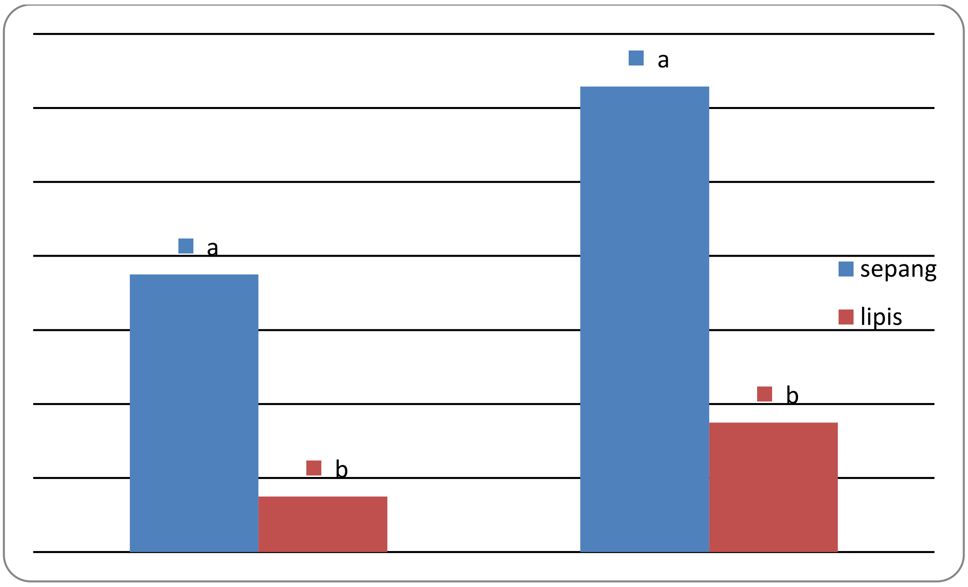

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Figure 12: Bioconcentration of iron in Sepang and Lipis soils at 20-40 cm soil depth

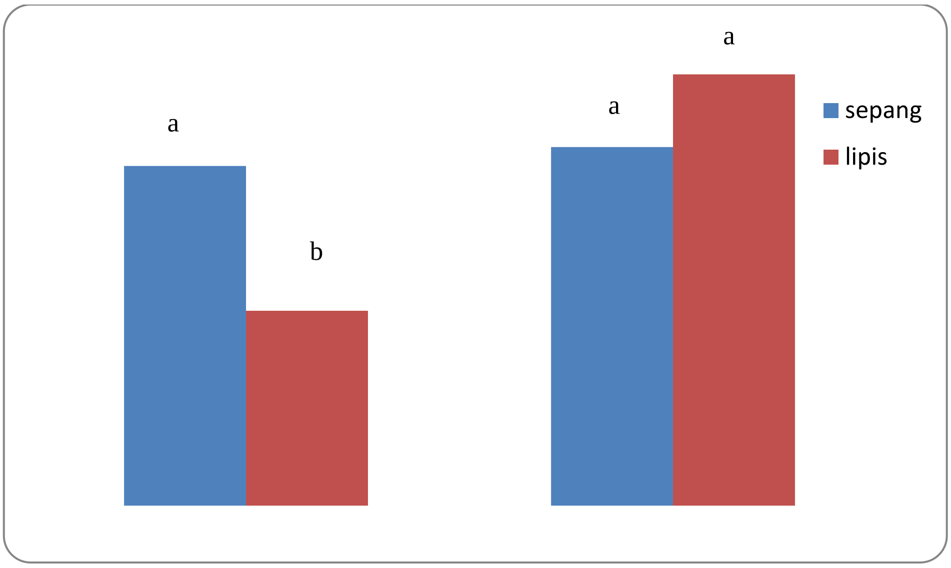

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Figure 13: Bioconcentration of zinc in Sepang and Lipis soils at 0-20 cm soil depth

