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Research Article

The potential of legume cover crops and soil microbes for gold mine tailings revegetation

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Abstract

Mercury (Hg) is commonly utilized in artisanal gold mining on Buru Island; the Hg-contaminated tailing possibly contaminates the agricultural land nearby. In general, tailings contain very low organic carbon and plant nutrients but are high in mercury and have extreme soil acidity. The objective of this study was to observe the growth of various legume cover crops (LCC) and the change of Hg in tailing inoculated with *Azotobacter-Trichoderma*. The field trial was conducted on Buru Island of Maluku by using a split-plot design with three replications. The main plot was LCC species, composed of *Centrosema pubescens* (CP), *Mucuna* sp. *(*MC), and *Crotalaria* sp. (CR); the subplots were microbial inoculants composed of two formulations of *Azotobacter-Trochoderma* inoculants. The results showed that the *Mucuna* sp. and consortia *Azotobacter-Trichoderma* (2:1) had the highest survival rate in the tailings and Hg uptake by 8.83 mg kg^{-1} per plant. Consortia *Azotobacter-Trichoderma* inoculant with the composition of 2:1 (v:v) was able to increase soil pH, total bacterial population, LCC biomass, and Hg uptake by LCC plants. The highest Hg removal effectivity was observed in the plot treated with *Crotalaria* sp. and in a plot with *Azotobacter-Trichoderma* (2:1), which was 34.0% and 33.6%, respectively.

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Introduction

Artisanal and Small-scale Gold Mining (ASGM) activities are commonly found in Indonesia, including Mount Botak and Gogorea, Buru Island, Maluku (Arifin et al., 2020; Pranoto and Budianta, 2021). The use of mercury (Hg) in the gold extraction process produces Hg-contaminated tailing that threatens soil and environment quality since the tailing was deposited in agricultural land (Manullang et al., 2021).

The soil around the ex-gold mining has changed its soil profile, the A and B horizons have disappeared, and the C horizon has become topsoil with a depth of 0-100 cm. Soil compaction (high density) occurs, and the soil becomes rough because it is dominated by the sand fraction, which causes drainage and permeability faster, macronutrient content, pH, and soil microbial population are low, and heavy metal contamination in ex-gold mining (Wasis and Fathia, 2011; Amri et al., 2020).

Gold mine tailings have poor physical and chemical properties. Tailings are dominated by the sand fraction with low organic matter content, very acidic soil pH, and low soil cation exchange capacity (CEC)

(Hindersah et al., 2018a). Moreover, the Hg content in the tailings was higher than in the natural soil. The total Hg content of soil on agricultural land in Waelata and Waeapo, Buru Island, is around 1.64-5.84 mg kg^{-1}, while in tailings, the Hg content is around 11.01-166.10 mg kg^{-1} (Hindersah et al., 2018b). Mercury can encounter biotransformation into organic compounds methyl mercury due to the decomposition process by microbes. Mercury (Hg^{2+}) can be methylated into gaseous methyl mercury by *Pseudomonas* sp., *Escherichia* sp., *Bacillus s*p., and *Clostridium* sp. Methylation by microbes plays an important role in the biogeochemical elements of metallic elements because methylated compounds are often volatile (Oh et al., 2015).

Tailings waste disposed on agricultural land needs to be remediated from excessive Hg levels so that it can be revegetated with legume plants. Bioremediation is an effective method to reduce Hg levels as well as soil nutrients. Inoculants of rhizobacterial biological agents (*Azotobacter*) can increase soil nitrogen availability and produce phytohormones, gibberellins, and indole acetic acid (IAA). *Azotobacter* strains also produce exopolysaccharides (EPS), an important metabolite to avoid metal toxicity. EPS on the surface of rhizobacterial cells forms coordination with Hg. The EPS-Hg chelate will be released from the cell surface and become mobile so that plants can absorb Hg (Hindersah et al., 2021a). Moreover, *Azotobacter* can also be used as an antagonist to plant pathogens. The well-known biological agent for controlling the soil-borne is *Trichoderma* (Hindersah and Asmiran, 2017). *Trichoderma spp*. can colonize the root surface and cortex and use nutrients from other fungi.

Organic matter amendment can improve tailing physical properties, enhance chemical fertility, and stimulate plant root exudates to provide organic compounds or exudates that can supply carbon sources like carbohydrates and organic acids for soil microbes. Revegetation is an important factor in improving the metabolic activity and community structure of microorganisms in tailings (Liu et al., 2018; Dhaliwal et al., 2019; Lin et al., 2021). Starting the revegetation with legumes is recommended on marginal land. Legume roots contain nodules with a synergistic association between roots and nitrogen-fixing rhizobia. Inside the nodules, rhizobium bacteria transport the fixed N to the xylem of plant roots in the form of asparagine (Schwember et al., 2019). Planting legume cover crops on tailing revegetation is reported to have advantages because of the extensive root system, good seedling vigor, fast-growing, large biomass, and high tolerance to unfavorable environmental conditions (Lin et al., 2021).

