Kayu Kuku (*Pericopsis mooniana* Thw.) Seedlings Growth Response to Tailing Media Added with Vermicompost, Rhizobium, and Arbuscular Mycorrhizal Fungi

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Abstract. Kayu kuku (*Pericopsis mooniana* THW.) has ability to grow excellently on former clay and nickel mining sites but it is unknown how this species can adapt on mercury-contaminated soil. The purpose of this study is to analyze the effectivity of vermicompost, rhizobium and arbuscular mycorrhizal fungi (AMF) on improving the seedling growth of *P. mooniana* on mercury-contaminated soil of gold mining tailings media. The study was conducted in a greenhouse using a factorial randomized complete design consisting of 3 factors (vermicompost, rhizobium, and AMF). The application of vermicompost, rhizobium, and AMF could not able to increase the heights, diameters, biomass, and number of root nodules. In contrast, the treatments could increase the amount of chlorophyll, leaf area, primary root length, secondary root number and seedling life percentage up to 92%. Based on root-shoot ratio of *P. mooniana* seedlings indicated that these seedlings are not suitable to be planted in the field. Symptoms of Hg toxicity seen in seedlings morphology are: leaf and root discoloration, reduction in leaf area, shortening of primary roots, reduction of secondary roots.

1. Introduction
Mining activities that are applied cause environmental damage in the form of decreased soil productivity, erosion, sedimentation and heavy metal pollution. One type of mine that is often encountered is the people's gold mine. The number of small-scale mining activities in Indonesia there are 713 locations spread across Java, Sumatra, Kalimantan and Sulawesi with 60,000 small-scale miners [1]. One of the people's mines that is still operating is in the village of Warungbanten, Lebak District, Banten. The mining uses mercury (Hg) in the mining process and then dumps tailings into the soil and river. The Environment which is contaminated by Hg can endanger human life because of the food chain. In addition to being harmful to health, tailings containing Hg damage the physical, chemical and biological nature of the soil, making it unproductive. Therefore, efforts are needed to increase soil productivity while cleaning Hg from the soil so that it is safe for life. One effort that can be done is reclaiming ex-mining land.

One of the criteria for successful reclamation according to Permenhut No. 60 of 2009 is the planting of local species of long-cycle. Kayu kuku (*Pericopsis mooniana* THW) is one of the typical types of local wood from Sulawesi. Uncontrolled exploitation and encroachment of natural habitats are the cause...
of the diminished presence of *Pericopsis mooniana* [2]. Based on the IUCN report [3], *Pericopsis mooniana* is considered as a vulnerable status, so that conservation efforts need to be carried out, namely by utilizing the land of the former gold mine as a place of growth. [4] Research shows that *Pericopsis mooniana* is a type that is suitable for use as a rehabilitation plant for ex-clay mines. However, there has been no research on the ability of *Pericopsis mooniana* to grow on land contaminated with Hg. The constraints in rehabilitation of ex-mining land, namely marginal land conditions such as dense soil, minimal nutrient content, potential for mineral poisoning, poor organic matter, low status of CEC (cation exchange capacity), and minimal population and microbial activity ground potential [5]. Chemically, mined soils lose organic material so that their fertility levels are low, pH is low, while the solubility of heavy metals increases [6].

Alternative treatments that can be used to help plant growth on lands that have poor physical, chemical, and biological soil properties, such as mining soils, are by creating suppressive soil conditions [7]. Improvement of the quality of ex-mining land can be done by adding soil amendments in the form of organic matter in the form of vermicompost fertilizer accompanied by the addition of rhizobium and mycorrhizae to meet crop nutrient needs. The *Pericopsis mooniana* seedlings inoculated by FMA are able to grow well on ex-nickel mining land [8]. This research aims to analyze the effectiveness of vermicompost, rhizobium and mycorrhizae in increasing the ingrown parts of *Pericopsis mooniana* seeds on gold mining tailings media containing Hg.

2. Method

2.1. Materials

The tools used in this research are autoclaves, hoes, sand sieve, sacks, masks, latex gloves, sheeting, mica, nail clippers, cup cups, heat resistant plastic, rubber, polybags size 20 cm x 15 cm, microscope, petri dish, filter paper, Centricities, multistage filters, labels, markers, scales, analytic balance overaccuracy 10-4, and sprinklers. The materials used in this research are the seeds of *Pericopsis mooniana*, vermicompost, Rhizobium intrums, FMA incarnate, media tailings, sand, water, and glucose solution.

