

Characteristics of post mining soils in Indonesia and its remediation

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Chapter I. Introduction

1.1. Indonesia's tropical rain forest

Indonesia tropical rainforests (Figure 1-1; Figure 1-2) illustrate belong to the world's abundant repertory of terrestrial biodiversity, and play an important role to the global climate. From 2002 to 2019, Indonesia lost 9.59 million ha of humid rainforest, making up 37% of its total tree cover loss in the same time period. Total of humid rainforest in Indonesia decreased by 10% in this time period (Global Forest Watch, 2020). The large-scale oil palm and timber plantations together contributed two-fifths of nationwide deforestation, conversion of forests to grasslands, which comprised an average of one-fifth of national deforestation and small-scale agriculture and small-scale plantations also contributed one-fifth of nationwide forest loss and were the dominant drivers of loss outside the major islands of Indonesia (Austin et al. 2019). Although relatively small contributors to total deforestation, logging roads were responsible for a declining share of deforestation, while mining activities were responsible for an increasing share, over the period (Austin et al. 2019). Mining activity caused a significant intervention on environmental conditions. The biomass is the main supply for nutrients in tropical rainforests and not the forest soil as is established in temperate forests. Study has indicated that there is an ecological relationship between soil fertility and plant biodiversity in a primary tropical rainforest. The abundance of higher tree species tended to be found on sites with lower soil chemical fertility, which is the complete opposite with temperate forests (Nadeau and Sullivan 2015). In tropical forest, the material organic content in the soil is low while the processes of decomposition on the forest surface and plants nutrient absorb are fast (Whitmore 1998).

1.2. Mining activity and its impact to the degradation of land

Indonesia's forested regions contain significant mineral and energy resources, the extraction of which contributes to the fulfillment of society's needs for goods and services and makes a significant contribution to the national economy. Large amounts of minable resources have been and will continue to be extracted. The

main sources of mining products are coal, nickel, gold, bauxite and tin. Mining activities concessions in Indonesia, however, are many in the tropical rainforest area (Figure 1-3A). The methods used to conduct mining is a lot with an opencast mining system of surface mining (Figure 1-3B). It is generally accepted that the surface mining operations are destructive to the environment. The activities not only disturb and eliminate the existing vegetation, animals and soil but also destroy the original ecosystem (Unanaonwi and Amonum 2017). The negative impact of the loss of vegetation and land degradation will lead to severe soil erosion, loss of biodiversity, damage of wildlife habitats, degradation of the watershed area, deterioration of life and reduction of the option for development (Asuoha et al. 2019). Following the regulation, the Indonesian government applies a rule that all mining companies operating in Indonesia is required to carry out the rehabilitation of its post-mining land (Figure 1-4; Figure 1-5). To remediated post-mining land however is not easily. Opencast mining activities may impact to the soil properties and decreasing soil fertility. The pre- and post-mining activities influence the soil quality due to removal of vegetation and topsoil cover (Paramasivam and Anbazhagan 2020). The soils deficiency of nutrients contents like nitrogen, phosphorus, potassium, and calcium (Paramasivam and Anbazhagan 2020). Arbuscular mycorrhizal (AM) fungi form symbiotic associations with roots in most land plants. AM symbiosis provide benefits to host plants by improving nutrition and fitness, and it has also been associated with increased resistance to pathogen infection in several plant species (Campo et al 2020).

1.3. Oil extraction and its impact to the degradation of land

Indonesia belong to world's oil producing countries with produce 825.000 barrel per day in 2015 (Indonesia-Investments 2015). Many countries currently, are delving into the potential of renewable energy, the global importance of - and dependency on - oil cannot be denied, nor neglected. Fossil fuels will remain to be the most important sources of global primary energy, with oil accounting for 33 percent, coal for 28 percent and natural gas for 23 percent of the total (IMF April 2011). Renewable sources only constitute a fraction of the total global primary energy supply (primary energy includes fossil fuels - oil, coal and natural gas-,

nuclear energy and renewable energy geothermal, hydropower, solar and wind). Petroleum is one of the dominant energy sources to maintain the economic and social development of a country, petroleum has become one of the most important types of organic pollutant and is caused by the main leakage of underground storage tanks and accidental spills during transportation and disposal. In carrying out its activities, oil extraction produces a lot of waste which degrades soil quality. Oil extraction can impact local soil, water, and air, which in turn can influence community health (Johnston et al. 2019). Bioremediation as an alternative tool for restoration petroleum-contaminated soils was set forth, and focusing on the phytoremediation plants, petroleum-biodegradable microorganism are responsible for the biodegradation of petroleum.

1.4. Land remediation after mining activity

1.4.1. Species selection of tropical fast growing tree species

Plantation of tropical fast growing species are a strategy to remediate post mining in degraded tropical lands. Such plantation may help to reduce the negative impacts of degraded lands, and they may contribute to the long-term livelihood of forest communities around the post-mining. Selecting tropical fast growing trees is important to increase successful rate of remediation in degraded mining lands. *Gmelina arborea* (Linn) Roxb., *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes, *Samanea saman* (Jacq.) Merr and *Enterolobium cyclocarpum* (Jacq.) Griseb are tropical fast growing species intensively grown in industrial forest plantations in Indonesia. *Gmelina arborea* is used as a raw material for matches, charcoal, light, construction, plywood, particleboard, pulp, and paper (Soerianegara et al. 1993). *Falcataria moluccana*, native to Molluca, New Guinea, the Bismark Archipelago and the Solomon Islands. This species has been recognized as a multipurpose species because it can be used for pulp and paper, fuel wood, ornamental plantings, shade trees and as a nitrogen supplier to soil (Yuskianti and Shiraishi, 2010) and fast-growing wood species for energy production in Indonesia (Amirta et al 2016). *Samanea saman* is important species used in furniture and craftwork industries. This species has moderate wood density, attractive wood

colour, excellent workability properties, natural durability, shade trees (Obando and Moya, 2013). *Samanea saman* has a massive root system with an equally large canopy. Most roots of *S. saman* expand horizontally while many fibrous roots expand vertically (Haq et al. 2012). *Enterolobium cyclocarpum* is used for forage trees, fuelwood, pulp, and paper (Akkasaeng et al. 1989). These species are used commonly for the re-vegetation of post mining sites and they are now commonly used for national programs of revegetation in Indonesia. Rehabilitation in the tropical forests takes place across a wide variety of climatic conditions and edaphic (Van Breugel et al., 2011). The chemistry properties of post mining can be strongly influenced by the existence of vegetation or tree species. Before implementing remediation in degraded mining lands, it needs to know the early growth of selected tropical tree seedlings under post mining land (Figure 1-4; Figure 1-5).

1.4.2. The role of arbuscular mycorrhizal fungi (AMF) for rehabilitation of post opencast mining land

1.4.2.1. Mycorrhizas and plant nutrition

Soil at remediation land are generally low in nutrient, being acidic and highly phosphate-fixing due to Al and Fe complexes. P-deficiency is a major factor limiting plant growth. At seedling stage, plants require a high demand for phosphate. To overcome this, the current strategy in Indonesia is to apply lime to the soil to increase the pH and to apply commercial fertilizer to overcome the phosphate deficiency. In low soil fertility, mycorrhizal fungal inoculation has potential evidence to promote plant nutrient acquisition and water uptake to alleviate abiotic stresses, such as drought (Aggarwal et al. 2011) (Figure 1-8). Results from Wang et al. (2019), showed that the growth parameters of the seedlings (height, basal diameter, dry biomass) were significantly enhanced by mycorrhizal fungi, associated with root colonization rates, greater than 75%. Concentrations of chlorophyll and photosynthetic rates were also increased by mycorrhizal fungi, and phosphorus (P), and potassium (K) content in the three organs (leaf, stem, and root), and nitrogen (N) content in the leaf and stem of AM seedlings were significantly higher than in non-AM seedlings. Mycorrhizal dependency of the AM seedlings

was greater than 350%, and significantly correlated with the increased P and K content in all three organs and increased N content in the leaf and stem.

1.4.2.2. Mycorrhizas and their effect on soil aggregation

It has been known that AMF improves soil aggregation and stabilizing soil aggregates. Soil aggregation is essential to maintain soil physical properties and facilitate biogeochemical cycling. Most AMF produce hyphae in the soil. These hyphae can stabilize soil macro-aggregates. Hyphae of AMF are considered to be primary soil aggregators and there is a positive correlation between AMF hyphae and aggregate stability in ecosystems. Recent evidence suggests that glomalin (GRSP), a glycoprotein produced by AMF hyphae which has a cementing capacity to maintain soil particles together, is mainly involved in such aggregation (Borie et al. 2008). The beneficial roles of AMF in improving soil structure may be particularly significant for soil conservation, in particular remediation of disturbed reforestation sites such as those that are commonly encountered in the eroded soil of mining spoils.

1.4.2.3. Mycorrhizas and plant-water relations

Water deficit is one of the major limiting factors affecting seedling survival in some remediation of post mining land. The seedling stage is typically the most vulnerable stage of the life cycle to drought. It has been suggested that AMF can improve plant water relations (Aggarwal et al. 2011). Colonization by AMF can increase drought resistance in certain host plants, by directly increasing water uptake through the hyphal network to the plant, by improving the P-supply, or by affecting stomatal conductance. The role of AMF in helping plants to cope with drought stress is well established. It is widely believed that the inoculation of AMF provides tolerance to host plants against various stressful situations like heat, salinity, drought, metals, and extreme temperatures (Begum et al. 2019). AMF may both assist host plants in the up-regulation of tolerance mechanisms and prevent the down-regulation of key metabolic pathways. AMF, being natural root symbionts (Begum et al. 2019). A likely effect of hyphae of AMF in improving drought tolerance is by binding the

rhizosphere soil to the roots, so preventing the separation of roots from the soil during periods of low water. This ability of mycorrhizas to improve the tolerance of seedlings to drought is of profound importance in ecosystems which have been chosen for reforestation in Indonesia.

1.4.2.4. Mycorrhizas and their effect on other microorganism

The failure of seedling establishment in adverse reforestation zones may not always be caused by unfavourable physical and chemical soil conditions, but may also be due to an ineffective population of beneficial microbes. Most current rehabilitation practices ignore the AMF-plant community dynamics and thus there has been a limited success in establishing self-sustaining plant communities. Synergistic interactions between AMF and Rhizobium, resulting in better utilization of phosphorus, increased nitrogenase activity in nodules, and increased growth and nitrogen fixation in the leguminous tree seedlings are well documented (Abd-Alla et al. 2013). The AMF symbiosis can contribute to the energy requirements of nitrogen fixation through its capacity to mobilize greater amounts of phosphorus (Soumare et al. 2015).

1.5. Land remediation after oil extraction

Soil pollution by petroleum hydrocarbons and their adverse effects create widespread environmental problem (Adipah 2018). Total petroleum hydrocarbons in the presence of soil have a negative impact on human health and the development of plant growth. With the expanding of the soil contaminated by petroleum hydrocarbons effort have been made to remediate total petroleum hydrocarbons contamination in soil (Figure 1-6). The government of Indonesia has the role, that all petroleum contaminated land have to be remediated (Figure 1-7). There are various methods of remediating oil-contaminated soil such as bioremediation, soil vapor extraction, soil washing, thermal treatment, and chemical oxidation but the most common conventional method is excavation followed by landfilling or incineration (Dadrasnia et al. 2013). However, excavation and landfilling is costly and does not only destroy contaminants but also cause secondary pollution such as

the formation of volatile organic compounds (VOCs). A number of bioremediation technologies such as composting, land farming, bioventing, and bioreactor treatment have been used (Kumar et al. 2018). Phytoremediation or plant-enhanced bioremediation consists of using the symbiotic relationship between plant and microorganism, along with the soil amendments and proper management practices to remediate contaminated soils in situ (Truua et al. 2015). It is cost effective process where plants are used to remediate contaminated matrices such as soil, sediment, surface and groundwater (Melinda et al. 2013). Native and indigenous plant is more effective than any other exotic plant species for the phytoremediation of hydrocarbon-contaminated soil for ecological and economic reasons, as they may not require long-term maintenance and they are better adapted to the environment. The use of the Fenton reaction (Figure 1-9) processes has been applied for removing organic hydrocarbons in the field. Compared with other methods, the Fenton reaction has several advantages such as cheaper cost, reduced reaction times and energy consumption, nontoxic compounds, and simple operation and control. This method can be used as a pre-treatment method for the oily sludge and their environmental hazards (Farzadkia et al. 2014). Chemical oxidation of fenton's reaction is in principle a method that can be utilized for all organic fuel residues thus making it a potential all-purpose, multi-contaminant, in situ application for cases in which storage and distribution of different types of fuels have resulted in contamination of groundwater or soil (Talvenmäki et al. 2019).

1.6. The objectives of the study

The objectives of this study were (1) to clarify the effects of opencast bauxite mining on the chemical characteristics of soil and its effect on the plant growth, (2) to clarify chemical characteristics of gold mine tailings and its effect to the growth of plants under greenhouse conditions, (3) to determine the impact of opencast nickel mining on soil chemical properties and its effect on the growth of two fast-growing tropical tree species under greenhouse conditions, (4) to determine the effect of inoculation with two indigenous AM fungi on the growth of plants in the nursery and post-opencast bauxite mining field conditions, (5) to clarify the effect of Asteraceae plants on degradation of petroleum hydrocarbon in contaminated

soils. The families of tropical tree species used in this investigation were Fabaceae, Verbenaceae, and Asteraceae. The families of Fabaceae and Verbenaceae are important for reforestation and providing timber for Industrial Forest Plantation in Indonesia while Asteraceae produce several products such as lettuce, cooking oils, sweetening agents, sunflower seeds, coffee substitutes, and herbal drinking. The plant species used in this investigation were *Gmelina arborea* (Verbenaceae), *Samanea saman* (Fabaceae), *Falcataria moluccana* (Fabaceae), *Enterolobium cyclocarpum* (Fabaceae). Aster family were also used, including *Achillea filipendulina* Lam., *Anthemis tinctorial* (L.) J. Gay ex Guss., *Tagetes erecta* L., *Chrysanthemum coronarium* (L.) Cass. Ex Spach., *Calendula officinalis* L., *Zinnia elegans* Jacq., and *Callistephus chinensis* L., *Cosmos caudatus* Kunth, and *Tagetes* sp. Soil pH, total carbon (C), nitrogen (N), and available phosphorus (P) concentrations, cation exchange capacity (CEC), C/N ratio and exchangeable K, Na, Mg, Ca, Fe and Ni concentrations of post mining soil from bauxite, nickel and gold tailings were analyzed. *F. moluccana* and *S. saman* were grown for 15 weeks and their shoot heights, shoot and root dry weights were calculated. Two native AM fungi, *Rhizophagus clarus* and *Gigaspora decipiens*, were inoculated into seeds of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum*. The seeds were sown in post-bauxite mining soil and grown in the nursery for three months. The seedlings were transplanted into a post-opencast bauxite mining field and grown for 12 months. Initial soils with 40 and 90 g kg⁻¹ of total petroleum hydrocarbon (TPH) were prepared. There were three treatments: (1) no addition, (2) addition of FeCl₃ and nitrilotriacetic acid (NTA) solution, and (3) addition of FeCl₃ + NTA and the cultivation of nine Asteraceae plants.

Chapter II. Post bauxite mining land soil characteristics and its effects on the growth of *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes and *Albizia saman* (Jacq.) Merr.

2.1. Introduction

The tropical rainforests on the Indonesian Islands of the Riau Archipelago (Sumatera) vary greatly in their biodiversity but have soils that are generally poor in nutrients. In tropical rainforests, however the biomass is the major pool for nutrients, and not the forest soil content, as is found in temperate forests. While the material organic content of tropical soils is low overall, the decomposition processes on the forest floor and nutrient uptake are fast (Whitmore 1998). The organic carbon in tropical forests declined with depth, in the topsoil layer of East Kalimantan, Indonesia (Wenzel et al. 1998). Information on soil characteristics in tropical rain forests, especially in Indonesia, is still very limited. Knowing the characteristics of the soil in tropical rainforests is important, to take a strategic step in the process of rehabilitation of degraded land.

Forest land cover has been decreasing due to forest conversion into cropland, oil palm, rubber plantations, and opencast mining, in Indonesia (Van der Laan et al. 2018). Bauxite is important for alumina production and one of its main producers globally, via opencast mines, is Indonesia. The process of opencast mining consists of clearing vegetation, topsoil stripping, and mining for the minerals (Ghose and Majee 2000). This process affects the landscape of the local environment and its social well-being, as it causes tremendous physical, chemical, and biological damage to forests, impacting on their vegetation and animal biodiversity (Craig 2010). Further, the removal of the top layer of soil causes loss of structure and functionality, and a subsequent reduction in its biodiversity, resulting in socio-economic impacts (Lad and Samant 2015 ; Daws et al. 2013). A study provides a selection of 20 forest plant native species for increasing biodiversity and restoration of area disturbed by petroleum extraction activities in the Ecuadorian Amazon (Villacis et al. 2016).

The rehabilitation of post-mining land is required to repair damage to local environments. Various methods are employed to achieve this, such as landscape

reclamation, planting ground cover crops, the utilization of fast-growing plants, and remediation of water and soil contaminants. As soil pH declines, the supply of most plant nutrients decreases, while aluminum and a few micronutrients become more soluble and toxic to plants. The problems are particularly acute in humic tropical forests that have been highly weathered. The soil pH beneath dipterocarp forests and plantations in East Kalimantan, Indonesia were on average below 4 (Sim et al. 1992). According to Sanchez and Logan (1992) for example, one-third of the tropical forests, or 1.7 billion hectares, are acidic enough for soluble aluminum to be considered toxic for most crop plants. In tropical forests, deep tropical weathering, and decomposition of rocks have caused excessive solution and leaching of bases, leaving insoluble Fe and Al sesquioxides.

The rehabilitation of tropical forest ecosystems disturbed by bauxite mining is the aim of an increasing number of programs in many parts of the world, including Indonesia. However, bauxite mining is an impermanent activity that has long term negative effects on tropical forests (Lad and Samant 2015). During the process of opencast mining, all vegetation and topsoils are removed and the soil fertility in each horizon is shuffled and chemical, physical, and biological conditions rapidly deteriorate. The removal of vegetation and the disturbance of the soil profile reduces the nutrient pools available for seedling trees during mine site rehabilitation (Daws et al. 2013). Forest soil compaction and erosion increase surface flow, flooding, and droughts in natural dipterocarp forests and the forest plantation industry in East Kalimantan, Indonesia (Sim et al. 1992). The number of plant species and the amount of cover vegetation is thus found to be lower in post bauxite mining land than in that of natural forests.

Typically, bauxite is covered by a thick lateritic zone. This lateritic zone is completely removed to access the bauxite ore. Bauxite mining processes consist of blasting, removal of ore, and the movement of heavy vehicles that alter the soils physical and chemical properties (Lad and Samant 2015). The chemical properties of post bauxite mining soil's can be strongly influenced by the presence of vegetation and tree species. It is necessary to know the chemical characteristics of the soil in post opencast mining land before implementing rehabilitation programs.

Selecting fast-growing tropical trees is important to increase the success rate of rehabilitation in degraded mining lands. *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes and *Albizia saman* (Jacq.) Merr. are two tropical fast-growing species that are intensively grown to support rehabilitation programs of industrial forest plantations in Southeast Asia. *F. moluccana* is native to Molluca, New Guinea, the Bismark Archipelago, and the Solomon Islands, and has been recognized as a multipurpose species as it can be used for pulp and paper, fuelwood, ornamental plantings, as a shade tree, and as a nitrogen supplier to soils (Krisnawati et al. 2011). *A. saman* is an important species used in the furniture and craftwork industries and has moderate wood density, an attractive wood color, excellent workability properties, natural durability, is considered a shade tree, and is also useful for animal feed (Delgado et al. 2014). It is necessary to select plant species that adapt to degraded post opencast mining lands. The rehabilitation of post-mining lands starts with rebuilding the soil's biological capacity, mineral nutrient cycling, and the establishment of plant communities (Huang et al. 2012). Dissemination of successful rehabilitation activities on post-mining land requires new collaborative science and technology-driven innovations (Cross et al. 2017).

The purpose of this chapter was to clarify the effects of opencast bauxite mining on the chemical characteristics of soil and its effect on the plant growth of *F. moluccana* and *A. saman* under greenhouse conditions.

2.2. Materials and Methods

2.2.1. Study site and soil sampling

Soil was collected from both a natural Adinandra belukar forest, a secondary succession after cleared land exploitation of the primary lowland rain forest (Sim et al. 1992), and a post bauxite mining site, on Bintan Island, Sumatera, on 10 May 2011 (Figure 2-1). The samples were collected from 3 samplings points at the natural forest site of Adinandra belukar (0°52'21"N, 104°39'02"E) and 3 samplings point at the post-mining site (0°49'22"N, 104°39'61"E) (Figure 2-2). Surface litter, fine roots, and stones were scraped away before taking the soil samples, and soils were collected from each sector at a depth of 0–25 cm. Fifteen soil samples from 3 randomized sampling points of the natural forest and 15 soil samples from 3

randomized sampling points of the post-mining land were collected with a hand scoop, mixed, and placed in a clean plastic bag. A smaller portion of these homogenized samples (approximately 1000 g) was diced and ground, before laboratory analysis.

2.2.2. Soil chemical analysis

The soil samples were air-dried, passed through a < 2 mm sieve, and used for the analysis of pH (H₂O) and pH (KCl). The use of the two methodologies for the measurement of pH was to indicate the presence of exchangeable protons or ions in the soil. The pH (H₂O) shows the actual pH of the solution in the soil while pH (KCl) is a potential pH of soil indicating pH in the soil solution and soil bonding complex. In pH potential, H⁺ ions are measured more than the actual pH. Furthermore, pH (KCl) was compared with pH (H₂O). Available phosphate (P) (Truog 1930) was extracted with a 0.001 M sulfuric acid solution and analyzed using the ammonium molybdate method. Total carbon (TC) and total nitrogen (TN) were determined by a C: N analyzer (Sumigraph NC-220F, Tokyo). Exchangeable potassium, sodium, magnesium, and calcium were extracted with 1 M (pH 7) ammonium acetate solution and their concentrations were determined using an atomic absorption spectrophotometer (Hitachi model Z-5000 series Polarized Zeeman, Tokyo). The sample was extracted with 100 g L⁻¹ potassium chloride solution and the supernatant was used to determine the CEC using the semi-micro Schöllenger method.

2.2.3. Plant growth

Two tropical forest trees, *Falcataria moluccana* and, *Albizia saman*, were selected for this study. Seeds of both species were purchased from a local seed company, Solo, Central Java, Indonesia. The seeds were soaked in water at 80 °C for 2 min and were then pre-germinated in a plastic bag using zeolite as a growth medium. After the radicles appeared, they were selected for uniformity before sowing. Two hundred grams of each soil was placed into polyethylene pots (7.5 cm height × 5 cm diameter), and the pots were placed in a randomized block design. Seedlings

(three weeks old) were transplanted for each treatment. The experiment was conducted from July – October 2011, in the greenhouse at the Forest and Nature Conservation Research and Development Centre, Bogor, West Java (6°36"S, 106°45"E). The temperature varied between 26 to 35 °C, relative humidity was 80 to 90 %, and the photoperiod was about 12 h. The plants were grown for 15 weeks. De-ionized water was added as required to maintain the moisture content to field capacity, by slowly pouring water over the pot that contains the plant, until the water excesses come out from the bottom of the pot.

2.2.4. Harvest and analysis of plants

Shoot height, from one cm above the soil surface, was measured every 2 weeks, until 15 weeks after transplanting when plants were harvested and the shoots and roots were separated. The shoots were oven-dried at 70 °C for 72 h and the shoot dry weight was measured.

2.2.5. Statistical analysis

All experiments were laid out in a random block design with fifteen replicates. All the data were analyzed with student's t-test, using the Minitab package (Minitab, U.S.A.). When F values were significant, the least significant difference test (LSD) was used to compare the significant differences between treatment means.

2.3. Results

2.3.1. Soil chemical characteristics of the natural forest and post bauxite mining land

The soil pH (KCl) of the post-mining land was higher than that of the natural forests (Table 2-1), but there was no difference between the soils for pH (H₂O). Total nitrogen, carbon, and available P concentrations in the soils of the post-mining lands were lower than those in the natural forests and the C/N ratio was higher. The CEC was not different between the post-mining land and the natural forest. The exchangeable Ca, Mg, and Na concentrations of the post-mining land were lower

than those of the natural forest, and there was no difference in the exchangeable K, Fe, and Ni concentrations.

2.3.2. Growth of *Falcataria moluccana* and *Albizia saman* in natural forests and post-mining land

Shoot height of the *F. moluccana* grown in both the natural forest and post-mining soils increased from 2 to 15 weeks after planting (Figure 2-3A.); however, 2, 4, 13, and 15 weeks after planting, those on the post-mining soil were shorter compared with those in the natural forest soils. Shoot height of the *A. saman* seedlings in both the natural forest and post-mining soils also increased from 2 to 15 weeks after planting (Figure 2-3B); however, 8, 10, 13, and 15 weeks after planting, those on the post-mining soil were shorter compared with those in the natural forest soils.

The shoot and root dry weights, 15 weeks after transplanting, for the *F. moluccana* grown in the post-mining soils, were lower than those in the natural forest (Figure 2-4). There was no difference in the shoot and root dry weights for *A. saman* for the different soil treatments (Figure 2-5).

2.4. Discussion

2.4.1. Effect of opencast bauxite mining on soil chemical characteristics

Opencast bauxite mining decreased the soil fertility overall and the TN concentrations by 75 % (Table 2-1). The reduction was due to the removal of tree cover, litter, and topsoil by the processes used to create the opencast mines. The reduction was higher than the 53 % reduction reported for soil from an opencast coal mining site in India (Singh et al. 2012) and the 53–80% reduction in soil from an opencast coal mining site in the U.S.A. (Shrestha et al. 2011). The effect of opencast mining on soil fertility in tropical areas is higher than that in non-tropical areas. The reduction in TN was also higher than the 45 % reduction in farmland compared with forests (Hajabbasi et al. 1997) and the 36 % reduction after forest fires (Sitlhou et al. 2014). Deforestation by opencast mining overall affected soil fertility more severely than the other causes of deforestation.