The application of compost and LCC effectively determines and maintains crop growth and biomass production and decreases heavy metal sorption by crop (Budianta et al., 2013; Prayogo and Ihsan, 2018). Species of LCC, such as *Centrosema pubescens* and *Peraria javanica* are tolerant to abiotic stress of tailing *Centrosema pubescens* is mainly found in tailing derived from gold mining (Budianta et al., 2013). *Centrosema mole* and *Calopogonium mucunoides* show that both legume species can significantly increase the phosphorus and organic matter content, as well as decrease heavy metal concentrations in mine tailings (Domingo and David, 2014).

Optimization of legume growth in low-nutrient and Hg-contaminated tailings need to be supported by good tailing fertility. Therefore, it is necessary to study the effectiveness of beneficial soil microbes to increase the growth of LCC. This study aimed to determine the effect of several types of LCC and the application of compost and the biological agent *Azotobacter-Trichoderma* in reclamation efforts and reducing mercury concentrations in the gold mine tailings in Buru Island, Maluku.

Materials and Methods

The research was conducted in Waelo Village, Waelata Subdistrict, Buru Regency, Maluku, Indonesia (Figure 1). The area is located in the tropics with a geographical position of 3°28'26"S-126°59'19"E. The experimental site is a tailing pile of gold extraction using the amalgamation method; the thickness of the tailing was more than 1 m which is not utilized due to the high mercury content.

Microbes and tailings

The compost was produced in Kairatu, Ambon Island; with a water content of 11.74%, pH 7.1, and organic carbon content of 26.66%, which follows the requirements of organic fertilizer regulatory by the Ministry of Agriculture No. 70 of 2011 (Permentan 70/2011). However, the content of N, P_2O_5, and K_2O in compost was low (1.10, 0.61, and 0.20%, respectively). The compost contains Pb, Cd, and Hg of 17.25, 1.21, and 0.40 mg kg^{-1}, respectively. The Azotobacter isolated from gold mine tailing in Buru was provided by Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. Trichoderma was a collection of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Pattimura.

The consortia of both microbes were formulated in organic-based solid inoculant with the composition of 2:1 and 1:2; the populations of Azotobacter and Trichoderma in solid inoculant were at least 10^7 CFU g^{-1} and 10^5 CFU $mL^{-1,}$ respectively. The tailing was dominated by a sand fraction (82%), the clay fraction was low (5%), and the silt fraction was 13%. The tailing has a pH of 2.7 (very acid), 0.71% of organic matter (low), 0.19% of total N (low), 10.80 mg kg^{-1} of available K (low), 5.07 mg kg^{-1} of available P_2O_5 (moderate), and 4.22 cmol kg^{-1} of cation exchange capacity (low). The low CEC was due to low clay and organic matter content in the tailing. The low nutrient content was caused by gold mining activities that caused land damage due to taking topsoil to a certain depth and the washing process, which contains toxic metal compounds to soil and plants. The concentration of Hg in the tailing at the study site was very high (566.9 mg kg^{-1}). According to Kabata-Pendias (2011), the maximum allowable concentration (MAC) of Hg in soil for agriculture is 0.5-5 mg kg^{-1}. So, efforts are needed to reduce Hg concentration on tailing and soil if used as agricultural land.

