Correlation Study of Rhizobium Bacteria and Legume Cover Crops (LCC) as Nutrient Providers and Bioremediators on Post-Mining Land at PT. Agincourt Resources

Yasmine Anggia Sari[1*], *Ade Gustina Hazar* Nasution[1], *Syaiful* Anwar[2,3], *Randyka* Nugraha[3], and *Sarah Patumona* Manalu[1]

[1]Department of Environmental Engineering, Universitas Sumatera Utara, Medan, Indonesia
[2]School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia
[3]PT. Agincourt Resources, Martabe Gold Mine, Jl. Merdeka Aek Pining, Batangtoru, North Sumatera, 22738, Indonesia

Abstract. Gold mining activities have a significant impact on land degradation, particularly in terms of the physical, chemical, and biological aspects of soil. Post-mining land reclamation is an important effort to restore environmental conditions, one of which is through the use of Legume Cover Crops (LCC) that are symbiotic with Rhizobium bacteria. This study aims to analyze the correlation between Rhizobium bacteria and LCC in nutrient supply and their potential as bioremediation agents on post-mining land at PT. Agincourt Resources. The methods used include macroscopic and microscopic observations of root nodules on Vigna unguiculata plants, Desmodium gyrans plants, and Mucuna Bracteata plants grown in the Melisa Dump reclamation area. The results show that Mucuna Bracteata samples had an average of 14 to 28 nodules per plant, while the Desmodium gyrans produced 11 to 23 nodules per sample. Vigna unguiculata had the lowest number of nodules, ranging from 3 to 11 nodules per sample. These findings indicate that the combination of Rhizobium and Mucuna bracteata plants has great potential for improving soil fertility and soil quality in post-mining areas. This study recommends that PT. Agincourt Resources prioritize Mucuna bracteata as a cover crop in their reclamation program.

1 Introduction

Mining activities, particularly open-pit gold mining, have a significant impact on environmental degradation, particularly in terms of the physical, chemical, and biological aspects of soil. Post-mining land typically experiences a drastic decline in fertility, characterized by reduced organic matter content, soil structure degradation, and heavy metal contamination. In Indonesia, the total area of former mining sites is estimated to exceed 1.3 million hectares and is spread across various regions such as Sumatra, Kalimantan, and

* Corresponding author: yasmineanggiasari@usu.ac.id

Papua. This situation poses a major challenge in efforts to restore ecosystem functions and land productivity [3].

Post-mining land reclamation is an important step in rehabilitating affected areas, with the aim of restoring environmental conditions and returning land functionality. One effective and sustainable approach to reclamation is the use of biological agents, such as Legume Cover Crops (LCC) that form a symbiotic relationship with Rhizobium bacteria. The symbiosis between Rhizobium and legume plants plays a crucial role in nitrogen fixation, which directly enhances soil fertility and supports the growth of new vegetation on degraded land. The formation of nodules on legume plant roots is the primary indicator of nitrogen fixation, which contributes to increased nutrient availability in the soil [1].

In addition to improving soil fertility, the interaction between Rhizobium and LCC also has potential in the bioremediation process through phytoremediation, which is the ability of plants to absorb and reduce heavy metal contamination in the soil [5]. Some legume species are known to accumulate heavy metals, making them effective in improving soil quality in reclamation areas [4].

PT. Agincourt Resources, one of Indonesia's gold mining companies, has implemented a reclamation program using various types of legumes, including *Vigna unguiculata*, *Desmodium gyrans*, and *Mucuna bracteata* in the Melisa Dump reclamation area. However, in-depth studies on the morphological characteristics of root nodules and the effectiveness of nitrogenase activity in each of these plants are still limited. Most existing studies on the symbiosis between Rhizobium and LCC have been conducted in agricultural or oil palm plantation systems, while research in post-gold mining soils remains very limited. Therefore, this study focuses on three legume species — *Mucuna bracteata*, *Desmodium gyrans*, and *Vigna unguiculata* — which have already been introduced by PT. Agincourt, but whose nodule efficiency and bioremediation potential have not yet been thoroughly evaluated.

This study aims to analyze the interaction between Rhizobium bacteria and LCC plants in the reclamation area of PT. Agincourt Resources, with a focus on observing root nodule morphology and their potential contribution to nutrient enhancement and soil bioremediation. The results of this study are expected to serve as a basis for optimizing sustainable biological reclamation strategies in post-mining areas.