The soil fertility of the post-mining soils in Bintan Island was very poor in comparison to the natural soil. The soil pH is important factor to support the growth of plants (Sena et al. 2018). The pH of the post-mining soils of the Bintan Island soils varied from 4.96 ± 0.05 for pH (H₂O) and 4.52 ± 0.09 for pH (KCl). The pH (KCl) was lower than that of the pH (H₂O). These results could indicate the presence of exchangeable protons or ions in the soil. The soil can hold cation exchanged of the plants. The pH (H₂O) and pH (KCl) of the post-mining soils of the Bintan Island, however, were higher compared to the average soil pH of 3.6 from the Adinandra belukar in Singapore (Sim et al. 1992), and the Acrisols beneath the dipterocarp forest and plantation in East Kalimantan, that had pH values from 3.70 to 3.75 (Wenzel et al 1998). Due to the soil pH of post-mining land of Bintan Island were higher compared to the Adinandra belukar in Singapore and the location of Bintan Island is geography adjacent to Singapore, thus reforestation of post bauxite mining land of Bintan Island may approachable by using a type of plant that grows in the Adinandra belukar in Singapore, such as *Adinandra Dumosa*, *Dillenia Suffruticosa*, and *Fragraea fragrans* (Sim et al. 1992). Reforestation may also approachable by the species plant from the dipterocarp forest. The total N of the post-mining of Bintan Island was 0.4 ± 0.2 (g kg⁻¹). It was lower than the total N of 0.8 (g kg⁻¹) of Adinandra belukar in Singapore, (Sim et al. 1992) and 1.5 ± 0.6 (mg/g) of N at heat forest on Gunung Keriong, Pahang, Malaysia (Chua et al. 1995). The species of nitrogen-fixing from leguminous trees could be used for reforestation of post bauxite mining land in Bintan Island with low of TN concentrations. The introduction of leguminous trees is able to form symbioses with nodulating N₂-fixing bacteria. The successful results using of nitrogen-fixing legume trees for the reclamation of areas degraded by mining activities in Brazil have been reported (Chaer et al. 2011). Soil physical characteristics can be different between natural soil and post-mining soil and can affect the growth of plants.

2.4.2. Reduction in carbon stock by bauxite mining

Total C concentrations of the post bauxite mining soils were lower than those of the natural forest soils. The removal of the topsoil from a post bauxite mining site and mixing it with the underlying soil considerably reduced the relative proportion of

organic carbon. The C concentration in the post bauxite mining soils was 7.0 ± 2.5 (g kg^{-1}) this range is included in the level of organic carbon in overburden was found to be 0.35 % to 0.85 % (Sheoran and Sheoran 2010). C is an important parameter for any soil, as it improves both the physical and chemical properties of the soil, and has several favorable effects on soil quality. The advantages and functions of natural forest ecosystems are, such as wildlife habitats, erosion and flood defenses, water regulator, carbon sequestration, protecting shallow water ecosystems such as coral reefs and seagrass beds and so forth (Wasis 2012). Therefore, the forest type ~~is~~ significantly affect soil Physico-chemical, of the soil microbial biomass carbon, nitrogen and phosphorous (Chandra et al. 2016). It could be applied to the organic compost to increase C concentration in post bauxite mining for the rehabilitation activities. The use of organic fertilizers of chicken manure increases organic C in the soil. It is better than that of cow manure, and organic letter compost on *Samanea saman* tree on Post mining land (Darma et al. 2017).

2.4.3. Growth of plant in post opencast mining land

Shoot dry weight of both *F. moluccana* and *A. saman* decreased in the post-mining soils, but the degree of the decrease was different between the two species (Figure 2-4; Figure. 2-5). *A. saman* seedlings were more tolerant than the *F. moluccana* in the post-mining soils after 15 weeks of growth under greenhouse conditions. The soil chemical characteristics, such as pH, are important factors to determine the growth of plants in soils post-mining (Sena et al 2018). *A. saman* adapts to a wide range of soil types and pH levels, has a massive root system that expands both horizontally and vertically (Haq et al 2012), can grow in soils that are free draining or those that have impeded drainage, can sometimes tolerate growth under waterlogged conditions, and finally, it can grow in slightly acidic to neutral pH (6.0-7.4) soils while tolerating pH values as high as 8.5 and as low as 4.7. These characteristics of *A. saman* indicate that it would be ideal to use for the reforestation of post bauxite mining land. Many studies of post-mining land in Africa have proven that pioneer leguminous trees show good adaptations and higher survival rates (Festin et al. 2019). After *F. moluccana* and *A. saman* are established and growing well in post bauxite mining lands, there will be enrichment plantation using

local species, including lots of local fast-growing trees species such as *Jacaranda obtusifolia*, *Macaranga bancana*, *Macaranga conifera*, and *Macaranga gigantean*. These species were identified as suitable in previous research if good quality and quantities of seeds can be sourced (Boo et al. 2014). The bauxite mining activities decreased the fertility of natural forest soil. The application of fertilizer to improve soil fertility of post opencast mining land and the inoculation of effective microorganisms to increase the initial growth of tree species are necessary for the rehabilitation of post bauxite mining land. This strategy of rehabilitation activity to develop bioindicators of hydro-geochemical and reconstruct root zones, which can enhance the recovery of the microorganism communities and ecological networks with rehabilitated plant communities in post bauxite mining land (Huang et al. 2012). *F. moluccana* and *A. saman* are widely used rehabilitation of disturbed land in Indonesia. The present result shows that *A. saman* is more suitable for the rehabilitation of post bauxite mining land. Growth response of plants in post opencast mining land should also be investigated under field conditions.

Chapter III. Chemical characteristic of forest soil and gold mine tailings and their effect to the plant growth of two leguminous trees

3.1. Introduction

Indonesia is one of the gold producing countries that belongs to the top 10 gold producing countries in the world. Production in the archipelago nation, with the number seven on the list of top global producers. In 2018, Indonesia gold mine production was reported 190 tons (Global Investor 2019).

One technique used in gold mining is underground mine which is referred as the processes and techniques to extract gold from the ground besides being recovered as a by-product of refining the ores of other metals (Aweng et al. 2013). Mining activities of gold however, produce large quantities of waste materials such as gold mine tailings. These are the materials left over after the process of separating the valuable fraction from the uneconomic fraction of mining ore. Tailings are the components of the primary mineral-bearing rock left after the extraction of minerals like gold, copper, and silver (Taberima et al. 2010). Mining processed residues such as gold mine tailings, may dump on forest land and occupy a large land area. The impacts that might occur as a result of poor processing of tailings is a disruption of natural ecosystem as indicated by a decline in quality and productivity of the environment due to changes in soil morphological, physical, chemical, and biological properties. The current global gold rush, driven by increasing consumption in developing countries and uncertainly in financial markets is an increasing threat for tropical forests (Alvarez et al. 2015).

On gold-mine tailings, certain extreme soil conditions may occur that prevent tree growth, referring particularly to physical, chemical properties, and extreme lack of certain nutrients for tree growth. The understanding of soil degradation and the tolerance of native tree species against the extreme conditions of mined area is still incomplete (Roman et al. 2015). It is still unclear how the relative importance of soil conditions influences species growth under varying degraded gold mine tailings. Soil is a vital natural resource, constituting a critical controlling component during the early stage of tropical forest ecosystem development (Mushia et al. 2016). Gold mining activities are invariably associated

with the removal of fertile soil organic layer enriched with vegetation cover and hence has environmental consequences. Important step of the activities for reclamation in post gold mining is revegetation, however revegetation is not easy. Since gold mine tailings area of post gold mine has limitation as medium for planting a successful revegetation would be quite challenging (Ekyastuti et al. 2016). These factors slow down or prevent the revegetation process and consequent stabilization of mine tailings (Aweng et al. 2013).

In order to increase the success of the re-vegetation of gold mine tailings, in addition to knowing the consideration of soil characteristics, the selection of types of tolerant trees species is indispensable. *Falcataria moluccana* is one of the most important pioneer multipurpose tropical tree species in Indonesia (Krisnawati et al. 2011). *Falcataria moluccana* is native to Indonesia, Papua New Guinea, Solomon Islands and Australia (Soerianegara et al. 1993). It is one of the tree species preferred for industrial forest plantations in Indonesia because of its very fast growth, its ability to grow on a variety soils. Its does not require fertile soil; it can grow well on dry soils, damp soils and even on salty to acid soils as long as drainage is sufficient. *Albizia saman* is a large leguminous tree found in the tropical forests and which is well known as a source of a wide range of useful products (Skerman et al. 1998). The ripe pods can be used as an edible pulp, or they can be dried and ground into a meal for animal feed, and the timber is used in furniture manufacture. *Albiza saman* is also valuable as a shade tree in pastures, where it stimulates the growth of grass. *Albizia saman* was the only tropical tree species in the study to perform close to plantation standards (Love et al. 2009).

The purpose of this chapter was to clarify chemical characteristics of gold mine tailings and its effect to the growth of *F. moluccana* and *A. saman* under greenhouse conditions.

3.2. Materials and Methods

3.2.1. Forest soil and gold mine tailings samples collection

Forest soil and gold mine tailings samples were collected from the rhizosphere of natural forest and gold mine tailings dumps in a gold mining at Pongkor, Bogor, West Java, Indonesia. The samples were collected in three sites of natural forest (6°39'06"S, 106°21'31"E; 6°39'25"S, 106°22'01"E ; 6°38'69"S, 106°23'21"E) and three sites of gold mine tailings dumps (6°39'56"S, 106°33'31"E; 6°38'40"S, 106°34'15"E; 6°38'40"S, 106°21'31"E) to characterize the chemical properties. Before taking the forest soil and gold mine tailings samples, surface litter, fine roots and stones were scraped away and samples were collected from each of this sectors at a depth of 0 – 25 cm. The samples were collected by hand scope and intimately mixed and placed in a clean and seal plastic bag. Five soil samples were collected from each site. A smaller portion of these homogenized samples (approximately 1000 kg) were drawn and ground, before laboratory analyzed.

3.2.2. Forest soil and gold mine tailings chemical analysis

Tailing samples from gold mine tailings dumps and forest soil from natural forest were air-dried and passed through a < 2 mm sieve. The passed dried forest soil and gold mine tailings was used for analysis of pH (H₂O) and pH (KCl). Available phosphate (P) (Truog 1930) was extracted with 0.001 M sulfuric acid solutions and analyzed by the ammonium molybdate method (Olsen and Sommers 1982). TC and TN were determined by a C:N analyzer (Sumigraph NC-220F, Tokyo). Exchangeable potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) were extracted with 1 M (pH 7) ammonium acetate solution and their concentrations were determined using an atomic absorption spectrophotometer (Hitachi model Z-5000 series Polarized Zeeman, Tokyo). After removing excess NH₄⁺, the sample was extracted with 100 g L⁻¹ KCl solution and the supernatant was used to determine CEC using the semi-micro Schöllenger method. Base saturation (BS) was calculated by dividing the sum of exchangeable cations (K, Na, Mg, Ca) by CEC and multiplying the result by 100%.

3.2.3. Tree growth

Two tropical leguminous tree seedling used were *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes and *Albizia saman* (Jacq.) Merr. Seed of both species were purchased from a local seed company, Solo, Central Java, Indonesia. The seeds soaked in water at 80°C for two minutes. Seeds of these two trees species were pregerminated on plastic bag container using zeolite as growth medium. After the radicles appeared, they were selected for uniformity before sowing. Two hundreds gram of each soil sample was placed into polyethylene pot (7.5 cm height x 5 cm diameter).

30 pots from 6 treatments (3 forest soils and 3 gold mine tailings) with 5 replications were placed in the randomized block design. Two different trees were transplanted for each treatment and replicate to get a total of 60 pots. The experiment was conducted in the greenhouse at the Forest Research and Development Centre, Bogor, West Java (6°36' S, 106°45' E). Temperature varied between 26 to 35°C, relative humidity was 80 to 90% and the photoperiod was about 12 h. The seedlings were grown for 15 weeks. De-ionized water was added as required to maintain moisture content to the field capacity.

3.2.4. Harvest and tree analysis

The tree seedlings were harvested 15 weeks after transplanting. Some growth parameters were measured. Shoot height at one cm from the soil surface were measured every two weeks until 15 weeks after transplanting. After 15 weeks, shoot and root dry weight were harvested and recorded. Shoots and roots were then separated. Shoots were oven-dried at 70°C for 72 h before dry weighing.

3.2.5. Statistical analysis

All experiments were laid out in a Completely Randomized Design with 15 replicates. All the data were analysis by using t-student analysis, using the package of minitab (Minitab, U.S.A). When F value were significant, the least significant different (LSD) was calculated to compare significant differences between treatment means.

3.3. Result

3.3.1. Soil chemical characteristics of forest soil and gold mine tailings

Total N, carbon and available P of gold mine tailings were lower than that of forest soil. CEC, Mg, K and Fe of gold mine tailings were lower than that of forest soil. C/N ratio of gold mine tailings were higher than that of forest soil. Soil chemical characteristics of pH (KCl), pH (H₂O), Ca and Na of gold mine tailings were higher than that of forest soil. There was no difference in Ni between forest soil and tailings on gold mining (Table 3-1)

3.3.2. Tree growth

Shoot height rate of *F. moluccana* seedling on 8, 10, 13 and 15 weeks after planting was lower on gold mine tailing than that of forest soil (Figure 3-1). There was no difference in shoot height between *F. moluccana* seedling grown on forest soil and gold mine tailings on 2, 4 and 6 weeks after planting. Shoot height rate of *A. saman* grown on gold mine tailings was higher 6, 8, and 10 weeks after planting than that of forest soil. There was no difference in shoot height between *A. saman* seedling grown on forest soil and gold mine tailings on 2, 4, 13 and 15 weeks after planting. Root dry weight of *A. saman* grown on gold mine tailings were higher than that of forest soil (Figure 3-2) while shoot dry weight of *A. saman* grown on gold mine tailings were tended to have higher than that on forest soil. Shoot and root dry weight of *F. moluccana* on gold mine tailings were lower than that of forest soil on gold mining (Figure 3-3).

3.4. Discussion

3.4.1. Forest soil and gold mine tailings chemical characteristics

The process of gold production in gold mining, produces many tailings that have lower fertility than that in forest soil. Total N (TN), and available P concentration in gold mine tailings were lower than that in original forest soil before mining. It was decreased by 91.3%, and 35.8% respectively. The reduction of soil fertility was due to the removal of soil, plant material, and litter that was resulted from the

processes of gold production. Total N and available P concentration of gold mine tailing were lower than that total N (0.429 g kg^{-1}) and P (552 mg kg^{-1}) of lead/zinc mine tailings in southern China (Shu et al. 2005), while total N of gold mine tailings similar to total N (0.36 g kg^{-1}) of gold mine tailings in Malaysia. Available P, however lower than that P (0.76 g kg^{-1}) of gold mine tailings in Malaysia (Tariq et al. 2016). On tailing, N is a major limiting nutrient and regular addition of fertilizer. N may be required to maintain healthy growth and persistence of tree seedlings (Sheoran and Sheoran 2010). An alternative approach might be to introduce legumes and other nitrogen-fixing species. Nitrogen fixing species have a dramatic effect on soil fertility through production of readily decomposable nutrient rich litter and turnover of fine roots and nodules. Also, native legume trees are more efficient in bringing out differences in soil properties than exotice legumes in the short term. Available P in gold mine tailings was decreased. After gold mining, the soil loses structure and fertility through the increased proportion of sand (lacking silt and clay particles) and deficiency in the organic matter content and CEC (Binkley and Fisher 2013). This causes low water and nutrient holding capacity, thereby decreasing soil fertility to levels insufficient to support normal tree growth. The loss and deficiency of soil phosphorus (P) and bases generally constrain biomass production; however, high productivity on nutrient-deficient soils of tropical forests is to be maintained by plant and microbes adaptation to an acidic soil environment (Fujii 2014).

Total C concentration in forest soil was higher almost three times. Total C in gold mine tailings decreased by 75% compared to forest soil. The gold production process that produces gold mine tailings, affects the loss of soil organic matter (SOM), including organic carbon. This problem has serious implications because of the role of SOM in fertility, water holding capacity and overall quality of soil used for site reclamation. Soils from natural ecosystem may have high carbon stock (Lal 2005). The high value of C concentration in forest soil is supplied by the amount of vegetation and fauna in the forest (Wasis 2012). It has been suggested that the soil C stock may comprise as much as 50% of the terrestrial C stock in the tropical rainforest (Dixon et al 1994). While carbon storage in above ground biomass of tropical forests is commonly measured (Jepsen 2006), this is not the case for other components, such as roots, understory vegetation, coarse woody

debris, fine litter and soil. All these components can play an important role in the carbon storage and cycling in tropical forest ecosystems.

Soil pH in forest soil was very low compared with soil pH in gold mine tailings of gold mining. A very drastic change in pH is influenced by the soil factor and the living components, such as fungi, which has symbiotic relationships with plant roots helping in nutrient absorption from the soil. In tropical forest ecosystems, a paradoxical relationship is commonly observed between massive biomass production and low pH (Fujii 2014). Soil acidification driven by plants and microbes does not simply mean “soil degradation” in tropical forests. Rather, this process reflects mineral weathering induced by plants and microbes and their acquisition of soil nutrients. The high biomass production by tropical trees is supported by the adaptations of plants and microbes to an acidic soil environment.

3.4.2. Growth of tree on gold mine tailings

Tree growth of *F. moluccana* seedlings was extremely lower in gold mine tailings than that in forest soil (Figure 3-1; Figure 3-3). Tree growth rate of *A. saman* grown on gold mine tailing, however was tend to have higher than that of forest soil even though there is no significant difference (Figure 3-1; and Figure 3-2). *A. saman* seedlings were more tolerant than *F. moluccana* in the gold mine tailings after 15 weeks of growth in the green house. *A. saman* has an indication to be ideal plant for being used on reclamation of gold mine tailings. The soil from gold mine tailings was characterized as having low levels of fertility sufficient to negatively affect tree growth. Young et al. (2015), reported that low levels of organic amendments improved soil fertility and plant cover on old mine tailings. This means that organic matters provide a source of soil biota including bacteria, fungi as well as invertebrates capable of mineralizing the organic matter into plant available nutrients. Furthermore, other studies have reported that addition of compost and microbes not only increased soil fertility and plant biomass, but also reduced the concentration of trace elements in plant species growing on metal-contaminated mine soils (Martinez et al 2014).

The soil chemical characteristic such as total N (TN), carbon and available P are very low on gold mine tailings .These are the factors that interfere with restrict

to the growth of reclamation plants in the area of gold mine tailings. The use of organic fertilizer such as chicken manure, cow manure or litter compost may increase the success of plant growth for land reclamation of gold mine tailings. Chicken manure is the best organics fertilizer to increase the growth of *A. Saman* on post coal mining land in Indonesia that has very low of total of N, C and available P in the soil (Darma et al. 2017).

Management of soils and lands subjected to human perturbation is crucial to post gold mining rehabilitation of the impacted area (Stahl et al. 2002). Because soils are a fundamental component of tropical forest ecosystems from which most organism obtain essential materials such as nutrients and energy, as well as habitat, successful restoration of a disturbed area is highly dependent on maintenance of soil quality. Therefore, management practices that minimize detrimental impacts of human activities to the soil resource can prevent further site degradation and facilitate site rehabilitation. Alternatively, it is advisable to use a clay cover as barrier of air and water infiltrations. Undisturbed top soil is recommended to be used for clay cover, and this also serves as a fertilizer source for revegetation (Changul et al. 2010).

Native grass species should be sown early to stabilize the top soil surface and to protech soil erosion. In addition, recommended practices/ managements for the company include the following: (i) Monitoring program of surface, deep well and shallow well waters should be performed every 6 months; (ii) pH plays a very important role for leaching of metals. Thus, it is strongly recommended to measure pH in all monitoring programs. Low soil fertility, species slow growth rates, and traces of heavy metal in plant tissues indicate that remediation and rehabilitation in areas degraded by gold mining can be very challenging.

Chapter IV. The impact of nickel mining on soil properties and growth of two fast-growing tropical trees species

4.1. Introduction

Nickel is one of the most important mining products in the world. In Indonesia, nickel is produced from opencast mines. Opencast nickel mining is an intensive process that has a significant impact on tropical rainforests, affecting both indigenous vegetation and soil fertility. Mining activity belongs to the land exploitation with consequent loss of ecological function and services (Mentis 2020). It results in wide environmental degradation of the mined area and tends to destroy terrestrial ecosystems. Furthermore, it results in the loss of structure and function of soil due to the removal of the top layer of soil, with subsequent reductions in biodiversity and socio-economic impacts (Lad and Samant 2015).

To reduce damage caused by nickel mining, land rehabilitation activities are required. Rehabilitation land after mining activity to a previous forested land condition can guarantee the services of these areas for economic, and ecosystem purposes (Metsaranta et al. 2018). Land should become a productive forested area for the sustainability of social authorization (Moffat et al. 2016). However, this is not easy. Revegetation of forests need time and take decades (Macdonald et al. 2015). Forests are the ecosystems that consist of community, interacting as a system between living organisms and the nonliving components of the environment. They have undergone successional changes, in many ways, which take years or decades (Taylor and Chen 2011). Returning disturbed land to become a forested area is required to guarantee the continuation of economic and ecosystem services to the environment.

Nickel mining mostly belongs to the geological of serpentinite regions. These are found contaminated with a tremendous amount of trace metals which include Cr, Ni, and associated metals (Mg, Pb, Co, Zn, etc.) with other elements (Kumar and Maiti 2013). Nickel mining also causes drastic changes to the physical and chemical characteristics of the land (Prasad et al. 2016). Mining activities can cause the release of toxic metals to the environment; damage to heritage; pollution; and acid mine drainage. In soils serpentinite regions, this condition results

unfavorable for most plants and habitat development that house certain plant biodiversity and communities with many endemic species (Chiarucci and Baker 2007).

One rehabilitation strategy for degraded tropical lands is to plant fast-growing tropical trees. Such plantations may help to reduce the negative impacts of degraded lands and may contribute to the long-term livelihood of forest communities following mining. It is important to select fast-growing tropical trees species for increasing the success rate of rehabilitation. *Falcataria moluccana*, also known as batay, is one of the most important fast-growing multipurpose tree species in Indonesia. It is intended for industrial forest plantations because *F. moluccana* plants are included in fast-growing species, high ability to grow on a different type of soil condition, favorable silvicultural characteristics, and marketable quality of wood for forestry industries (Krisnawati et al. 2011). This species can be used for pulp and paper, fuelwood, shade trees, and as a nitrogen supplier to soil (Yuskianti and Shiraishi 2010). Moreover, *Falcataria moluccana* plays an important role in both commercial and traditional farming systems that commonly called as huma in Indonesia. The plant has been adopted and cultivated by the village people, such as integrated into the development of traditional agroforestry in huma (Iskandar et al. 2017). *Albizia saman* (*Fabaceae*), with the preferred common name as rain tree, is originally from Northern South America and has become naturalized in the tropics, grows in a wide range of climatic conditions, best in the lowlands from sea level to 300 m with rainfall 600-3000 mm, and mainly use on agroforestry system for silvopasture and crop shade (Staples and Elevitch 2006). *Albizia saman* is an important species, a multi-purpose tree with potentialities as an alternative feed for animals (Delgado et al. 2014). It also produces variation in breast diameter, attractive wood color, and physical properties of wood, such as green density and volume shrinkage (Obando and Moya 2013). *Albizia saman* has a massive root system with an equally large canopy. Most roots of *A. saman* expand horizontally, but many fibrous roots expand vertically (Haq et al. 2012). Both species belongs to the nitrogen- fixing trees commonly used for the revegetation of post-mining land and for national programs of revegetation in Indonesia. The species of nitrogen-fixing trees are able to form

symbioses with nodulating nitrogen-fixing bacteria. Rehabilitation of post-mining land takes place across a wide variety of climatic and edaphic conditions (Van Breugel et al. 2011). For example, soil chemical properties of post-nickel mining sites can be strongly influenced by the presence of vegetation or tree species. Before implementing rehabilitation in degraded mining lands, it is necessary to select tropical tree seedlings that are adapted to post-mine soil.

The objective of this chapter was to determine the impact of opencast nickel mining on soil chemical properties and, subsequently, its effect on the growth of two fast-growing tropical tree species, *F. Moluccana*, and *A. saman*, under greenhouse conditions.

4.2. Materials and methods

4.2.1. Soil sampling

Soil samples were collected from the top layer at PT Vale Indonesia, a nickel mining site in Sorowako, East Luwu, South Sulawesi, Indonesia (Figure 4-1). PT Vale Indonesia, previously named PT International Nickel Indonesia (INCO) was founded in 1968. The company, currently has mining concessions belong to Wallace's line of almost 120.00 hectares, most of which are still in the form of natural forests. The samples were collected from 3 natural forest sites (2°34'06"S, 121°20'52"E; 2°34'20"S, 121°25'03"E; and 2°34'20"S, 121°25'03"E) and 3 post-nickel mining sites (2°34'26"S, 121°21'37"E; 2°35'19"S, 121°22'30"E; and 2°31'36"S, 121°29'47"E), to characterize soil properties. Litter, roots, and stones, were scraped away from the surface before soil samples were taken. The samples were collected using a hand scope and mixed thoroughly before being placed in a clean and seal plastic bag. Five soil samples were collected from each site at a depth of 0 – 25 cm. A small sub-sample was taken and ground for chemical analysis. The remaining soil was kept for the plant growth experiment.

4.2.2. Analysis of soil properties

The soil was air-dried and passed through a < 2 mm sieve. Soil pH was analyzed two ways: using H₂O and using KCl. Available phosphorus (P) (Truog 1930) was

extracted with 0.001 M sulfuric acid and analyzed using the ammonium molybdate method (Olsen and Sommers 1982). Total carbon (TC) and total nitrogen (TN) were analyzed using a C: N analyzer (Sumigraph NC-220F, Tokyo). Exchangeable potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) were extracted with 1 M (pH 7) ammonium acetate analyzed using an atomic absorption spectrophotometer (Hitachi model Z-5000 series Polarized Zeeman, Tokyo). To determine CEC, excess NH_4^+ was removed, and an extraction was performed with 100 g L^{-1} KCl. The supernatant was analyzed using the semi-micro Schöllenger method.

4.2.3. Growth of two fast-growing tropical tree species

Two fast-growing tropical tree species, *F. moluccana* and *A. Saman*, were selected for this study. Seeds of both tree species were purchased from a local company, Central Java, Indonesia. The seeds were soaked in water at $80 \text{ }^\circ\text{C}$ for 2 min. They were then pre-germinated in plastic containers using zeolite as a germination medium. After radicle growth, individual plants were selected for sowing based on uniformity. Trees were grown in polyethylene pots (height: 7.5 cm, diameter: 5 cm) containing 200 g of soil. Each pot contained soil from a different soil sample, resulting in 30 pots, 15 with natural forest soil, and 15 with post-mine soil. Two tree seedlings were transplanted for each soil sample, resulting in a total of 60 pots. Pots were positioned in a greenhouse in a randomized block design. The greenhouse was located in the Forest Microbiology Laboratory, Research and Development Agency (FORDA), the Ministry of Environment and Forestry, Bogor, West Java, Indonesia ($6^\circ 36'' \text{ S}$, $106^\circ 45'' \text{ E}$). The temperature varied between 25 and $37 \text{ }^\circ\text{C}$, relative humidity was $80\% - 90\%$, and the photoperiod was approximately 12 h. The plants were grown for 15 weeks, and watering by deionized water was applied to maintain a moisture content similar to field capacity.