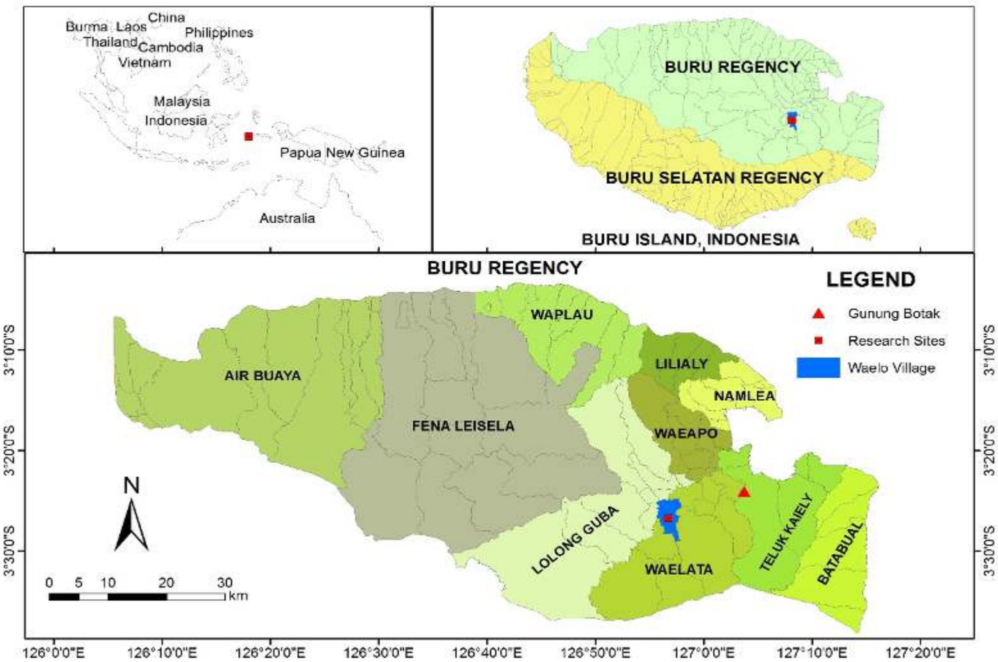

Figure 1**.** Map of research location in Waelo Village, Waelata Subdistrict, Buru Regency, Maluku Province, Indonesia.

Experimental methods

The research used a split-plot design with three replications. The main plots were LCC consisting of *Centrosema pubescens* (CP), *Mucuna* sp. (MC), and *Crotalaria* sp. (CR), and the subplots were soil microbial inoculation consisted of control (M0), inoculants of *Azotobacter*-*Trichoderma* (1:2) (M1), and inoculants of *Azotobacter*-*Trichoderma* (2:1) (M2).

The size of individual plots was 1 m x 1 m with 30 cm space between plots. Plowing of all plots was carried out by hoe at the dept of 30 cm, and then a total of 2 t ha^{-1} of lime was added and incubated for two weeks to increase soil pH. A compost of 40 t ha^{-1} was then incorporated evenly and incubated for three days. The inoculation of *Azotobacter*-*Trichoderma* was carried out after the planting medium was added with compost and incubated for three days. LCC plants were seeded first and then transplanted 30 days after seedling at a distance of 30 cm x 30 cm. The LCC plants were maintained for 8 weeks after planting (WAP).

Parameters and data analysis

Research parameters included microbial population, legume biomass, as well as tailing characteristics, total Hg in tailing, and plant after experiments. Tailing samples were taken before and 8 WAP using a composite on top layer (0-20 cm) for each treatment plot. Biomass samples were taken at the LCC plant 8 WAP. The bacterial and fungal populations in the rhizosphere were counted by serial dilution plate method by using nutrient agar and potato dextrose, respectively. The intact LCC was weight to determine the fresh biomass total, The fresh biomass total was then stored at 60 ^{o}C for 3 days until constant weight prior to the dry weight.

The soil pH, organic carbon, total N, and CEC of tailing were analyzed proximately by the method of ISRI (2009). Analysis of total Hg in tailing and plants was performed using HNO_3 and $HClO_4$ extracts, and the Hg content was determined by an atomic absorption spectrophotometer (AAS).

The uptake of heavy metal by plants was calculated with the formula = metal concentration in the plant (mg kg^{-1}) x plant biomass (kg), according to Hadi and Purnomo (2022). The removal effectivity (RE) in this study was calculated by reducing metal concentration in soil (Khoiriyah et al., 2015). The formula used is:

$$RE = \frac{(A-B)}{A} \text{ x } 100\%$$

where: RE is removal effectivity, A is the initial metal concentration in soil (mg kg^{-1}), and B is the last metal concentration in soil (mg kg^{-1}).

The data were subjected to analysis of variance at the 5% level and continued with the Tukey test at $p \leq 0.05$. All statistical analyses were performed using Minitab Ver 16 software.

Results and Discussion

The total population of rhizosphere microbes

Table 1 shows the total population of bacteria and fungi. The total population of bacteria and fungi in tailing with *Mucuna* sp. (MC) and Azotobacter: Trichoderma (2:1) treatment were 121 x 100 CFU g^{-1} and 43 x 100 CFU g^{-1}, respectively. *Mucuna* sp. plants had larger seeds and wide roots than other LCC. *Mucuna* sp. produced root exudate, which caused the microbial population to grow and increase around the roots. The population of bacteria was higher than fungi, which agreed with a higher population of bacteria in soil than other microorganisms (Wijayanti et al., 2020). Bacteria are more resistant to changes in soil and environmental conditions, unlike fungi which are easily influenced by soil pH, nutrients, and extreme environmental conditions (Feketová et al., 2021).

Table 1. Total population of total bacteria and fungi in the rhizosphere of LCCs grown in Hg-contaminated tailing of 8 weeks after planting.

