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Impact of Underground Gold Mining on Soil Chemistry and Biology: Indigenous Microbe-Driven Rehabilitation?

INDAH FATHIKASARI[1], DEWI WULANDARI[2,*], HAPPY WIDIASTUTI[3], JOCELYN T. ZARATE[4]

[1]*Master Program, Department of Environmental Engineering, Faculty of Civil & Planning Engineering, Universitas Islam Indonesia (UII), Indonesia*
[2]*Department of Environmental Engineering, Faculty of Civil & Planning Engineering, Universitas Islam Indonesia (UII), Indonesia*
[3]*Indonesian Research Institute for Biotechnology and Bioindustry, Jl Taman Kencana No 1 Bogor, Indonesia*
[4]*National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Banos, College, Laguna 4030, Philippines*

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ABSTRACT

Gold mining boosts Indonesia's GDP and exports. However, gold mining changes soil chemical and biological qualities, and therefore harming the environment. Thus, rehabilitating post mining land using environmentally friendly and chap method are essential for soil and ecosystem health. Nitrogen (N), and phosphorus (P) are the most limiting factors in degraded mining area. *Azotobacter* sp. and *Rhizobium* sp. are well-known in assisting plant by providing N. *Paraserianthes falcataria* is well-known as a fast-growing tree species that have good symbiosis with *Rhizobium* sp. This study examines the chemical and biological effects of underground gold mining on soil and the ability of indigenous *Azotobacter* sp. and *Rhizobium* sp., to improve the soil of post gold mining under greenhouse condition. Soils were sampled from natural forest (NF), tailing (T), and 3 different ages of rehabilitated area: 1 year old, 2 year old, and 5 year old. According to this study, gold mining negatively effect on soil organic carbon and nitrogen levels. One *Rhizobium* sp. isolate and 12 *Azotobacter* spp. isolates were found in post mining soil. All *Azotobacter* spp. isolates fixed nitrogen by NFB test and produced IAA. All *Azotobacter* spp. was determined as gram negative bacteria. A greenhouse study found that *Azotobacter* sp. inoculation with 10% compost improved the soil quality by increasing soil organic carbon, soil N, soil available P, and exchangeable K. Therefore, this improvement on soil condition increased seedlings height, diameter, and biomass growing in gold tailings soil. These findings highlight the necessity of employing indigenous microorganisms and organic materials to improve soil quality and plant growth on former gold mining areas.

Key words: *Azotobacter* sp.; *Rhizobium* sp.; post-gold mining; *P. falcataria*

INTRODUCTION

Gold mining is a significant contributor to the Indonesian economy, playing a vital role in the country's gross domestic product. In 2019, the mining and quarrying sector accounted for approximately 7.1% of Indonesia's Gross Domestic Product, as reported by the Central Statistic Agency. According to the US Geological Survey in 2020, Indonesia is a major global producer of gold, with a production volume of approximately 130 tonnes in 2019. The gold mining industry not only has a substantial impact on the country's gross domestic product, but also plays a crucial part in the nation's export of minerals. According to the Central Statistic Agency (2021) Indonesia's gold export value exceeded USD 5 billion in 2020, establishing it as a significant export commodity,

* *Corresponding author:*
Department of Environmental Engineering, Faculty of Civil & Planning Engineering, Universitas Islam Indonesia (UII), Indonesia. Jl. Kaliurang Km. 14,5 Yogyakarta 55585 Indonesia. E-mail: dewi.wulandari@uii.ac.id

following coal and palm oil. However, despite its considerable economic advantages, the gold mining sector also presents environmental obstacles that must be effectively addressed to ensure long-term viability and minimize its adverse effects on the ecosystem.

Underground gold mining has a tremendous impact on the environment, particularly soil's chemical and biological qualities. Mining activities frequently alter the soil's physical structure and introduce contaminants such as heavy metals, disrupting the equilibrium of the soil ecosystem (Koch, 2022). Using toxic chemicals in gold extraction, such as cyanide and mercury, degrades soil quality and reduces the soil's ability to support microbial and plant life (Tarras-Wahlberg *et al*., 2001). The mining process damages the physical structure of the soil, reduces the soil's ability to hold water and air, which are essential for the life of microorganisms and plant growth(Wulandari *et al*., 2022), and removes the organic-rich top layer of soil essential nutrients, drastically reducing land fertility (Hilson, 2002). This condition diminishes the productivity of previously mined gold land and renders it unsuitable for optimal plant development. The extensive mining operations render the ground incapable of sustaining healthy plant growth. In the absence of significant rehabilitation measures, including the incorporation of organic matter, soil reconfiguration, and the establishment of protective flora, the land persists in its infertility and lack of productivity. Substantial measures are necessary to rehabilitate soil fertility and render the area suitable for agricultural or ecological applications. In the absence of these interventions, the land will remain unsuitable for supporting optimal plant development.

Pioneer plants like *Paraserianthes falcataria* can help with initial rehabilitation degraded land by improving soil structure and providing habitat for soil microorganisms (Wulandari *et al*., 2016), particularly in tropical settings. This is due to its rapid growth, ability to increase soil structure and quality, and providing organic matter (Mulyadi *et al*., 2022), and broad and deep root system, which can assist in preventing soil erosion and boost water infiltration (Hartemink, 2005). *P. falcataria* can interact with nitrogen-fixing bacteria, which promotes soil fertility (Prayoga *et al*., 2018). As a result, *P. falcataria* could be used as part of a rehabilitation program for degraded mining land (Wulandari *et al*., 2016).

Gold mining has a negative influence on soil biodiversity. Heavy metals and hazardous compounds can impact soil microbial populations, essential for nutrient cycling and soil health maintenance (Fagariba *et al*., 2024). Soil microorganisms such as *Azotobacter* sp. and *Rhizobium* sp. are susceptible to environmental changes. The presence of pollutants can impede their metabolic activity, reducing soil production (Khan *et al*., 2007). *Azotobacter* sp. is a bacterium capable of readily binding nitrogen from the environment and converting it into a form that plants can use (Bhattacharyya & Jha, 2012). These bacteria also produce phytohormones and enzymes that aid plant growth and soil health (Sumbul *et al*., 2020). *Azotobacter* sp. is very resistant to adverse environmental conditions, including the presence of heavy metals, which are commonly found on post-mining sites (Suryatmana *et al*., 2024).

Rhizobium sp. creates symbiotic relationships with legumes to aid in nitrogen fixation, which is critical for soil fertility (Alemayehu *et al*., 2018). *Rhizobium* sp. treatment is particularly beneficial because it can promote the growth of legume plants, which are frequently utilized in revegetation operations. According to research, inoculating soil with *Rhizobium* sp. can boost plant yields while improving deteriorated soil's chemical and physical qualities (Abd-Alla *et al*., 2023).

