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Literature Review and Experiment: Diversity of Bacteria in Forest, Revegetated Post-Mining Land, and Active Tin Mining with A Metagenomic Approach

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ABSTRACT

The objective of this study was to investigate the diversity of bacteria in the rhizosphere of *Acacia* grow in the forest and different ages of revegetated land and non-rhizospheric bacteria from active tin mining soil on Bangka Island with a metagenomic approach and to investigate its relationship with physicochemical properties of the soil and its metal content. 17 species/genera were found in the four fields, and *Bradyrhizobium* is the dominant genus. Bacterial communities of the forest (more than 5 years in revegetated post-tin mining land containing C, N, P, and Fe) are *Paraburkholderia* sp., *Bacillus* sp., *Candidatus-Xiphinematobacter*, and *Bacterium enrichment.* The physicochemical properties of soil from less than 5 years of revegetated contain Cu, Cr, and Mo, and the dominant species in this soil were *Bulkhorderia* sp., *Bacillus lehensis*, *B. fordii*, *B. thermolactis*, *Chloroflexi bacterium*, and *Thermonosporaceae bacterium*. The physicochemical properties of soil from the mining were dominated by Cd and Ni, and the dominant species in this soil were *Sinomonas atrocyanea*.

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1. INTRODUCTION

Tin mining industry activities on Bangka Island produce various metal wastes: Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Fe, Mn, Cu, Zn, and Al. Several researchers report metal waste generated in industrial activities to have polluted the soil environment. Sn was found in the tailings soil of Nibung Village, Central Bangka, at 350 ppm. Tin mine tailings left for 40 years in Pemali, Bangka produced Pb of 60.1 ppm. Sari *et al.* (2016a) examined soil from land reclamation of the Pemali tin mine, Bangka, exposed to a Pb component of 50.53 ppm. The tin mine reclamation land in Bencah Village, South Bangka, Indonesia, contains a Pb of 10.80 ppm (Sari *et al.,*2017).

The metals produced from the tin mining industry are harmful to the environment, and if transferred into the food chain, they will affect the health of animals and humans as final consumers. According to Ali *et al.* (2019), Cr, Ni, Cu, Zn, Cd, Pb, Hg, and as metals are the most dangerous for the environment, especially if they enter the food chain. Pb, Cd, As have the potential to cause non-carcinogenic diseases (Baghaie & Fereydoni, 2019) and have an impact on kidney damage (Anyanwu *et al.,*2018), blood and liver (Andjelkovic *et al.,*2019). Nickel accumulation increases the risk of lung cancer (Chiou *et al.,*2014). Metals can also cause toxicity to soil microbes, such as bacteria. The toxicity of metals in the soil from the revegetation area of the Bangka Island tin mining can cause adverse effects on soil bacteria. Bacteria can detoxify metals through electrostatic interactions, ion exchange, precipitation, redox processes, and surface complexation (Igiri *et al.,* 2018). Some effects of metals on soil bacteria, such as: reacting as redox catalysts in the production of reactive oxygen species (ROS), impairing ion regulation, and directly affecting DNA and protein formation (Tarekegn *et al.,*2020) and affecting the activity and composition of soil bacteria (Li *et al.,*2017). Therefore, efforts are needed to reduce the availability of metals in the post-tin mining area of Bangka Island.

One of the efforts that can be made to accelerate the reduction of metal toxicity in post-tin mining areas is bioremediation. The bioremediation method is tremendously effective because it is cheap and environmentally friendly. The bioremediation agent used to reduce the toxicity of metal contaminants in post-tin mining land is in the form of associations of soil bacteria and plants. Soil bacteria have a mechanism that causes changes in the mobility of metal elements (http://faperta.ugm.ac.id/download/publikasi_dosen/irfan_dp/pdf/Peranan%20Mikroorganisme%20Pada%20Fitoremediasi%20Tanah%20Tercemar%20Logam%20Berat.pdf).

Thus, they become more difficult or easier for plants to absorb, namely redox changes from inorganic metals and changes in metal forms from inorganic or vice versa. The physical and chemical properties of the soil, the chemical species of the adsorbed metal, and the nature of the plant's absorption are also factors that cause the efficiency of metal uptake by plants. Adaptive plants in post-tin mining areas show that there is an interaction with soil bacteria in remediating metals. The diversity of bacteria in the soil environment plays an essential role in the bioremediation process.