Treatments	Bacteria	Fungi
	x 100 (CFU g^{-1})	
CP x M0	11	8.7
CP x M1	56	15
CP x M2	110	75
MC x M0	15	4.3
MC x M1	64	9.5
MC x M2	121	43
CR x M0	7.3	1.4
CR x M1	31	9.1
CR x M2	87	37

Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1).

The total population of bacteria was low, and compost amendment did not affect the total rhizosphere microbes. Before the experiment, the tailing contained low N but had high C/N resulting in a lower rate of microbial activity to decompose organic matter in the soil (Zainudin and Kesumangwati, 2021). The prominent factor that can reduce the population of microorganisms in the soil is land use, such as the conversion of agricultural land to mining land and primary forest to secondary forest. Land use also affects the vegetation type, where soil microorganisms and vegetation influence each other.

The low population of rhizobacteria could also be due to the root growth of LCC had not been intensive until 8 weeks after planting (WAP). Nonsymbiotic heterotrophic nitrogen-fixing bacteria require carbon provided by root exudates as an energy source. The effect of LCC and Azotobacter, in general, has not induced fungal populations in the soil, so callus formation resulted in the stagnation of fungal populations in the soil. Inoculation of *Azotobacter* spp. has the potential to inhibit fungal growth and play a role in controlling plant diseases (Kasa et al., 2015; Hindersah et al., 2018c).

Total biomass produced by legume cover crops (LCC)

The LCC grew in all treatments/plots with lime and compost (40 t ha^{-1}). Organic matter enables to increase soil nutrient content. The sand fraction of >60% dominated the tailing causing the soil to be very porous; watering twice a day was not optimal because water easily leaches out of the root zone in porous soil, and the plant cannot withstand the harsh environment. The high concentration of Hg at the study site might not directly cause the retarded growth of LCC. Ex-mining areas generally have low organic carbon content. This is because the gold ore undergoes a reshuffle of the soil layer. The tailing pH was very acidic after liming, and the LCC was difficult to grow on ex-gold mine land.

Table 2 shows that the interaction between LCC plants and microbial inoculants on total biomass was significant ($p<0.05$). *Mucuna* sp. is a plant species that can adapt well, indicated by higher plant biomass compared to *Centrosema pubescens* and *Crotalaria* sp., and the addition of *Azotobacter-Trichoderma* (2:1) inoculant was able to increase plant biomass. The combination of *Mucuna* sp. and *Azotobacter-Trichoderma* (2:1) inoculant resulted in fresh and dry plant biomass weights of 427.63 g per plant and 259.23 g per plant, respectively. The application of *Azotobacter-Trichoderma* inoculants, in addition to increasing plant nutrient absorption, also increased plant biomass. Inoculation of these two microbes can competitively colonize plant roots and can be used as biofertilizers or biopesticides, or both simultaneously.

The most common symptoms of Hg toxicity are stunted, limited root development, and photosynthesis inhibition, which results in decreased yields. The accumulation of Hg in root tissue also inhibits the uptake of K^+ by plants. *Centrosema pubescens* tolerance to low N and P content in tailings enriched with nitrogen-fixing bacteria (NFB) and phosphate-solubilizing bacteria (PSB). The LCC can growth were slower than annual food crops, so nutrient uptake is limited. Therefore, LCC can grow naturally without fertilizer since rhizobia in the root nodules provide available N through nitrogen fixation (Hindersah et al., 2021b).

Soil chemical properties in the research site

The effect of the LCC plant did not significantly affect soil pH, organic carbon, total N, and CEC (Table 3). Growing LCC for 8 weeks and the application of microbial have not improved the chemical properties of tailing. Nonetheless, the addition of compost increased the organic carbon content by 1.99% up to 2.40%. The addition of compost enhanced the total N content in the former gold mine in Sijunjung, West Sumatra. The ex-gold mine land has decreased in land quality and limited vegetation growth, so it is necessary to add organic matter in large quantities (Oktabriana and Syofiani, 2021). Prayogo and Ihsan (2018) reported that combining LCC plants with bokashi fertilizer improves soil pH, organic carbon, available P, and available K. The classified soil pH at the study site is very acidic. The lower pH causes the increase of acidic compounds, which hence increases the solubility of mercury so that the level of toxicity in the environment is also higher (Hernandez-Flores et al., 2018; Lutfi et al., 2018).

Table 2. Interaction of LCC and microbial on total biomass weight of LCCs grown in Hg-contaminated tailing 8 weeks after planting.