However, indigenous bacteria have considerable promise for rehabilitating past gold mining land. These bacteria can survive in hostile environments and detoxify heavy metals, restoring soil fertility (Rajkumar et al., 2010). The employment of *Azotobacter* sp. and *Rhizobium* sp. in bioremediation systems provides an environmentally beneficial and long-term addressing soil deterioration induced by gold mining (Glick, 2010). Combining these bacteria

with planting *P. falcataria* can accelerate soil regeneration (Singh *et al*., 2011).

More research is required to understand the influence of underground gold mining on soil chemical and biological qualities and the potential of indigenous microbes in land rehabilitation. This study analyzed soil chemistry changes and evaluated soil microbial communities. It examined the usefulness of indigenous bacteria such as *Azotobacter* sp. and *Rhizobium* sp. in promoting *P. falcataria* growth for future rehabilitation of post-mined land. It is envisaged that this strategy will yield an effective way to improve the state of post-gold mining soil and restore the soil ecosystem's function sustainably.

MATERIALS AND METHODS

Study site and soil sampling

Soil samples were taken from an underground post gold mine in Pongkor West Java, Indonesia. Soils were collected from natural forests, rehabilitated areas of varying ages (1, 2, and 5 year), and abandoned 10 years old tailing in post-gold mining areas. Soils were obtained from the rhizospheres of plants growing in each region. In each of these samples, we selected five different sites. Chemical properties were analyzed using a composite sample from each location, totaling five samples.

Analysis of soil chemical characteristics

Soil Composite soil samples were analyzed for several chemical characteristics such as soil pH H2O, pH KCl, Soil Organic Carbon (SOC) (Walkley-Black Method), N Total (Kjedahl method), Available P (Bray Method), Exchangeable K (AAS), and Hg (AAS).

Header and footer isolation of *Rhizobium* from root nodule

In mining area, only abandoned gold tailings had leguminous plants. Leguminous plants are pulled carefully to check root nodules. Red inner root nodules are gathered and packaged in tissue paper for the lab. The healthy nodule is gently removed from the root and collected for root nodulating bacteria isolation. After sterilizing in 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds, the removed root nodules are cleansed with tap water to eliminate soil particles. Transfer the sterilized nodule to a test tube with 5 ml sterilized deionized water, crash it with a sterilized glass rod, mix well, and isolate nodulating bacteria. Aseptically streaking the suspension into Congo Red Yeast Mannitol Agar (CRYEMA) plates isolates nodulating bacteria. Parafilm is used to seal plates and incubate them at 28 °C for 24–48 hours to prevent contamination. After 24–72 h, *Rhizobium* sp. colonies remain white, transparent, raised, and mucilaginous, while contaminations turn crimson. The colonies are transferred to Yeast Mannitol Agar (YEMA) for further analysis.

Isolation of *Azotobacter* from soil

The soil samples from natural forests, reclamation areas of varying ages, and post-gold mining areas are categorized as serial soil dilution. A total of 0.1 ml of soil suspension at 10-4 and 10-5 dilutions was disseminated on Ashby medium (20 g mannitol, 0.2 g K_2HPO_4, 0.2 g $MgSO_4$ –$7H_2O$, 0.2 g NaCl, 0.1 g K_2SO_4, 5 g $CaCO_3$, and 15 g agar per L). Under dark conditions at room temperature, the inoculated Ashby agar medium is incubated at 28°C for 5-7 days. This process is repeated for each sample. A single colony is sub-cultured and stored after all bacteria exhibiting the morphological characteristics of *Azotobacter* sp. are purified after incubation.

N-fixation of *Azotobacter* sp. by NFB indicator

The efficiency of nitrogen fixation in isolated *Azotobacter* sp. was assessed qualitatively using Nitrogen Free Bromothymol Blue (NFB) semi-solid medium (DL-malic acid 5 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2.2H_2O$ 0.02 g, micronutrient solution 2 ml, Bromothymol blue solution (0.5 % in 0.2N KOH) 2 ml, Fe (III) EDTA (1.6%) 4 ml, vitamin solution 1 ml, distilled water 1 L, pH 6.8, agar 0.5 g). Semi-solid NFB was autoclaved at 121°C 1.5 atm for 5 minutes. A single colony of *Azotobacter* sp. was aseptically

transferred into a 10 ml semi-solid NFB media. Inoculated NFB was incubated at 33°C under dark condition for 3 days. Uninoculated NFB was prepared as control. Three replications were created. A positive result in nitrogen fixation was indicated by the change in color of the inoculated NFB from green to blue.

Analysis of indole-3-acetic acid (IAA)

Preparation of nutrient broth and inoculums of Azotobacter sp.

The nutrient broth (NB) medium was enriched with 5 mM tryptophan and further sterilized by autoclaving at 121°C for 15 minutes. Following that, a volume of approximately 100 µL of *Azotobacter* spp. bacterial culture was added into 100 mL of previously prepared media. The NB culture was treated with an inoculum and then placed in an incubator at a temperature of 30°C. The culture was shaken at a speed of 150 revolutions per minute for a duration of 72 hours. This method was described by Gordon and Weber (1951) and later by Loper & Schroth (1986).

Extraction of IAA

Following incubation, the bacterial culture was centrifuged at a speed of 10,000 rpm per minute for a duration of 15 minutes in order to separate the supernatant from the bacterial cells. In the meantime, the liquid portion was extracted and subjected to filtration using a 0.22 µm membrane filter in order to eliminate cellular remnants (Patten & Glick, 2002).

Color reaction with Salkowski Reagent

About 2 mL of the filtered supernatant was combined with 2 mL of Salkowski reagent. Subsequently, the combination was subjected to incubation at room temperature for a duration of 30 minutes in a light-deprived environment to facilitate the development of colour. IAA undergoes a reaction with Salkowski's reagent, resulting in the formation of a pink to red colour. This colour change can be quantified using a spectrophotometer (Gordon and Weber, 1951).

Measurement of IAA concentration

The concentration of IAA can be determined by measuring the absorbance at a wavelength of 530 nm using a UV-Vis spectrophotometer. Following the measurement, the subsequent procedure involves comparing the absorbance outcomes with the previously established IAA standard curve in order to ascertain the concentration of IAA in the sample (Bric *et al.*, 1991).

Preparation of growth media

The tailings from gold mining were collected and utilized as a plant substrate to evaluate the effectiveness of isolates in enhancing plant development. Tailings were autoclaved for five minutes at 121°C and 1.5 atm, either with or without a 10% compost addition. There were 5 replications made.

Preparation of seedling

Paraserianthes falcataria was used in this study. The percentage of seed germination was increased by immersing *P. falcataria* seeds at 40°C for one night prior to sowing. Seeds were planted in sterilized sand. Uniformed two-week-old germinated seedlings were selected for inoculation testing.