2.2. Procedure

2.2.1. Growing media

Tailings are taken at a depth of 30 cm. Tailings were analyzed for chemical and physical properties in the form of nitrogen (N), phosphorus (P), potassium (K), cation exchange capacity (CEC), C-organic and texture carried out at the Chemical and Soil Fertility Laboratory, pH was measured using a digital pH meter, and Hg were analyzed by the APHA method in the Productivity and Aquatic Environment Laboratory of IPB. The growing media was sterilized using an autoclave at 121 oC pressure of 1 atm for 30 minutes. Then the planting media is inserted into the polybag. Each polybag contains 1 kg of tailings. The amount of water used to flush is 25 mL.

2.2.2. *Pericopsis mooniana* Seed

The seeds come from the *Pericopsis mooniana* tree located in the Faculty of Forestry of IPB. Downloading the fruit is done in 2 ways, namely taken directly from the tree (climbed) and taken from under the stand. The seeds are extracted from the fruit manually. The seeds are cleaned by immersing them in 5% NaOCl solution for 15 minutes, then rinsed thoroughly. Breaking dormancy is done by scarification using nail clippers on one part of the seed coat. Then the seeds are soaked with 2 treatments namely: rhizobium solution and plain water for 24 hours.

2.2.3. Vermicompost, Rhizobium and FMA inoculum

The amount of vermicompost used was 50 g / seed. Rhizobium used is rhizobium which has been formulated by Wish Indonesia with the composition: (1) *Rhizobium* sp., (2) *Bacillus polymixa*, and (3)
Pseudomonas flourescens. Rhizobium concentration used is 5 g / L water. The inoculum used was the FMA type Glomus aggregate obtained from the SEAMEO BIOTROP mycorrhizal collection. The number of FMA inoculums used was 50 spores / seedlings.

2.2.4. Research implementation, Preservation and Observation
Placing media that have been inserted into the polybags were doused to moist then made a planting hole and treated with vermicompost and FMA. The prepared seedlings are put into the planting hole. The planting hole is then covered with planting media. As a control, Pericopsis mooniana were not treated with vermicompost, rhizobium and AMF inoculation. Watering is done once a day in the afternoon or adjusting to the humidity of the media. Preservation is carried out by cleaning weeds that grow on the media and pests on the seeds. The variables observed in this study were: (1) seedling height (cm) measured once a week, (2) diameter (mm) measured every 4 weeks, (3) amount of chlorophyll (unit), (4) leaf area (cm²), (5) number of root nodules, root to shoot ratio (NPA), (6) primary root length (cm), (7) number of secondary roots, (8) total dry weight (BKT), (9) FMA colonization (%), and (10) percentage of live seedlings (%) was measured at 15 weeks. Soil nutrient analysis was carried out on the tailings media before being treated and after being treated. Hg element analysis was carried out on the tailings media before being treated.

2.2.5. Experimental Design and Data Analysis
The experimental design used was a completely randomized factorial design (RAL) consisting of three factors, namely vermicompost, rhizobium, and FMA. Data analysis was performed using SAS v9.3.1 software. Before the data were analyzed normality was done with SPSS v16 and Stata v11 software on data that had a variance coefficient > 20. The confidence interval used is 95%. The treatment that showed significant difference was followed by Duncan Multiple Range Test (DMRT).

3. Result and discussion

| Soil characteristics | Method | Before Treatment | Criteria | After treatment¹ | Criteria | Alteration |
|----------------------|--------|------------------|----------|------------------|----------|------------|
| C-Organic (%)        | Walkley & Black  | 0.2 | Very lowᵇ | 1.43 | Lowᵇ | +1.19 |
| Total N (%)          | Kjeldahl | 0.06 | Very lowᵇ | 0.07 | Very lowᵇ | +0.01 |
| P (ppm)              | Bray I/Olsen | 186.84 | Very highᵇ | 46.72 | Highᵇ | -140.12 |
| K (ppm)              | Bray I/Olsen | 120.45 | Very highᵇ | 1472.8 | Very highᵇ | +1352.38 |
| pH                   | Digital pH meter | 7.00 | Netralᵇ | 7.00 | Netralᵇ | 0.00 |
| KTK (cmol⁹/kg)       | N NH₄OAc pH 7.0 | 4.80 | Very lowᵇ | 6.45 | Lowᵇ | +1.65 |
| Mo (ppm)             | AAS | 4.99 | Very highᶜ | 2.84 | Very highᶜ | -2.15 |
| Hg (ppm)             | APHA | 18.831 | >Tresholdᵈ | 24.12 | +22.86 |
| Moisture content     | Gravimetri | 1.26 | 41.22 | 37.00 | 4.22 |
| Sand (%)             | Pipette | 37.00 | 28.02 | 52.00 | +23.98 |
| Silt (%)             | Pipette | 1.26 | 41.22 | 37.00 | 4.22 |
| Clay (%)             | Pipette | 1.26 | 41.22 | 37.00 | 4.22 |