The interaction between bacteria and plants on metal-contaminated soil in the tin mine revegetation area is the initial data. Thus, it can be used in further research to obtain rhizobacteria that are always present and superior as bioremediation agents. Acacia is often used to revegetate the tin mines of Bangka Island because several studies mention its potential as a metal phytoremediator. Sari *et al.* (2016a) reported that *acacia auriculiformis has the* potential as a phytoremediator of heavy metals Pb and Sn in the post-tin mining area of Bangka. According to Jeannne *et al.* (2019), *Acacia mangium* is a Pb metal phytoremediator.

Bacteria that live in the rhizosphere, especially on the root surface (rhizoplane), were chosen because they are thought to be closely related to the metal bioremediation process. Bacteria require more amino acids in rhizoplane and rhizosphere soils than in soils outside the rhizosphere. Soil bacteria could change chemical properties in the rhizosphere to increase polluting metal's mobility (http://faperta.ugm.ac.id/download/publikasi_dosen/irfan_dp/pdf/Peranan%20Mikroorganisme%20Pada%20Fitoremediasi%20Tanah%20Tercemar%20Logam%20Berat.pdf). Bioremediation is also influenced by environmental factors, such as pH, nutritional requirements, temperature, and humidity (Jin *et al.,* 2018). An interaction between rhizobacteria and plants in the bioremediation process needs to be studied with a metagenomic approach.

Bacterial diversity can be analyzed by using a metagenomic approach. It is due to the weakness of culture analysis, which can only isolate 1% of bacteria from a sample. The limitations of this culture method can be overcome by using metagenome analysis using the 16S rRNA gene as a molecular marker. Methods with the latest sequencing technology with coverage of reading DNA sequences are determined as complete, detailed, and accurate genetic information. Therefore, metagenomes are believed to be able to analyze the diversity of soil bacteria from post-tin mining fields. Efforts to improve soil reclamation through biological below-ground treatment based on the interaction of soil bacteria and plants will show a specific diversity of soil bacteria. Therefore, it is necessary to do a metagenomic analysis of bacterial diversity in different lands to determine the profile of soil bacterial diversity.

Investigation of metagenomic diversity of soil bacteria has never been reported from Bangka Island to date, particularly in four different areas, namely forest land, tin mine revegetation land > 5 years, tin mine revegetation land < 5 years, and active tin mine. This effort is exceptionally vital to know to measure the success of post-tin mining land reclamation on Bangka Island.

2. LITERATURE REVIEW

Metagenomics applies a set of genomic technologies and bioinformatics tools to directly access the genetic content of entire communities of organisms (Thomas *et al.,* 2012). Metagenomics has become an indispensable tool for studying environmental microbes' diversity and metabolic potential, most of which cannot be cultured in the laboratory (Teeling & Glockner, 2012). Analysis of bacterial diversity with a metagenomic approach has more advantages than bacterial analysis by culture. These advantages including:

(i) metagenomics has the main advantage of being functional and not merely a taxonomic characterizing the ability of a particular community, while 16S rRNA sequencing provides a taxonomy based on the conserved region of one gene. Metagenomics can provide higher taxonomic resolution than 16S rRNA sequencing, reaching species and strain levels (Ellerman *et al.,* 2017).

(ii) the problem with the bacterial culture in the laboratory is that most bacteria are not cultured and may not be cultured, so ±99% of bacterial biodiversity has been lost and may be beyond the reach of traditional methods (Trash 2019).

From previous studies until now, no research has been conducted on measuring soil bacterial diversity using a metagenomic approach to soil bacteria from metal-contaminated post-tin mining areas on Bangka Island (**Table 1**). Previous studies on the diversity of soil bacteria from post-tin mining areas on Bangka Island only used the culture method. This method is weak because it can only interpret 1% of the diversity of bacteria isolated from the soil.