Inoculation of *Azotobacter* and *Rhizobium*

The inoculation of *Azotobacter* sp. was performed due to its nitrogen-fixing capabilities, as confirmed by the NFB test. A two-week-old *P. falcataria* seedling was transplanted into 300 grams of sterilized growing medium. *Azotobacter* sp. was injected two weeks after being transplanted. The *Azotobacter* sp. and *Rhizobium* sp. inoculums were generated by propagating a single colony of *Azotobacter* sp. from Ashby agar, and *Rhizobium* sp. from YEMA media into the nutritional broth. The broth was incubated until the population of *Azotobacter* sp. and *Rhizobium* sp. reached a concentration of 108. Once the inoculums are prepared, the seedlings get 1 ml of each inoculum. The seedlings were cultivated for three months in a greenhouse. There was no application of fertilizer. Water was provided daily. Seedling

height, diameter, leaf number, and biomass were assessed three months after planting.

Data analysis

The statistical significance of soil chemical characteristics, plant growth (leaf, height, and diameter), and shoot dry weight (SDW) were assessed using KaleidaGraph 4.1 software (Synergy Software 2012, USA) by analysis of variance (ANOVA). A post hoc analysis was conducted using the Tukey HSD test, with a significance level of $p<0.05$.

RESULT AND DISCUSSION

Result

The table 1 presents the soil chemical properties from five different locations. Mining activities led to a significant decrease in the amount of soil organic carbon and N. Meanwhile, the older period of rehabilitation resulted in an improvement in these levels. All soil origins exhibited deficiencies in available phosphorus and exchangeable potassium.

The inoculation of *Rhizobium* sp. had a substantial impact on the leaf number and shoot dry weight of *P. falcataria* growing in tailings, as shown in Table 2. Furthermore, the use of 10% compost resulted in enhanced seedling height, diameter, and shoot dry weight when combined with the application of *Rhizobium* sp., as shown in Table 3. These findings suggest that the use of compost in combination with *Rhizobium* sp. can potentially enhance the growth of some plants in areas that have been affected by gold mining.

Among different land use change, *Azotobacter* sp. was successfully isolated from soil collected in natural forest, post gold mining area, and 5 years old rehabilitated area (Table 4). There was no *Azotobacter* sp. could be obtained from 1 and 2 years old rehabilitated area. There were 3 *Azotobacter* spp. isolated from natural forest (NF 1, NF2, NF 3), 4 *Azotobacter* spp. isolated from 5 years old rehabilitated area (R5.1, R5.2, R5.3, R5.4), and 5 *Azotobacter* spp. isolated from tailing of post gold mining area (T1, T2, T3, T4, T5).

The application of compost along with *Azotobacter* sp. inoculation significantly increased seedling height (Figure 1A). *Azotobacter* 8 exhibits the superior performance in seedling height, succeeded by *Azotobacter* 4, 1, 2, and 5. *Azotobacter* strains 2 and 6 produced a substantial increase in height compared to the control in tin tailings without the addition of compost. The inoculation of *Azotobacter* 8 and 4 resulted in a greater leaf number compared to the control (Figure 1B).

Azotobacter sp. inoculation markedly enhanced seedling diameter in tailings supplemented with 10% compost (Figure 2A). Only *Azotobacter* strains 6 and 7 did not influence this parameter. Simultaneously, *Azotobacter* 8 exhibited the highest efficacy in augmenting shoot dry weight, succeeded by *Azotobacter* 4 (Figure 2B).

Generally, *Azotobacter* sp. strains obtained from undisturbed forests exhibited greater indole-

Table 1. Soil chemical characteristics collected from natural forest, tailing gold mining, and three different ages of rehabilitated area in Pongkor West Java, Indonesia.

Soil Origin	Soil chemical characteristics						
	pH		SOC	N Total	Av. P	Ex. K	Ex. Hg
	H2O	KCl	(%)	(%)	(%)	(cmol/kg)	(ppm)
NF	4.50 a	3.70 d	4.21 a	0.299 a	0.013 c	0.410 bc	0.9 a
Tailing	7.40 a	6.70 a	0.88 c	0.096 d	0.019 c	0.538 ab	0.9 a
R 1 yr	7.35 a	6.70 a	1.30 bc	0.115 cd	0.019 c	0.333 c	0.1 b
R 2 yr	5.60 a	4.50 c	1.03 bc	0.126 bc	0.043 b	0.513 b	0.3 b
R 5 yr	6.40 a	5.50 b	1.62 b	0.142 b	0.142 a	0.667 a	1.0 a

*Different letters within the same column indicate statistically significant differences according to the Tukey HSD test ($P<0.05$), with a sample size of 5.

3-acetic acid (IAA) synthesis compared to isolates from land that had undergone 5 years of rehabilitation, with the lowest IAA production observed in tailings area that had been rehabilitated for 10 years (Table 5).

Inoculation of *Azotobacter* sp. into NFB media resulted on the change color of NFB from green turned into blue (Figure 3). This result indicates that all *Azotobacter* sp. are free living microorganism that capable in fixing N2 from atmosphere and change it into ammonium or nitrate.

Discussion

Gold mining significantly decreased the soil biochemical properties. Corresponds to this results, Misebo *et al.*, 2022 reported that soil on post mining land has significantly reduced levels of soil organic carbon (SOC) and nitrogen (N) compared to undisturbed land.

One of the primary obstacles in rehabilitating post mining land is the insufficient levels of soil organic carbon (SOC) and nitrogen (N) concentration. Mining frequently leads to the physical, chemical, and biological deterioration of soil, which ultimately leads to the depletion of organic matter and vital nutrients. This decrease in SOC and N content has a detrimental effect on soil productivity and its capacity to support vegetation growth (Qiu *et al.*, 2018). The depletion of indigenous flora and soil microorganisms, crucial for the breakdown of organic material, further exacerbates the low levels of soil organic carbon (SOC) and nitrogen (N) content, hence further deteriorating soil conditions in this region (Hu *et al.*, 2020). This situation hampers the productivity of post mining land and necessitates human involvement to expedite the process of ecosystem restoration (Misebo *et al.*, 2022).

Leguminous plants were only discovered near by abandoned tailings among multiple sampling sites. The plant's root nodule has a finger-like shape and is coloured red on its inside. In addition, one *Rhizobium* sp. isolate was successfully obtained from the root nodule. *Rhizobium* sp. bacteria colonies that are isolated are white and exhibit rapid growth, typically within a timeframe of less than five days. Among nodules, only one *Rhizobium* sp. was successfully isolated and used in this research.