4.2.4. Harvest

Shoot height, measured 1 cm from the soil surface in the pot, was determined every 2 - 3 weeks. After 15 weeks, the shoots were harvested and oven-dried at $70 \text{ }^\circ\text{C}$ for 72 h before dry weight was recorded.

4.2.5. Statistical analysis

Data on laboratory tests of soil chemical properties and plant growth were analyzed using a statistical test, student's T-tests at 95% confidence interval ($P < 0.05$) in Minitab (Minitab Inc. USA). When the F value was significant, the least significant difference (LSD) was calculated to compare treatment means.

4.3. Results and Discussion

4.3.1. Impact of nickel mining on soil chemical properties

We found that the chemical properties of post-nickel mine soil differed significantly from nearby natural forest soil (Table 4-1). Total N, TC, available P, CEC, exchangeable Ca, and Na were lower in post-nickel mine soil than natural forest soil. Conversely, soil pH and C/N ratio were higher in post-nickel mine soil than natural forest soil. There were no differences in Mg, K, Fe, and Ni between natural forest soil and post-nickel mine soil.

Opencast nickel mining activities have an impact on soil fertility. In opencast mining, rock or minerals are extracted from an open pit or burrow. Topsoil and vegetation are seriously damaged during opencast nickel mining, thus decreasing soil fertility. Nickel ultramafic soils are commonly known as serpentines in the botanical and ecological literature (Chiarucci and Baker 2007). The serpentized and ultramafic soil/rock are distinguished by high levels concentration of heavy metals and unbalanced Ca/Mg ratio (Kumar and Maiti 2013), poor plant nutrient content such as N, P, K (Chiarucci and Baker 2007). Nitrogen and phosphorous are the most important nutrients for soil productivity and plant development. It significantly enhances plant growth and productivity, chlorophyll and carotene contents, and promotes root morphology (Razaq et al. 2017). Most studies have demonstrated the influence of nitrogen enrichment on plant communities. Soils are known to have heterogeneous physical, chemical, and biological properties. Soil heterogeneity is closely related to nitrogen enrichment to determine plant growth and nutrient status (Palacios et al. 2012). The availability of N in the soil directly influences a wide range of ecological processes, both above

and below ground, at the physiological, community, environment, ecosystem services, and global levels (Frank and Groffman 2009). In our study, we found that post-nickel mine soil had 98% less TN than a nearby natural forest soil. This indicates a greater decline in TN compared with gold mine tailings in Indonesia (91.3%) (Table 3-1), an opencast bauxite mine in Bintan Island, Indonesia (75% reduction) (Table 2-1.) an opencast coal mine in India (53% reduction in TN) (Singh et al. 2012) and the USA (53%-80% reduction (Shrestha and Lal 2011).

Phosphorus is another essential plant macronutrient. In the present study, the available P of post-nickel mine soil was $11.00 \pm 0.02 \text{ mg P}_2\text{O}_5/\text{kg}^{-1}$, which was lower than that in natural forest soil ($12.30 \pm 0.02 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$). Soil phosphorus is the elements considered important in determining the biodiversity and biomass of natural ecosystems (Cramer 2010). Production of many ecosystems especially in subtropical and tropical regions is strongly considered to be P rather than N limited (Vitousek et al. 2010). Recent literature indicates that in tropical forests, a large fraction of P found as organic and microbial P in the soil; plant adaptations to absorb organic P, including the phosphatase enzymes. Plants also cope with low P availability in the soil through enhances in P use-efficiency resulting from increased retention time of P in biomass and decreased tissue P concentration (Dalling et al. 2016).

The impact of surface mine activity involves drastic disturbances to the ecosystem and soil properties including the reduction of soil organic material (SOM) and organic carbon (Liu et al. 2016). Soil organic matter is lost as a result of the initial stripping of the soil from the site. Further losses occur while the soil is stored in stockpiles during replacement to the reclaimed site. This has serious implications because SOM plays an important role such as in soil fertility and water holding capacity. Soil is the primary store of terrestrial carbon (Tibbett 2010). Topsoil management plays an important role that rehabilitation of post-mining land lead to prevent carbon losses. Soils surface after reclamation of post-coal mines in Wyoming are sequestering C at a rapid rate. For example, soil organic C content at one reclaimed mine site near Hanna, WY increased from 10.9 g C kg^{-1} in 1983 to 20.5 g C kg^{-1} in 2002 (Stahl et al. 2003). Carbon is an important soil parameter, it improves soil physical and chemical properties and overall soil quality. Soil carbon

exists in various forms that are functionally different and have contrasting residence times. Removal of topsoil from mining sites and subsequent replacement and mixing with underlying soil considerably reduces the concentration of soil organic C. In the current study, we found that TC was 93% lower in post-nickel mine soil compared to natural forest soil, with concentrations of $2.90 \pm 0.05 \text{ g kg}^{-1}$ and $40.20 \pm 0.25 \text{ g kg}^{-1}$, respectively. This decline in TC is greater than that of oil palm plantations, in which TC can decline by 42% (Rahman et al. 2018).

Soil pH was higher in post-mine soil compared with natural forest soil, by 26% for the H₂O method and by 35% for the KCl method. This happens because of the loss of vegetation cover on the top layer of soil on post-mining land. Most post-mine land is categorized as dry land which contains metals such as Mg, Na, K, and Ca which are very high soil pH of 9.0 (Wasis and Andika 2017). In their natural forest environment, however, soil chemicals such as Mg, Na, Ca, K, and other chemical characteristics produced from the decomposition process of organic soil material will be absorbed by the plants. It results in the conservation and efficiency of the nutrient with a closed ecological nutrient cycle (Barot et al. 2007; Wasis et al. 2018). The higher pH of post-mine soil could support mining rehabilitation activities. Soil pH is influenced by various soil biological, chemical, and physical properties that affect the growth of plants and biomass yield (Neina 2019). For example, many N and C mineralization processes occur at a pH between 6.5 and 8. The application of dolomite may be considered to increase soil pH even further. It can also increase many other soil nutrients, including Mg (Pawlikowski and Kozi n 2019).

Soil CEC is a major soil chemical property. It reflects the surface properties of soil colloids, and the retention and supply proportions of soil fertilizer. CEC is a key indicator for evaluating soil fertility, plant growth, and pollutants partition and transport in soils. It is also an important parameter that influences the adsorption of heavy metals and organic pollutants in soils (Yunan et al. 2018). We found that CEC in the present study was 62% lower in post-nickel mining, in comparison with natural forest soil. The decrease in CEC is greater compared to post bauxite mining (Table 2-1) and is lower compared to gold tailings (Table 3-1), in which CEC can decrease by 30% and 76% respectively. Some of the important micronutrients that

are essential for plant growth are Ni and Fe. The micronutrient is available in the soil due to the continuous weathering of minerals mixed with primary minerals. Nickel contributes to the nitrogen fixation in legume plants and is the component of the urease enzyme which brings about hydrolysis of urea (Seregin et al. 2006), while Fe is a major micronutrient for almost all living organisms which plays important role in metabolic processes such as photosynthesis, DNA synthesis, and respiration. Furthermore, many metabolic pathways are stimulated by Fe, and it is a prosthetic group constituent of many enzymes (Rout and Sahoo 2015). In high-level concentration, however, Fe is toxic. It can act catalytically through the Fenton reaction to generate hydroxyl radicals, which can destroy proteins, lipids, and DNA. Consequently, plants must respond to Fe stress because of both Fe deficiency and Fe overload (Rout and Sahoo 2015). In the current study, we found that Fe was 1707% tend to higher in post-nickel mine soil compared to natural forest soil, with concentrations of $38.30 \pm 18.73 \text{ mg kg}^{-1}$ and $2.12 \pm 0.09 \text{ mg kg}^{-1}$, respectively.

4.3.2. Growth of fast-growing tropical tree species

The shoot height of *F. moluccana* seedlings grown in both the natural forest soil and post-nickel mine soil increased from 2 to 15 weeks after planting (Figure 4-2). In comparison with natural forest soil, shoot height at 10, 13, and 15 weeks after planting was significantly lower in post-nickel mine soil. No significant difference in shoot height was showed between natural forest and post-nickel mine soil at 2, 4, 6, and 8 weeks after planting. The shoot dry weight of *F. moluccana* grown in post-nickel mine soil was significantly ($P < 0.05$) lower than that of natural forest soil (Figure 4-4). Root dry weight of *F. moluccana* grown in natural forest soil was generally higher in comparison with post-nickel mine soil without statistically significance. The shoot height of *A. saman* seedlings of natural forest and post-nickel mine soil increased from 2 to 15 weeks after planting (Figure 4-3). Shoot height 15 weeks after planting was significantly lower in the post-nickel mine soil than in the natural forest soil. Shoot dry weight in natural forest soil was generally higher than that in post-nickel mine soil (Figure 4-5), while root dry weight in post-nickel mine soil was generally higher than in natural forest soil without statistically significance.

The rehabilitation of land after nickel mining is a mandatory activity for all mining companies in Indonesia. One rehabilitation approach is to plant fast-growing tropical leguminous trees that have a high level of adaptation and survival on post-nickel mining land and improve the fertility of the soil. Our results, as shown in figure 4.5, suggest that *A. saman* is more tolerant to growth on post-nickel mining land than *F. moluccana*. *Albizia saman* is a fast-growing tropical leguminous tree that is highly adapted to various types of soil with a wide pH range and poor drainage (Haq et al. 2012). Planting leguminous trees that can grow on post-nickel mining land can improve the ability of the soil to retain water. Large pores in the surface layer of natural forest soils (due to the activity of microbes and roots) allow infiltration of rainwater into the soil. In post-nickel mining land with low nitrogen concentration, the leguminous trees as nitrogen-fixing species could be used for revegetation. Several studies on revegetation of post-mining land in Africa have shown that leguminous tree species have a high survival rate (Festin et al. 2019). The successful use of leguminous trees for post-mining land reclamation has also been demonstrated in Brazil. Leguminous trees form a symbiosis with nodulating N-fixing bacteria (Chaer et al. 2011). Several leguminous trees, including *Caesalpinia sappan* L., *Enterolobium cyclocarpum* (Jacq.) Griseb., *Gliricidia sepium* (Jacq.) Walp., *Delonix regia* (Hook.) Raf., and *Cassia siamea* Lamk., have been used in the rehabilitation of a former tin mining area in Bangka Island, Indonesia (Narendra and Pratiwi 2016).

The use of organic amendments and microbial inoculants could increase soil fertility and help plant growth in post-nickel mine soil. For example, chicken manure, cow manure, mulch, municipal green waste, and litter compost might increase the success of rehabilitation. The application of chicken manure to post-coal mining land in Indonesia, which had very low soil nutrient concentrations, increased the growth of *Samanea saman* (Darma et al. 2017). The treatment of municipal green waste had growth rates comparable to untreated plants for mine site rehabilitation. The use of municipal green waste on degraded opencast coal land in South East Wales, the United Kingdom had significantly greater survival rates, compared with trees planted without green waste (Haigh et al. 2019). Other studies have shown that the addition of compost not only increases soil fertility and plant

biomass but also reduces the concentration of trace elements in plant species grown in metal-contaminated mine soils (Martinez et al 2014). Oyebamiji et al. (2018) reported the distribution of heavy metal such as, Pb, Zn, Cu, Ni, Cr and Fe in active mining soils in Southwestern Nigeria. Incorporation of compost provides benefits for remediating trace elements (Cu, Pb, Zn, and As) in polluted soil (Tandy et al. 2008). The dissolution of organic matter can increase the solubility of Al, Fe, and Pb within the reclaimed soils (Miller et al. 2019). The application of microbial inoculants, such as AMF, could improve the growth and survival of trees on post-nickel mining land. Plants are part of the ecosystem with many and diverse microorganisms in the soil. It has been established that some of these microbes, such as mycorrhizal fungi or nitrogen fixing bacteria, play important roles in plant development by improving mineral nutrition (Jacoby 2017). Several investigations have shown good results; the application of AMF increased the growth and survival of *P. falcataria* and *A. saman* in post-coal mining land in Indonesia (Wulandari et al. 2016). Additionally, the use of coconut powder inoculated with AMF increased the survival of *Anadenanthera colubrina* seedlings in post-mine soil in Brazil (Fernandes et al. 2019). The application of AMF and leguminous trees might be used to increase the success of revegetation programs in post-nickel mining land. In our study, Fe content in post-mine soil was seventeen times higher than natural forest soil. Agus et al. (2018) reported that revegetation with fast-growing legume species of *Pongamia pinnata* and AMF application can not only increase nutrient contents of post-coal mining soil but also increases Fe absorption, which is mostly accumulated in the root system.

Fast-growing tropical leguminous trees that belong to the N-fixing species may contribute to improving soil quality on degraded soil of post-nickel mining land. Some results indicate that legumes plant may increase the resistances of soil physicochemical and biological properties to the ecosystem disturbance (Gao et al. 2017). Legumes fix the atmospheric nitrogen, release in the soil high-quality organic matter, facilitate soil nutrients' circulation and water retention (Stagnari et al. 2017). It could be investigated in future studies, in which fast-growing tropical leguminous trees of *F. moluccana* or *Albizia saman* have a better impact to increase soil quality on post-nickel mining land.

Chapter V. Effect of Arbuscular Mycorrhiza Fungal Inoculation on Growth of Tropical Tree Species under Nursery and Post-Opencast Bauxite Mining Field in Bintan Island, Indonesia

5.1. Introduction

Indonesia is a fast-growing, emerging economic country in Southeast Asia (Tiess and Mujiyanto 2019). Energy resources and mineral mining provide the largest contribution to the country's foreign exchange. Major mining commodities produced in Indonesia's tropical rainforests are coal, nickel, bauxite, gold, copper, and tin, which are extracted by opencast mining. Forest exploitation by opencast mining causes erosion, flooding, loss of the top layer of soil, reduction of soil pH, lowering of soil organic matter content, and a reduction in soil fertility (Ghose 2004). Opencast mining damages the environment (Feng et al. 2019) and destroys ecological functions and services (Mentis 2020). Opencast mining operations have effects on nearby landscapes and influence soil quality due to the removal of the vegetation and soil surface (Paramasivam and Anbazhagan 2020).

A mining company in Indonesia must rehabilitate its post-mining land. However, the result of rehabilitation activities in opencast mining in most companies can be regarded as unsuccessful. Many transplanted tree seedlings die in the first year. Moreover, the seedlings have poor growth performance and become stunted in the field because of the low quality of transplanted seedlings. Soil degradation makes the rehabilitation of degraded post-mining land extremely challenging (Parrotta 2002). Soils in post-opencast mining land have low fertility, low organic matter, and poor soil properties that limit their ability to sustain vegetation growth and development (Mushia et al. 2016).

The application of chemical fertilizers is required to promote the success of post-mining land rehabilitation. Fertilizer utilization is important for silvicultural management to maintain forest seedling growth and health (Deng et al. 2019). However, inappropriate fertilization can also lead to unexpected effects, such as inhibiting forest-tree seedling growth, contaminating the environment, and enhancing production costs. Application of chemical fertilizers in industrial

plantations or mining companies is carried out several times a year, and fertilization is carried out until the trees are three years old in the field. Utilization of symbiotic microorganisms such as nitrogen-fixing bacteria, ectomycorrhizal fungi, AMF, endophytic fungi, and plant growth-promoting rhizobacteria can increase tree growth under pot culture conditions (Maulana et al. 2018; Singh et al. 2012; Wildman 2014).

AMF are important components of soil microorganisms that contribute to the stability and heterogeneity of natural ecosystems (Van der Heijden et al. 1998) AMF provide a direct biological and physical link between the host plant root and the soil. These fungi can increase production and plant growth under degraded post-mining land, despite low pH, water stress, nutrient deficiency, and soil toxicity (Smith and Read 2008). Moreover, the domination of AM fungi in tropical forests demonstrates that AM fungi play an important role in these forests (Tresseder and Cross 2006). There are several efficient and effective ways to promote the early growth of tree seedlings in post-mining sites (Setyaningsih et al. 2020; Wulandari et al. 2016; Gardner and Malajczuk 1988). A symbiosis between host tree seedlings and AM fungi has a real influence on the success of rehabilitation activities in the damaged areas.

A proven strategy to enhance the success of revegetation in degraded post-opencast bauxite mining fields is to select fast-growing tropical tree species. *Gmelina arborea* (Linn.) Roxb., *Samanea saman* (Jacq.) Merr. *Falcataria moluccana* (Miq.) Barneby & J. W. Grimes, and *Enterolobium cyclocarpum* (Jacq.) Griseb are four tropical forest tree species that are grown to support sustainable forest rehabilitation programs in Indonesia. They have a variety of uses as industrial wood. *Gmelina arborea* is used as a raw material for matches, charcoal, light construction, plywood, particleboard, pulp, and paper (Soerianegara and Lemmens 1993). *Falcataria moluccana* is a multipurpose tree widely planted in forest community gardens on Java Island. This species can be used for packing boxes, housing construction, furniture, pulp, paper, and other purposes (Krisnawati et al. 2011). *Enterolobium cyclocarpum* is used for forage trees, fuelwood, and shade trees (Akkasaeng et al. 1989). *Samanea saman* is usually planted as an urban

forestry species to provide shade and minimize air pollution on roadsides, around office buildings, and in parks and schoolyards in urban areas (Kabir et al. 2012).

Opencast bauxite mining reduces soil fertility; total carbon (C), nitrogen (N), available phosphorus (P) concentrations, and exchangeable sodium (Na), calcium (Ca) and magnesium (Mg) concentrations were reduced by 75.7%, 75%, 15.7%, 52%, 92%, and 100%, respectively, compared to the forest soils (Table 2-1). The use of beneficial soil microorganisms has been recommended to promote the successful restoration of the post-mining sites (Jasper 2007; Taheri and Bever 2010). Inoculation of mycorrhizal fungi and nitrogen-fixing bacteria can increase nutrient absorption by plants (Jasper 2007). Application of AM fungi to post-bauxite mining land is a biological approach that ensures good practices and is effective in enhancing plant growth (Asmelash et al. 2016).

To increase the growth of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum*, two AM fungal species, *Rhizophagus clarus* and *Gigaspora decipiens* were inoculated into seeds. These AM fungal species were adopted because they are native to Indonesia and previous studies have shown that they have the capacity to promote plant growth in Indonesia's tropical peat-swamp forests (Turjaman et al. 2006), coal mining (Wulandari et al. 2014; Wulandari et al. 2016). This technique, combining fast-growing tree species and native AM fungi, should be relatively easy for staff in the environmental divisions of bauxite mining companies to apply. The objective of this chapter was to determine the effect of inoculation with two indigenous AM fungi on the growth of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* in the nursery and post-opencast bauxite mining field conditions.

5.2. Materials and Methods

5.2.1. Nursery site and soil substrate preparation

The experiment was conducted in the nursery at the Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. The site was a post-opencast bauxite mining area managed by a national mining company. Ultisol soil was collected near the nursery area and stored in a nursery. The soil was air-dried and sieved through a < 5 mm sieve. The soil chemical characteristics were

as follows: pH (H₂O), 4.96; total carbon, 7.00 g kg⁻¹; total N, 0.4 g kg⁻¹; available P, 11.30 mg P₂O₅ kg⁻¹ (Table 2-1). The soil substrate was prepared by mixing river sand with the soil (1:3, v/v) to increase drainage and porosity.

5.2.2. Inoculum propagation and inoculation of AMF

Two AM fungi, namely, *Rhizophagus clarus* Nicholson & Schenk (Figure 5-5) and *Gigaspora decipiens* Hall & Abbott (Figure 5-6), were isolated from peat soil in Kalampangan, Palangkaraya, Central Kalimantan, Indonesia (Turjaman et al. 2006). *Rhizophagus clarus* and *G. decipiens* were propagated in pot cultures of *Pueraria javanica* Benth (Figure 5-7). Plastic pots (7.5 cm height × 4 cm diameter) were filled with 175 g of sterilized zeolite. Two 7-day-old *P. javanica* were placed in pots, and 5 g of AM fungal inoculum was inoculated surrounding the roots of *P. javanica*. The inoculum of AMF contained a substrate of zeolite with external hyphae, spores, and mycorrhizal roots from a pot culture of *P. javanica* grown in a greenhouse with no humidity and temperature control in the Forest Research and Development Centre (FRDC), FORDA, The Ministry of Environment and Forestry, Bogor, West Java, Indonesia. Pot cultures were irrigated daily to field capacity using sterilized water to maintain the moisture content. After 120 days, an AM fungal inoculum with colonized roots, spores, and hyphae of *R. clarus* and *G. decipiens* was observed under a microscope in the zeolite media. Ten grams of inoculum was then mixed with 500 g of soil in a polyethylene bag (15 cm height × 10 cm diameter), and 10 g of zeolite was placed into no-inoculated pots as a control treatment.

5.2.3. Seed germination

Four tropical forest trees, *Gmelina arborea*, (Linn.) Roxb., *Falcataria moluccana* (Miq.) Barneby & J. W. Grimes, *E. cyclocarpum* (Jacq.) Griseb, and *Samanea saman* (Jacq.) Merr. were selected for this investigation. Tropical forest-tree seeds were purchased from a local seed company in Solo, Central Java, Indonesia. The seeds were soaked in hot water at 85 °C for 2 min. Five-hundred grams of soil substrate was poured into a polyethylene bag (15 cm height × 10 cm diameter). Three seeds were sown and, after germination, one forest-tree seedling was allowed to

grow in the polyethylene bag. The pots were placed in a randomized block design on the bench in a nursery. Tap water was applied two times a day. The source of tap water from groundwater, with water quality index (WQI) analysis, shows lightly polluted (WQI = 0.59) (Marganingrum et al. 2020). There was no application of chemical fertilizer or pesticides. Because these tree species require shade conditions, the seedlings were grown under 55% shading intensity net to control solar radiation for four months in the nursery of Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. The tree seedlings were then transplanted into the field. The experiment consisted of three treatments for *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* seedlings at nursery (a) control, (b) *R. clarus*, and (c) *G. decipiens*. There were 20 replicates of four tree species per treatment.

5.2.4. Field plantation and growth parameters

The field experiment was conducted on post-opencast bauxite mining land at the nursery of the Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. A planting field experiment without vegetation was covered with disposal waste and overburden from bauxite mining activity. Commercial organic compost was obtained from PT Green Planet Indonesia at the local market on Bintan Island. The organic compost contained: N, 1–3%; P₂O₅, 2–5%; K₂O, 1–3%; water content, 9–11%; and C-organic, 15–17%. A complete randomized block design with three treatments and six replications per treatment was used in this experiment. The field experiment consisted of three treatments: (1) control, (2) *R. clarus* AM fungal inoculation, and (3) *G. decipiens* AM fungal inoculation. On flat areas with similar soil conditions, six (24 m × 6 m) blocks with a distance between blocks of 5 m were prepared in the post-opencast bauxite mining field. Planting holes (30 cm × 30 cm × 30 cm) with 2 m distance between holes were laid out in each block. Five hundred grams of organic compost was then applied to the planting hole. Each block contained a treatment area (6 m × 6 m) with a distance between the treatment areas of 3 m. Three-month-old *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* were transplanted into the holes for each treatment. The seedlings were irrigated with tap water once a day for two weeks.

The source of tap water from groundwater, with water quality, shows lightly polluted (WQI = 0.59) (Marganingrum et al. 2020). There was no weeding or fertilizer application to the seedlings after transplanting. The seedlings were grown for 12 months in the mining field.

5.2.5. Data collection and arbuscular mycorrhizal fungal colonization

Three months after sowing in the nursery, each treatment consisting of six replicates of seedlings was harvested. Twelve months after transplanting into the mining field, the seedlings from each treatment with six replications were also harvested. After separation, shoots and roots were oven-dried at 70 °C for 72 h. The dry shoots and roots were then weighed. After harvest in the nursery, roots of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* were washed gently under running tap water over a 2-mm sieve to separate them from soil particle debris. Using the methods of (Brundrett et al. 1996), the roots were cleared with KOH (100 g l⁻¹) for 1 h, acidified with dilute HCl, and stained with 500 mg l⁻¹ trypan blue in lactoglycerol. The roots were de-stained in 50% glycerol, and 30 1-cm segments were viewed under a compound microscope at 200 × magnification. The percentage of colonization by the AM fungi (Figure 5-8) was calculated using the gridline intersect method (Giovannetti and Mosse 1994).

5.2.6. Statistical analysis

The Minitab package (Minitab, USA) was used to analyze all collected data from the nursery and mining fields. The least significant difference (LSD) test was used to compare the significant differences between the means of the treatments when F showed a significant value.

5.3. Results

5.3.1. Arbuscular mycorrhizal colonization of seedlings under nursery conditions

AMF colonized all tree seedlings of *G. arborea*, *S. saman*, and *E. cyclocarpum*, and there was no significant difference in AM fungal colonization among the treatments

in the nursery (Table 5-1). Colonization of *F. moluccana* inoculated with *R. clarus* was lower than that of control seedlings and the seedlings inoculated with *G. decipiens*.

5.3.2. Shoot and root dry weights of seedlings under nursery conditions

Shoot and root dry weights of *G. arborea* inoculated with *G. decipiens* were higher than those of the control seedlings (Table 5-1). There was no significant difference in shoot and root dry weights between seedlings inoculated with *R. clarus* and control seedlings. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* was higher than that of control seedlings. In comparison with control forest-tree seedlings, the root dry weight of *E. cyclocarpum* inoculated with *R. clarus* was higher than that of control seedlings. Shoot dry weight of seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens*. Shoot and root dry weights of *S. saman* and *F. moluccana* inoculated with AM fungi were not different from that of control seedlings.

5.3.3. Arbuscular mycorrhizal colonization of seedlings under field conditions

AMF colonized all *G. arborea* seedlings (Figure 5-1A.), *S. saman* (Figure 5-2A), *F. moluccana* (Figure 5-3A), *E. cyclocarpum* (Figure 5-4A), and control seedlings in the post-opencast bauxite mining field. Arbuscular mycorrhizal fungal colonization did not differ among treatments in any species.

5.3.4. Shoot dry weight of seedlings under field conditions

There was no significant difference in *G. arborea* and *S. saman* among treatments (Figure 5-1B.; Figure. 5-2B). Shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings (Figure 5-3B). There was no significant difference in shoot dry weight between seedlings inoculated with *R. clarus* and control seedlings. Shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* and *G. decipiens* was higher than that of control seedlings (Figure 5-4B).