LCC plants	**Fresh weight (g plant^{-1})**			**Dry weight (g plant^{-1})**		
	Microbes			**Microbes**		
	M0	**M1**	**M2**	**M0**	**M1**	**M2**
CP	60.77 cd	62.27 cd	64.30 cd	31.50 cd	33.63 cd	34.63 cd
	A	A	A	A	A	A
MC	118.40 bc	162.30 b	427.63 a	72.07 bc	93.10 b	259.23 a
	B	B	A	B	B	A
CR	28.40 d	53.07 cd	32.57 d	16.47 d	31.07 cd	20.40 cd
	A	A	A	A	A	A

Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1). The mean in the different letters indicated a significantly different at Tukey test $p \leq 0.05$. Low case letters compared the value in columns, and capital letters compared the value in rows.

Table 3. The main effect of LCC plants and microbes in Hg-contaminated tailings 8 weeks after planting on soil chemical properties

Treatments	**Soil pH**	**Organic carbon (%)**	**Total N (%)**	**CEC (cmol$_{(+)}$ kg^{-1})**
LCC Plants				
CP	3.96 a	2.29 a	0.12 a	21.1 a
MC	4.09 a	2.23 a	0.13 a	20.2 a
CR	3.86 a	2.12 a	0.11 a	38.6 a
Microbial				
Mo	3.62 b	1.99 a	0.12 a	36.4 a
M1	4.32 a	2.26 a	0.11 a	22.3 a
M2	3.96 ab	2.40 a	0.13 a	21.1 a

Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1). The mean in the same letter is not significantly different at Tukey test $p \leq 0.05$.

The treatment of LCC and microbial application increased soil pH due to the addition of lime during soil tillage. Damage to the soil layer due to gold mining and soil leaching resulted in the loss of some cations in the soil colloid and decreased pH. Therefore, reclamation of ex-mining tailings land needs liming. Organic carbon content enhancement after harvesting explained that the addition of compost enables to increase in the ability of the tailing to provide nutrients and support plant growth. Likewise, the total N content and soil CEC were increased compared to initial values. Allo (2016) reported that mining decreased soil organic matter, so it is necessary to add a topsoil layer and soil organic matter due to the loss of nutrients in the subsoil.

Hg concentrations in soil and plants

LCC was planted during 8 weeks; the Hg concentration in the treatment of three LCC plants showed no significant effect, while the treatment with *Azotobacter-Trichoderma* inoculants (2:1) increased Hg concentration in plants (Figure 2). The rhizobacteria can increase Hg uptake by LCC plants. Plant roots are inhabited by various microbes where there is a symbiosis between plant bacteria in heavy metals contaminated soil. Microbes play an important role in plant nutrient cycling, soil structure improvement, detoxifying harmful toxic substances, pest control, and plant growth (Jing et al., 2007). The type of LCC did not significantly affect the Hg concentration in plants (Figure 2a). However, the treatment of *Azotobacter-Trichoderma* (2:1) increased the accumulation of Hg concentration in plants by 35.5 mg kg^{-1} (Figure 2b).

The accumulation of Hg in *Centrosema pubescens*, *Mucuna* sp., and *Crotalaria* sp. plants was 25.9 mg kg^{-1}, 23.5 mg kg^{-1}, and 26.7 mg kg^{-1}, respectively. This value was already very high and caused plant toxicity to Kabata-Pendias (2011); the permissible Hg concentration in plants is 0.2 mg kg^{-1}.

Tailings were tolerant and showed higher Hg uptake. Heavy metal uptake is only sometimes correlated with plant biomass production. *Micania cordata* and *Calopogonium mucunoides* plants were able to absorb Hg, Cd, and Pb higher, despite their lower biomass production. On the other hand, *Centrosema pubescens* plants produced high biomass in waste contaminated with heavy metals. However, the ability to absorb heavy metals was lower than *Miconia cordata* and *Calopogonium mucunoides* (Hidayati et al., 2006).

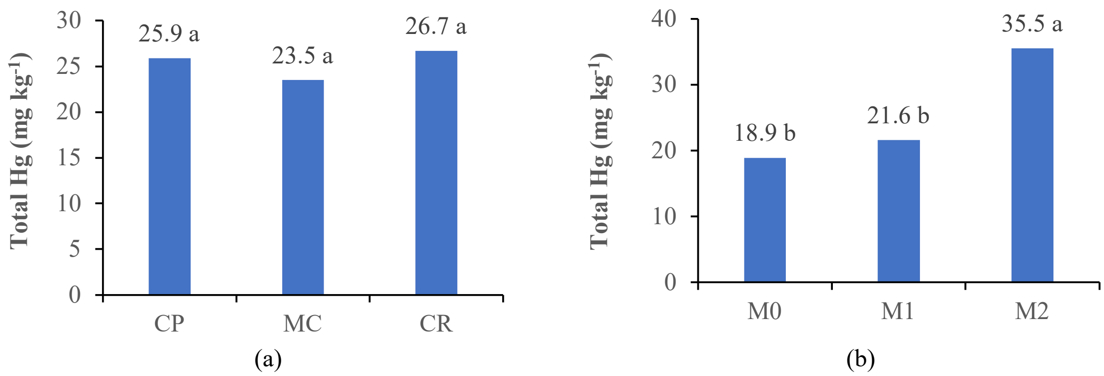

Figure 2. The main effect of LCC plants (a) and microbes (b) on Hg concentration in plants 8 weeks after planting Different letters indicate significantly different at the Tukey test ($p \leq 0.05$). Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1).