The application of compost alongside *Rhizobium* sp. may augment the growth of certain plants in regions impacted by gold mining (Table 3). *Rhizobium* sp., a symbiotic bacterium with the ability to perform nitrogen fixation by turning atmospheric nitrogen into a usable form for plants, provides significant advantages to legumes that engage in a mutualistic association with this bacterium. Studies indicate that introducing *Rhizobium* sp. into soil that has been supplemented with a little amount of compost can enhance nutrient accessibility, enhance soil composition, and boost soil microbial activity (Ahmed *et al.*, 2023). Compost serves as an organic material that promotes the growth and activity of *Rhizobium* sp., leading to an enhancement in nitrogen fixation and the growth of legume plants (Tortosa *et al.*, 2023).

Furthermore, the utilization of *Rhizobium* sp. inoculation in combination with the application of compost contributes to the enhancement of soil

Table 2. Growth of 3 months old *P. falcataria* with or without inoculation of *Rhizobium* sp. growing in tailing of post-gold mining in greenhouse.

Treatment	Leaf number	Height (cm)	Diameter (mm)	SDW (g)
Control	4.14 b	5.93 a	1.21 a	3.58 b
Rhizobium	5.28 a	5.13 a	1.35 a	4.93 a

*Different letters within the same column indicate statistically significant differences according to the Tukey HSD test (P<0.05), with a sample size of 5.

Table 3. Growth of 3 months old *P. falcataria* with or without inoculation of *Rhizobium* sp. growing in mixture 10% compost+tailing of post-gold mining in greenhouse.

Treatment	Leaf number	Heigh t (cm)	Diameter (mm)	SDW (g)
Control	5.86 a	4.73 b	1.43 b	4.55 b
Rhizobium	5.86 a	7.37 a	1.65 a	6.63 a

*Different letters within the same column indicate statistically significant differences according to the Tukey HSD test (P<0.05), with a sample size of 5.

quality in post mining areas. Compost enhances the soil's organic matter level and supplies extra microorganisms and vital nutrients necessary for plant development. Studies have demonstrated that legume plants cultivated on post mining area and subjected to this specific treatment exhibit noteworthy enhancement in biomass, nodulation, and leaf nitrogen content in comparison to plants that were not subjected to this treatment (Koziel, 2023). This demonstrates that this method not only promotes the development of legume plants but also enhances the state of depleted soil, expediting the rehabilitation of post mining land ecosystems.

The absence of *Azotobacter* sp. during the early stages of land restoration is due to the unstable soil conditions and insufficient organic matter necessary for the sustenance of microbial life. *Azotobacter* sp. grows well in a soil environment

Table 4. Morphological characteristics of *Azotobacter sp* growing in medium of Ashby Mannitol Agar 3 days after incubation isolated from three different land use.

Land Use	Morphology of *Azotobacter* colony						Gram	Colony
	Form	Elevation	Size	Texture	Color	Margin	Staining	Number
NF1	Round	Flat	Medium	Slimy	Clear white	Entire	-	50×10^3
NF2	Round	Convex	Medium	Slimy	Clear white	Entire	-	160×10^4
NF3	Round	Flat	Medium	Slimy	Clear white	Entire	-	10×10^3
T1	Round	Flat	Medium	Slimy	Clear white	Entire	-	2.5×10^3
T2	Round	Flat	Medium	Slimy	Clear white	Entire	-	100×10^3
T3	Round	Flat	Medium	Slimy	Clear white	Entire	-	6×10^3
T4	Round	Flat	Medium	Slimy	Clear white	Entire	-	15×10^3
T5	Round	Flat	Medium	Slimy	Clear white	Entire	-	20×10^3
R5.1	Round	Convex	Small	Slimy	Clear white	Entire	-	160×10^3
R5.2	Round	Convex	Medium	Slimy	Clear white	Entire	-	3×10^3
R5.3	Round	Convex	Medium	Slimy	Clear white	Entire	-	90×10^4
R5.4	Round	Flat	Small	Slimy	Clear white	Entire	-	240×10^3

* NF: Natural Forest; T: Tailing soil of post-gold mining; R5: 5 years old rehabilitated area. -: indicates gram negative.

Table 5. Characteristics of *Azotobacter* sp. growing in nitrogen free contain bromothymol blue (NFB) semi solid media in 3 days after incubation isolated from three different land use soil.

Code	Surface			Sediment	NFB	IAA	Concentration of IAA
	Pellicle	White ring	Membrane			qualitative	(mg/ml)
NF1	-	+	-	-	+	+++	117
NF2	-	-	+	++	+++	+	17
NF3	-	+	-	+	+++	+++	191
T1	-	-	-	+	+	++	50
T2	-	-	-	+	+++	+	10
T3	-	-	-	++	+++	++	8.5
T4	-	-	-	++	++	+	13.5
T5	-	-	-	++	+++	++	62
R5.1	-	+	-	++	++	++	52
R5.2	-	+	-	+	++	++	42.4
R5.3	-	-	-	+	+++	++	73.4
R5.4	-	+	-	+	++	++	53.9

*Notes: NF: Natural Forest; T: Tailing soil of post gold mining; R5: 5 years old rehabilitated area. -: indicates not forming on surface media and sediment; +: indicates forming on surface media and sediment; +, ++, +++: number of positive indicates darker blue color in NFB or darker red color in IAA solution.

that is abundant in organic matter and provides favourable microhabitats for its reproduction and optimal functioning. During the initial phases of rehabilitation, soil that was previously used for mining typically has a deteriorated structure, a low amount of organic matter, and a deficiency in necessary nutrients. As a result, these conditions are not optimal for the survival and functioning of microorganisms (Koziel, 2023). Furthermore, recently rehabilitated mine soil frequently exhibits a pH imbalance and increased levels of heavy metal contamination (Fodoué *et al*., 2022), both of which might impede the growth and functioning of *Azotobacter* sp. (Diaconu *et al*., 2020). This clarifies the rationale behind the greater number of *Azotobacter* sp. populations in natural forests and 5-year-old restored areas compared to tailing soil in post gold mining sites, as evidenced in Table 1, where there is a substantial presence of soil organic carbon in both regions.

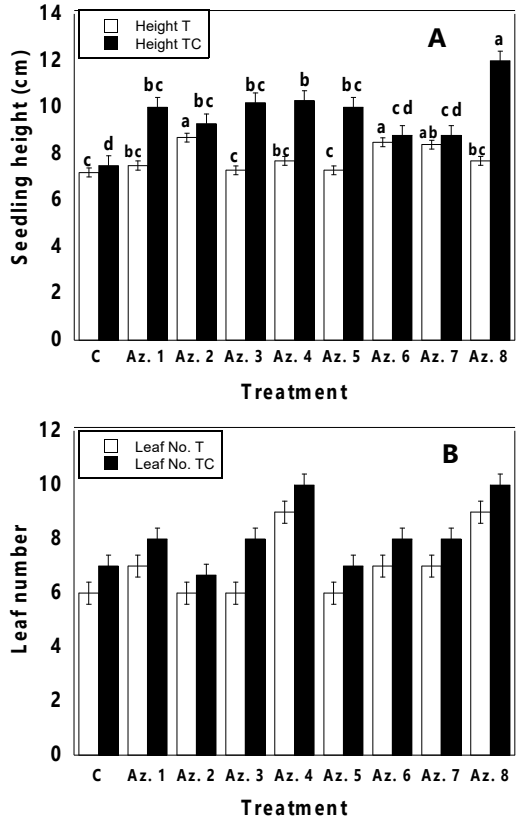

Figure 1. The height (A) and leaf number (B) of *P. falcataria* growing in tailing of post gold mining with or without *Azotobacter* sp. and 10% compost.