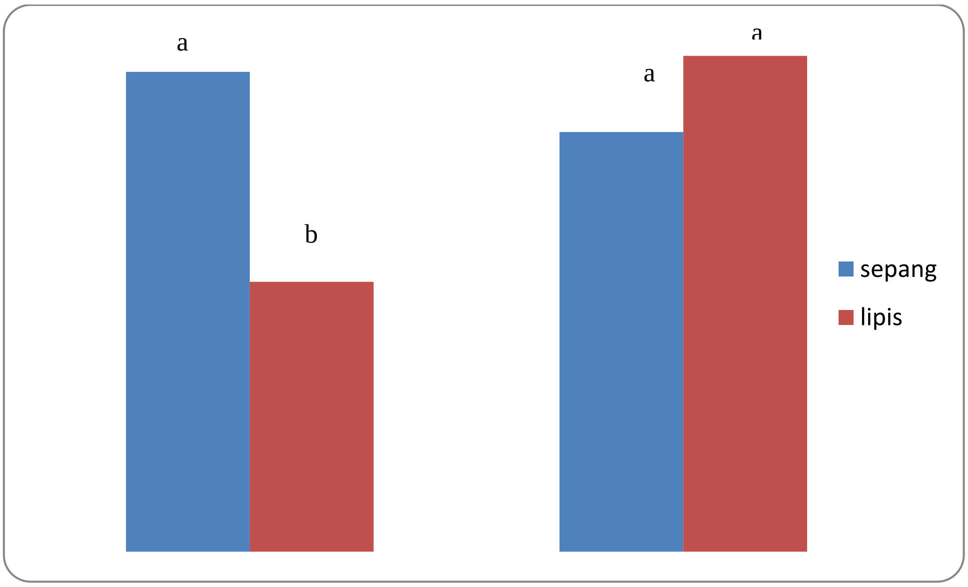

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Figure 14: Bioconcentration of zinc in Sepang and Lipis soils at 20-40 cm soil depth

Actual stand productivity at any given time was determined by how well the plant can capture soil mineral resources (Mead, 2004). Zinc is an important cation for growth of *A. Malaccensis*, as shown by a much higher uptake than iron. Trees in Lipis plantation showed higher zinc uptake in induced trees whereas trees growing in the Sepang rubber agroecosystem showed less distinctive trend.

Plant growing under stressful condition such as water deficit, nutrient deficiency and under attack by pathogens may often show reduction in photosynthesis (Fracheboud, 1999). Increment in plant productivity may indicate physiological efficiency, and physiological efficiency may manifest itself in increase plant productivity. Site factor values such as nutrient content may also contribute to this hypothesis, thus based on zinc bioconcentration values in both Sepang and Lipis trees it can be said that the small difference in zinc uptake was giving a big impact on mass (or growth) of leaves in the Lipis plantation.

Plant responses are not only dependent on species and silviculture treatment, but also on stage of plant growth. Seedlings planted at Kluang plantation has shown lower N, P and K nutrients uptakes compared to seedlings growing in Bukit Katil plantation, due to the age of these seedlings (Dayana-Aisyah *et al.*, 2009). In contrast, the Sepang trees showed more zinc nutrient uptake although trees growing in Lipis plantation showed better growth than trees growing in Sepang rubber agroecosystem in terms of leaf biomass. This could be due to the adaptation between physiological process and nutrient uptake that are already fully established in matured plant as compared to seedling at the early stage of growth and development. At the early stage of growth, nutrient uptake is possibly geared more towards the enhancement of seedlings to use the energy during the process of photosynthesis.

## CONCLUSION

In *Aquilaria malaccensis* trees, leaves from Lipis plantation showed higher growth or biomass values as compared to leaves from Sepang rubber agroecosystem regardless of treatment given (control or induced). Iron content in matured leaves at Sepang was higher than leaves at Lipis area. Iron content in control leaves at Sepang habitat (149.6 ± 0.7 ppm) showed significantly different concentration as compared to leaves at Lipis habitat (57 ± 0.6 ppm). However, the iron content in leaves of induced trees at both habitats was nearly similar. On the other hand, zinc contents in leaves of both control and induced trees at both habitats showed no significant difference, which were 59.6 ± 1.0 ppm and 67.6 ± 0.8 ppm, respectively. The uptake of Zinc nutrient was more in Sepang leaves although plant produced leaf with less weight. Leaves in Lipis plantation showed higher zinc uptake in induced trees whereas trees growing wild in the Sepang rubber agroecosystem showed less distinctive trend between control and induced trees.

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