Shoot dry weight of seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens*.

5.4. Discussion

5.4.1. Growth of colonized forest tree seedlings in the nursery

Successful post-bauxite mine land rehabilitation requires a large number of forest tree seedlings with high-quality performance. For this purpose, several bauxite mining companies in Indonesia have built nurseries to produce seedlings that can be used for post-mining land rehabilitation activities. Production of forest tree seedlings in nurseries, however, is not easy. Slow growth limits the quantity and quality of seedling production. In addition to seed quality, some factors required to produce high-quality seedlings for mine site rehabilitation, are knowledge of the breakdown of seed dormancy, seed germination, and seed storage tolerance (Salazar et al 2015; Ramos et al 2019), plant growth, and nutrient requirements (Carvalho et al. 2018). It is also important to select a suitable medium to support seed growth and produce seedlings in the pots. In Indonesia, compost is one of the most frequently used pot-substrates for growing forest-tree seedlings in the nursery. However, not all mining sites can provide good quality compost because mining companies in Indonesia are scattered in remote areas.

To save costs, the fresh soil near the bauxite mining site could be used as an alternative growing medium to produce forest tree seedlings in the nursery. However, due to the opencast mining system, the fresh soil near bauxite mining is often limited and categorized as post-mine soil with low fertility. The process of opencast mining, including surface soil stripping, excavation, transportation, and dumping, causes physical, chemical, and biological damage to the forest surface. Furthermore, the re-established landscape generates small-scale spatial heterogeneity of soils after mining (Feng et al. 2019).

It has been demonstrated that colonization by AM fungi enhances the growth of forest tree seedlings in the nursery. Inoculation with AM fungi is highly recommended to promote the growth of mycorrhizal tree seedlings in the nursery before transplantation into the degraded land of the mining field. AMF improved the early growth of *Mallotus paniculatus* in the nursery (Wulandari et al. 2014).

These fungi also enhance leguminous seedling growth and P uptake of *P. falcataria*, *Calliandra calothyrsus*, *Cassia siamea*, and *Sesbania grandifolia* (Maulana et al. 2017). Arbuscular mycorrhizal fungal inoculation with *Funneliformis mosseae* (syn. *Glomus mosseae*), *Rhizophagus intraradices* (syn. *Glomus intraradices*), and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*) increased the growth and drought tolerance of *Acacia seyal* Del. Seedlings (Abdelmalik et al. 2020). The inoculated seedlings of *Eucalyptus tereticornis* with various bioinoculants, Azospirillum+Phosphobacterium (PGPR), *Glomus fasciculatum* (AM fungi), and pink-pigmented facultative methylotrophic bacteria (PPFM), have shown improved performance in terms of seedling survival, shoot length, and collar diameter in the nursery (Murugesan et al. 2016).

Bauxite mining soil contains pollution of heavy metals. The elements, such as iron ($194,912 \pm 30,229 \text{ mg kg}^{-1}$), mercury ($2.63 \pm 0.40 \text{ mg kg}^{-1}$), arsenic ($25.17 \pm 37.49 \text{ mg kg}^{-1}$), lead ($108.06 \pm 78.88 \text{ mg kg}^{-1}$), copper ($100.09 \pm 32.79 \text{ mg kg}^{-1}$) were detected in bauxite mining soil in Kuantan, Pahang, Malaysia (Ismail et al. 2018). Aluminum was determined in the formation of High-Grade Al Deposits of the Dopolan Karst Type Bauxite, Iran (Ellahi et al. 2017) while pollutant of arsenic, lead, and copper were also detected in bauxite mining soil from Bintan Island, Indonesia (Putra et al. 2018). AMF ameliorated metal toxicity as they intensify the plant's ability to tolerate metal stress (Dhalaria et al. 2020). Agus et al. (2018) reported that fast-growing legume species of *Pongamia pinnata* and AM fungi application can not only increase nutrient contents of post-coal mining soil but also increases iron absorption, which is mostly accumulated in the root system. AMF *Gigaspora margarita* and *E. cyclocarpum* seedlings can tolerate up to $375 \mu\text{M}$ Hg supply (Ekamawanti et al. 2013). Root colonization by symbiotic AM fungi enhances plant resistance to acidity and phytotoxic levels of aluminum in the soil environment (Seguel et al. 2013). Arbuscular mycorrhizal fungal treatment could reduce the toxic effects of arsenic on the growth of *G. arborea* in degraded soil during the nursery stage (Barua et al. 2010), on phyto-extraction by Corn (*Zea mays*) of lead-contaminated soil (Hovsepian and Greipsson 2014). Research has established the potential of carbonized rice hull (CRH) and AM fungi inoculation

to improve the health of *Paraserianthes falcataria*, grown under the Cu-stressed soil in the nursery (Rollon et al. 2017).

Our study showed that using the Ultisol soil near the bauxite mining site, mixing with river sand as a growing medium for the seedlings combined with AM fungal inoculation, irrigating with lightly polluted tap water (WQI = 0.59) (Marganingrum et al. 2020), enhanced tree seedling growth of *G. arborea* and *E. cyclocarpum* at the nursery stage, three months after planting. In *G. arborea* seedlings, inoculation with *G. decipiens* increased both shoot and root growth. *Rhizophagus clarus* and *G. decipiens* showed similar responses by enhancing the shoot and root growth of *S. saman* and *F. moluccana*. In *E. cyclocarpum* seedlings, both *R. clarus* and *G. decipiens* improved shoot growth. In comparison with the previous investigation, this result showed that *R. clarus* and *G. decipiens* enhanced shoot growth of *S. saman* and *M. paniculatus* (Wulandari et al. 2014), *A. saman*, and *P. falcataria* (Wulandari et al. 2016). This result confirms that AM inoculation could promote the growth of seedlings on low-soil-fertility media in the nursery.

5.4.2. Growth of colonized seedlings under post-opencast bauxite mining conditions

Information about the use of AM fungal colonization to increase plant growth in post-bauxite mining fields is still limited. The success of rehabilitation in the post-bauxite mining field is dependent on the plant's growth; however, there are no studies to clarify the effect and utilization of AM fungal inoculation to improve the growth of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* in post-bauxite mining fields in Indonesia. This study showed the importance of AM fungal inoculation in promoting the growth of tropical tree seedling species in the post-bauxite mining field in Bintan Island, Riau Archipelago, Indonesia.

Inoculation with *R. clarus* and *G. decipiens* demonstrated the positive effects of increasing the growth of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum*, by using low soil fertility as a growth medium under nursery conditions. The effect of these AM fungi was also examined under field conditions

to ensure consistency. Twelve months after transplanting into the field, *R. clarus* and *G. decipiens* tended to increase the shoot growth of *G. arborea* (Figure 5-1B). In *S. saman* seedlings, both *R. clarus* and *G. decipiens* seem to have a beneficial effect on shoot growth (Figure 5-2B). Inoculation with *G. decipiens* increased the shoot growth of *F. moluccana* seedlings (Figure 5-3B). Both *R. clarus* and *G. decipiens* enhanced shoot growth in *E. cyclocarpum* seedlings (Figure 5-4B).

AMF colonization is important for promoting the growth of mycotrophic plant species in the field. Arbuscular mycorrhizal fungal inoculation has been reported to enhance early growth and nutrient absorption by some tropical forest-tree species in a nursery and in the field (Tawaraya and Turjaman 2014), *P. falcataria* and *A. saman* growth in a post-opencast coal mining field in Kalimantan, Indonesia (Wulandari et al. 2016). *Enterolobium cyclocarpum* was classified as a highly mycorrhizal-dependent plant species in response to low soil P concentration (Habte and Musoko 1994). A combination of AM fungi and *Rhizobium* sp. increased the growth of *A. saman* in degraded gold-mining land in Pongkor, West Java, Indonesia (Setyaningsih et al. 2020). Some studies on the application of AM fungi in poor soil and post-mining have shown that this treatment was very effective in increasing the growth of *G. arborea* under salt stress (Dudhane et al. 2011). AMF association also influence soil fertility through the enhancement of chemical, biological, and physical properties. AMF have a positive correlation with organic carbon, organic matter, total phosphorus, CEC, water level, soil fungi, and soil bacteria (Syib'li et al. 2013). There is a plentiful scientific confirmation to indicate that AM fungi significantly promote soil attributes, improve above and belowground biodiversity, significantly enhance tree seedlings survival, growth, and establishment on moisture and nutrient stressed soils after the restoration of degraded lands (Asmelash et al. 2016). It could be expected that, after rehabilitation, trees affected on soil conditions. Rehabilitation through forest vegetation is one of the efficient means of restoring soil fertility through improved soil organic matter content, available nutrients, CEC, increase biological activities as well as improvement in physical conditions of the soil (Mensah 2015). A study in the jarrah forest for the rehabilitation of bauxite mines in south-west Australia shows that level of total nitrogen of soil in rehabilitated lands enhanced from around 0.04 –

0.05% after 8.5 years, while soil pH decreased after rehabilitation (Ward 2000). After rehabilitation, using *Eucalyptus camaldulensis* and *Brachiaria decumbens*, in a soil contaminated with Zn, Cu, Cd, and Pb can enhance the soil pH, phosphorus (P) concentration, and exchangeable K by 31%, 40%, and 98%, respectively while decreased Ca, Mg, Al, and Mn by 97%, 96%, 93% and 96%, respectively (Leal et al. 2016).

The inoculation of *R. clarus* and *G. decipiens* enhanced growth of tropical tree species in post-opencast bauxite mining. *Gmelina arborea* inoculated with *G. decipiens* increased shoot and root dry weights by 1,431 and 359 %, respectively, while shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* increased by 510 and 220%, respectively, in comparison with control seedlings, under nursery conditions. Root dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 224%, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 90%, in comparison with seedlings inoculated by *G. decipiens*. Under field conditions, the shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings by 188%. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* increased by 198% and 149%, respectively, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* seedlings inoculated with *R. clarus* was higher by 20% than that of seedlings inoculated with *G. decipiens*. Our study showed that regarding the enhancement of plant growth, *R. clarus* was found to be superior to *G. decipiens*. Growth of *E. cyclorapum* seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens* under both nursery and post-opencast bauxite mining field. In contrast to our results, growth of seedlings, *Mallotus paniculatus* and *Albizia saman* inoculated with *G. decipiens* was found to be superior to *R. clarus* (Wulandari et al. 2014). It is well known that AM fungi can exhibit a considerable level of selectivity in their association with different plants species or plant ecological groups (Varelo-Cevero et al. 2015). Moreover, different AMF strains displayed different colonization rates, which suggest that AMF strain has certain selectivity to their host plants (Xiaoying et al. 2014).

This study demonstrated the consistent effect of *R. clarus* and *G. decipiens*, which are AM species indigenous to Indonesia, in increasing plant growth, not only in post-bauxite mining but also in tropical peat-swamp forests (Turjaman et al. 2006), post-coal mining in nursery conditions (Wulandari et al 2014), and post-coal mining in both nursery and field conditions (Wulandari et al 2016). It appears that native AM inoculation increases plant growth thus saving time and increasing cost efficiency during the rehabilitation of post-bauxite mining land. Further research should be conducted to determine the capability of native AM species to increase plant growth on other post-mining land in Indonesia.

Chapter VI. Petroleum hydrocarbons degradation in contaminated soil using the plants of the Aster family

6.1. Introduction

The oil extraction is one of the causes of soil contamination with the total petroleum hydrocarbons. The contamination is mainly caused by transport loss, disposal of petroleum waste, storage leaks and intentional oil spillage (Gerhardt et al. 2009). Petroleum products contain toxic compounds which are believed to be soil contaminants (Euliss et al. 2008). Contaminated soils with petroleum are unsuitable for agriculture lands, recreational, housing, human health and ecosystem sustainability. The qualities of groundwater and agricultural product are also largely affected by petroleum contaminated soils (Wang et al. 2008). Therefore, it is necessary to remediate petroleum hydrocarbon-contaminated soil.

Many technologies, such as bioremediation and chemical oxidation, have been applied for degradation of petroleum in contaminated soil, for the restoration of environments. Bioremediation is more preferable and greener than chemical oxidation for the remediation of TPH-contaminated soil (Huang et al. 2016). Phytoremediation or enhanced biological degradation is an accepted approach to clean up soil contaminated with gasoline (Al-Mansoori et al. 2017). Phytoremediation is a method that utilize living plants to restore contaminated media to regulatory mandated levels. This can be used to restore contaminated air, soil, surface water, and groundwater (Landmeyer 2012). This technology has also been demonstrably effective and has ability to degrade petroleum contaminated soils in laboratory and field experiments (Euliss et al. 2008). The plant roots give an appropriate environment for the degradation of contaminated materials. Plant root systems allow rapid movement of gases and water thorough the soil due to the change of soil physical characteristics. Rhizosphere soil also gives an environment to increase microbial activity and enhance contaminant bioavailability (Gerhardt et al. 2009). Plants may contribute to the dissipation of contaminants in rhizosphere soil. They can have great possibility for remediation in natural environments (Boyajian & Carreira 1997). The problem is inhibition of root growth due to higher concentrations of contaminants (Harvey et al. 2002). The resulting stress limits the

rate of phytoremediation *in situ* (Huang et al. 2005). Contaminated soils have poor nutrients poor and low diversity of microbes. It contributes to low plant growth and impedes rates of remediation (White et al. 2006). Soil loosening is necessary prior to phytoremediation so that the length and biomass of plant root systems can develop properly. The mean root length of herbaceous plants is 500 mm (Pilon-Smits 2005).

Microbial remediation in the soil rhizosphere has also been expanding. Microbe helped phytoremediation thorough naturally existing microbe and seed inoculated microbe (Huang et al. 2005). However, this microbial remediation technique has proven to be limited (Pilon-Smits 2005). Some possible reasons for this un success are failure of establishment of inoculated microbes to compete with indigenous microbes in the soil environment (Van Dillewijn et al. 2007).

The use of the Fenton reaction processes has been applied for removing organic hydrocarbons in the field (Figure 1-9). Compared with other methods, The Fenton reaction has several advantage such as cheaper cost, reduced reaction times and energy consumption, non-toxic compounds, and simple operation and control (Flotron et al. 2005). The basis of the Fenton method is the decomposition of hydrogen peroxide (H_2O_2) and the production of hydroxyl radicals in the presence of Fe^{3+} ion as a catalyst (Gu et al. 2012). Studies have shown that the produced hydroxyl radicals have the ability to decompose and degrade organic contaminants of petroleum hydrocarbons (Kang and Hwang 2000). Liu et al. (2010) studied that the application of the Fenton reaction process on petroleum contaminated soil increase the efficiency of the biological process. The combination of biodegradation and a modified Fenton reaction has removal efficiency of polycyclic aromatic hydrocarbons (PAHs) in the range of 70–98%. It depends on the chemical characteristics of the PAHs (Nam et al. 2001).

Asteraceae plants are almost found everywhere, except in Antarctica and the extreme Arctic. It is also an economically important family which could be produced as several products such as lettuce, cooking oils, sweetening agents, sunflower seeds, coffee substitutes, and herbal drinking. Asteraceae are also used for some industrial purposes. For instance, marigold (*Tagetes patula*) is usually used for commercial poultry feeds and its oil is commonly extracted for uses in

cigarette and cola industries. Besides their commercial and economic importance, Asteraceae plants can be used for phytoremediation of petroleum hydrocarbon in soils. Erect bur marigold, an Asteraceae plant, has shown methane (CH₄) decomposing ability through rhizosphere Fenton reaction (Wagatsuma et al. 2007). Nevertheless, information is scarce about the effect of Asteraceae plants on degradation of petroleum hydrocarbon in contaminated soils. The objective of this chapter was to clarify the effect of Asteraceae plants on degradation of petroleum hydrocarbon in contaminated soils.

6.2. Materials and Methods

6.2.1. Preparation of contaminated soil

The used fresh soil used for this study was obtained from the oil exploited land at Riau Province, Indonesia (Latitude 0°48'20.81" N; Longitude 101°25'14.24" E). The composite soil samples were taken from a depth of 0 to 25 cm, and homogenized by mixing the soil. The homogenized soil was then sifted through a 5 mm stainless steel sieve after air-drying for 7 days. The light oil waste was collected from the oil exploited land and was homogenized with fresh soil to create low (40 g kg⁻¹) and high (90 g kg⁻¹) concentrations. We prepare 10 kg each of 40 g kg⁻¹ TPH soil and 90 g kg⁻¹ TPH soil. The soil pH was measured with the electrode using pH meter machine (Thermo orion model 410, USA).

6.2.2. Plant materials and plant growth condition

The seeds of nine plants of Aster family including, *Achillea filipendulina* Lam., *Anthemis tinctorial* (L.) J. Gay ex Guss.; *Tagetes erecta* L.; *Chrysanthemum coronarium* (L.) Cass. Ex Spach; *Calendula officinalis* L.; *Zinnia elegans* Jacq.; and *Callistephus chinensis* L., were purchased from a local company in Japan. Seeds of *Cosmos caudatus* Kunth and *Tagetes* sp. were purchased from a local company in Bogor, Indonesia. The seeds were immersed in tap water for 30 min and sterilized by dipping them in a 5% sodium hypochlorite solution for 2 minutes. Afterward, seeds were thoroughly washed twice using sterile distilled water. The seeds were placed in a plastic vat filled with sterilized zeolite and germinated in

greenhouse at the Forest Research and Development Center, Bogor, West Java (6°36' S, 106°45' E) for 7 days. Polyethylene pots (7.5 cm × 5 cm), with a drainage hole at the bottom, were then filled with 100 g of contaminated soil. A plate was placed under each pot to collect any leachate, and the soil was irrigated using this leachate. The experiment consisted of 3 treatments: (1) control (initial contaminated soil); (2) contaminated soil with 20 ml of 10 mM FeCl₃ solution + 10 mM nitrilotriacetic acid (NTA) solution; (3) contaminated soil with Asteraceae plants and 20 ml of 10 mM FeCl₃ solution + 10 mM NTA solution. There were five replicates per treatment, and three seedlings were grown per pot. Seven-day-old seedlings of *A. filipendulina*, *A. tinctoria*, *C. officinalis*, *C. chinensis*, *C. coronarium*, *C. caudatus*, *T. erecta*, *Tagetes* sp. and *Z. elegans* were transplanted into the pots containing contaminated soil and 20 ml of 10 mM FeCl₃ solution + 10 mM NTA solution was applied. The seedlings were irrigated everyday with tap water to field capacity. The number of seedlings per pot was reduced to one seedling per pot, one week after transplanting. Fertilizer was not applied to the soil during the course of the experiment. Pests and weeds were taken manually. The seedlings were planted for 90 days in the greenhouse at the Forest Research and Development Center.

6.2.3. TPH concentration and plant growth

The TPH concentration in the soils was measured using infrared spectrophotometer according to USEPA Method 418.1. and APHA 5520. The plants were harvested two and three months after transplanting (MAT), and the shoots and roots were separated. These were oven-dried at 70°C for 72 h after which the shoot and root dry weights were measured.

6.2.4. Statistical analysis

The data were analyzed using Minitab package (Minitab Inc. USA). The least significant difference was calculated to compare the significant differences between treatment means, when F values were significant.

6.3. Results and Discussion

6.3.1. Petroleum hydrocarbons degradation in soil using the plants of the Aster family

Concentrations of TPH in soils without FeCl₃ and NTA and soils with FeCl₃ and NTA were similar to that in the initial soil (40 g kg⁻¹ TPH), 2 MAT (Figure 6-1). Concentration of TPH in soil cultivated with *C. caudatus* was lower than that in the initial soil, 2 MAT. Concentrations of TPH in soil without FeCl₃ and NTA and soil with FeCl₃ and NTA, were similar to that in the initial soil, 3 MAT. Concentrations of TPH in soils cultivated with *C. officinalis*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. were lower than that in the initial soil.

Concentrations of TPH in soils cultivated with *A. filipendulina*, *A. tinctoria*, *T. erecta*, *C. coronarium*, *C. officinalis*, *C. chinensis*, and *C. caudatus* were lower than that in the initial soil, 2 MAT (Figure 6-2). Concentration of TPH in soil with FeCl₃ and NTA was similar to that in the initial soil (90 g kg⁻¹ TPH). However, concentrations of TPH in soils without plants and without FeCl₃ and NTA were lower than that in the initial soil, 2 MAT. Concentrations of TPH in soils cultivated with *T. erecta*, *A. tinctoria*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. were lower than that in the initial soil, 3 MAT. Concentrations of TPH in soils without plants, without FeCl₃ and NTA, and with FeCl₃ and NTA were similar to that in the initial soil, 3 MAT.

TPH degradation was 24–38% and 17–34% in the treatments with plants at 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively. The degradation was 9–13% and 14% in unvegetated controls at 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively (Figure 6-3; Figure 6-4). The highest decrease was 38% and 34% for 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively. The degradation values were higher than 27% at 20 g kg⁻¹ TPH with *Pharbitis nil* for 127 days (Zhang et al. 2010), 20% at 20 g kg⁻¹ TPH with *Mirabilis jalapa* for 127 days (Peng et al. 2009), and 8% at 7.5 g kg⁻¹ TPH with *Samanea saman* for 101 days (Bento et al. 2012).

Some plants decomposed TPH through the rhizosphere Fenton reaction. Hydrogen peroxide and Fe²⁺ produce hydroxyl radicals (H₂O₂ + Fe²⁺ → Fe³⁺ + OH⁻ + *OH). These hydroxyl radicals scavenge organic compounds such as petroleum hydrocarbon (RH + *OH → R* + H₂O); H₂O₂ is necessary for this reaction. The

TPH concentrations did not decrease in the control treatments with FeCl₃ and NTA because of the lack of H₂O₂. Erect bur marigold (*Bidens tripartita* L., Asteraceae) had high H₂O₂ concentrations in their root-tips (>1 mM) and decomposed CH₄ through the rhizosphere Fenton reaction (Wagatsuma et al. 2007). Similarly, the Asteraceae plants in the present study could have high H₂O₂ concentrations in the rhizosphere and could decompose TPH through the rhizosphere Fenton reaction.

Asteraceae plants may also absorb petroleum hydrocarbon from contaminated soil. Phytoremediation has provided valuable insights into the types and specific mechanisms of the removal of organic contaminants (Pilon-Smits 2005). Across plant membranes some organic compounds can be transported. Low molecular weight compounds can be absorbed from the soil and transported to the plant leaves, which can be released through evapotranspiration. Some non-volatile compounds can be degraded to nontoxic compounds through enzymatic modification and sequestration in plants. The rhizosphere provides an active ecosystem where plants can interact through the Fenton reaction for effective degradation of TPH in soil. The hydroxyl radical produced by Asteraceae plants may also decompose the hydrocarbon to CO₂.

One of the most important functions of plant root is the production of organic compounds in the rhizosphere zone called root exudates which is a secondary metabolites photosynthesis product. Root exudates is supporting in the development of microbes so that the rhizoremediation process occurs. The Root exudates produced by Asteraceae plants may also play an important role in the rhizoremediation process. The success of rhizoremediation application is highly dependent of the capacity of contaminant degraders or plant growth that promote microbes to efficiently colonize growing roots (Lugtenberg et al. 2001). The aromatic plant compounds such as flavonoids and coumarins are the most important components in root colonization. These are structurally similar organic contaminants such as petroleum hydrocarbons, thereby providing a means to exploit natural processes in the rhizosphere for the remediation of contaminants (Holden & Firestone 1997).

6.3.2. Relationship between biomass of Asteraceae plants and degradation of TPH in the soil

A positive correlation was noted between root biomass of Asteraceae plants and degree of TPH degradation in the initial 40 g kg⁻¹ TPH soil (Figure 6-5). Root growth was necessary for remediation, and therefore higher levels of root biomass could increase degradation of TPH in the soil (Gurska et al. 2009). The large root biomass may also produce more hydroxyl radicals through the Fenton reaction. It has been shown that these hydroxyl radicals are capable of decomposing and degrading organic contaminants such as petroleum hydrocarbons (Chamarro et al. 2001). There was no positive correlation between the root biomass of Asteraceae plants and the degree of TPH degradation in the initial 90 g kg⁻¹ TPH soil (Figure 6-6). The root biomass of Asteraceae plants smaller than that in the initial 40 g kg⁻¹ TPH soil (Table 6-2). High TPH concentrations inhibit root growth of Asteraceae plants, because the toxic contaminants restrain the proliferation of plant roots.

Shoot dry weight of *C. officinalis* in 40 g kg⁻¹ TPH soil was higher than that of *A. tinctoria*, 3 MAT and tended to be higher than that of *C. chinensis*, *C. caudatus*, and *Tagetes* sp. In 90 g kg⁻¹ TPH soil, shoot dry weight of *T. erecta* was higher than that of *A. tinctoria*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. and tended to be higher than that of *Z. elegans* (Table 6-1). Root dry weight of *C. caudatus* in 40 g kg⁻¹ TPH soil was higher than that of *A. tinctoria* and tended to be higher than that of *C. officinalis*, *C. chinensis*, and *Tagetes* sp. There were no differences in root dry weights among *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in 90 g kg⁻¹ TPH soil (Table 6-2). These species maintained growth rate in the initial TPH soil concentration and may be the most tolerant Asteraceae plants. Growth of *A. tinctoria* greatly decreased in the presence of 40 g kg⁻¹ TPH in the soil and did not degrade the TPH. This species is possibly sensitive to TPH, causing stress and limiting its rate of phytoremediation (Huang et al. 2005). Contaminated soils also tend to be nutrient poor and/or lack microbial diversity, which contributes to sub-optimal plant biomass accumulation, as well as impedes rates of remediation (White et al. 2006). However, in 90 g kg⁻¹ TPH soil, *A. tinctoria* degraded the TPH. This suggests that the abilities of plants to degrade soil TPH are not only directly

related to their growth but also their adaptive abilities to different contamination conditions (Bento et al. 2012).

The root dry weight of *C. caudatus* was higher than that of *A. tinctoria* in 40 g kg⁻¹ TPH soil. There was no difference in root dry weight of *C. officinalis*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. However, root dry weight of *C. caudatus* was higher than that of the other Asteraceae plants, 3 MAT (Table 6-2). There was no difference in root dry weight of *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in the initial 90 g kg⁻¹ TPH soil. However, root dry weight of *C. caudatus* tended to be higher than that of the above-mentioned plants, 3 MAT. These data suggest that *C. caudatus* has the most potential among the studied Asteraceae plants for rhizosphere Fenton reaction to degrade TPH concentration in the soil. In fact, *C. caudatus* was consistent among the Asteraceae plants in its ability to degrade TPH in the initial 40 and 90 g kg⁻¹ soil, 2 and 3 MAT.