*Different letters in the same color bar indicate a significant difference using Tukey HSD ($p < 0.05$), with n=5. The vertical bars represent standard errors of mean (SE). T: tailing without compost; TC: tailing with 10% compost addition.

Meanwhile, *Azotobacter* sp. can be discovered in the 10-year-old tailings of a former gold mining site that has been abandoned. This is a result of natural recovery processes and reclamation efforts that have enhanced soil conditions to be more conducive for soil microorganisms. Over a decade, the natural process of leaves, roots, and other organic matter being deposited can lead to the accumulation of organic matter. This accumulation enhances the structure of the soil and supplies essential nutrients for bacteria (Prescott & Vesterdal, 2021). Furthermore, implementing reclamation measures such as including compost or organic fertilizer can enhance the organic matter concentration and nutrient equilibrium in the soil. This, in turn, fosters a more favourable environment for the survival and function of *Azotobacter* sp. bacteria (Diaconu *et al*., 2020). The enhancements in soil quality encompass the elevation of pH levels and the reduction of heavy metal pollution, hence facilitating the establishment and functioning of nitrogen-fixing microorganisms like *Azotobacter* sp. *Azotobacter* sp. can thrive and actively participate in the process of nitrogen fixation in post mining land, due to the presence of stable and nutrient-rich soil conditions (Sumbul *et al*., 2020). This is crucial for maintaining soil fertility and promoting the growth of plants.

The variation of IAA production of each isolate *Azotobacter* sp. from different land use may be related to the quality and quantity of organic material, along with the soil conditions, is responsible for this phenomenon in different types of land. Typically, natural forests exhibit a significant amount of organic matter and a consistent soil environment, which supports a greater variety and more active microbial populations. Among these microorganisms are

Azotobacter sp., which produce indole-3-acetic acid (IAA) (Sumbul *et al*., 2020).

On the other hand, land that has undergone rehabilitation for a period of 5 years is still undergoing the process of recovery and is beginning to exhibit a rise in the amount of organic matter present and the stabilisation of soil conditions. *Azotobacter* sp. is able to establish itself and generate IAA, eventhough at lower levels compared to those found in natural forests (Singh *et al*., 2020). After 10 years of rehabilitation, tailings fields show some improvement in soil condition compared to the initial stage. However, there are still limitations in the quality of organic matter and ecosystem stability needed to support optimal microbial activity, similar to that found in a natural forest environment.

In summary, the *Azotobacter* spp. that were introduced to the nitrogen-free bromothymol blue (NFB) medium exhibited a transition in color from green to blue (Figure 3). This is due to their capacity to generate and excrete ammonium (NH_4^+) as a consequence of the nitrogen fixation process. Bromothymol blue is a pH indicator that undergoes a color transition from green to blue in the presence of alkaline circumstances. *Azotobacter* sp. captures nitrogen from the atmosphere and transforms it into ammonium. This conversion leads to an increase in the pH of the surrounding environment, which is indicated by a change in colour to blue (Ekowati *et al*., 2021). The magnitude of this color alteration can fluctuate based on the nitrogen fixation activity of each *Azotobacter* sp. isolate, which is impacted by factors such as the availability of nutrients and microenvironmental circumstances (Cordova-Rodriguez *et al*., 2022).

Variations in nitrogen fixation efficiency among the investigated *Azotobacter* sp. isolates are also reflected in the differences in the amount of blue colour concentration in NFB media. Isolates with higher nitrogen fixation efficiency will generate greater amounts of ammonium, resulting in more pronounced colour variations in the media. In addition, this variation may also be attributed to variances in the metabolic and physiological states of each isolate, encompassing their growth rate and ability to adjust to the environmental circumstances of NFB semi-solid media (Arsita *et al*., 2020). Therefore, the transition of colour from green to blue in NFB media serves as both a signal of the existence of *Azotobacter* sp. and a quantitative measure of their nitrogen fixing activity.

The successful isolation of *Azotobacter* sp. can be determined by observing numerous different characteristics that manifest when this bacterium is cultivated in liquid media (Table 5). One of the primary indicators is the deposition of sediment at the base of the culture tube and the development of a white ring at the surface of the liquid medium (Arsita *et al*., 2020). Sediment is produced as a

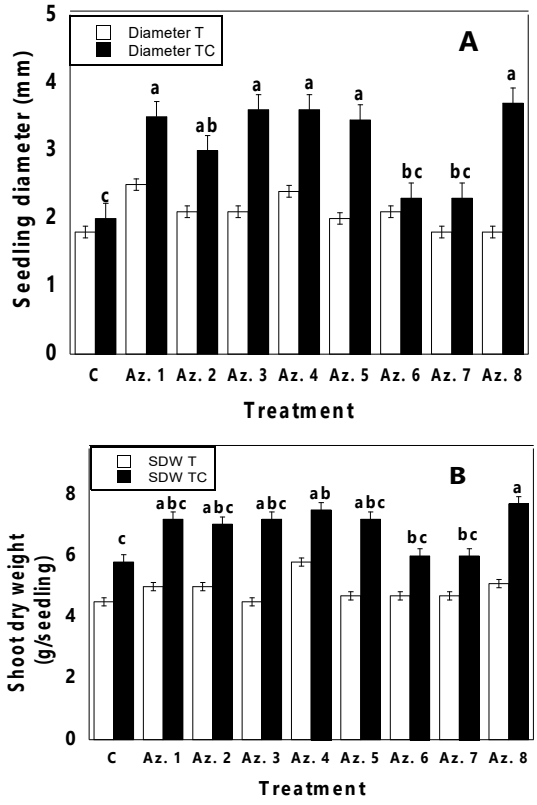

Figure 2. The diameter (A) and shoot dry weight (B) of *P. falcataria* growing in tailing of post gold mining with or without *Azotobacter* sp. and 10% compost.
*Different letters in the same color bar indicate a significant difference using Tukey HSD ($P < 0.05$), with n=5. The vertical bars represent standard errors of mean (SE). T: tailing without compost; TC: tailing with 10% compost addition.

result of the growth of *Azotobacter* sp., a bacterium that is obligate aerobic and relies on oxygen for its development. *Azotobacter* sp. exhibits enhanced growth when cultivated in liquid media, particularly on or in close proximity to the surface where the oxygen levels are more abundant. Consequently, nonviable or dormant cells will precipitate to the bottom of the tube (Bhattacharyya *et al.*, 2016).