The concentration of Hg in the soil decreased at 8 WAP. LCC and microbial application have no significant effect on total Hg in soil and removal effectivity (RE) value at $p \leq 0.05$ (Table 4). The highest RE values showed by *Crotalaria* sp. (34.00%) and *Azotobacter-Trichoderma* (1:2) (33.56%).

Table 4. The main effect of LCC plants and microbes in Hg-contaminated tailings on total Hg concentration in soil 8 weeks after planting and removal effectivity (RE).

Treatments	Total Hg (mg kg^{-1})	Removal Effectivity (%)
LCC Plants		
CP	160.73 a	24.44 a
MC	152.91 a	30.50 a
CR	149.58 a	34.00 a
Microbial		
M0	157.64 a	30.87 a
M1	152.52 a	33.56 a
M2	153.01 a	27.52 a

Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1). The mean in the same small letter is not significantly different at Tukey test $p \leq 0.05$.

The LCC were able to reduce the concentration of Hg in the soil after 8 WAP from 566.9 mg kg^{-1} to 149.58-160.73 mg kg$^{-1,}$ and microbial application also reduced the concentration of Hg to 153.01-157.65 mg kg^{-1}. Microbial application increased the root depletion zone so that plant roots can absorb Hg and translocate it to other plant parts. The low soil pH conditions at the study site increased the availability of Hg, so the uptake of Hg by the LCC plant also increased.

The interaction effect between LCC plants and microbes on Hg uptake was significant (Table 5). Interaction of *Mucuna* sp. and *Azotobacter-Trichoderma* (2:1) resulted in the highest Hg uptake of 8.83 mg per plant. The total biomass of *Mucuna* sp. is more considerable than *Centrosema pubescens* and *Crotalaria* sp.

Table 5. Interaction effect of LCC plants and microbes in Hg-contaminated tailings on Hg uptake by legume cover crop 8 weeks after planting.

LCC plants	Hg uptake (mg plant^{-1})		
	Microbes		
	M0	M1	M2
CP	0.56 b	0.95 b	1.21 b
	A	A	A
MC	1.40 b	1.55 b	8.83 a
	B	B	A
CR	0.34 b	0.68 b	0.78 b
	A	A	A

Notes: CP = *Centrosema pubescens*; MC = *Mucuna* sp.; CR = *Crotalaria* sp.; M0 = control; M1 = *Azotobacter-Trichoderma* (1:2); M2 = *Azotobacter-Trichoderma* (2:1). The mean in the different letters indicated a significantly different at Tukey test $p \leq 0.05$. Low case letters compared the value in columns, and capital letters compared the value in rows.

The extensive root system causes the *Mucuna* sp. to absorb more Hg and accumulate it in the plant tissue. Several factors which can affect the uptake mechanisms of heavy metals include plant species, soil properties, root zone, environmental conditions, chemical properties of the contaminant, metal bioavailability, and chelating agent added (Tangahu et al., 2011). The characteristics of plant species in the phytoextraction mechanism are plants: fast growth and high biomass, extended root system, good tolerance to high metal concentrations, high translocation factor, adaptability to specific environments, ability to adapt to certain environmental conditions, and access to agricultural management (Sheoran et al., 2016).

Conclusion

Mucuna sp. was more survive to grow in the tailings of the ex-gold mine and increased Hg uptake in the tailing compared to the legumes *Censtrosema pubescen*s and *Crotalaria* sp. The application of the biological agent *Azotobacter-Trichoderma* in the composition 2:1 increased soil pH, total bacterial population, the biomass of legumes, and Hg uptake by LCC.

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References

Allo, M.K. 2016. Conditions of physical and chemical properties of the soil in the former nickel mine and its influence on the growth of trengguli and mahogany. *Jurnal Hutan Tropika* 4 (2):207-217 (*in Indonesian*).

Amri, F., Hindersah, R., Kurnani, B., Sunardi, Cahyandito, M. and Nursyamsi, D. 2020. Physical and chemical natures of post-artisanal gold mine area at Kebunlado Village of Riau Province. *Indonesian Mining Journal* 23(1): 9-19, doi:10.30556/imj.vol23.no1.2020.1062.