Table 1. Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
1	Sari *et al.* (2016)	Rhizosphere soil from the post-tin mining area, Bangka, Indonesia	*Acacia auriculiformis, Eragrostis chariis*	Pb, Sn	*Bacillus subtilis* strain 2C-62, *Enterobacter aerogenes* strain KNUC5001, *Paenibacillus* sp.	Culture
2	Ponce *et al.* (2017)	Tailings rhizosphere soil mined from Santa Mar´ıa de la Paz	*Prosopis laevigata, Spharealcea angustifolia*	As, Pb, Cu, Zn,	*Alcaligenes, Bacillus, Curtobacterium, Microbacterium*	Culture
3	Hansda *et al.* (2017)	Rhizosphere soil from Copper mines in India	*Saccharum spontaneum*	Cu, Cd, Zn, Pb, Ni	*Kocuria* sp. *CRB15*	Culture
4	Feng *et al.*(2018)	Non-Rhizosphere soil from Shuangsheng town, Shifang County, Sichuan province, China	-	Cd	Proteobacteria, *Sulfuricella, Thiobacillus*	Metagenomic
5	Mitra *et al.* (2018)	Rhizosphere soil from India	*Oryza sativa*	Cd	*Klebsiella michiganensis MCC3089*	Culture
6	Tipayno *et al.* (2018)	Non-rhizosphere and rhizosphere soil from a nonferrous metal industry area surrounding the Janghang smelter in Seocheon city, Chungnam, South Korea	Paddy	As, Cd, Cu, Ni, Pb	*Desulfatibacillum, Desulfovirga, Bacillus*	Metagenomic
7	Sun *et al.* (2018)	Non-rhizosphere and rhizosphere soil from copper mine tailings in Jiangxi province, china	*Alnus cremastogyne*	Cu, As	*Alphaproteobacteria, Deltaproteobacteria, Chloroflexi, Acidobacteria, Gemmatimonadetes*	Metagenomic

Table 1 (Continue). Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
8	Ren *et al.* (2019)	Cu-contaminated rhizosphere soil in Zheijiang, China	*Brassica napus* L.	Cu	*Microbacterium oxydans* JYC17, *Pseudomonas thivervalensis* Y1-3-9, *Burkholderia cepacia* J62	Culture
9	Guo *et al.* (2019)	Rhizosphere soil from the Xikuangshan mine in southern China	*Broussonetia papyrifera* *Ligustrum lucidum*	Sb, As, Cr	Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes	Metagenomic
10	Jiang *et al.* (2019)	Non-rhizosphere soil from an e-waste dumpsite at Alaba International Market, Lagos State, Nigeria	-	Cd, Cr, Cu, Ni, Pb, Zn,	Proteobacteria, Firmicutes, Acidobacteria, Planctomycetes	Metagenomic
11	Ramírez *et al.*(2019)	Rhizosphere soil from the region of Nexapa River Chietla, Puebla, Mexico	*Prosopis laevigata*	Cr	*Bacillus* sp.	Culture
12	Marwa *et al.* (2020)	Non-rhizosphere and Rhizosphere soil from the region of Baripur district of West Bengal, India.	*Adiantum capillus-*veneris	As	Proteobacteria, Actinobacteria, Firmicutes	Culture and Metagenomic
13	Wolĭ nska *et al.*(2020)	Non-rhizosphere soil from the vicinity of the abandoned “Staszic” mine in Rudki village (south-central Poland)	-	Fe	Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, Alphaproteobacteria, Acidimicrobia, Nocardioides, Strepromyces, Pseudonocardia, Jatrophihabitants, Alkanibacter, Ferrithrix	Metagenomic

Table 1 (Continue). Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
14	Akhtar *et al.* (2021)	Rhizosphere soil from Rawat industrial area Rawalpindi, Pakistan	*Chenopodium album* L.	Cr	*Bacillus cereus*	Culture
15	Abdullahi *et al.* (2021)	Rhizosphere soil from an ex-tin mining area that had been abandoned for more than 30 years in Perak, Malaysia	*Mimosa pudica*	As, Cr, Co, Zn, Ni, Cu, Cd, Fe, Hg	*Paraburkholderia, Bradyrhizobium, Bacillus, Candidatus, Acidothermus, Acidibacter Nitrospira*	Metagenomic
16	Costa *et al.* (2021)	Rhizosphere soil from a native shrub canga and in waste piles of an iron mine land in the canga, with a revegetation The program started in 2014 in Serra dos Carajás, Pará, Brazil	*Mimosa acutistipula* var. ferrea	Fe	*Bradyrhizobium, Rhodoplanes, Paraconiothyrium*	Metagenomic
17	Li *et al.* (2021)	Non-Rhizosphere soil from a gold mining area in the northwest of Beijing, China	-	As	Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi	Metagenomic
18	Sun *et al.* (2021)	Rhizosphere soil from two Pb/Zn mine tailings, Gudan and Silang, China	*Miscanthus sinensis*	As, Cd, Cr, Cu, Ni, Pb, Sb,Zn	Alphaproteobacteria, Gammaproteobacteria , Acidobacteria	Metagenomic