6.3.3. Plant survival

Plants for phytoremediation must survive in contaminated soil. The plant is a connecting line with contaminated material, which is an extension of another phase that will interact with certain contaminants, as the plant offers phases that include solid, organic, organic lipids, water and gas phase (Landmeyer 2012). There are differences in the tolerance to TPH concentration among plant species (Kaimi 2007). *A. filipendulina*, *T. erecta*, *Z. elegans*, and *C. coronarium* in 40 g kg⁻¹ TPH soil died between 2 and 3 MAT (Table 6-1). Other plants grew until 3 MAT. The shoot dry weight of *C. officinalis* was higher than that of *A. tinctoria*. There was no difference in shoot dry weight among *C. chinensis*, *C. caudatus*, and *Tagetes* sp. *C. officinalis*, *C. coronarium*, and *A. filipendulina* in 90 g kg⁻¹ TPH soil died between 2 and 3 MAT. Other plants grew until 3 MAT. The shoot dry weight of *T. erecta* was higher than that of *A. tinctoria*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. There was no difference in the shoot dry weights between *T. erecta* and *Z. elegans*.

The root dry weight of *C. caudatus* was higher than that of *A. tinctoria* in 40 g kg⁻¹ TPH soil. There was no difference in root dry weights among *C. officinalis*, *C. chinensis*, and *Tagetes* sp. (Table 6-2). There was no difference in the root dry

weights among *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in 90 g kg⁻¹ TPH soil.

A. tinctoria, *C. officinalis*, *Tagetes* sp., *C. chinensis*, *C. caudatus*, *T. erecta*, and *Z. elegans* were hydrocarbon tolerant. However, *A. filipendulina* and *C. coronarium* cultivated in the initial 40 and 90 g kg⁻¹ TPH soils died during the experiment (Table 6-1; Table 6-2). This could be because they are sensitive to the TPH concentrations in the soil. In severely polluted soils, high concentrations of TPH inhibited seed germination and created nutrient deficient conditions, in which the plants could not survive (Peng et al. 2009). These plants could also be sensitive to the soil pH, as in this study the soil pH levels were approximately 4.5–5.5. Studies have shown that acidic condition is the optimum condition for the Fenton's process (Farzadkia et al. 2014). By adding Fenton's reagent, the pH is reduced, which is accompanied by the production of intermediates such as carboxylic acid. Therefore, the maximum reduction rate occurred at a high pH (Kumar et al. 2012).

The concentration of contaminants tended to inhibit plant growth, including root growth, in part due to oxidative stress (Huang et al. 2005). However, we showed that Asteraceae plants had high potential for use in the phytoremediation of petroleum contaminated soil.

Chapter VII. General discussion

7.1. Impact of opencast mining to the soil chemical properties

Opencast mining causes significant damage to the environment. The removal of top layers of soil causes loss of structure and functionally, with a subsequent reduction in biodiversity. The main waste products of ore processing operations are crushed rock material, referred to as tailings (Young et al. 2015). Extraction activities in bauxite, nickel and gold mining have a significant impact on degraded tropical rain forest, affecting both indigenous vegetation and soil fertility. The soil fertility of the post-mining soils in bauxite, nickel and gold mining were very poor in comparison to the natural forest soil. Opencast mining decreased the soil fertility overall. Opencast mining activity decreased soil fertility of bauxite mining soil (Table 2-1) and nickel mining soil (Table 4-1). Moreover, the process of gold extraction in gold mining, produces many tailings, that have lower fertility in comparison to the forest soil (Table 3.1).

The activity of post bauxite, nickel and gold tailings mining land increased the soil pH. Shrestha et al (2011), stated that Mining and reclamation activities increased soil pH and electrical conductivity (EC). This is caused by the loss of vegetation cover on opencast mining soil, causing dry soil and dissolved metals and minerals due to mining activities (Wasis et al. 2018). Total nitrogen concentration decreased by 75, 98 and 90% on post bauxite, nickel and gold mining, respectively while opencast bauxite mining, opencast nickel mining and gold tailings decreased carbon concentration by 76, 93 and 77%, respectively. Similar investigation by Wasis et al (2018) reported that the reduction nitrogen and carbon concentration were 86% and 94%, respectively on opencast bauxite mining in Indonesia. Mining and reclamation activities decreased soil organic carbon (SOC) and nitrogen (N) pools (Shrestha and Lal 2011). In comparison with opencast mining in non-tropical area, the reduction of nitrogen and carbon concentration were higher than the 53% reduction of N concentration and the 66% reduction of C concentration, in opencast coal mining in India (Singh et al. 2012). The impact of opencast mining on soil fertility in tropical areas is higher than that in non-tropical areas. Available phosphorus concentration on post bauxite, nickel and gold mining, also significant

decreased by 16, 39, and 11%, respectively. Huang et al. (2018), stated that according to soil nutrient grading standards, the level abundance and deficiency of soil available phosphorus on reclamation site of post coal mining in China was categorized as very lacking (3-5 mg kg⁻¹) and totally lacking (<3 mg kg⁻¹).

Improvement of soil fertility is necessary for success of post-mining land rehabilitation activities in Indonesia. Increasing soil fertility could be done by improving soil acidity to approach normal soil pH by applying dolomite (Neina 2019), soil amendment by chicken manure (Darma et al. 2017), and organic compost (Santibanez et al. 2012). Improvement of soil physic is also required to increase porosity and drainage (Amri et al. 2020). Selection of fast-growing tree species that have a high degree of adaptation to grow and survive in post-mining land is very necessary in addition to microbial biotechnology approaches. Leguminosae family plants are highly recommended for planting in post-mining land rehabilitation activities (Skousen 2018), followed by the application of mycorrhizal and rhizobium microorganisms (Setyaningsih et al. 2020).

7.2. The growth of fast growing tropical leguminous trees on opencast mining land

The shoot height of *Falcataria moluccana* seedlings grown in the forest soil and post mining soil become greater from 2 to 15 weeks after planting on bauxite mining (Figure 2-3A.), nickel mining (Figure 4-2) and gold tailings (Figure 3-1B). The shoot height of *A. saman* seedlings of forest soil and post-mine soil increased from 2 to 15 weeks after planting on bauxite mining (Figure 2-3B), nickel mining (Figure 4-3) and gold tailings (Figure 3-1A). Shoot height 15 weeks after planting was lower in the post-mine soil than in the forest soil. Shoot dry weight of *F. moluccana* in natural forest soil was higher than that in post-bauxite mining (Figure 2-4), post-nickel mine soil (Figure 4-4) and gold tailings (Figure 3-3).

The rehabilitation of land after mining in Indonesia is a mandatory activity for all mining companies. Planting fast-growing tropical leguminous trees that have a high level of adaptation and survival on post mining land and increase the fertility of the soil is the strategy for improving the success of post mining rehabilitation activities. Our investigation shows that *A. saman* is more tolerant to growth on post-

mining land than *F. moluccana*. *Albizia saman* is a fast-growing tropical leguminous tree that is highly adapted to various types of soil with a wide pH range and poor drainage (Haq et al. 2012). Planting leguminous trees that can grow on post-mining land can improve the ability of the soil to retain water. Large pores in the surface layer of natural forest soils (due to the activity of microbes and roots) allow infiltration of rainwater into the soil. In post-nickel mining land with low of nitrogen concentration, the leguminous trees as nitrogen-fixing species could be used for revegetation. Several studies on revegetation of post-mining land in Africa have shown that leguminous trees species have a high survival rate (Festin et al. 2019). The successful use of leguminous trees for post-mining land reclamation has also been demonstrated in Brazil. Leguminous trees form a symbiosis with nodulating N-fixing bacteria (Chaer et al. 2011). Several leguminous trees, including *Caesalpinia sappan* L., *Enterolobium cyclocarpum* (Jacq.) Griseb., *Gliricidia sepium* (Jacq.) Walp., *Delonix regia* (Hook.) Raf., and *Cassia siamea* Lamk., have been used in the rehabilitation of a former tin mining area in Bangka Island, Indonesia (Narendra and Pratiwi 2016).

The use of organic amendemnts and microbial inoculants could increase soil fertility and help plant growth in post-mine soil. For example, chicken manure, cow manure, mulch, municipal waste compost, and litter compost might increase the success of remediation. The application of chicken manure to post-coal mining land in Indonesia, which had very low soil nutrient concentrations, increased the growth of *Samanea saman* (Darma et al. 2017). The treatment of municipal waste compost had growth rates comparable to untreated plants for mine site rehabilitation. The use of municipal green waste compost on degraded opencast coal land in the United Kingdom had significantly greater survival rates, compared with trees planted without green compost (Haigh et al. 2019). Other studies have shown that the addition of compost not only increases soil fertility but also reduces the concentration of trace elements in plant species grown in metal-contaminated mine soils (Martinez et al. 2014). Composting provides benefit for remediating by immobilizing trace elements (Cu, Pb, Zn, and As) in mine spoil (Tandy et al. 2008). Oyebamiji et al. (2018) reported the distribution of heavy metal such as, Pb, Zn, Cu, Ni, Cr and Fe in active mining soils in Southwestern Nigeria. The dissolution of

organic matter can increase the solubility of Al, Fe, and Pb within the reclaimed soils (Miller et al. 2019).

7.3. Effect of arbuscular mycorrhiza fungal inoculation on growth of tropical tree species under nursery and post-opencast mining land

All seedling (Table 5-1) of *G. arborea*, *S. saman*, and *E. cyclocarpum* including control were colonized by AM fungi in the nursery. No difference in AM colonization was noted among the seedlings. All seedling of *F. molucana* including control were colonized by AM fungi. Seedling inoculated with *R. clarus* had lower AM colonization than control seedlings and seedlings inoculated with *G. decipiens*. Shoot dry weight and root dry weight of *G. arborea* colonized by *G. decipiens* was higher than that of control seedlings. There was no difference in shoot dry weight and root dry weight between seedlings colonized by *R. clarus* and control seedlings. Shoot dry weight of *E. cyclocarpum* colonized by *R. clarus* and *G. decipiens* was higher than that of control seedlings. Shoot dry weight of seedlings colonized by *R. clarus* was higher than seedlings colonized by *G. decipiens*. Root dry weight of *E. cyclocarpum* colonized by *R. clarus* was higher than that of control seedling. There was no difference in root dry weight between seedlings colonized by *G. decipiens* and control seedlings. No difference in shoot dry weight and root dry weight were showed among the seedlings of *S. saman* and *F. molucana*.

AMF colonized all *G. arborea* seedlings (Figure 5-1A.), *S. saman* (Figure 5-2A.), *F. moluccana* (Figure 5-3A.), *E. cyclocarpum* (Figure 5-4A.), and control seedlings in the post-opencast bauxite mining field. Arbuscular mycorrhizal fungal colonization did not differ among treatments in any species. There was no significant difference in *G. arborea* and *S. saman* among treatments (Figure 5-1B; Figure 5-2B). Shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings (Figure 5-3B). There was no significant difference in shoot dry weight between seedlings inoculated with *R. clarus* and control seedlings. Shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* and *G. decipiens* was higher than that of control seedlings (Figure 5-4B). Shoot dry weight of seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens*.

The application of microbial inoculants, such as AMF, could improve the growth and survival of trees on post-nickel mining land. Plants are part of the ecosystem with many and diverse microorganisms in the soil. It has been noted that some of these microbes, such as mycorrhizal fungi or nitrogen fixing bacteria, play important roles in plant development by improving mineral nutrition (Jacoby et al. 2017). Several investigations have shown good results; the application of AMF increased the growth and survival of *P. falcataria* and *A. saman* in post-coal mining land in Indonesia (Wulandari et al. 2017). Additionally, the use of coconut powder inoculated with AMF increased the survival of *Anadenanthera colubrina* seedlings in post-mine soil in Brazil (Fernandes et al. 2019). The application of AMF and leguminous trees might be used to increase the success of revegetation programs in post-nickel mining land. In our study, Fe content in post-mine soil was seventeen times higher than natural forest soil. Agus et al. (2018) reported that revegetation with fast-growing legume species of *Pongamia pinnata* and AMF application can not only increase nutrient contents of post-coal mining soil but also increases Fe absorption, which is mostly accumulated in the root system.

7.4. Remediation of contaminated soil after oil extraction using the plants

7.4.1. Petroleum hydrocarbons degradation in soil using the plants of the Aster family

Concentrations of TPH in soils without FeCl₃ and NTA and soils with FeCl₃ and NTA were similar to that in the initial soil (40 g kg⁻¹ TPH), 2 MAT (Figure 6-1). Concentration of TPH in soil cultivated with *C. caudatus* was lower than that in the initial soil, 2 MAT. Concentrations of TPH in soil without FeCl₃ and NTA and soil with FeCl₃ and NTA, were similar to that in the initial soil, 3 MAT. Concentrations of TPH in soils cultivated with *C. officinalis*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. were lower than that in the initial soil.

Concentrations of TPH in soils cultivated with *A. filipendulina*, *A. tinctoria*, *T. erecta*, *C. coronarium*, *C. officinalis*, *C. chinensis*, and *C. caudatus* were lower than that in the initial soil, 2 MAT (Figure 6-2). Concentration of TPH in soil with

FeCl₃ and NTA was similar to that in the initial soil (90 g kg⁻¹ TPH). However, concentrations of TPH in soils without plants and without FeCl₃ and NTA were lower than that in the initial soil, 2 MAT. Concentrations of TPH in soils cultivated with *T. erecta*, *A. tinctoria*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. were lower than that in the initial soil, 3 MAT. Concentrations of TPH in soils without plants, without FeCl₃ and NTA, and with FeCl₃ and NTA were similar to that in the initial soil, 3 MAT.

TPH degradation was 24–38% and 17–34% in the treatments with plants at 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively. The degradation was 9–13% and 14% in unvegetated controls at 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively (Figure 6-3; Figure 6.4). The highest decrease was 38% and 34% for 40 g kg⁻¹ and 90 g kg⁻¹ TPH, respectively. The degradation values were higher than 27% at 20 g kg⁻¹ TPH with *Pharbitis nil* for 127 days (Zhang et al. 2010), 20% at 20 g kg⁻¹ TPH with *Mirabilis jalapa* for 127 days (Peng et al. 2009), and 8% at 7.5 g kg⁻¹ TPH with *Samanea saman* for 101 days (Bento et al. 2012).

Some plants decomposed TPH through the rhizosphere Fenton reaction. Hydrogen peroxide and Fe²⁺ produce hydroxyl radicals (H₂O₂ + Fe²⁺ → Fe³⁺ + OH⁻ + *OH). These hydroxyl radicals scavenge organic compounds such as petroleum hydrocarbon (RH + *OH → R* + H₂O); H₂O₂ is necessary for this reaction. The TPH concentrations did not decrease in the control treatments with FeCl₃ and NTA because of the lack of H₂O₂. Erect bur marigold (*Bidens tripartita* L., Asteraceae) had high H₂O₂ concentrations in their root-tips (>1 mM) and decomposed CH₄ through the rhizosphere Fenton reaction (Wagatsuma et al. 2007). Similarly, the Asteraceae plants in the present study could have high H₂O₂ concentrations in the rhizosphere and could decompose TPH through the rhizosphere Fenton reaction.

Asteraceae plants may also absorb petroleum hydrocarbon from contaminated soil. Phytoremediation has provided valuable insights into the types and specific mechanisms of the removal of organic contaminants (Pilon-Smits 2005). Across plant membranes some organic compounds can be transported. Low molecular weight compounds can be absorbed from the soil and transported to the plant leaves, which can be released through evapotranspiration. Some non-volatile compounds can be degraded to nontoxic compounds through enzymatic

modification and sequestration in plants. The rhizosphere provides an active ecosystem where plants can interact through the Fenton reaction for effective degradation of TPH in soil. The hydroxyl radical produced by Asteraceae plants may also decompose the hydrocarbon to CO₂.

7.4.2. Relationship between biomass of Asteraceae plants and degradation of TPH in the soil

A positive correlation was noted between root biomass of Asteraceae plants and degree of TPH degradation in the initial 40 g kg⁻¹ TPH soil (Figure 6-5). Root growth was necessary for remediation, and therefore higher levels of root biomass could increase degradation of TPH in the soil (Gurska et al. 2009). The large root biomass may also produce more hydroxyl radicals through the Fenton reaction. It has been shown that these hydroxyl radicals are capable of decomposing and degrading organic contaminants such as petroleum hydrocarbons (Chamarro et al. 2001). There was no positive correlation between the root biomass of Asteraceae plants and the degree of TPH degradation in the initial 90 g kg⁻¹ TPH soil (Figure 6-6). The root biomass of Asteraceae plants smaller than that in the initial 40 g kg⁻¹ TPH soil (Table 6-1). High TPH concentrations inhibit root growth of Asteraceae plants, because the toxic contaminants restrain the proliferation of plant roots.

Shoot dry weight of *C. officinalis* in 40 g kg⁻¹ TPH soil was higher than that of *A. tinctoria*, 3 MAT and tended to be higher than that of *C. chinensis*, *C. caudatus*, and *Tagetes* sp. In 90 g kg⁻¹ TPH soil, shoot dry weight of *T. erecta* was higher than that of *A. tinctoria*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. and tended to be higher than that of *Z. elegans* (Table 6-2). Root dry weight of *C. caudatus* in 40 g kg⁻¹ TPH soil was higher than that of *A. tinctoria* and tended to be higher than that of *C. officinalis*, *C. chinensis*, and *Tagetes* sp. There were no differences in root dry weights among *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in 90 g kg⁻¹ TPH soil (Table 6-2). These species maintained growth rate in the initial TPH soil concentration and may be the most tolerant Asteraceae plants. Growth of *A. tinctoria* greatly decreased in the presence of 40 g kg⁻¹ TPH in the soil and did not degrade the TPH. This species is possibly sensitive to TPH, causing stress and limiting its rate of phytoremediation (Huang et al. 2005). Contaminated

soils also tend to be nutrient poor and/or lack microbial diversity, which contributes to sub-optimal plant biomass accumulation, as well as impedes rates of remediation (White et al. 2006). However, in 90 g kg⁻¹ TPH soil, *A. tinctoria* degraded the TPH. This suggests that the abilities of plants to degrade soil TPH are not only directly related to their growth but also their adaptive abilities to different contamination conditions (Bento et al. 2012).

The root dry weight of *C. caudatus* was higher than that of *A. tinctoria* in 40 g kg⁻¹ TPH soil. There was no difference in root dry weight of *C. officinalis*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. However, root dry weight of *C. caudatus* was higher than that of the other Asteraceae plants, 3 MAT (Table 6-1). There was no difference in root dry weight of *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in the initial 90 g kg⁻¹ TPH soil. However, root dry weight of *C. caudatus* tended to be higher than that of the above-mentioned plants, 3 MAT. These data suggest that *C. caudatus* has the most potential among the studied Asteraceae plants for rhizosphere Fenton reaction to degrade TPH concentration in the soil. In fact, *C. caudatus* was consistent among the Asteraceae plants in its ability to degrade TPH in the initial 40 and 90 g kg⁻¹ soil, 2 and 3 MAT.

7.4.3. Plant survival

Plants for phytoremediation must survive in contaminated soil. The plant is a connecting line with contaminated material, which is an extension of another phase that will interact with certain contaminants, as the plant offers phases that include solid, organic, organic lipids, water and gas phase (Landmeyer 2012). There are differences in the tolerance to TPH concentration among plant species (Kaimi 2007). *A. filipendulina*, *T. erecta*, *Z. elegans*, and *C. coronarium* in 40 g kg⁻¹ TPH soil died between 2 and 3 MAT (Table 6-1). Other plants grew until 3 MAT. The shoot dry weight of *C. officinalis* was higher than that of *A. tinctoria*. There was no difference in shoot dry weight among *C. chinensis*, *C. caudatus*, and *Tagetes* sp. *C. officinalis*, *C. coronarium*, and *A. filipendulina* in 90 g kg⁻¹ TPH soil died between 2 and 3 MAT. Other plants grew until 3 MAT. The shoot dry weight of *T. erecta* was higher

than that of *A. tinctoria*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. There was no difference in the shoot dry weights between *T. erecta* and *Z. elegans*.

The root dry weight of *C. caudatus* was higher than that of *A. tinctoria* in 40 g kg⁻¹ TPH soil. There was no difference in root dry weights among *C. officinalis*, *C. chinensis*, and *Tagetes* sp. (Table 6-2). There was no difference in the root dry weights among *A. tinctoria*, *T. erecta*, *Z. elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. in 90 g kg⁻¹ TPH soil.

A. tinctoria, *C. officinalis*, *Tagetes* sp., *C. chinensis*, *C. caudatus*, *T. erecta*, and *Z. elegans* were hydrocarbon tolerant. However, *A. filipendulina* and *C. coronarium* cultivated in the initial 40 and 90 g kg⁻¹ TPH soils died during the experiment (Table 6-1; Table 6-2). This could be because they are sensitive to the TPH concentrations in the soil. In severely polluted soils, high concentrations of TPH inhibited seed germination and created nutrient deficient conditions, in which the plants could not survive (Peng et al 2009). These plants could also be sensitive to the soil pH, as in this study the soil pH levels were approximately 4.5–5.5. Studies have shown that acidic condition is the optimum condition for the Fenton's process (Farzadkia et al. 2014). By adding Fenton's reagent, the pH is reduced, which is accompanied by the production of intermediates such as carboxylic acid. Therefore, the maximum reduction rate occurred at a high pH (Kumar et al. 2012).

The concentration of contaminants tended to inhibit plant growth, including root growth, in part due to oxidative stress (Huang et al. 2005). However, we showed that Asteraceae plants had high potential for use in the phytoremediation of petroleum contaminated soil.

Chapter VIII. Summary

Tropical rainforests in Indonesia supply the world's abundant terrestrial biodiversity and play an important role in the global climate. However, area of rainforest in Indonesia has been decreasing from time to time. Opencast bauxite mining, opencast nickel mining, gold tailings and oil extraction have significant impacts on tropical rainforests, affecting their vegetation and animal biodiversity, soil fertility, ecological function and services. Land remediation activities are required for reduction of damage caused by opencast mining, gold tailings and oil extraction. The remediation of damaged forests in Indonesia is difficult, due to effect of mining and oil extraction process on the landscape of the local environment. It is necessary to select plant species, and to use microbial activity for remediation of degraded areas. The aims of this study were (1) to clarify the effects of opencast bauxite mining on the chemical characteristics of soil and its effect on the plant growth, (2) to clarify chemical characteristics of gold mine tailings and its effect on the growth of plants under greenhouse conditions, (3) to determine the impact of opencast nickel mining on soil chemical properties and its effect on the growth of two fast-growing tropical tree species under greenhouse conditions, (4) to determine the effect of inoculation with two indigenous arbuscular mycorrhizal (AM) fungi on the growth of plants in the nursery and post-opencast bauxite mining field conditions, and (5) to clarify the effect of Asteraceae plants on degradation of petroleum hydrocarbon in contaminated soils.

Soils were collected from both natural forest and post mining land in Indonesia, nearby bauxite mining site in Bintan Island, Sumatera; nickel mining site at Sorowako, East Luwu, South Sulawesi and gold mining site at Pongkor, Bogor, West Java. Soil pH, total carbon (C), nitrogen (N), and available phosphorus (P) concentrations, cation exchange capacity (CEC), C/N ratio and exchangeable K, Na, Mg, Ca, Fe and Ni concentrations of post mining soil from bauxite, nickel and gold tailings were analyzed.

The plant species used in this investigation were *Gmelina arborea* (Verbenaceae), *Samanea saman* (Fabaceae), *Falcataria moluccana* (Fabaceae), *Enterolobium cyclocarpum* (Fabaceae). Aster family plants were also used,

including *Achillea filipendulina* Lam., *Anthemis tinctorial* (L.) J. Gay ex Guss., *Tagetes erecta* L., *Chrysanthemum coronarium* (L.) Cass. Ex Spach., *Calendula officinalis* L., *Zinnia elegans* Jacq., and *Callistephus chinensis* L., *Cosmos caudatus* Kunth, and *Tagetes* sp. *Falcataria moluccana* and *S. saman* were grown for 15 weeks and shoot heights, shoot and root dry weights were measured. Two native AM fungi, *Rhizophagus clarus* and *Gigaspora decipiens*, were inoculated into seeds of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum*. The seeds were sown in post-bauxite mining soil and grown in the nursery for three months. The seedlings were transplanted into a post-opencast bauxite mining field and grown for 12 months. AM fungal colonization and shoot and root dry weights were measured. Initial soils with 40 and 90 g kg⁻¹ of total petroleum hydrocarbon (TPH) were prepared. There were three treatments: (1) no addition, (2) addition of FeCl₃ and nitrilotriacetic acid (NTA) solution, and (3) addition of FeCl₃ and NTA and the cultivation of nine Asteraceae plants. The concentration of (TPH) was measured using infrared spectrophotometer, 2 and 3 months after transplanting (MAT). Shoot and root dry weight were measured 3 MAT.

Total N, C, and available P concentrations and exchangeable Ca, Mg, and Na concentrations in the post bauxite mining soils decreased by 75, 75.7, 15.7, 92, 100, and 52%, respectively, in comparison with the natural forest soils. The shoot and root dry weights of *F. moluccana* when grown in the post bauxite mining soils were also lower than those from the natural forest soils. There was no difference in the shoot and root dry weights of *A. saman* when grown in the two soil types. Total N, C and available P of gold mine tailings were lower than that of forest soil. CEC, Mg, K and Fe of gold mine tailings were lower than that of forest soil. C/N ratio of gold mine tailings were higher than that of forest soil. The pH (KCl), pH (H₂O), Ca and Na concentration of gold-mine tailings were higher than that of forest soil. There was no difference in Ni concentration between forest soil and gold mine tailings. Shoot dry weight and root dry weight of *F. moluccana* on gold mine tailings were lower than that of forest soil. Shoot and root dry weight of *A. saman* grown on gold mine tailings were higher than that of forest soil. The post nickel mining soils TN, TC, available P, CEC, and exchangeable Ca and Na concentrations decreased by 98%, 93%, 11%, 62%, 85%, and 74%, respectively, in comparison

with the natural forest soils. The pH of post nickel mining soil was higher than natural forest soil. Shoot dry weight of *F. moluccana* seedlings grown in post mining soil was lower than that of seedlings grown in natural forest soil. There was no difference in shoot dry weight between *A. saman* seedlings grown in natural forest soil and post mining soil, as well as root dry weights of both species. Under nursery conditions, *G. arborea* inoculated with *G. decipiens* increased shoot and root dry weights by 1,431 and 359 %, respectively, while shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* and *G. decipiens* increased by 510 and 220%, respectively, in comparison with control seedlings. Root dry weight of *E. cyclocarpum* inoculated with *R. clarus* increased by 224%, in comparison with control seedlings. Shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* increased by 90%, in comparison with seedlings inoculated by *G. decipiens*. Twelve months after transplanting into post-opencast field conditions, the shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings by 188%. Shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* and *G. decipiens* increased by 198% and 149%, respectively, in comparison with control seedlings. Shoot dry weight of *E. cyclocarpum* seedlings inoculated with *R. clarus* was higher by 20% than that of seedlings inoculated with *G. decipiens*.