Arifin, Y.I., Sakakibara, M., Takakura, S., Jahja, M., Lihawa, F. and Sera, K. 2020. Artisanal and small-scale gold mining activities and mercury exposure in Gorontalo Utara Regency, Indonesia. *Toxicology and Environmental Chemistry* 102(10):521-542, doi:10.1080/02772248.2020.1839074.

Budianta, G., Gofar, D. and Andika, N. 2013. Improvement of sand tailing fertility derived from post-tin mining. *Journal of Tropical Soils* 18(3):217-223, doi:10.5400/jts.2013.18.3.217.

Dhaliwal, S.S., Naresh, R.K., Mandal, A., Singh, R. and Dhaliwal, M.K. 2019. Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators* 1-2:100007, doi:10.1016/j.indic.2019.100007.

Domingo, J.P.T. and David, C.P.C. 2014. Soil amelioration potential of legumes for mine tailings. *Philippine Journal of Science* 143(1): 1-8.

Feketeová, Z., Hrabovský, A. and Šimkovic, I. 2021. Microbial features indicating the recovery of soil ecosystem strongly affected by mining and ore processing. 2021. *International Journal of Environmental Research and Public Health* 18(6):1-16, doi:10.3390/ijerph18063240.

Hadi, A.N.K.H. and Purnomo, T. 2022. Potential *Cordyline fructicosa* and *Sanseviera trifasciata* as Cd heavy metal absorption agents in soil. *Lentera Bio* 11(3): 59-368, doi:10.26740/lenterabio.v11n3.p359-368 (*in Indonesian*).

Hernández-Flores, J.L., Melo, J.G.B., Hernández, A.C., López, M.A.R., Gutiérrez, C.S., Gomez, S.R., Moreno, V.P., Medina, R.P.C., Soto, J.H.V., Hernández, S.P., Jones, G.H., Flores, S.C. and Campos-Guillen, J. 2018. Isolation and characterization of mercury resistant *Trichoderma* strains from soil with high levels of mercury and Its effects on *Arabidopsis thaliana* mercury uptake. *Advances in Microbiology* 08(07):600-613, doi:10.4236/aim.2018.87040.

Hidayati, N., Syarif, F. and Juhaeti, T. 2006. Potency of *Centrocema pubescence*, *Calopogonium mucunoides*, and *Micania cordata* for cleaning metal contaminants of gold mines waste. *Biodiversitas Journal of Biological Diversity* 7(1):4-6, doi:10.13057/biodiv/d070102.

Hindersah, R. and Asmiran, P. 2017. Role of biological agents Azotobacter-Trichoderma on the growth and yield of red chili (*Capsicum annum* L.) in a pot experiment. Agric 29(2):137-146, doi: 10.24246/agric.2017.v29.i2.p137-146 (*in Indonesian*).

Hindersah, R., Asda, K.R., Herdiyantoro, D. and Kamaluddin, N.N. 2018a. Isolation of mercury-resistant fungi from mercury-contaminated agricultural soil. *Agriculture* 8 (33):1-8, doi:10.3390/agriculture8030033.

Hindersah, R., Handyman, Z., Indriani, F.N., Suryatmana, P. and Nurlaeny, N. 2018b. Azotobacter population, soil nitrogen and groundnut growth in mercury-contaminated tailing inoculated with Azotobacter. *Journal of Degraded Mining Lands Management* 5(53):1269-1274, doi:10.15243/jdmlm.2018.053.1269.

Hindersah, R., Adityo, B. and Suryatmana, P. 2018c. Bacterial and fungal populations and growth of tea plants (*Camellia sinensis* L.) on two types of growing media after Azotobacter inoculation. *Agrologia* 5(1):1-9, doi:10.30598/a.v5i1.191 (*in Indonesian*).

Hindersah, R., Nurhabibah, G. and Harryanto, R. 2021a. Azotobacter inoculation and application of compost for bioremediation of mercury-contaminated tailings. *Jurnal Teknologi Mineral dan Batubara* 17(1):39-46, doi:10.30556/jtmb.vol17.no1.2021.1142 (*in Indonesian*).

Hindersah, R., Sunarya, Y., Arifin, M. and Priyadi, R. 2021b. Performance of Centrocema grown on mercury-contaminated tailing inoculated with beneficial bacteria: Preliminary study. *IOP Conference Series Earth and Environmental Science* 882(1), doi:10.1088/1755-1315/882/1/012061.

ISRI (Indonesian Soil Research Institute). 2009. Analyses of Soil, Plants, Water and Fertilizers. Indonesian Soil Research Institute, Bogor, Indonesia (*in Indonesian*).