Table 1 (Continue). Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
19	Jarosławiecka & Piotrowska-Seget (2022)	Rhizosphere soil from the proximity of the former Mining and Metallurgical Plants in Piekarý Sl̨ askie (Upper Silesia) and ‘Bolesław’ in Bukowno (Lesser Poland), Poland	‘Warý nski’	Zn, Cd, Cu, Ni	Proteobacteria, Actinobacteria, Bacteroidetes	Metogenomik
20	Mu˜noz-García *et al.*(2022)	Rhizosphere soil from a semi-arid mangrove of La Guajira-Colombia	*Avicennia germinans*	Cu, Ni, Zn, Cd	*Vibrio fluvialis*	Metagenomic
21	Sun *et al.* (2022)	Rhizosphere soil from Enshi City, in the Hubei Province of China	*Cardamine violifolia*	Se	*Rhodanobacter, Nitrospira*	*Metagenomic*
22	Jiang *et al.* (2022)	Rhizosphere soil from Yangshuo Lead-Zinc Mine, 81 km south of Guilin, a world-famous Karst scenic city in South China	*Miscanthus sinensis, Pueraria phaseoloides*	Pb, Zn	*Conexibacter, Thiobacillus, Rubrobacter, Nitrospira*	*Metagenomic*
23	Liu *et al.* (2022)	Rhizosphere soil from Xinli tailing pond of Sanshandao Gold Mine, Shandong province, China	*Phragmites australis* (Cav.) Trin. Exteud., *Chrysopogon zizanioides* (L.) Roberty , *Amorpha fruticosa* l., *Festuca elata* Keng ex E.B.Alexeev, *Artemisia scoparia* Waldst. & Kit , *Cynanchum chinense* R.Br.	Cu, Mn, Pb, As, Zn	Actinobacteria	*Metagenomic*

Table 1 (Continue). Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
24	Geng *et al.* (2022)	Rhizosphere soil from the Majuanzi and Qian'anling iron tailingponds in the Huairou region, Beijing, China	*Salsola collina, Setaria viridis, Xanthium sibiricum*	As,Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn,	Proteobacteria, Actinobacteria, Ascomycota, Thaumarchaeota.	*Metagenomic*
25	Parsarnia *et al.* (2022)	Non-rhizosphere soil from tailings dam effluent (TDE) of Zarshuran gold mine, Takab, northwest of Iran	-	As	Bacteroidetes Proteobacteria Actinobacteria Firmicutes	*Metagenomic*
26	Pradhan *et al.*(2022)	Non-rhizosphere soil from Sukinda chromite mines, Odisha, India	-	Cr, Fe, Mn, Pb, Co, Ni	Proteobacteria, Actinobacteria, Bacteroidetes	*Metagenomic*
27	Koner *et al.* (2022)	Non-rhizosphere soil from the Eastern part of the Central Range (Eurasian plate-tectonic plain) and on the Longitudinal Valley (Philippines sea plate)	-	Ca, Mg, Ni,Cr, Co, Mn	Proteobacteria, Actinobacteria, Cyanobacteria, Planctomycetes, Chloroflexi	*Metagenomic*
28	Kalu *et al.*(2022)	Rhizosphere soil from two mine tailing dams, Lancaster in Gauteng province, South Africa	*Phragmites australis*	Cu, Cr, Fe, Zn	Firmicutes, Proteobacteria	*Metagenomic*

Table 1 (Continue). Research on soil bacterial diversity using culture and metagenomic methods in several countries during the last six years.

No	Library	Habitat	Dominant Plant	Type of Metal	Bacterial Dominant	Method
29	Yin *et al.* (2022)	Non-rhizosphere soil from the Yaopo mountain mining district, southwest of Shaoguan, Guangdong Province of China	-	Mn, Pb, Zn, Cu, As, Cr, Co, Ni, Cd	Chloroflexi Proteobacteria Actinobacteriota Acidobacteriota	*Metagenomic*
30	Xie *et al.* (2022)	Non-rhizosphere and rhizosphere soil from Qinglong County in the southwest of Guizhou Province, China	*Trifolium repens* L, *Polygonum capitatum*, *Cynoglossu mlanceolatum* Forssk, *Rumex acetosa* L. *Plantago asiatica* L., *Conyza canadensis* (L.) Cronq., *Brassica juncea* (L.) Czern. et Coss., *Raphanus sativus* L	Sb, As	Proteobacteria, Bacteroidota, Acidobacteriota, Actinobacteriota	*Metagenomic*