The concentration of TPH in soil cultivated with *Cosmos caudatus* was lower than that of the initial soil (40 g kg⁻¹ TPH), 2 MAT. The concentrations of TPH in soils cultivated with *Calendula officinalis*, *Callistephus chinensis*, *C. caudatus*, and *Tagetes* sp. were also lower than that in the initial soil, 3 MAT. The concentrations of TPH in soils cultivated with *Achillea filipendulina*, *Anthemis tinctoria*, *Tagetes erecta*, *Chrysanthemum coronarium*, *C. officinalis*, *C. chinensis*, and *C. caudatus* were lower than that in the initial soil (90 g kg⁻¹ TPH), 2 MAT. The concentrations of TPH in soils cultivated with *T. erecta*, *A. tinctoria*, *Zinnia elegans*, *C. chinensis*, *C. caudatus*, and *Tagetes* sp. were lower than that in the initial soil, 3 MAT. *A. filipendulina* and *C. coronarium* died at both 40 and 90 kg⁻¹ TPH soils.

These findings suggest that opencast bauxite mining, opencast nickel mining and gold tailings decreased the soil fertility, low fertility inhibited the initial growth of two leguminous tree, *F. moluccana* and *A. Saman*. and *A. saman* were

adapted better to post mining land. AM fungal inoculation promoted the growth of tropical tree species on post opencast bauxite mining land both in the nursery and field conditions. The roots of Asteraceae plants degraded petroleum hydrocarbon in contaminated soil and *C. chinensis* and *Z. elegans* were more suitable for TPH remediation. Plant survival and extensive root system were important factors for the remediation of TPH in contaminated soil.

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Table 2-1. Chemical characteristics of the soils from the natural forest and post bauxite mining land.

Location	pH (H ₂ O)		pH (KCl)		Total carbon (g kg ⁻¹)		Total nitrogen (g kg ⁻¹)		C/N ratio		Available P (mgP ₂ O ₅ kg ⁻¹)		CEC (cmol kg ⁻¹)		Ca (mg kg ⁻¹)		Mg (mg kg ⁻¹)		K (mg kg ⁻¹)		Na (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Ni (mg kg ⁻¹)			
Natural forest	5.06	a	3.99	b	2.88	a	1.6	a	19.0	b	13.4	a	12.75	a	11.24	a	1.82		38.04	a	1.35	a	4.49	a	7.82	a		
SE	0.07		0.04		4.1		0.3		0.9		0.2		1.71		0.29		0.74		19.39		0.52		0.52		1.02			
Postmining	4.96	a	4.52	a	7.0	b	0.4	b	39.4	a	11.3	b	8.90	a	0.92	b	bdl*		81.54	b	0.65	b	3.72	a	5.72	a		
SE	0.05		0.09		2.5		0.2		8.8		0.1		2.44		0.22				45.05		0.08		0.18		1.52			

Different letters within column indicate a significant differences ($P < 0.05$) by t-test. Mean \pm standard error (SE) are shown (n=15)

*bdl: below the detection limit

Table 3-1. Soil chemical characteristic between forest soil and tailing of gold mining.

Location	Total		C/N ratio	pH (H ₂ O)		pH (KCl)		Available																		
	nitrogen (%)	carbon (%)				P (mgP ₂ O ₅ 100 g ⁻¹)	CEC (cmol kg ⁻¹)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	K (mg kg ⁻¹)	Na (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Ni (mg kg ⁻¹)													
Forest soil	a	5.53	a	24.46	a	4.28	a	3.77	a	2.15	a	30.96	a	57.09	a	10.30	a	89.65	a	2.07	a	3.21	a	4.86	a	
SE	0.04	0.53		7.71		0.11		0.08		0.19		2.91		10.98		1.49		25.64		0.25		0.05		1.00		
Tailing	0.04	b	1.26	b	38.77	b	6.48	b	6.74	b	1.31	b	7.58	b	347.29	b	5.41	b	28.21	b	12.28	b	0.89	b	1.60	a
SE	0.00	0.16		5.91		0.04		0.06		0.07		0.80		5.61		5.41		5.34		2.12		0.38		0.80		

Different letters within column indicate significant difference ($P < 0.05$) by t-test. Mean \pm standard error are (SE) are shown (n=15)

Table 4-1. Chemical properties of soils from natural forest and post nickel mining land.

Chemical properties	Natural forest		Post mining Land		Change (%)
pH (H ₂ O)	5.02 ± 0.10	b	6.31 ± 0.05	a	+1.29 (26)
pH (KCl)	4.66 ± 0.10	b	6.31 ± 0.07	a	+1.65 (35)
Total carbon (g kg ⁻¹)	40.20 ± 0.25	a	2.90 ± 0.05	b	-37.30 (93)
Total nitrogen (g kg ⁻¹)	2.60 ± 0.02	a	0.057 ± 0.01	b	-2.54 (98)
C/N (ratio)	15.84 ± 0.29	b	65.12 ± 8.72	a	+49.28 (311)
Available P (mg P ₂ O ₅ kg ⁻¹)	12.30 ± 0.02	a	11.00 ± 0.02	b	-1.30 (11)
CEC (cmol kg ⁻¹)	8.23 ± 0.67	a	3.15 ± 0.71	b	-5.08 (62)
Ca (mg kg ⁻¹)	5.85 ± 0.93	a	0.86 ± 0.29	b	-4.99 (85)
Mg (mg kg ⁻¹)	7.32 ± 0.81	a	6.27 ± 1.18	a	-1.05 (14)
K (mg kg ⁻¹)	10.84 ± 6.23	a	5.25 ± 5.25	a	-5.59 (52)
Na (mg kg ⁻¹)	0.81 ± 0.19	a	0.21 ± 0.08	b	-0.6 (74)
Fe (mg kg ⁻¹)	2.12 ± 0.09	a	38.30 ± 18.73	a	+36.18 (1707)
Ni (mg kg ⁻¹)	3.30 ± 0.33	a	3.17 ± 0.45	a	-0.13 (4)

Different letters within row indicate significant difference ($P < 0.05$) by t-test.

Mean ± standard error are shown (n=15)

Table 5-1. Mycorrhizal colonization, shoot and root dry weight of *Gmelina arborea*, *Samanea saman*, *Falcataria moluccana* and *Enterolobium cyclocarpum* grown with or without mycorrhizal fungi under nursery conditions adjacent to a bauxite mine in Bintan Riau Islands, Indonesia, three months after sowing.

Plant Treatment	Mycorrhizal Colonization			Shoot Dry Weight			Root Dry Weight					
	(%)			(g/plant)			(g/plant)					
<i>Gmelina arborea</i>												
Control	92.77	±	5.33	a	0.48	±	0.45	b	0.32	±	0.13	b
<i>Rhizophagus clarus</i>	89.93	±	12.22	a	4.47	±	3.86	ab	0.88	±	0.61	ab
<i>Gigaspora decipiens</i>	74.90	±	22.17	a	7.35	±	3.45	a	1.47	±	0.23	a
<i>Samanea saman</i>												
Control	10.13	±	15.35	a	0.84	±	0.61	a	0.50	±	0.20	a
<i>Rhizophagus clarus</i>	24.03	±	28.77	a	1.09	±	0.47	a	0.42	±	0.23	a
<i>Gigaspora decipiens</i>	2.63	±	3.55	a	0.44	±	0.07	a	0.35	±	0.12	a
<i>Falcataria moluccana</i>												
Control	82.08	±	14.86	a	1.08	±	0.57	a	0.27	±	0.13	a
<i>Rhizophagus clarus</i>	25.80	±	15.50	b	1.93	±	0.74	a	0.41	±	0.18	a
<i>Gigaspora decipiens</i>	63.13	±	6.88	a	1.22	±	0.42	a	0.21	±	0.03	a
<i>Enterolobium cyclocarpum</i>												
Control	26.50	±	24.44	a	2.29	±	1.06	c	1.11	±	0.49	b
<i>Rhizophagus clarus</i>	3.05	±	3.54	a	13.97	±	3.22	a	3.60	±	1.09	a
<i>Gigaspora decipiens</i>	5.65	±	7.06	a	7.34	±	0.88	b	2.38	±	0.28	ab

For each plant species, different letters within the column indicate significant difference ($P = .05$) by Tukey HSD test. Means \pm standard error are shown ($n = 6$)

Table 6-1. Shoot and root dry weight of nine plants of Asterales in 40 g kg⁻¹ total petroleum hydrocarbon concentration, 3 months after planting.

Plant species	Shoot dry weight (g/plant)				Root dry weight (g/plant)			
<i>Achillea filipendulina</i>	dead				dead			
<i>Anthemis tinctoria</i>	0.007	±	0.002	b	0.013	±	0.003	b *
<i>Calendula officinalis</i>	0.039	±	0.006	a	0.045	±	0.015	ab
<i>Callistephus chinensis</i>	0.021	±	0.009	ab	0.040	±	0.018	ab *
<i>Chrysanthemum coronarium</i>	dead				dead			
<i>Cosmos caudatus</i>	0.030	±	0.003	ab	0.138	±	0.057	a *
<i>Tagetes erecta</i>	dead				dead			
<i>Tagetes sp.</i>	0.027	±	0.004	ab	0.064	±	0.017	ab *
<i>Zinnia elegans</i>	dead				dead			

Different letter in the columns indicate significant differences ($P < 0.05$) by Tukey HSD test. Data are shown as mean \pm standard error (n=5)

* indicates significant difference between 40 and 90 g kg⁻¹ petroleum hydrocarbon concentrations

Table 6-2. Shoot and root dry weights of nine plants of Asterales in 90 g kg⁻¹ total petroleum hydrocarbon concentration, 3 months after planting.

Plant species	Shoot dry weight (g/plant)			Root dry weight (g/plant)		
<i>Achillea filipendulina</i>	dead			dead		
<i>Anthemis tinctoria</i>	0.009	± 0.002	c	0.01	± 0.004	a
<i>Calendula officinalis</i>	dead			dead		
<i>Callistephus chinensis</i>	0.016	± 0.003	bc	0.02	± 0.003	a
<i>Chrysanthemum coronarium</i>	dead			dead		
<i>Cosmos caudatus</i>	0.018	± 0.018	bc	0.05	± 0.025	a
<i>Tagetes erecta</i>	0.061	± 0.024	a	0.03	± 0.005	a
<i>Tagetes sp.</i>	0.014	± 0.004	bc	0.03	± 0.021	a
<i>Zinnia elegans</i>	0.043	± 0.010	ab	0.01	± 0.002	a

Different letters in the columns indicate significant differences ($P < 0.05$) by Tukey HSD test. Data are shown as mean ± standard error (n=5)



Figure 1-1. Tropical rain forest in Indonesia



Figure 1-2. Surface land of tropical rain forest



Figure 1-3. Nickel mining in natural forest (A) and opencast nickel mining land (B) in Sorowako, East Luwu, Sulawesi, Indonesia



Figure 1-4. Post nickel mining after land preparation activity before remediation at Sorowako, East Luwu, Sulawesi, Indonesia.



Figure 1-5. Tailings land at Pongkor, West Java, Indonesia



Figure 1-6. Soil contaminated by petroleum hydrocarbon



Figure 1-7. Phytoremediation land

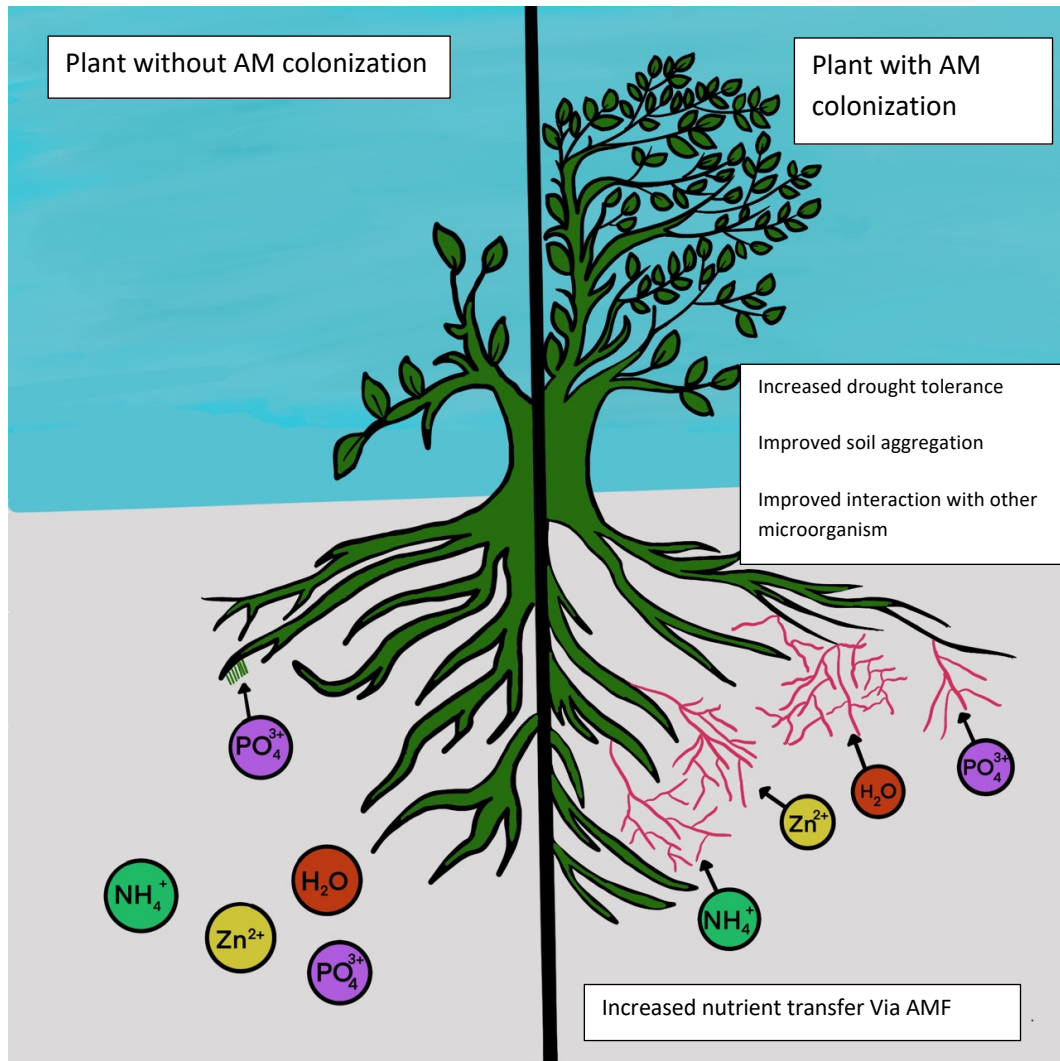


Figure 1-8. Plant without and with AM colonization. AM colonization increased nutrient transfer, drought tolerance to the plant, interaction with other microorganism and soil agregation

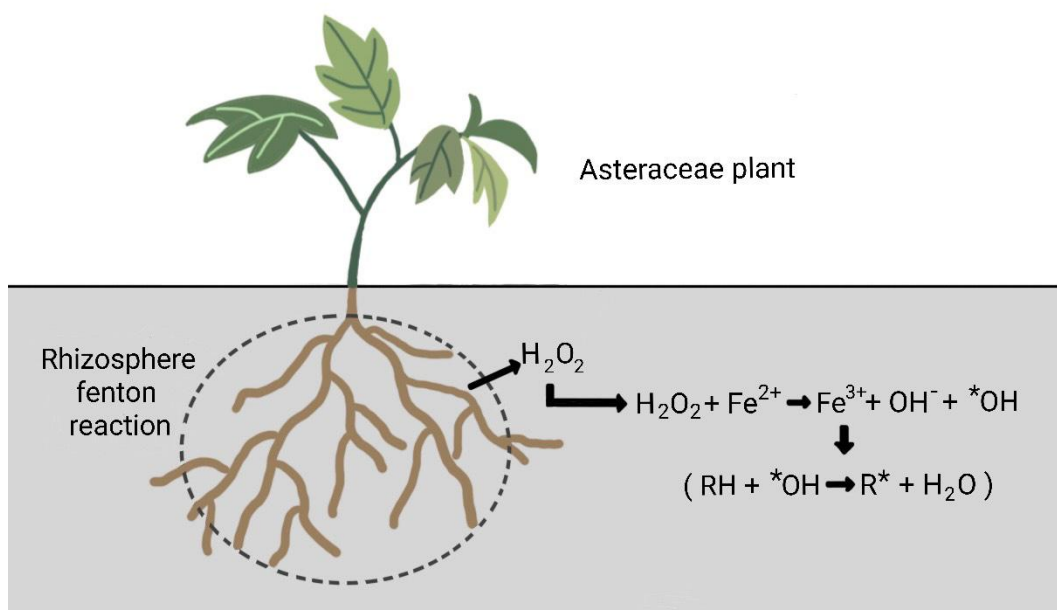


Figure 1-9. The rhizosphere fenton reaction. Hydrogen peroxide and Fe^{2+} produce hydroxyl radicals. These hydroxyl radicals scavenge petroleum hydrocarbon



Figure 2-1. Map of Bintan Island, Indonesia.

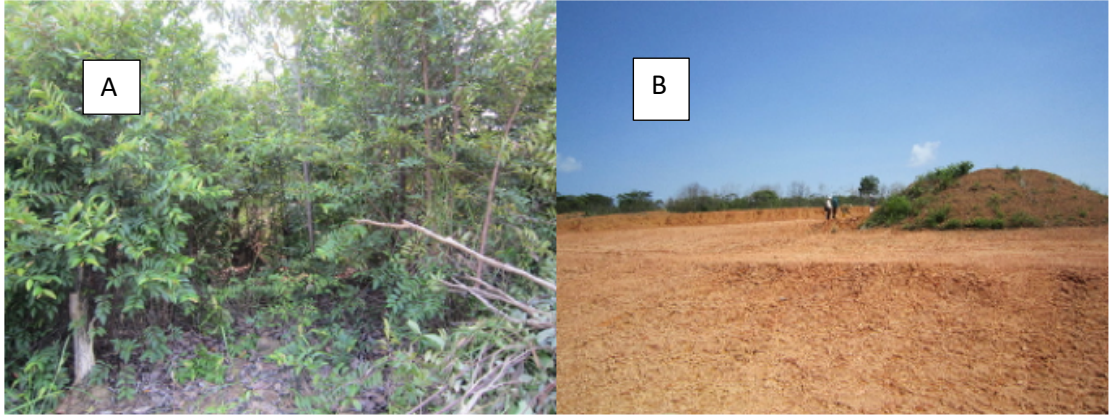


Figure 2-2. Natural forest (A) and post opencast (B) bauxite mining land, Bintan Island, Indonesia.

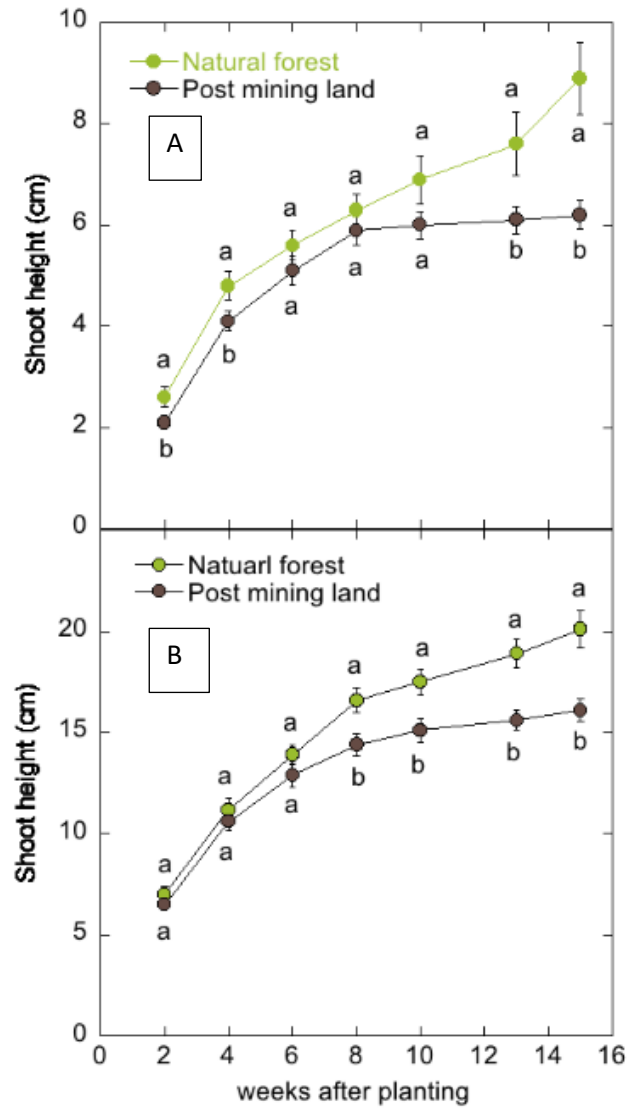


Figure 2-3. Shoot height of *Falcataria moluccana* (A) and *Albizia saman* (B) grown on natural forest soils and post bauxite mining soils for 15 weeks under greenhouse conditions (n=15).

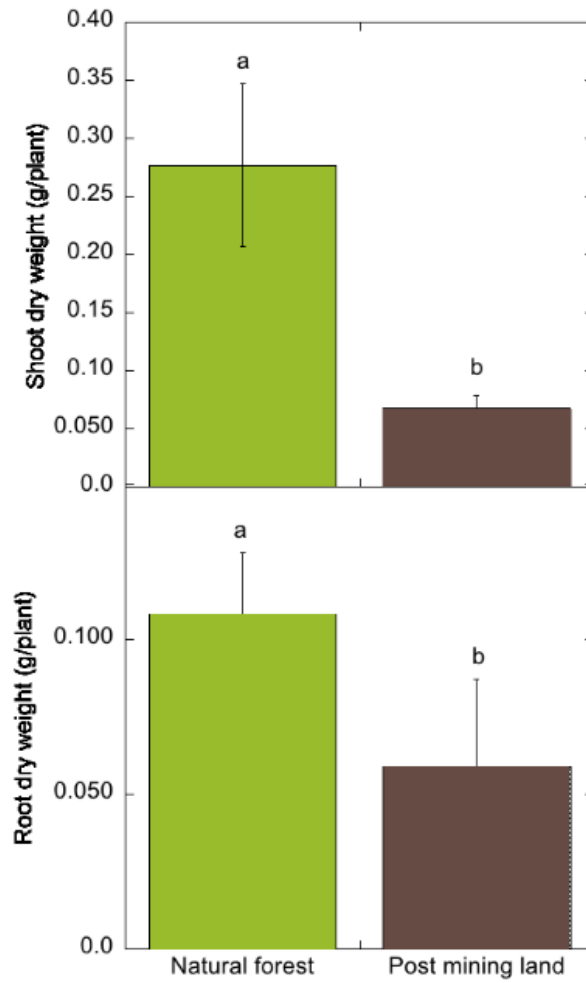


Figure 2-4. Shoot dry weight and root dry weight of *Falcataria moluccana* grown on natural forest soils and post bauxite mining soils for 15 weeks under greenhouse conditions (n=15).

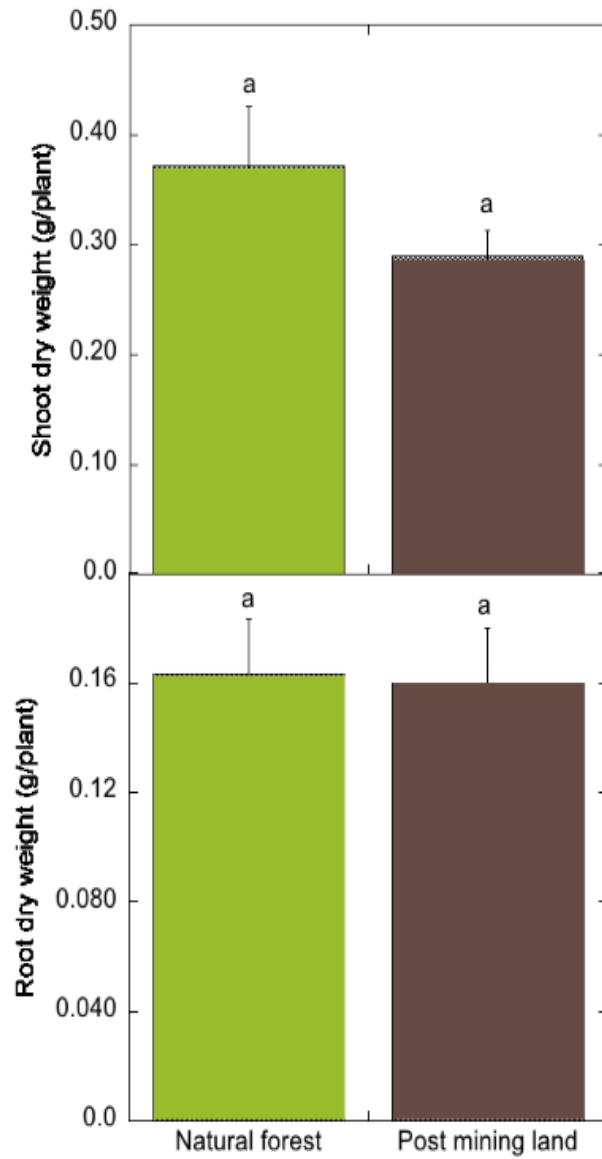


Figure 2-5. Shoot dry weight of *Albizia saman* grown on natural forest soils or post bauxite mining soils for 15 weeks under greenhouse conditions (n=15).