Jing, Y., He, Z. and Yang, X. 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University- Science* B 8 (3):192-207, doi:10.1631/jzus.2007.B0192.

Kabata-Pendias, A. 2011. *Trace Elements in Soils and Plants*. CRC Press.

Kasa, P., Modugapalem, H. and Battini, K. 2015. Isolation, screening, and molecular characterization of plant growth promoting rhizobacteria isolates of Azotobacter and Trichoderma and their beneficial activities. *Journal* *of Natural Science, Biology, and Medicine* 6(2):360-363, doi:10.4103/0976-9668.160006.

Khoiriyah, A., Samang, L. and Zubair, A. 2015. Phytoremediation of soil contaminated with Cd and Pb using *Vetiver* sp. plants. *Jurnal Universitas Hasanuddin Makassar* 9(2): 57-69 (*in Indonesian*).

Lin, H., Jiang, X. Li, B., Dong, Y. and Qian, L. 2020. Soilless revegetation: An efficient means of improving physicochemical properties and reshaping microbial communities of high-salty gold mine tailings. *Ecotoxicology, and Environmental Safety* 207:111246, doi:10.1016/j.ecoenv.2020.111246.

Liu, C., Lin, H., Dong, Y., Li, B. and Liu, Y. Investigation on microbial community in remediation of lead-contaminated soil by *Trifolium repens* L. 2018. *Ecotoxicology and Environmental Safety* 165:52-60, doi:10.1016/j.ecoenv.2018.08.054.

Lutfi, S.R., Wignyanto, and Kurniati, E. 2018. Mercury bioremediation using indigenous bacteria from gold mining waste in Tumpang Pitu, Banyuwangi. *Jurnal Teknologi Pertanian* 19(1):15-24 (*in Indonesian*).

Manullang, D.W.S., Gusmini, and Rezki, D. 2021. Characteristics of soil chemical properties and mercury content in ex-gold mining land and oil palm plantation land (*Elaeis guineensis* Jacq.) *Jurnal Riset Perkebunan* 2(1):1-10 (*in Indonesian*).

Oh, K., Takahi, S., Wedhastri, S., Surarmawan, H.L., Rosariastuti, R. and Prijambada, I.D. 2015. Phytoremediation of mercury-contaminated soils in a small-scale artisanal gold mining Region of Indonesia. *International Journal. of Bioscience and Biotechnology* II(1):14-21.

Oktabriana, G. and Syofiani, R. 2021. Efforts to improve the chemical properties of ex-gold mining land by administering ameliorant to soybean growth in Sijunjung Regency. *Jurnal Agrium* 18(1):57-62, doi:0.29103/agrium.v18i1.3843 (*in Indonesian*).

Pranoto, B.S.M. and Budianta, W. 2021. Phytoremediation of heavy metals contaminated soil in artisanal gold mining at Selogiri, Wonogiri District, Central Java, Indonesia. *Journal of Applied Geology* 5(2):64-72, doi:10.22146/jag.54586.

Prayogo, C. and Ihsan, M. 2018. Utilization of LCC (legume cover crop) and bokashi fertilizer for the efficiency of Fe and Mn uptake of former coal mine land. *Journal of Degraded and Mining Lands Management* 6(1):1527-1537, doi:10.15243/jdmlm.2018.061.1527.

Schwember, R., Schulze, J., Pozo, A. and Cabeza, R. A. 2019. Regulation of symbiotic nitrogen fixation in legume root nodules. *Plants* 8:333, doi:10.3390/plants8090333.

Sheoran, V., Sheoran, A.S. and Poonia, P. 2016. Factors affecting phytoextraction: a review. *Pedosphere* 26(2):148-166, doi:10.1016/S1002-0160(15)60032-7.

Tangahu, B.V., Abdullah, S.R.S., Basri, H. Idris, M. Anuar, N. and Mukhlisin, M. 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. I*nternational Journal of Chemical Engineering* Article ID 939161:1-3131, doi:10.1155/2011/939161.

Wasis, B. and Fathia, N. 2011. Growth of Gmelina seedling with various compost fertilizers in ex-gold mining land media. *Jurnal Manajemen Hutan Tropika* 17(1):29-33 (*in Indonesian*).

Wijayanti, M., Kapp, G. and Mansur, I. 2020. Evaluation of revegetation practices in post mined of Indonesia. 2020. *Biotropia* 27(3):189-198, doi:10.11598/btb.2020.27.3.1031.

Zainudin, Z. and Kesumaningwati, R. 2021. Identification of fungi and bacteria in several land uses in Samarinda City. *Ziraa'ah* 46(2):165-174, doi:10.31602/zmip.v46i2.4352 (*in Indonesian*).