Table 1 shows that the addition of vermicompost, rhizobium, and mycorrhiza on the media tailings are able to improve the physical and chemical properties of soil, namely C-Organic, Total N, available K, KTK, and moisture content; As well as revamp the soil texture of the clay-grade clays (clay loam)
into a dusty clay (silty loam). The results of the analysis of Hg on tailings before the treatment showed a value of 18,831 ppm, it signifies that the Hg on the ground has exceeded the threshold according to CCME [11], the allowable threshold is 0.8 ppm for farmland. The high concentration of Hg on the tailings is due to the process of separation of gold using Hg. Generally, miners use excess Hg for the purpose of obtaining more gold [1].

Table 2. Growth of *P. mooniana* seedlings are given the treatment of vermicompost, Rhizobium, and Mycorrhiza age 15 weeks

| Treatment       | Height (cm) | Diameter (mm) | Total Dry Weight (g) | Percentage of Life (%) | Root to Shoot Ratio |
|-----------------|-------------|---------------|----------------------|------------------------|---------------------|
| Control         | 1.40a       | 0.13a         | 0.38a                | 63.00                  | 3.44                |
| Vermicompost (K)| 1.90a       | 0.14a         | 0.37a                | 75.00                  | 4.68                |
| Rhizobium (R)   | 1.30a       | 0.15a         | 0.37a                | 67.00                  | 4.48                |
| Mycorrhiza (M)  | 2.40a       | 0.13a         | 0.35a                | 71.00                  | 4.99                |
| K x R           | 1.30a       | 0.13a         | 0.37a                | 79.00                  | 3.86                |
| K x M           | 2.24a       | 0.14a         | 0.42a                | 71.00                  | 5.39                |
| R x M           | 1.40a       | 0.14a         | 0.42a                | 67.00                  | 4.25                |
| K x R x M       | 2.40a       | 0.15a         | 0.38a                | 92.00                  | 4.76                |

*The same letter indicates the treatment is not a real difference in confidence level 95%*

Table 3. *Pericopsis mooniana* seedlings are given treatment vermicompost, Rhizobium, and mycorrhiza age 15 weeks

| Treatment       | Number of Chlorophyll (unit) | Leaf Area (cm²) | Primary root length (cm) | Number of Secondary root | Number of Root Nodules (Colonization (%)) |
|-----------------|------------------------------|----------------|--------------------------|--------------------------|------------------------------------------|
| Control         | 48.15abc                     | 117.38c        | 6.01ab                   | 74.50bc                  | 0.00a                                    |
| Vermicompost (K)| 74.35abc                     | 192.75abc      | 6.37ab                   | 111.25ab                 | 0.00a                                    |
| Rhizobium (R)   | 33.65bc                      | 134.25bc       | 5.29b                    | 59.75c                   | 0.00a                                    |
| Mycorrhiza (M)  | 98.325ab                     | 208.88abc      | 5.35b                    | 114.50ab                 | 0.00a                                    |
| K x R           | 55.35abc                     | 147.38bc       | 6.36ab                   | 136.00a                  | 0.00a                                    |
| K x M           | 138.5a                       | 212.25ab       | 4.83b                    | 143.50a                  | 0.00a                                    |
| R x M           | 41.95c                       | 122.38c        | 7.77a                    | 95.25abc                 | 0.25a                                    |
| K x R x M       | 120.35ab                     | 268.63a        | 7.50a                    | 149.00a                  | 24.00                                    |

*The same letter shows treatment is not a real difference in confidence level 95%*

Table 2 shows that the granting of vermicompost, Rhizobium, and mycorrhiza in the tailing media has not been able to increase the seedling height, diameter, and total dry weight; But it can already increase the life percentage of seedlings. The best life percentage obtained on *Pericopsis mooniana* seedlings are given a combination of vermicompost treatment, Rhizobium, and Mycorrhiza. Although it already has a good percentage of life, the *Pericopsis mooniana* seedlings of the research results are less appropriate if planted in the field.