2 Materials and Method

This study was conducted from November to December 2024 in the Melisa Dump reclamation area, PT. Agincourt Resources, South Tapanuli. Morphological observations of root nodules were carried out at the PT. Agincourt Resources Microbiology Laboratory. This study used several tools such as shovels, hoes, buckets, petri dishes, razors, microscopes, and cameras. The materials used included clean water, distilled water, and a 0.5% safranin solution used for microscopic staining.

The samples observed were roots from three types of leguminous plants grown in the reclamation area, namely Vigna unguiculata, Desmodium gyrans, and Mucuna Bracteata. Sampling was conducted by digging the plant roots 5–20 cm deep from the soil surface. Three samples were taken from each plant species, and the roots were washed thoroughly with running water to remove any remaining soil.

Observations were conducted macroscopically and microscopically. Macroscopic observations aimed to observe the number, shape, and color of root nodules. The parameters observed included the number of nodules per sample, the shape of the nodules (round, oval, or branched), and the color of the nodules (pink, white, or brown). Pink color in the nodules indicated active nitrogenase activity, while white and brown colors indicated inactive nodules. Microscopic observations were conducted by slicing the nodules using a razor blade to obtain thin sections. The nodule slices were then placed on a Petri dish that had been

dripped with distilled water and observed under a microscope. To clarify the internal structure of the nodules, simple staining was performed by immersing the nodule slices in a 0.5% safranin solution for 2–3 minutes. After that, the nodules were rinsed with clean water and observed again under a microscope.

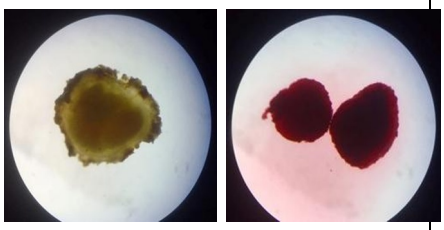
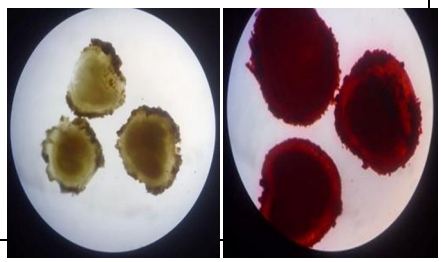
The observation data are recorded and analyzed descriptively. The analysis is conducted by comparing the number of nodules, the color of the nodules as an indicator of nitrogenase activity, and the shape of the nodules as morphological variations in each plant. The results of this analysis are used to evaluate the effectiveness of each type of legume plant in nodule formation and its potential as a soil quality improvement agent in post-mining reclamation areas.

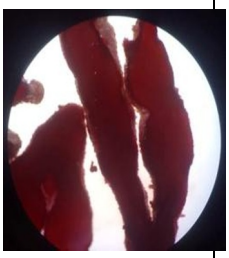
3 Results and Discussion

3.1 Observation of Root Nodules

The observation of root nodules on Vigna unguiculata, Desmodium gyrans, and Mucuna Bracteata planted in the reclamation area of Melisa Dump at PT. Agincourt Resources shows variation in the number, shape, and color of nodules for each plant species. The detailed results are presented in Table 1.

Table 1. Observation of root nodules on legume plants.

Plant Species	Sample	Number of Nodules	Nodule Color	Nodule Shape	Plant Species Images
Vigna Unguiculata	1	11	White & Brown	Round & Oval	
	2	8	White & Brown	Round & Oval	
	3	3	White & Brown	Round & Oval	
Desmodium Gyrans	1	11	Pink, White, & Brown	Round & Oval	
	2	13	Pink, White, & Brown	Round & Oval	

Plant Species	Sample	Number of Nodules	Nodule Color	Nodule Shape	Plant Species Images
	3	23	Pink, White, & Brown	Round & Oval	
Mucuna Bracteata	1	28	Pink, White, & Brown	Round & Branched	 
	2	14	Pink, White, & Brown	Round & Branched	
	3	18	Pink, White, & Brown	Round & Branched	

3.2 Nodule Characteristics Analysis

Based on Table 1, Mucuna Bracteata plants showed the highest number of nodules compared to the Vigna unguiculata, Desmodium gyrans,. Mucuna Bracteata samples had an average of 14 to 28 nodules per plant, while the Desmodium gyrans produced 11 to 23 nodules per sample. Vigna unguiculata had the lowest number of nodules, ranging from 3 to 11 nodules per sample.