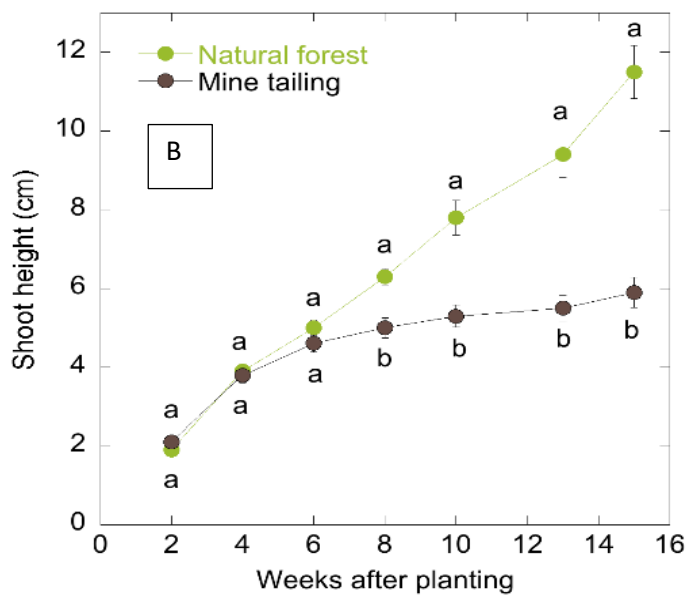
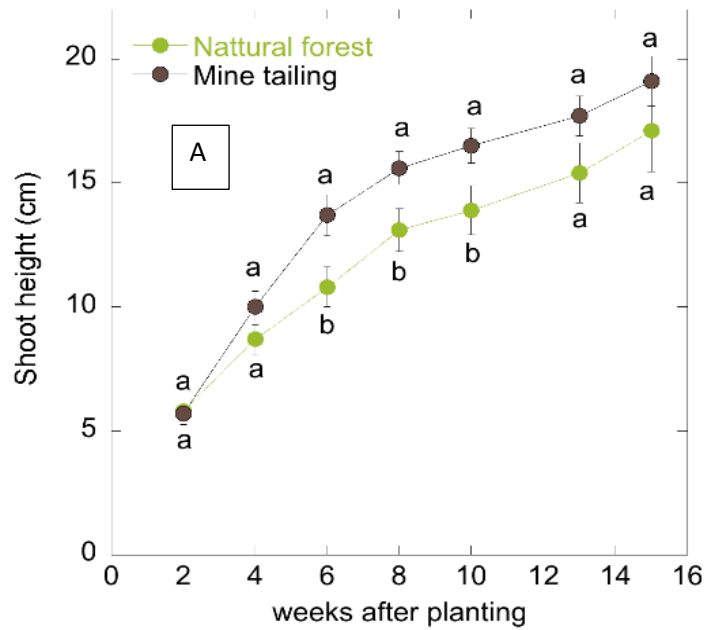


Figure 3-1. Shoot height of *Albizia saman* (A) and *Falcataria moluccana* (B) grown on forest soil and gold mine tailings for 15 weeks under greenhouse conditions. Different letters of each week indicate significant difference ($P = .05$) by t-test. Data are shown as mean \pm standard error (n=15).

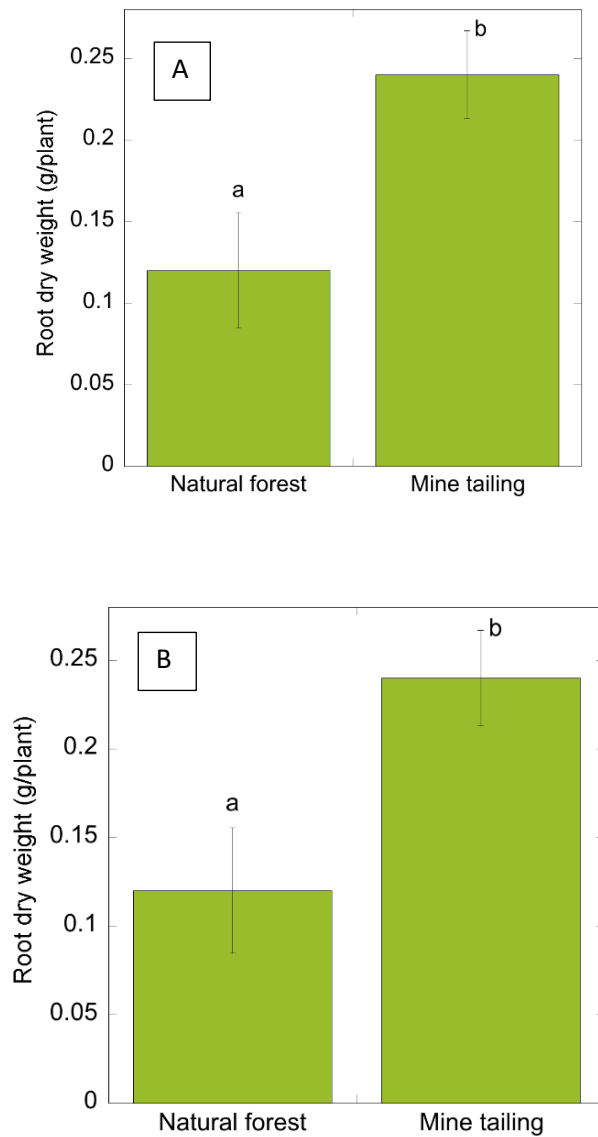


Figure 3-2. Shoot (A) and root (B) height of *Albizia saman* grown on natural forest soil and gold mine tailing for 15 weeks. Different letters of each column chart indicate significant difference ($P = .05$) by t-test. Data are shown as mean \pm standard error (n=15).

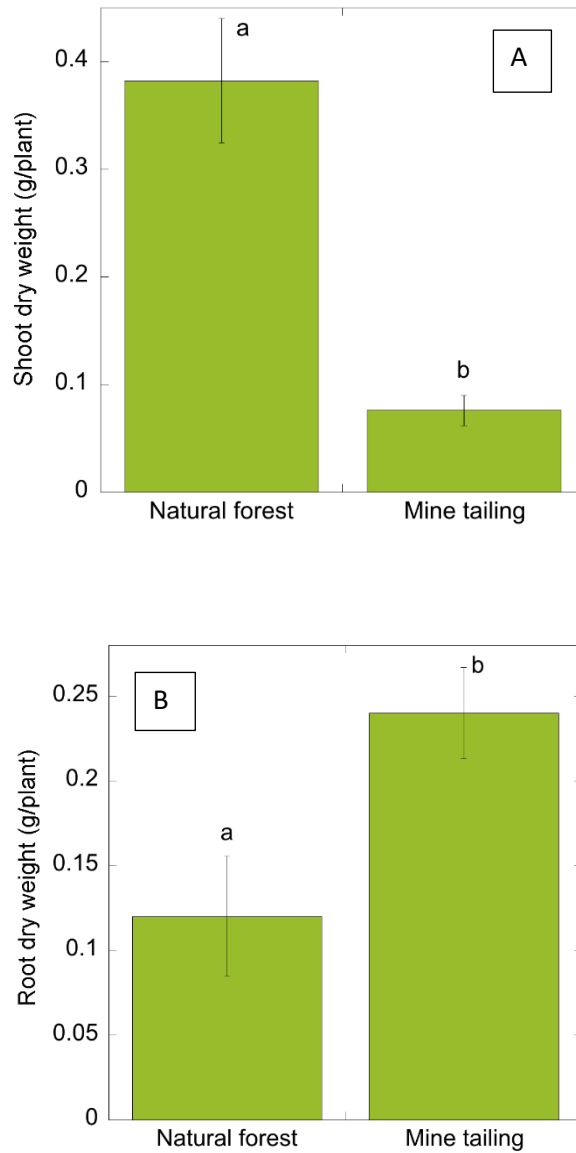


Figure 3-3. Shoot (A) and root (B) height of *Falcataria moluccana* grown on natural forest soil and gold mine tailing for 15 weeks. Different letters of each column chart indicate significant difference ($P = .05$) by t-test. Data are shown as mean \pm standard error (n=15).



Figure 4-1. The location of soil sample collection at PT Vale Indonesia, Sorowako, East Luwu, South Sulawesi, Indonesia.

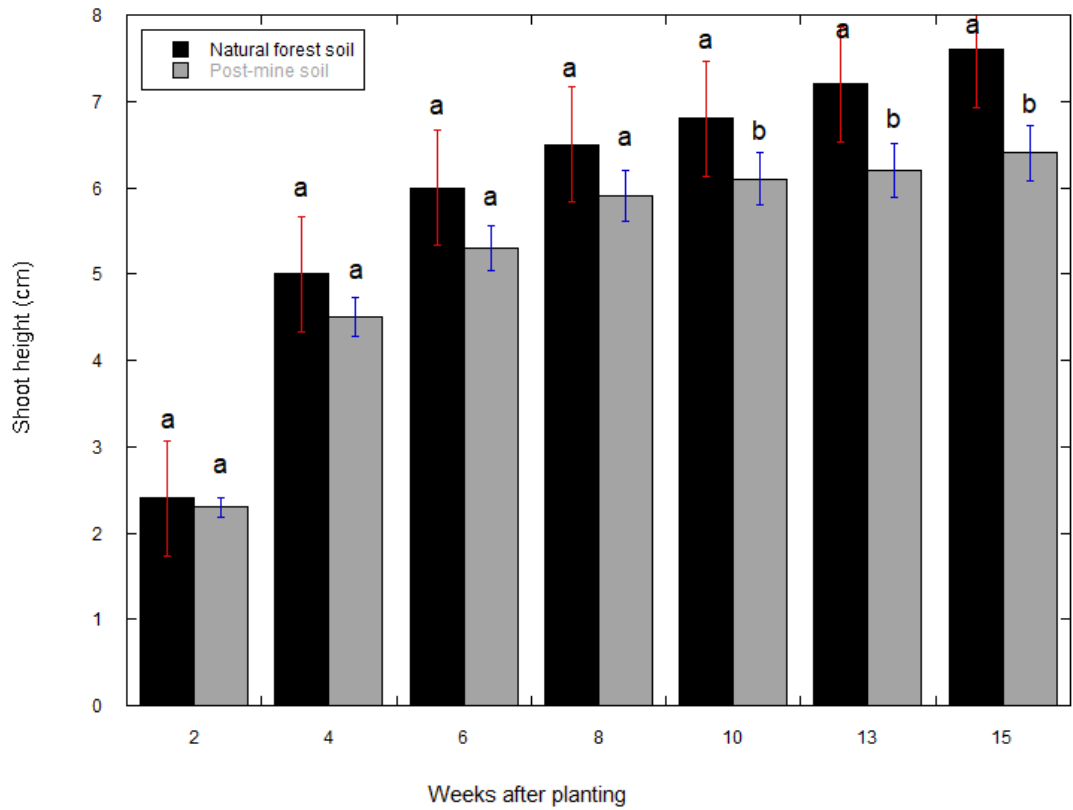


Figure 4-2. Mean shoot height of *Falcataria moluccana* grown in soil from post-nickel mining land and natural forest for 15 weeks under greenhouse condition. Different letter indicated significant differences ($p=0.05$). Error bars indicate standard error ($n=15$).

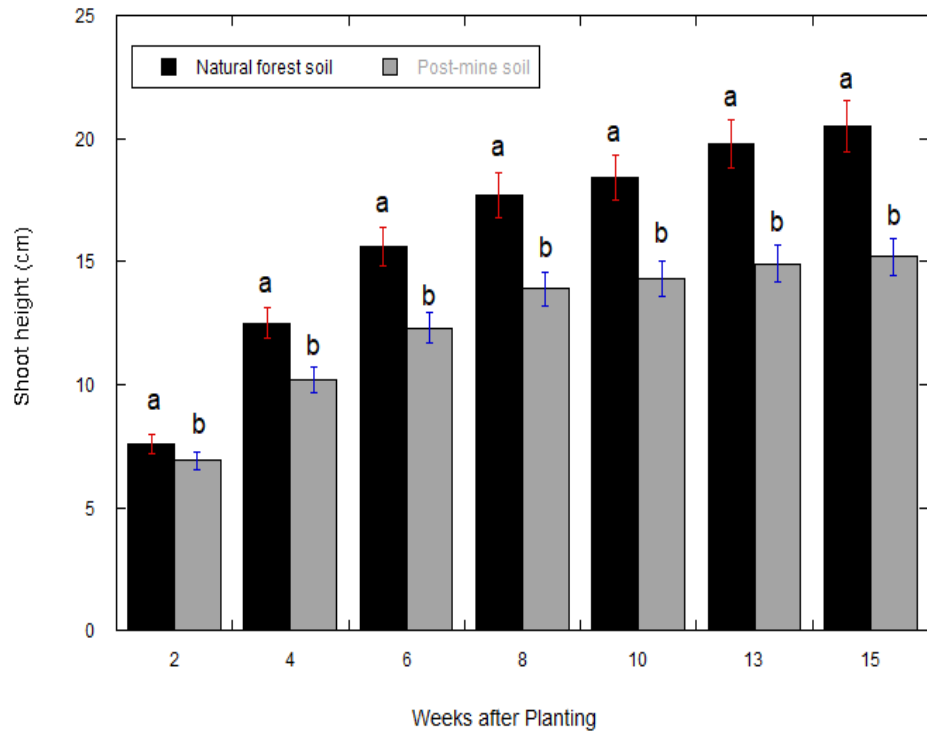


Figure 4-3. Mean shoot height of *Albizia saman* grown in soil from post-nickel mining land and natural forest for 15 weeks under greenhouse condition. Different letter indicated significant differences ($p=0.05$). Error bars indicate standard error ($n=15$).

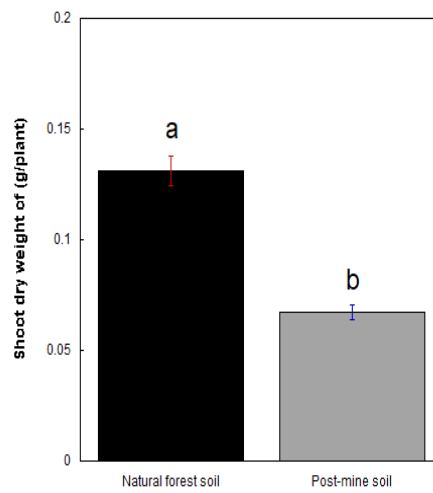
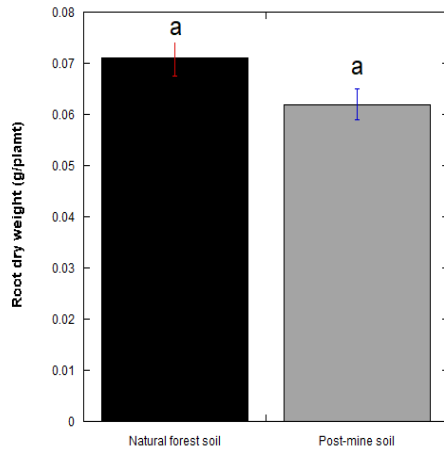


Figure 4-4. Mean shoot and root dry weight of *Falcataria moluccana* 15 weeks after planting in soil from post-nickel mining land and natural forest. Different letter indicated significant differences ($p=0.05$). Error bars indicate standard error ($n=15$).

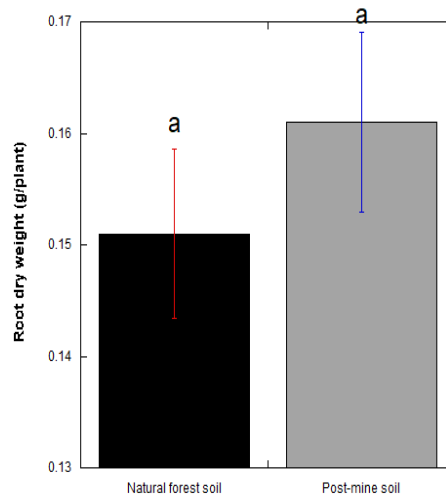
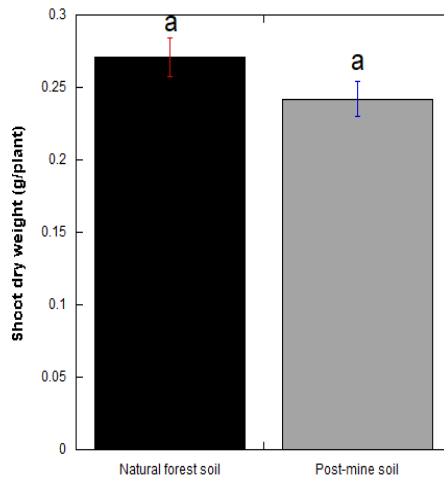


Figure 4-5. Mean shoot and root dry weight of *Albizia saman* 15 weeks after planting in soil from post-nickel mining land and natural forest. Different letter indicated significant differences ($p=0.05$). Error bars indicate standard error ($n=15$).

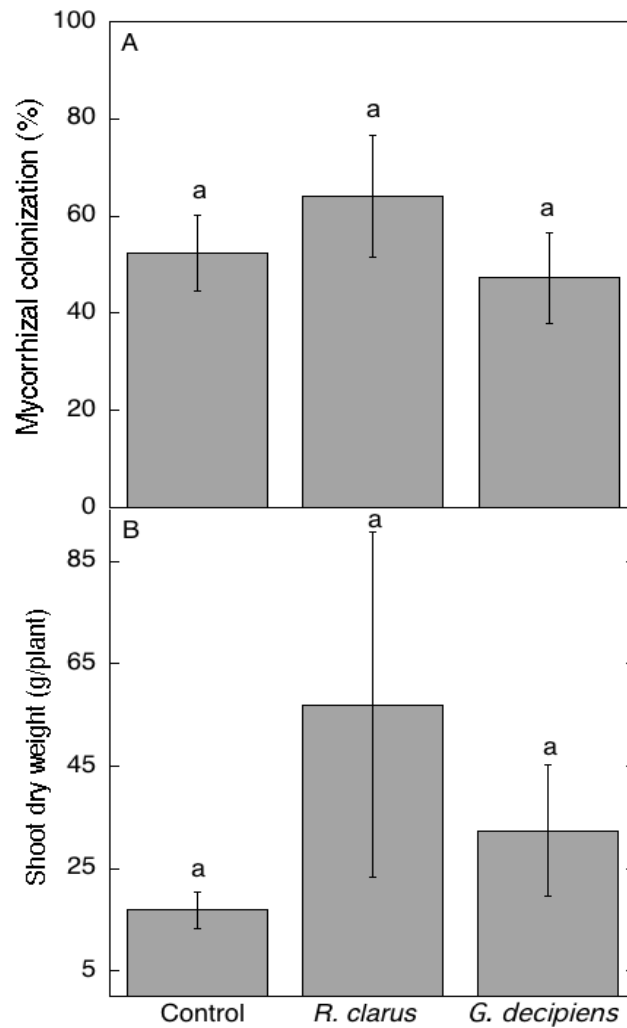


Figure 5-1. Mycorrhizal colonization (A) and shoot dry weight (B) of *Gmelina arborea* grown on post opencast bauxite mining 12 months after transplanting into the field. On each column, different letters indicate a significant difference ($P = .05$) by t test. Data are shown as mean \pm standard error ($n = 6$).

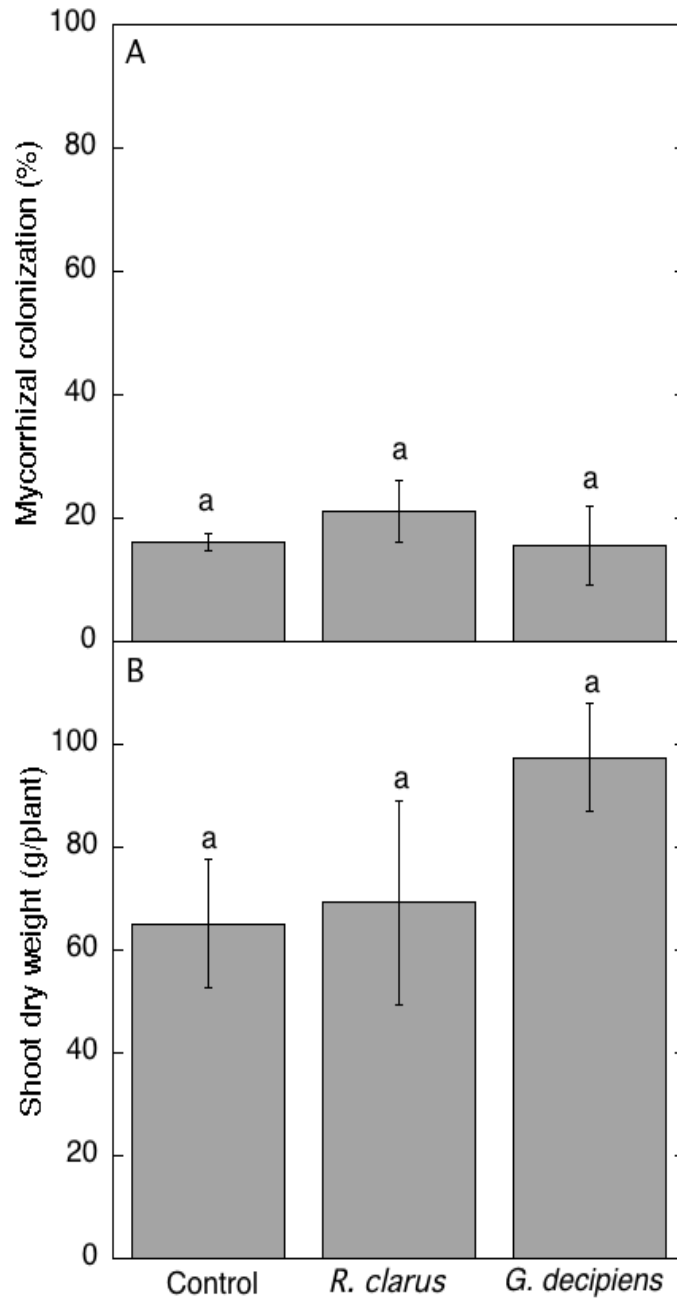


Figure 5-2. Mycorrhizal colonization (A) and shoot dry weight (B) of *Samanea saman* grown on post opencast bauxite mining 12 months after transplanting into the field. On each column, different letters indicate a significant difference ($P = .05$) by t test. Data are shown as mean \pm standard error ($n = 6$).

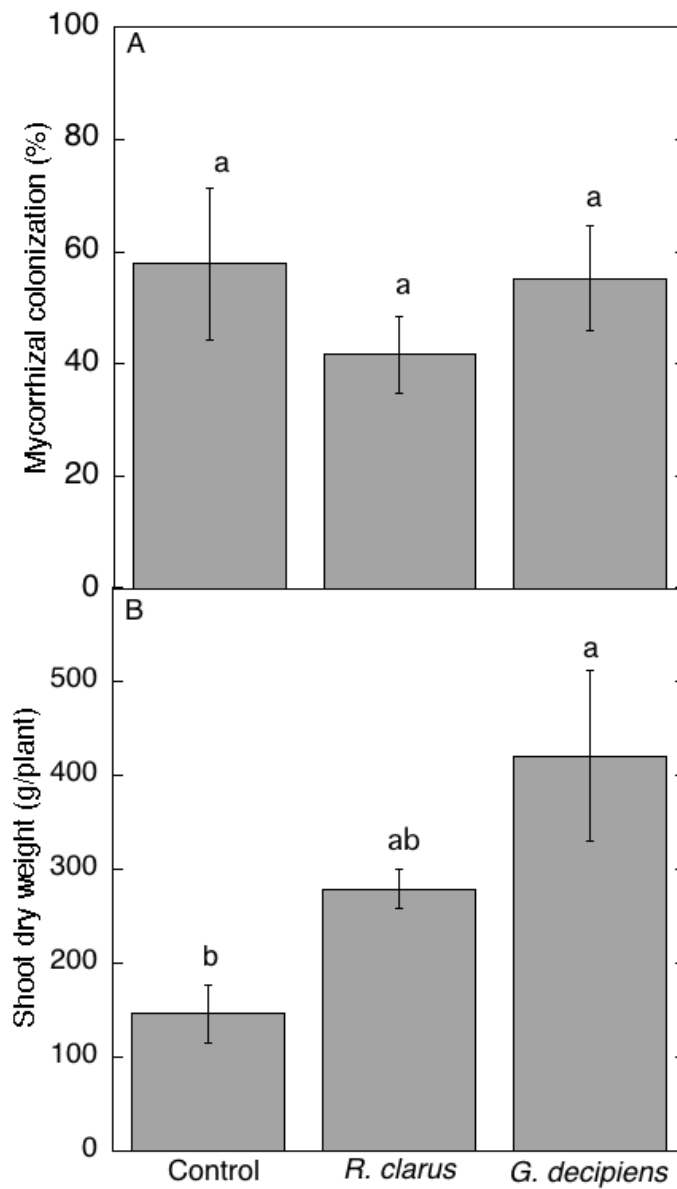


Figure 5-3. Mycorrhizal colonization (A) and shoot dry weight (B) of *Falcataria moluccana* grown on post opencast bauxite mining 12 months after transplanting into the field. On each column, different letters indicate a significant difference ($P = .05$) by t test. Data are shown as mean \pm standard error ($n = 6$).

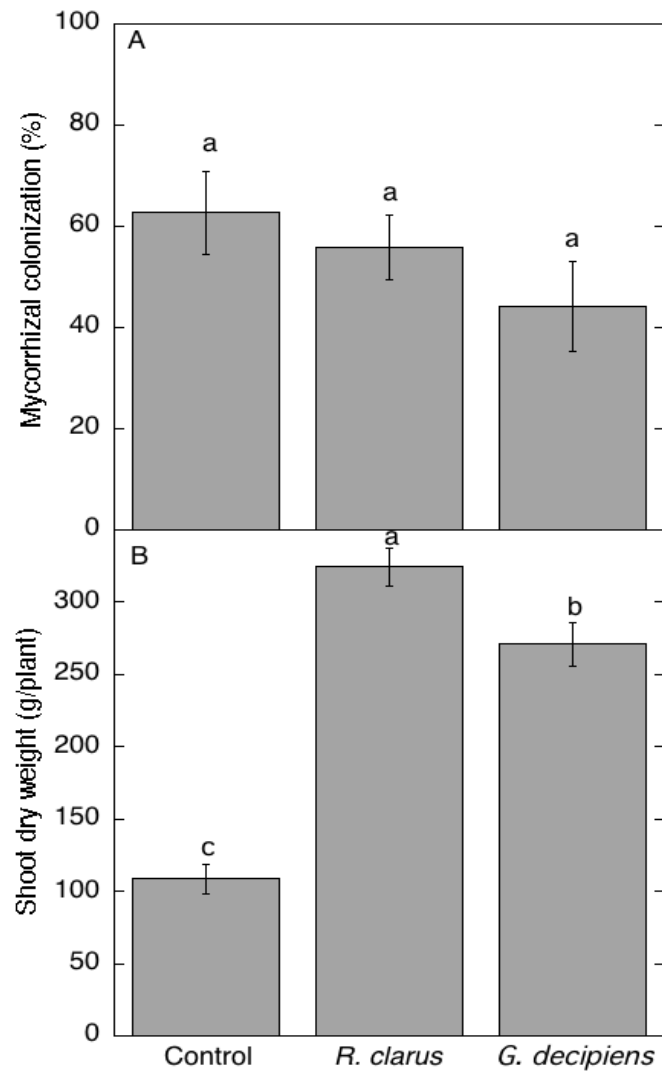


Figure 5-4. Mycorrhizal colonization (A) and shoot dry weight (B) of *Enterolobium cyclocarpum* grown on post opencast bauxite mining 12 months after transplanting into the field. On each column, different letters indicate a significant difference ($P = .05$) by t test. Data are shown as mean \pm standard error ($n = 6$).