Table 3 shows that the interaction treatment between vermicompost, rhizobium, and mycorrhizal provides the best growth in leaf area, primary root length, and number of secondary roots. The interaction between vermicompost and mycorrhizal provides the best treatment on the amount of chlorophyll. The addition of height and diameter are not noticeable in both of each treatment. The least three environmental factors (the place of growing) and one genetic factor (internal) that is very real effect on growth in diameter and high, namely nutrient content of soil minerals, moisture soil and sunlight, as well as a balance of genetic properties between high growth and the diameter of a tree [12].

Metal Hg provides an influence on the leaf area, primary root length, number of secondary roots, root to shoot ratio and number of chlorophyll plants. The interactions between vermicompost, rhizobium, and mycorrhiza have the best influence on the broad-scale of leaves. The leaf area of the interaction between vermicompost, rhizobium, and mycorrhiza is caused by the number of leaves that
grow on the treatment at most among other treatments. The factor of the cause is due to the nutrients obtained from the vermicompost, coupled with rhizobium colonization and mycorrhiza at the roots of plants. Vermicompost that was made using *Eisenia fetida* containing N 0.63%, P 0.35%, K 0.20%, Ca 0.23%, Mg 0.26%, Na 0.07%, Cu 17.58%, Zn 0.007%, Mn 0.003%, Fe 0.79%, Bo 0.21% and the capacity of storing water 41.23% [13]. Rhizobium is able to slow down nitrogen from the air [14]. Mycorrhiza has a role in supporting plant growth because it can increase the availability of nutrients in particular P [2]. However, when compared with the area of *Pericopsis mooniana* seedlings age 15 weeks on ordinary land of 375 CM², it appears that Hg inhibits plant parherbs. Heavy metals can cause retarding of cell cleavage and extension, water and nutrient absorption, and enzymatic activity so that the growth rate is stunted [15].

In addition, the effect of Hg is also seen in the changes in the colour of the leaves that become younger when compared with the colour of the other seedlings of the *Pericopsis mooniana* planted in normal soil medium (not polluted Hg). This is caused by damage to the chlorophyll as the color pigment of the leaf. The element of Hg can replace the Mg element of the chlorophyll structure thereby causing a decrease in chlorophyll content [16]. The influence of mercury at a concentration of 5 ppm to a decrease in chlorophyll a 23% and chlorophyll B as much as 24% in *Cicer arietanum* L [17]. Chlorophyll is a green pigment found in most plants, algae, and Cyanobacteria [18]. The highest amount of chlorophyll is in the interaction treatment between vermicompost and mycorrhiza as much as 138.5 units. This occurs because in the vermicompost there are nutrients coupled with mycorrhiza function as a nutrient absorber and water, and do not happen competition with other microb in utilizing the nutrients and energy needed for its activities. Noticeable differences in the comparison of the amount of chlorophyll *Pericopsis mooniana* seedlings planted in normal soil of 360 units. The color change of the leaves also indicates that the *Pericopsis mooniana* seedlings are able to absorb Hg. All plants have the ability to absorb metals but in varying quantities [19].

Hg also affects the growth and development of *Pericopsis mooniana* roots. It is seen that the interaction between rhizobium and mycorrhiza has the longest primary root length of 7.77 cm, but not a real difference with the interaction between vermicompost, rhizobium, and mycorrhiza 7.50 cm. Number of secondary roots found in the treatment of interactions between vermicompost, rhizobium, and mycorrhiza but not real difference with the treatment of interactions between vermicompost and mycorrhiza. The concentration of soil contaminated with tailings is higher than the Hg content is higher, so it can inhibit the growth of roots because there is no nutrients needed [20]. The tailings condition around the condensed holes caused a poor water system that could directly bring a negative impact on the development of the roots, causing the roots to be unable to develop perfectly and function as a tool of absorption of nutrients and water will be interrupted [21]. As a result, the plant cannot develop normally, and its growth remains dwarf. Hg poisoning causes the color of the *Pericopsis mooniana* to change into brownish, short, and easily broken. It is in accordance with the statements Patra *et al.* [16] Hg poisoning also causes the roots of the plant to be brown, the amount and size of the roots decreases, and the root hood is broken. The root of the plant has very important role because of the root function as an absorber of plant nutrients and translocation of elements from the roots to stems, leaves, or fruits [10].