The formation of a white ring at the top of the liquid medium is a result of *Azotobacter* sp. producing mucus capsules, which is a distinctive trait of this bacterium. These capsules, composed of polysaccharides, serve as a protective shield against adverse environmental conditions, shielding the bacterial cells while also aiding in the absorption of essential nutrients (Das *et al.*, 2024). The presence of a distinct white ring at the upper portion of the liquid medium in which *Azotobacter* sp. is cultivated is a direct consequence of this unique trait.

The *Azotobacter* sp. colonies identified in this investigation exhibit distinct morphological characteristics, as shown in Table 4. *Azotobacter* sp. colonies exhibit a round form with either convex or flat elevations, which is contingent upon the specific species and growth conditions, as indicated by previous studies (Arsita *et al.*, 2020). The colony's texture is typically viscous or slimy, resulting from the secretion of exopolysaccharides. These substances serve to safeguard bacterial cells from desiccation and other microbial predators. *Azotobacter* sp. colonies typically exhibit a translucent white hue (Hala & Ali, 2019), signifying the lack of pigments commonly seen in numerous other soil bacteria.

Furthermore, the periphery or boundaries of *Azotobacter* sp. colonies typically remain undamaged (intact), devoid of any protrusions or lobes. The physical properties of *Azotobacter* sp. colonies render them readily identifiable in the microbiology laboratory. Haerani *et al*. (2021) conducted a study that revealed *Azotobacter* sp. colonies isolated from agricultural soil exhibited a circular form with raised surfaces, a slimy texture, a distinct white hue, and undamaged edges. These findings align with the traditional morphological characterization of *Azotobacter* sp., which is commonly employed in bacterial identification for soil microbiology research.

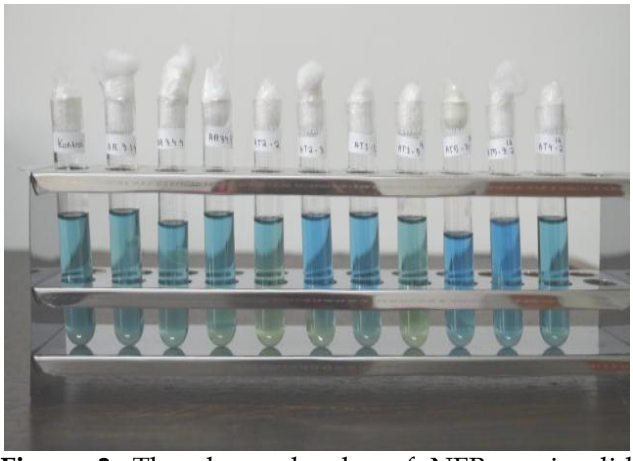

Figure 3. The changed color of NFB semi solid media when inoculated with *Azotobacter* sp. isolated from natural forest, 5 years old rehabilitated area, and 10 years old abandoned tailing of post gold mining.

The investigation involved doing gram staining on microorganisms, which revealed that 100% of the bacteria were gram negative (Table 4). Prior research has verified that *Azotobacter* sp. are categorized as Gram-negative bacteria on the basis of their Gram stain. In this regard, (Wakarera *et al.*, 2022) conducted a study where they particularly isolated and identified *Azotobacter* sp. from agricultural soil. The researchers used Gram stain analysis to determine that this bacterium displayed the typical characteristics of Gram-negative bacteria. The characteristic is distinguished by a reduced cell wall thickness in comparison to Gram-positive bacteria, together with the existence of an outer membrane layer composed of lipopolysaccharide, which sets them apart from Gram-positive bacteria (Dadook *et al.*, 2014). Gram staining is a crucial method for identifying bacteria and comprehending their cellular structure. This knowledge is vital for studying microbial ecology and applying it to agricultural practices like biofertilization.

Overall, the addition of 10% compost to *Azotobacter* sp. inoculation resulted in superior development, as depicted in Figure 2A. When

Azotobacter sp. was inoculated without using compost, only 2 Az isolates were observed. *Azotobacter* strain 2 and *Azotobacter* strain 6 exhibited superior height compared to the control and other *Azotobacter* strains. With the exception of Az. 6 and 7, nearly all *Azotobacter* spp. inoculations yielded superior plant height results compared to the control when 10% compost was introduced. However, the growth characteristics of leaf number did not show any significant difference when comparing the presence or absence of *Azotobacter* sp. inoculation, regardless of whether 10% compost was added or not (Figure 2B). Nevertheless, there was a tendency for an enhancement in leaf count when *Azotobacter* sp. inoculation and 10% compost were introduced.

Consistent with the findings of this research, a study conducted by Singh *et al.* (2020) corroborates this discovery. The study found that the application of *Azotobacter* sp. bacteria to tomato plants (*Solanum lycopersicum*) led to a significant growth enhancement in plant height when compared to the control plant. Furthermore, Albureikan (2024) studied the effects of *Azotobacter* sp. inoculation and compost on the growth of *Phaseolus vulgaris*, revealing significant positive impacts on plant development and soil fertility. The findings demonstrated a substantial growth enhancement in plants that were subjected to *Azotobacter* sp. inoculation, as opposed to the control plant that did not receive any inoculation. The researchers proposed a correlation between the rise in plant height with the greater availability of nitrogen fixed by PGPR, which the plant subsequently utilizes for the growth of its stem and roots. However, additional factors such as plant genetics and climatic conditions may have a more significant impact on the quantity of leaves that are produced. These data indicate that while *Azotobacter* sp. is successful in enhancing certain elements of plant growth, its impact on leaf number may be more unpredictable and susceptible to other influences.

Generally, compost addition helped six out of the eight types of *Azotobacter* sp. in improving plant growth performance particularly diameter and biomass of the seedlings. Consistent with this study, (Mahato & Kafle, 2018) demonstrated that the use of *Azotobacter* sp. alone, without the addition of compost, did not result in a substantial increase in the plant height and biomass of wheat (variety Gautam) seedlings. This study suggests that while *Azotobacter* sp. can contribute to nitrogen fixation, the absence of supplementary organic nutrients like compost may restrict the bacteria's ability to fully enhance plant growth. On the other hand, when *Azotobacter* sp. is used in conjunction with compost, notable enhancements in plant growth characteristics are frequently noted. Nalini *et al*. (2017) conducted a study shown that when *Azotobacter* sp. was introduced to chili (*Capsicum annuum*) seedlings along with compost, there was a notable enhancement in both seedling height and biomass. The findings are supported by Kumar *et al*. (2008). The researchers used a mixture of *Azotobacter* sp. and fertilizer to *Zea mays* plants in their experiment. The findings demonstrated a notable enhancement in plant's height, leave number, leaf length, dry biomass of plant, number of grain and grains yield, in comparison to the treatment using *Azotobacter* sp. alone.