Figure 5-5. *Rhizophagus clarus*



Figure 5-6. *Gigaspora decipiens*



Figure 5-7. Arbuscular mycorrhizal inocula production by pot culture in the greenhouse

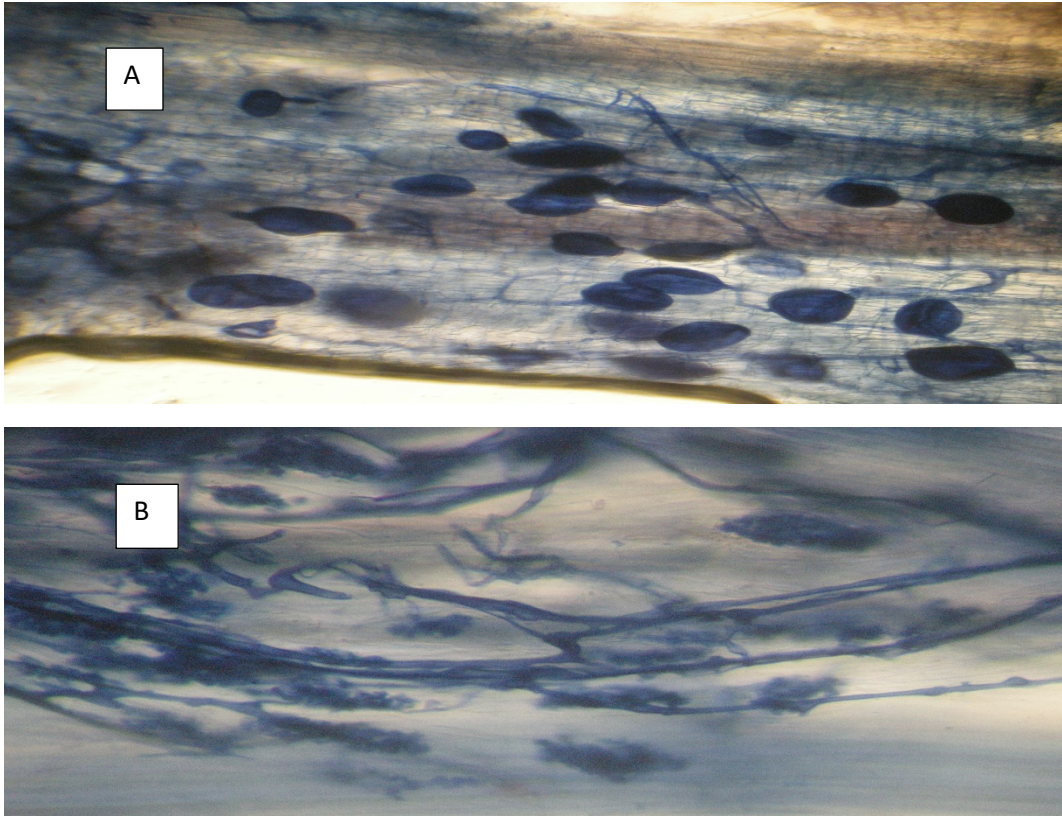


Figure 5-8. Arbuscular mycorrhizal fungi colonization. *Rhizophagus clarus* colonized root of *Falcataria moluccana* (A) vesicles and (B) arbuscules 3 months after transplanting in the nursery.

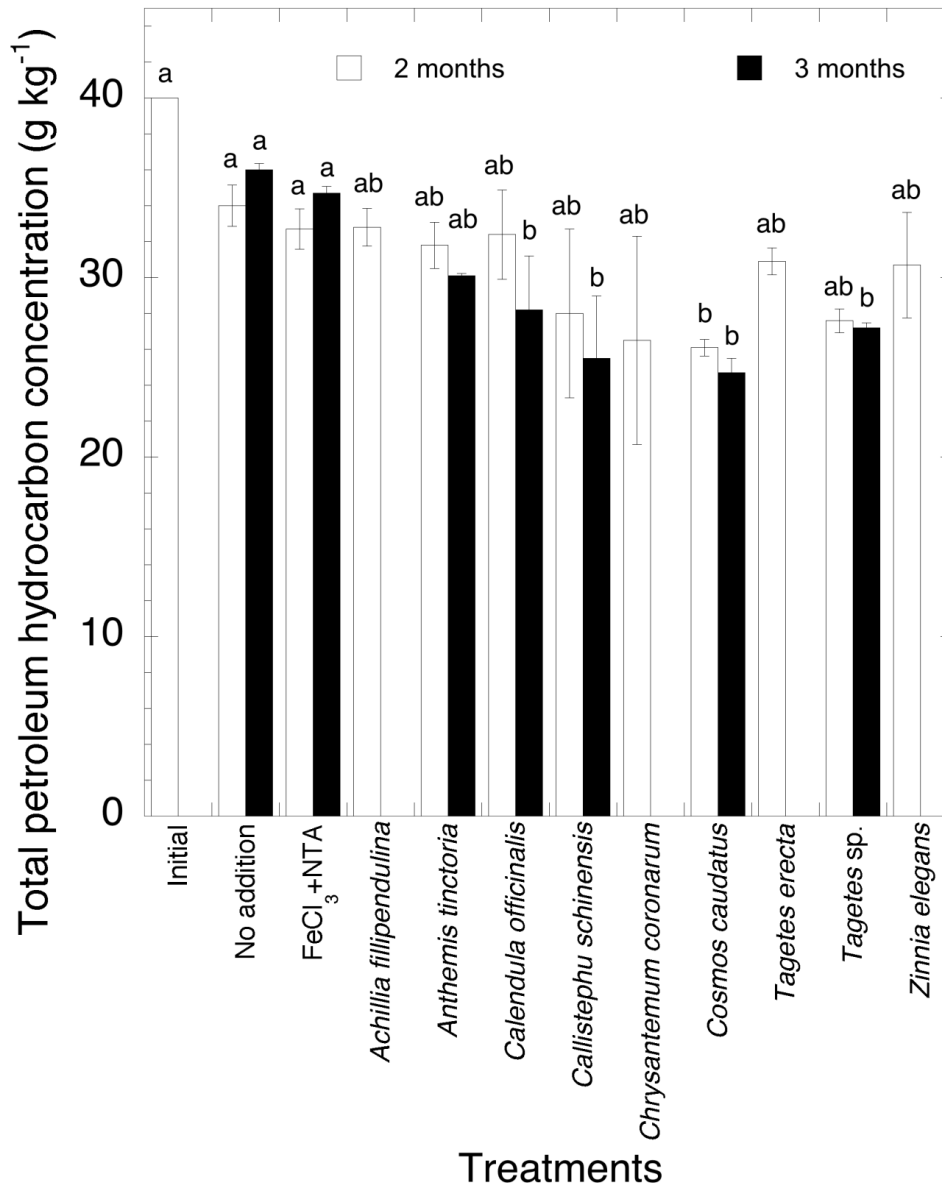


Figure 6-1. Total petroleum hydrocarbon (TPH) concentrations in initial soil, soil without FeCl₃ and NTA, soil with FeCl₃ and NTA, and soil with FeCl₃ and NTA cultivated with *Achillea filipendulina*, *Anthemis tinctoria*, *Calendula officinalis*, *Callistephus chinensis*, *Chrysanthemum coronarium*, *Cosmos caudatus*, *Tagetes erecta*, *Tagetes sp.*, and *Zinnia elegans* in 40 g kg⁻¹ TPH soil, 2 (white bar) and 3 (black bar) months after transplanting. Different letters indicate significant difference among treatments, each month (P<0.05) by Tukey HSD test. Data are shown as mean ± standard error (n=5).

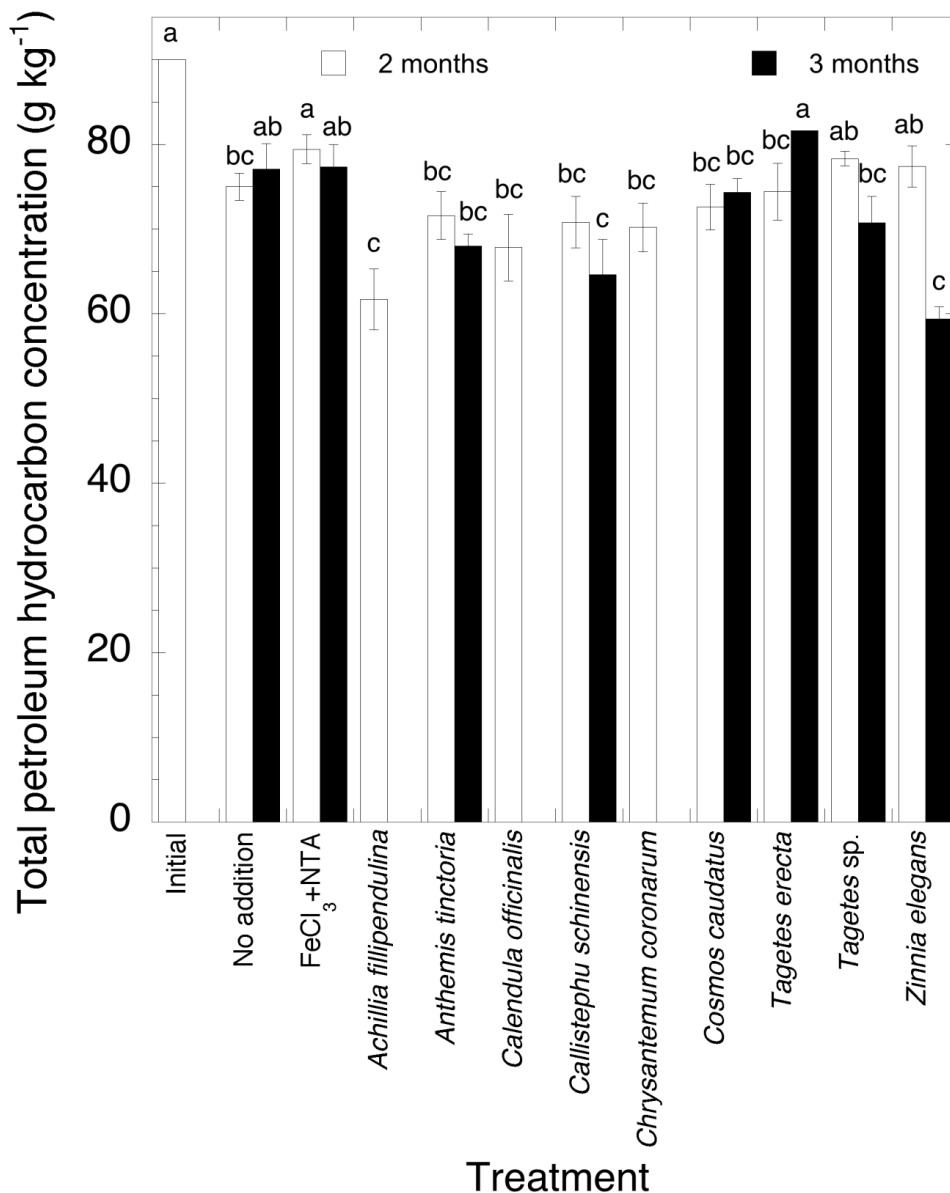


Figure 6-2. Total petroleum hydrocarbon concentration in initial soil, soil without FeCl₃ and NTA, soil with FeCl₃ and NTA, and soil with FeCl₃ and NTA cultivated with *Achillea filipendulina*, *Anthemis tinctoria*, *Calendula officinalis*, *Callistephus chinensis*, *Chrysanthemum coronarium*, *Cosmos caudatus*, *Tagetes erecta*, *Tagetes sp.*, and *Zinnia elegans*, in 90 g kg⁻¹ TPH soil, 2 (white bar) and 3 (black bar) months after transplanting. Different letters indicate significant differences among treatments for each month (P<0.05) by Tukey HSD test. Data are shown as mean ± standard error (n=5).

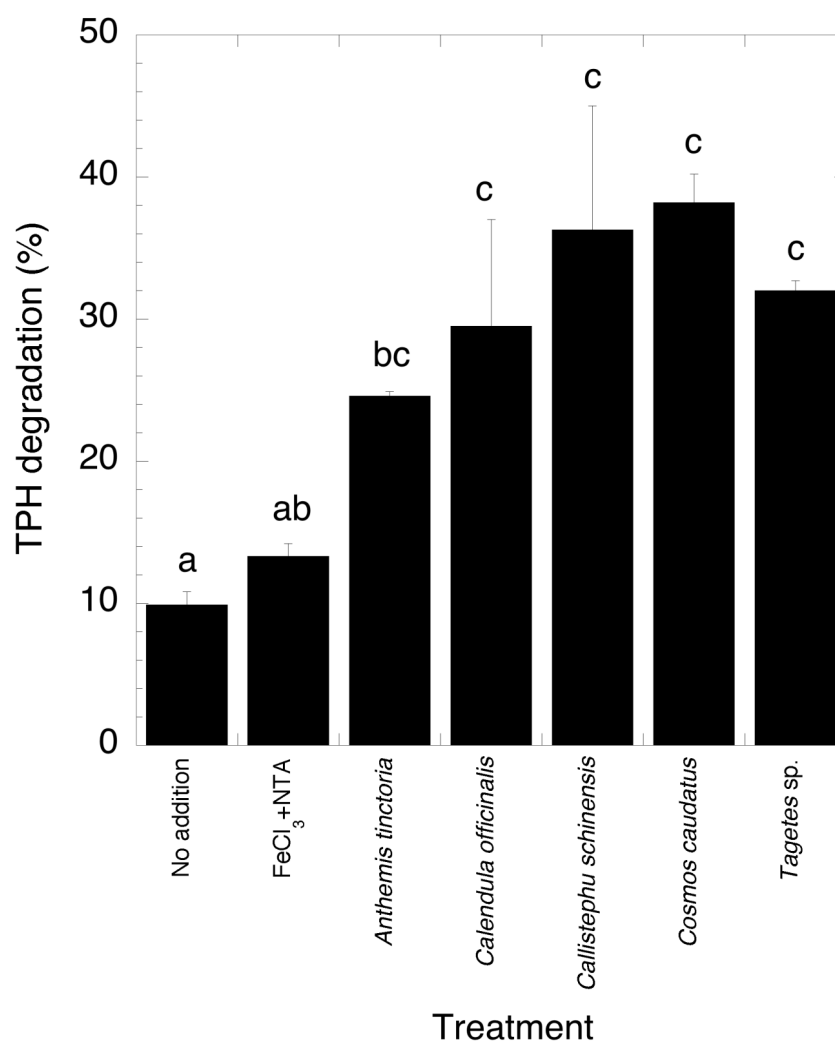


Figure 6-3. Total petroleum hydrocarbon (TPH) degradation in 40 g kg⁻¹ TPH soil without FeCl₃ and NTA (no addition), soil with FeCl₃ and NTA, and soil with FeCl₃ and NTA cultivated with *Anthemis tinctoria*, *Calendula officinalis*, *Callistephus chinensis*, *Cosmos caudatus*, and *Tagetes sp.*, 3 months after transplanting. Different letters indicate significant differences among treatments (P<0.05) by Tukey HSD test.

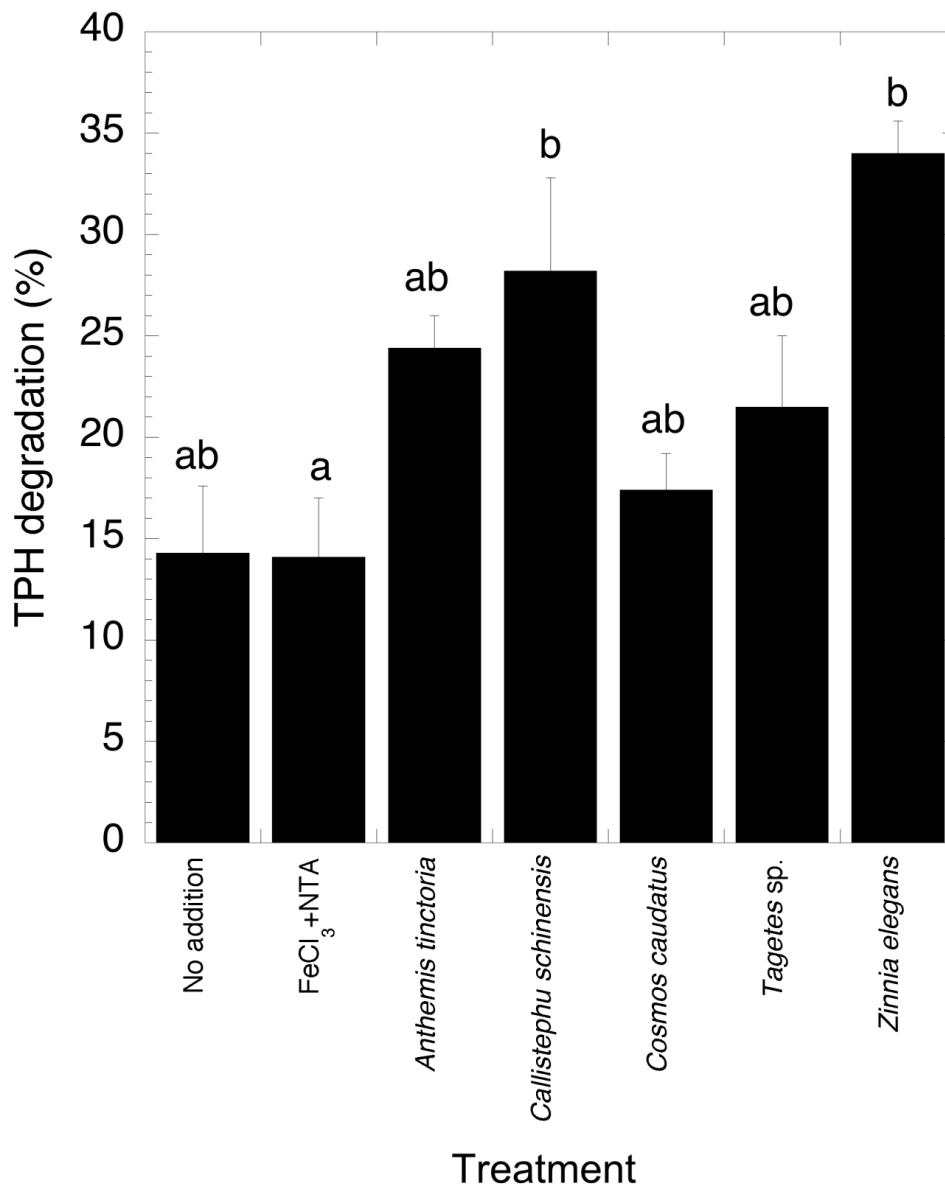


Figure 6-4. Total petroleum hydrocarbon (TPH) degradation in 90 g kg⁻¹ TPH soil without FeCl₃ and NTA (no addition), soil with FeCl₃ and NTA, and soil with FeCl₃ and NTA cultivated with *Anthemis tinctoria*, *Calendula officinalis*, *Callistephus chinensis*, *Cosmos caudatus*, and *Tagetes sp.*, 3 months after transplanting. Different letters indicate significant differences among treatments (P<0.05) by Tukey HSD test.

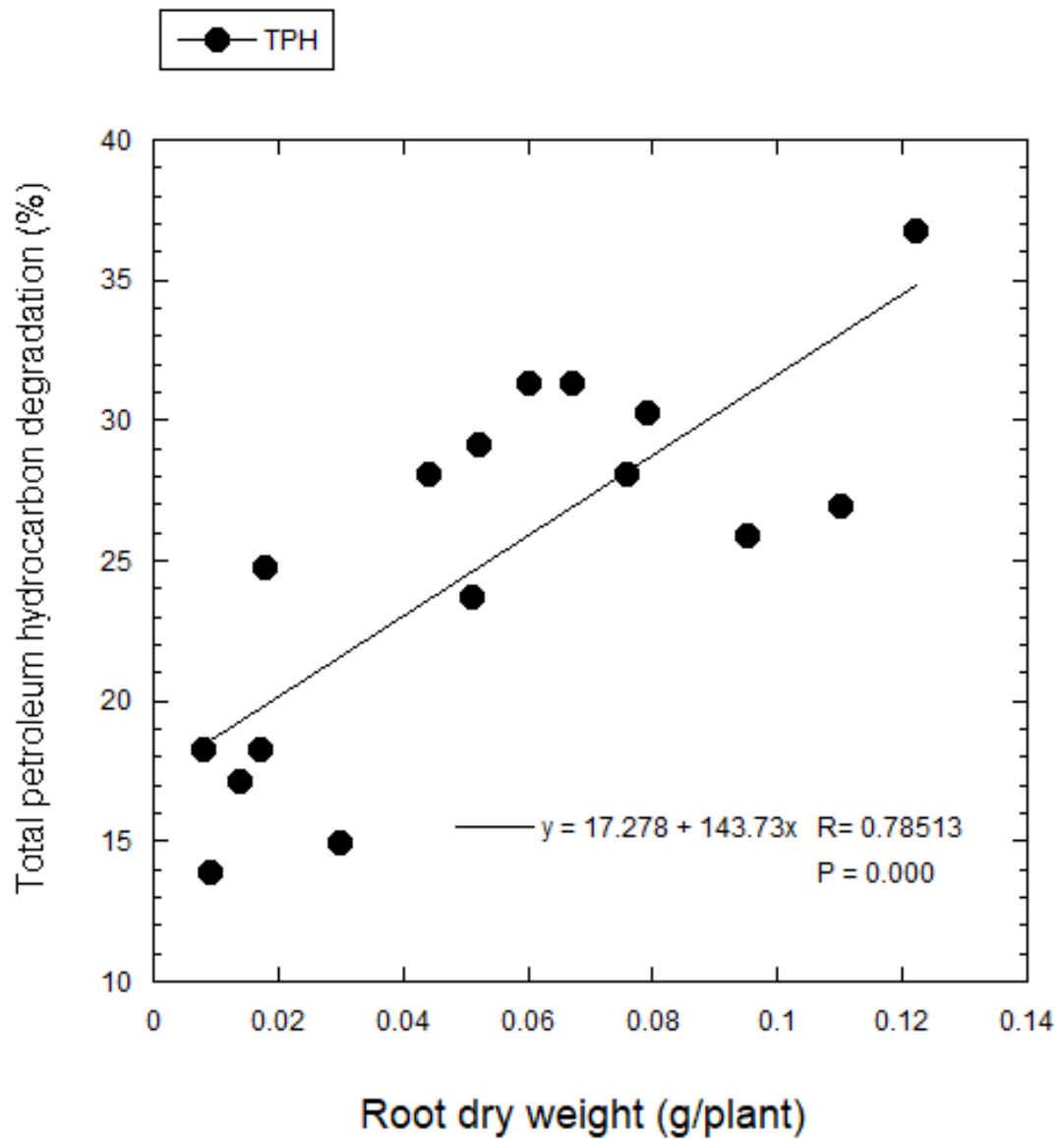


Figure 6-5. Relationship between root dry weight of Aster family and total petroleum hydrocarbon degradation (TPH) in 40 g kg^{-1} TPH soil, three months after transplanting.

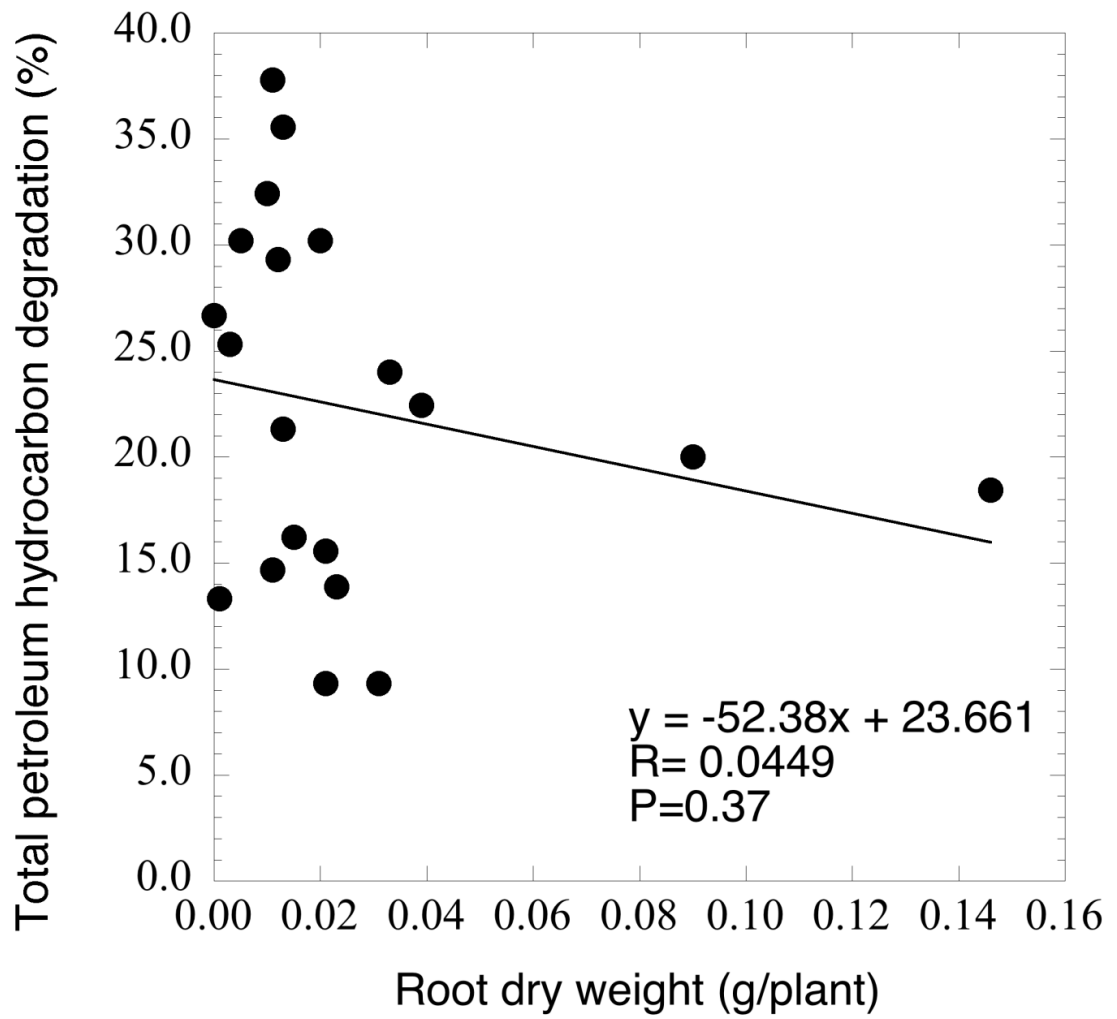


Figure 6-6. Relationship between root dry weight of Aster family and total petroleum hydrocarbon (TPH) degradation in 90 g kg⁻¹ TPH soil, three months after transplanting.