The root tip ratio shows the growth index of a plant in the balance of the bud and the roots. The undeveloped rooting system causes limited growth of shoots due to insufficient supply of nutrients and minerals, while decreasing photosynthesis activities can inhibit root growth due to reduced carbohydrate [22]. High root to shoots ratio values indicate that the tip has better growth when compared to the roots. Similarly, the low root to shoots ratio value indicates that the root part has better growth compared to the growth of the bud [23]. A good root to shoots ratio value ranges from 1–3. The root to shoots ratio value located at > 3 indicates less suitable crops if planted in the field [24].

There are only 1 root star found after 15 weeks of planting of *Pericopsis mooniana* in planting media. This is thought to be due to the type of rhizobium used in this study does not correspond to the type of *Pericopsis mooniana*. The suitability of plants with Rhizobium strains determines the success of symbiosis between the two [25].
The supply of inorganic nutrients, soil type, the percentage of sand and clay is another factor affecting the formation of root nits [26]. Table 1 shows that after the addition of vermicompost, rhizobium, and mycorrhiza on the planting medium, C-Organic and Total N status is at low and very low status based on the table of the chemical properties of the LPT [9]. The value of C-organic indicates the efficacy of organic matter in soil. Indications of organic matter in soil can be seen from the content of C-organic and Total N so that the C/N ratio can be used to suspect the availability of nutrients from mineralization of organic matter [27]. In addition, the effect of Hg that causes root shortening makes rhizobium has no good place to thrive.

Mycorrhizal colonisation suggests that a single mycosis has the highest infection rate of 51.38%. Infections decreased in the presence of treatment interactions. It is related to the role of mycorrhiza on plants. Mycorrhiza has four fungsional roles, namely bioprosessor (nutrient and water absorbent), bioprotectors (protective of biotic and abiotic rings), bioaktivator (increases carbon rhizosphere uptake and activity of the body renic), and Bioagregator (fixing land aggregates) [28]. Decreased mycotic infections in the treatment consisting of the interaction between the vermicompost with mycorrhiza, rhizobium with mycorrhiza, and vermicompost with rhizobium and mycorrhiza due to the state of planting media better, as well as the occurrence of competition in gain energy for its development so that the infection of mycosis decreases.

The percentage of life of the \textit{Pericopsis mooniana} seedlings that were given the interaction between vermicompost, rhizobium, and mycorrhiza reached 92%. The seedlings that are given the interaction between vermicompost, Rhizobium, and mycorrhiza have exceeded the conditions of the percentage life of seedlings in the nursery as much as 80% [29]. One function of mycorrhiza is to protect plants from toxins in the soil such as heavy metals by means of filtration of toxins, disabling them chemically, or stockpiling in the Hypha mycorrhizal itself [2]. The addition of vermicompost, Rhizobium, and mycorrhiza improve soil structure and provide nutrients for seedlings so that it can grow better.

In addition, the bribing that is done at the age of one-month of seedlings causes the many dead seeds in the control treatment, single, and 2-factor interactions. It is suspected because the plant needs nutrients that are much in the early days of growth to optimize its growing, especially the N. Total N available on very low media so that the plant is stressed and dead. N serves as a major component of proteins, hormones, chlorophyll, vitamins, and essential enzymes for the helivelihood of plants. Plants that get N are quite characterized by a characteristic of dark green, while the lack of supply N causes yellow leaves, dwarf parherbs, and failed to harvest [30].

4. Conclusion
Toxic Hg affects the morphology and physiology of the roots and leaves of the \textit{Pericopsis mooniana} so that the roots are brown, short and fragile. Toxic Hg on the leaves appears to be a younger color change, the leaves area shrinks, and contains fewer chlorophyll. The provision of vermicompost, rhizobium, and mycorrhiza is able to increase the percentage of life of \textit{Pericopsis mooniana} on the tailings that contain Hg up to 92%. The granting of vermicompost, rhizobium, and mycorrhiza has not been enough to increase the height and diameter of the \textit{Pericopsis mooniana} seedlings. The root to shoots ratio value is at the number > 3 indicates the \textit{Pericopsis mooniana} seedlings still cannot be planted in the field.

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