CONCLUSION

Mining activities exert significant impacts on the quality of soil, resulting in diminished levels of soil organic carbon, total soil nitrogen (N), and deficits in nutrients. Within this investigation, out of the 12 *Azotobacter* spp. isolates that were obtained, a total of 8 isolates were chosen for assessment in terms of their potential to enhance plant growth. The selection was based on their distinctive physical traits as well as their capacity to both fix nitrogen and generate indole-3-acetic acid (IAA). The research findings indicate that the growth of *Paraserianthes falcataria* planted in mine tailings can be enhanced by inoculating *Azotobacter* sp. with a 10% compost mixture. This approach, which utilizes organic materials and employs a biological method, demonstrates significant promise in rehabilitating post mining land.

REFERENCES

Abd-Alla, M.H., Al-Amri, S.M. and El-Enany, A.W.E., 2023. Enhancing rhizobium–legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. *Agriculture.* 13(1): *2092*. Doi: 10.3390/agriculture13112092.

Ahmed, T., M. Noman, Y. Qi, M. Shahid, S. Hussain, H.A. Masood, L. Xu, H.M. Ali, S. Negm, A.F. El-Kott, Y. Yao, X. Qi, and B. Li. 2023. Fertilization of microbial composts: A technology for improving stress resilience in plants. *Plants.* 12(20): 3550. Doi: 10.3390/plants12203550.

Albureikan, M.O.I. 2024. Enhancement of plant growth with plant-based compost and the heterotrophic *Azotobacter* and *Streptomyces* inoculation under greenhouse conditions. *Journal of Pure and Applied Microbiology.* 18(3): 1632–1647. Doi: 10.22207/jpam.18.3.13.

Alemayehu, D., A. Zerihun, and B. Solomon. 2018. Limitations and strategies to enhance biological nitrogen fixation in sub-humid tropics of Western Ethiopia. *Journal of Agricultural Biotechnology and Sustainable Development*. 10(7): 122–131. Doi: 10.5897/jabsd2018.0318.

Arsita, R., H. Karim, Y. Hala, N. Iriany, and O. Jumadi. 2020. Isolation and identification of nitrogen-fixing bacteria in the corn rhizosphere (*Zea mays* L.*)* originating from Jeneponto Regency, South Sulawesi. *IOP Conference Series: Earth and Environmental Science*. 484: 012051. Doi: 10.1088/1755-1315/484/1/012051.

Bhattacharyya, P.N., M.P. Goswami, and L.H. Bhattacharyya. 2016. Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. *Journal of Phytology*, 8: 26. Doi: 10.19071/jp.2016.v8.3022.

Bhattacharyya, P.N., and D.K. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology.* 28(4): 1327-1350. Doi: 10.1007/s11274-011-0979-9.

Bric, J.M., R.M. Bostock, and S.E. Silverstone. 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology.* 57(2): 535–538. Doi: 10.1128/aem.57.2.535-538.1991.

Central Statistic Agency. 2019. *Produk domestik bruto Indonesia*. [online] Available at: <https://www.bps.go.id/id/pressrelease/2020/02/05/1755/ekonomi-indonesia-2019-tumbuh-5-02-persen.html> [Accessed 21 March 2025].

Central Statistic Agency. 2021. *Statistik Ekspor*. [online] Available at: <https://www.bps.go.id/id/publication/2022/07/06/d40cf02ad71801b227c81310/statistik-perdagangan-luar-negeri-indonesia-ekspor-menurut-kode-sitc-2020-2021.html> [Accessed 21 March 2025].

Cordova-Rodriguez, A., M.E. Rentería-Martínez, C.A. López-Miranda, J.M. Guzmán-Ortíz, and S.F. Moreno-Salazar. 2022. Simple and sensitive spectrophotometric method for estimating the nitrogen-fixing capacity of bacterial cultures. *MethodsX.* 9. Doi: 10.1016/j.mex.2022.101917.

Dadook, M., S.Mehrabian, M. Salehi, and S. Irian. 2014. Morphological, biochemical and molecular characterization of twelve nitrogen-fixing bacteria and their response to various zinc concentration. *Jundishapur Journal of Microbiology*. 7(4): e9415. Doi: 10.5812/jjm.9415.

Das, A., S. Ghosh, and N. Pramanik. 2024. Chitosan biopolymer and its composites: Processing, properties and applications- A comprehensive review. *Hybrid Advances.* 6: 100265. Doi: 10.1016/j.hybadv.2024.100265.

Diaconu, M., L.V. Pavel, R.M. Hlihor, M. Rosca, D.I. Fertu, M. Lenz, P.X. Corvini, and M. Gavrilescu. 2020. Characterization of heavy metal toxicity in some plants and microorganisms—A preliminary approach for environmental bioremediation. *New Biotechnology*. 56: 130–139. Doi: 10.1016/j.nbt.2020.01.003.

Ekowati, C.N., M. Mirani, K. Handayani, and R. Agustrina. 2021. Detection of nitrogenase producing bacteria from the soil of Liwa Botanical Garden. *Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati*. 8(2): 53–58.

Fagariba, C.J., J.B.B. Sumani, and A.S.Mohammed. 2024. Artisanal and small-scale gold mining impact on soil and agriculture: Evidence from upper Denkyira East Municipality, Ghana. *European Journal of Environment and Earth Sciences.* 5(3): 12–20. Doi: 10.24018/ejgeo.2024.5.3.443.

Fodoué, Y., A. Ismaila, M. Yannah, M.J. Wirmvem, and C.B. Mana. 2022. Heavy metal contamination and ecological risk assessment in soils of the Pawara Gold Mining Area, Eastern Cameroon. *Earth.* 3(3): 907–924. Doi: 10.3390/earth3030053.

Glick, B.R. 2010. Using soil bacteria to facilitate phytoremediation. *Biotechnology Advances.* 28(3): 367-74. Doi: 10.1016/j.biotechadv.2010.02.001.

Gordon, S.A., and R.P. Weber. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiology*. 26(1): 192–195.

Haerani, N., E. Syam'un, B. Rasyid, and F. Haring. 2021. Isolation and characterization of n-fixing and IAA producing rhizobacteria from two rice field agro-ecosystems in south sulawesi, Indonesia. *Biodiversitas.* 22(5): 2497–2503. Doi: 10.13057/biodiv/d220506.