There are 30 literature studies over the last six years discussing the diversity of soil bacteria in metal-contaminated soil using culture methods and metagenomic approaches from several countries. The metagenomic approach is the most widely used to study the diversity of soil bacteria, especially from China. There are seven metals out of a total of 16 metals have been studied the most in this research. The seven metals from highest to lowest order, namely: Cu, As, Zn, Cd, Ni, Cr, and Pb. Three dominant bacterial phyla in metal-contaminated soils from several parts of the world, especially with a metagenomic approach: Proteobacteria, Actinobacteria, and Acidobacteria. *Bacillus* is the dominant bacterial genus in metal-contaminated soil using both culture and metagenomic methods.

One of the contaminants commonly found in soil due to tin mining activities is metal. Metals often found in tin mines on Bangka Island and outside Bangka Island (Singkep Island and Malaysia) are Ag, Zn, Cd, Cr, Cu, Pb, Se, As, Hg, Sn, Ni, Fe, Mn, V, and Ba. The most abundant Pb metal was found in Bangka Island tin mines, with the highest levels reaching 60.1 ppm, while the highest metals in Singkep Island and Malaysia were Cr (84.32 ppm) and Sn (404 ppm) (Nurtjahya *et al.,* 2009, Sari *et al.*2016, Sari *et al.*2017, Irzon *et al.,* 2018).

According to Ojuederie & Babalola (2017), there are three mechanisms by which bacteria can remediate metals in the environment. This method consists of the following:

(i) Sequestration of toxic metals by cell wall components or intracellular metal-binding proteins and peptides. Examples: metallothioneins and phytochelatins, along with catechollic siderophores.
(ii) Changes in biochemical pathways to block metal uptake,
(iii) Convers#ion of metals to harmless forms by enzymes, and finally, reduction of intracellular metal concentrations using an appropriate efflux system.

3. METHODS

The research was conducted on four different lands on Bangka Island. First, the forest that has never been mined for tin (HN) in Rebo Village, Bangka Regency, Indonesia. Second, tin mine revegetation land > 5 years (RG) in Jelitik Village, Bangka Regency, Indonesia (representing non-natural revegetation). Third, tin mine revegetation land < 5 years (RT) Kampoeng Air Jangkang Reclamation, Riding Panjang Village, Bangka Regency, Indonesia (representing natural revegetation). Fourth, an active tin mine (TF) in Jelitik Village, Bangka Regency, Indonesia (**Figure 1**).

On forest land and both revegetation areas, samples of Acacia rhizosphere soil were taken at a depth of ± 20 cm, especially on soils still attached to plant roots. From an active tin mine, non-rhizosphere soil samples were taken at 0-40 cm depth. Three sampling points were taken from each land, so there were 12 samples from four different lands. All soil samples were analyzed for chemical properties of soil (C, N, and P), metals (Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Fe, Mn, Cu, Zn, and Al), and bacterial diversity with the metagenomic approach. Soil metals were analyzed by using HNO_3 with Atomic Absorption Spectrophotometer. Hence, carbon content was analyzed by the Walkey & Black method. Nitrogen content uses the Kjeldahl method. P-potential uses 25% HCl with a spectrophotometer. K-potential uses HCl 25% with a flame photometer. P-available uses the Bray method, while K-available uses the Morgan method.

Identification of bacterial diversity utilizes the 16S rRNA Next Generation Sequencing (NGS) metagenome method. The platform used is Illumina NovaSeq 6000. Amplicon sequencing in the V3-V4 region with a fragment length of 470 bp. The primers used were 341F (CCTAYGGGRBGCASCAG) and 806 R (GGACTACNNGGGTATCTAAT). The metagenome sequencing workflow consists of: sample preparation, PCR amplification, mixing and purification of PCR products, library preparation, sequencing, and bioinformatics analysis.