In terms of nodule color, both Mucuna Bracteata and the Desmodium gyrans showed the presence of pink nodules, which indicate active nitrogenase activity. This suggests that these two plants were able to fix nitrogen effectively in the reclamation area. On the other hand, most nodules on Vigna unguiculata were white and brown, which indicates low or inactive nitrogenase activity.

The nodule shapes also varied among plant species. Mucuna Bracteata tended to produce round and branched nodules, while the Desmodium gyrans and Vigna unguiculata formed round and oval nodules. The presence of branched nodules in Mucuna Bracteata indicates more advanced morphological development, which can potentially increase nitrogen fixation efficiency.

The results show that Mucuna Bracteata has a better ability to form a large number of nodules with high nitrogenase activity. This indicates that Mucuna Bracteata is highly adaptable to post-mining soil conditions and can effectively contribute to nitrogen availability through biological nitrogen fixation. This finding is consistent with the study by Fakhriyansyah (2021), which states that Mucuna Bracteata has strong adaptability to various soil types and is effective as a ground cover plant [2].

The Desmodium gyrans also demonstrated good performance in forming active nodules. Although the number of nodules was lower than in Mucuna Bracteata, the Desmodium gyrans still has potential as an alternative in revegetation programs because it produced nodules with significant nitrogenase activity.

Conversely, Vigna unguiculata was less effective in forming active nodules in the study area. The low number of nodules and the dominance of white and brown colors suggest minimal nitrogenase activity, possibly due to environmental factors such as soil pH, low organic matter content, or the absence of compatible Rhizobium strains in the soil.

Overall, this study confirms that the interaction between Rhizobium bacteria and legume plants, particularly Mucuna Bracteata and the Desmodium gyrans, has great potential to

improve soil fertility and quality in post-mining reclamation areas. The use of legume species capable of forming effective nodules with high nitrogenase activity should be prioritized in sustainable reclamation strategies at PT. Agincourt Resources.

4 Conclusion

Based on the results of this study, it can be concluded that the interaction between Rhizobium bacteria and legume cover crops (LCC) plays a significant role in improving soil fertility in post-mining reclamation areas. Among the three plant species observed, Mucuna Bracteata demonstrated the highest potential in forming abundant root nodules with active nitrogenase activity, as indicated by the predominance of pink nodules. The branched nodule morphology further suggests the ability of Mucuna Bracteata to fix nitrogen efficiently and adapt well to the soil conditions of the reclamation area.

Desmodium gyrans also showed promising results, producing a relatively high number of nodules with sufficient nitrogenase activity, although not as high as that of Mucuna Bracteata. Meanwhile, Vigna unguiculata formed fewer nodules, with most of them inactive as indicated by their white and brown coloration, which implies that this plant may have lower nitrogen fixation capacity under the given site conditions.

The findings suggest that Mucuna Bracteata is the most effective legume species for use as a cover crop to support soil recovery in post-mining reclamation areas, while the Desmodium gyrans may serve as an alternative. The use of Vigna unguiculata may not be optimal without further investigation or soil enhancement.

Suggestions

1. Mucuna Bracteata should be prioritized as the primary legume cover crop in the revegetation and soil rehabilitation programs at PT. Agincourt Resources, considering its superior nodule formation and nitrogen-fixing capabilities.
2. The Desmodium gyrans can be used as a complementary or alternative cover crop due to its satisfactory nitrogenase activity and adaptability.
3. Further research is recommended to explore the specific strains of Rhizobium associated with each plant species to optimize symbiotic performance.
4. It is advisable to conduct controlled inoculation of effective Rhizobium strains to increase nodule formation and nitrogen fixation in species such as cowpea, which showed low activity in this study.
5. Environmental factors such as soil pH, nutrient availability, and moisture content should be further evaluated to maximize the success of the reclamation efforts.
6. Additional studies focusing on the long-term monitoring of nitrogen fixation rates and soil quality improvements are necessary to ensure the sustainability of biological reclamation strategies.

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