Hala, Y., and A. Ali. 2019. Isolation and characterization of *Azotobacter* from neems rhizosphere. *Journal of Physics: Conference Series*. Doi: 10.1088/1742-6596/1244/1/012019.

Hartemink, A.E. 2005. Plantation agriculture in the tropics: Environmental issues. *Outlook on Agriculture.* 34(1): 11–21.

Hilson, G. 2002. An overview of land use conflicts in mining communities. *Land Use Policy*. 19: 65–73.

Hu, Y., Z. Yu, X. Fang, W. Zhang, J. Liu, and F. Zhao. 2020. Influence of mining and vegetation restoration on soil properties in the eastern margin of the qinghai-tibet plateau. *International Journal of Environmental Research and Public Health.* 17(12): 1–12. Doi: 10.3390/ijerph17124288.

Khan, M.S., A. Zaidi, and P.A. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. *Agronomy for Sustainable Development.* 27(1): 29-43. Doi: 10.1051/agro:2006011.

Koch, J. 2022. *The Alteration of Mine Tailings through Chemical, Physical and Biological Amelioration Aimed at Improving Soil Aggregation*. [Ph.D. Thesis]. North-West University.

Koziel, M. 2023. Factors determining the occurrence and number of bacteria of the genus Azotobacter in the soil environment. *Polish Journal of Agronomy.* 52: 14–21.

Kumar, K.V., N. Singh, H.M. Behl, and S. Srivastava. 2008. Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. *Chemosphere.* 72(4): 678–683. Doi: 10.1016/j.chemosphere.2008.03.025.

Loper, J.E., and M.N. Schroth. 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Physiology and Biochemistry.* 76: 386–389.

Mahato, S., and A. Kafle. 2018. Comparative study of Azotobacter with or without other fertilizers on growth and yield of wheat in Western hills of Nepal. *Annals of Agrarian Science.* 16(3): 250–256. Doi: 10.1016/j.aasci. 2018.04.004.

Misebo, A.M., M. Pietrzykowski, and B. Woś. 2022. Soil carbon sequestration in novel ecosystems at post-mine sites—A new insight into the determination of key factors in the restoration of terrestrial ecosystems. *Forests.* 13(1): 63. Doi: 10.3390/f13010063.

Mulyadi, D. Ruhiyat, M.I. Aipassa, and S. Hardwinanto. 2022. The growth of *Paraserianthes falcataria* at three different plant ages and soil thickness classes on reclamation sites of post-coal mining areas in East Kalimantan, Indonesia. *Biodiversitas.* 23(4): 1930–1937. Doi: 10.13057/ biodiv/d230427.

Nalini, B.S., L.K. Naik, N. Saba, P. Prasad, and G. Ashiwini. 2017. Studies on the effect of microbial inoculants on growth and yield of capsicum (*Capsicum annuum* L.). *Journal of Pure and Applied Microbiology.* 11(2): 913–919. Doi: 10.22207/JPAM.11.2.31.

Patten, C.L., and B.R. Glick. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology.* 68(8): 3795–3801. Doi: 10.1128/AEM.68.8.3795-3801.2002.

Prayoga, D., M. Riniarti, and Duryat. 2018. The application of *Rhizobium* and urea on *Paraserianthes falcataria* seedling growth. *Jurnal Sylva Lestari.* 6(1): 1–8.

Prescott, C.E., and L. Vesterdal. 2021. Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. *Forest Ecology and Management.* 498: 119522. Doi: 10.1016/j.foreco. 2021.119522.

Qiu, K., Y. Xie, D. Xu, and R. Pott. 2018. Ecosystem functions including soil organic carbon, total nitrogen and available potassium are crucial for vegetation recovery. Sci Rep. 8: 7607. Doi: 10.1038/s41598-018-25875-x.

Rajkumar, M., N. Ae, M.N.V. Prasad, and H. Freitas. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology.* 28(3): 142-149. Doi: 10.1016/j.tibtech. 2009.12.002.

Singh, B.K., P. Trivedi, E. Egidi, C.A. Macdonald, and M. Delgado-Baquerizo. 2020. Crop microbiome and sustainable agriculture. *Nature Reviews Microbiology.* 18: 601–602. Doi: 10.1038/s41579-020-00446-y.

Singh, J.S., V.C. Pandey, and D.P. Singh. 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. *Agriculture, Ecosystems and Environment.* 140(3): 339-353. Doi: 10.1016/j.agee.2011.01.017.

Sumbul, A., R.A. Ansari, R. Rizvi, and I. Mahmood. 2020. Azotobacter: A potential bio-fertilizer for soil and plant health management. *Saudi Journal of Biological Sciences.* 27(12): 3634-3640. Doi: 10.1016/j.sjbs.2020.08.004.

Suryatmana, P., S. Handayani, S. Bang, and R. Hindersah. 2024. Screening and profiling of mercury-resistant Azotobacter isolated from gold mine tailing in Pongkor, West Java. *Journal of Degraded and Mining Lands Management.* 11(2): 5287–5300. Doi: 10.15243/jdmlm. 2024.112.5287.

Tarras-Wahlberg, N.H., A. Flachier, S.N. Lane, and O. Sangfors. 2001. Environmental impacts and metal exposure of aquatic ecosystems in rivers contaminated by small scale gold mining: the Puyango River basin, southern Ecuador. *The Science of the Total Environment.* 278(1): 239-61.

Tortosa, G., S. Mesa, M.J. Delgado, and C.V. Amaya-Gómez. 2023. "Alperujo" compost improves nodulation and symbiotic nitrogen fixation of soybean inoculated with *Bradyrhizobium diazoefficiens*. *Nitrogen.* 4(2): 223–230. Doi: 10.3390/nitrogen4020015.

US Geological Survey. 2020. *Mineral Commodity Summaries 2020.* https://pubs.usgs.gov/periodicals/mcs2020/ mcs2020.pdf.

Wakarera, P.W., P. Ojola, and E.M. Njeru. 2022. Characterization and diversity of native *Azotobacter* spp. isolated from semi-arid agroecosystems of Eastern Kenya. *Biology Letters.* 18(3): 20210612. Doi: 10.1098/rsbl.2021.0612.

Wulandari, D., C. Agus, R. Rosita, I. Mansur, and A.F. Maulana. 2022. Impact of tin mining on soil physio-chemical properties in Bangka, Indonesia. *Jurnal Sains & Teknologi Lingkungan.* 14(2): 114–121. Doi: 10.20885/jstl.vol14.iss2.art2.

Wulandari, D., Saridi, W. Cheng, and K. Tawaraya. 2016. Arbuscular mycorrhizal fungal inoculation improves *Albizia saman* and *Paraserianthes falcataria* growth in post-opencast coal mine field in East Kalimantan, Indonesia. *Forest Ecology and Management.* 376: 67–73. Doi: 10.1016/j.foreco.2016.06.008.