Data were analyzed descriptively and presented in the form of tables or graphs. The correlation analysis (Pearson correlation) between the physical and chemical properties of the soil and the metals in the soil was carried out using Past 4.03 at a significance level of $p < 0.05$. Metagenome bioinformatics analysis, including Alpha diversity analysis, Beta diversity analysis, NMDS analysis, MRPP analysis, Anosim, Adonis, and AMOVA, LefSE analysis, and follow-up analysis of *Canonical Correspondence Analysis* (CCA). Six indices are measured in Alpha diversity: *Observed species, Goods coverage, Chao1, ACE, Shannon, and Simpson Index*. Beta diversity consists of *Unifrac distance heatmap, PCA, PCoA,* and *UPGMA*.

No	Code	Land Area (Ha)	Location	Description
1	HN	5	Rebo Village, Sungailiat District, Bangka Regency, Indonesia	Forest
2	RG	6,2	Jelitik Village, Sungailiat District, Bangka Regency, Indonesia	Tin Mine Revegetation Land > 5 Years
3	RT	2	Riding Panjang Village, Merawang District, Bangka Regency, Indonesia	Tin Mine Revegetation Land < 5 Years
4	TF	30,68	Jelitik Village, Sungailiat District, Bangka Regency, Indonesia	Active Tin Mine

Figure 1. Map of the entire research location on four different land types in Bangka, Bangka Belitung Islands, Indonesia (taken using Google Earth on September 2022).

3. RESULTS AND DISCUSSION

3.1 Soil and Metal Chemistry Physics in the Soil in Forest and Revegetation Land for tin mines > 5 years and < 5 years, and Active Tin Mines on Bangka Island

The physical and chemical characteristics of the soil can determine the success rate of reclamation of ex-tin mining land, especially seen in the organic matter content, represented in the C-organic or N-total content. The content of C-organic, total N, potential P, and available P is still low in the soil in the tin mine, but with revegetation and over time, the content has increased, especially in revegetation land > 5 years, which almost resembles the soil properties in the forest (**Figure 2**). The same thing was reported by Nurtjahya *et al.* (2009), soil properties, such as C, N, and P content, are higher in the forest than in post-tin mining land that has been abandoned for a long time. The high content of C-organic and total N in the soil in the forest indicates the high organic matter is sourced from various vegetation in the forest compared to the three locations in tin mining. The land clearing by forest encroachment causes a decrease in the amount of soil organic matter, especially: C-organic, total N, and P. Therefore, reclamation activities in tin mine revegetation land > 5 years in Jelitik Village-Bangka have a higher success rate than reclamation activities in tin mine revegetation land < 5 years in Kampoeng Reclamation, Riding Panjang Village-Bangka.

The existence of tin mining activities on the three lands causes tin ore, namely Sn and its associated minerals such as Co, Mo, Ag, Fe, and Al, to decrease at the mining site and leaves only a few minerals, such as Pb, Cd, Cr, Ni, Mn, Cu and Zn (**Figure 3**) The metals in the soil were highest in all types of land, namely Sn, Mn, Pb, Zn, and Mo (**Figure 4**).

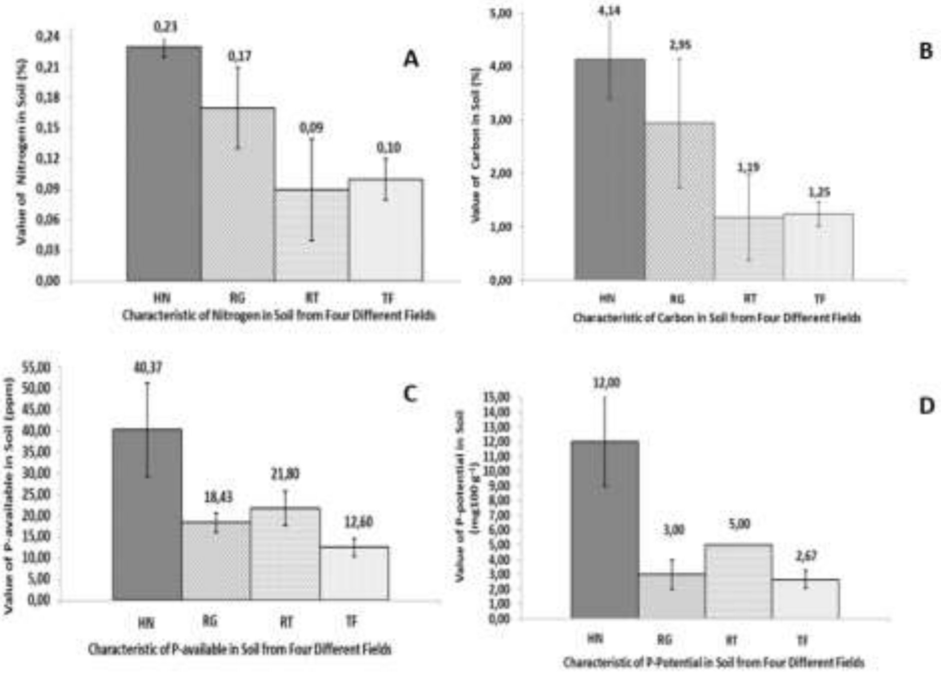

Figure 2. Mean and standard deviation of the C, N, and P content of four fields. (A) C-organic, (B) N-total, (C). P-potential, (D) P-available. Note: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine).

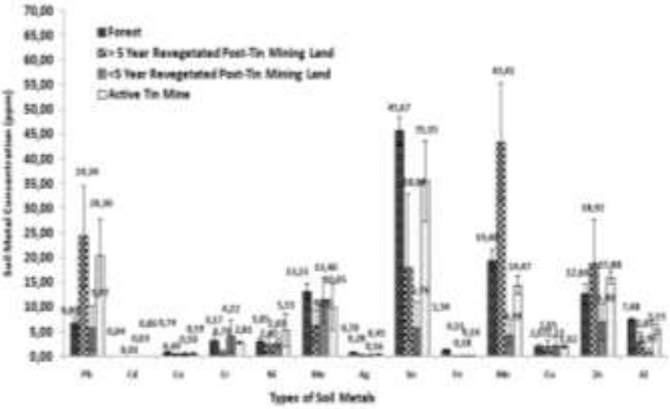

Figure 3. Mean and standard deviation of metals in soil from four different fields

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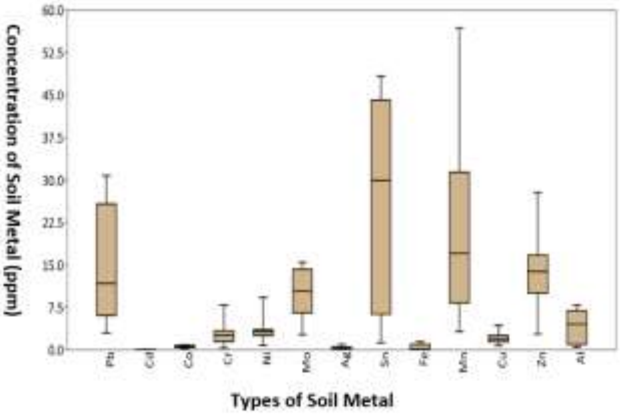

Figure 4. Boxplot Metal content in soil from four different fields on Bangka Island.

The average soil metal content was highest in the forest, namely Co, Cr, Mo, Ag, Sn, Fe, and Al; in tin mine revegetation land > 5 years, namely: Pb, Mn, and Zn; in tin mine revegetation land < 5 years, namely Cu; and in active tin mines, namely Cd and Ni. Sari *et al.* (2016a) and Sari *et al.* (2017) reported that Pb was the highest metal from post-tin mining land in Pemali Village and Bencah Village, Bangka Island. The high Sn in the forest is due to the possibility that Tin (Sn) reserves are still available in the land because tin mining has not been carried out. It is different from the case in active tin mines because a lot of Sn has been taken, then only Cd and Ni are left on the land.

The physicochemical properties of the soil are thought to affect the metal content in the soil. The Pearson Correlation plot found that the potential P-potential and P-available in the soil had a significant relationship with the Fe content in the soil. The relation they have is directly proportional and very strong, with a correlation coefficient value of 0.97995. Therefore, the higher the potential P-potential and available P-content in the soil, the higher the Fe content in the soil. This condition is probably because the decomposition of organic matter in the form of organic acids can help release P bound by acidic soils (such as Al and Fe). The increase in available P could occur due to the release of P from added organic matter and the indirect effect of organic matter on P in the soil adsorption complex.

3.2 Diversity of Rhizosphere and Non-Rhizosphere Bacteria in Forest and Revegetation Land for Tin Mines > 5 Years and < 5 Years, and Active Tin Mines on Bangka Island

Rarefaction curves and rank abundance curves are widely used to show sample biodiversity. These curves identify sample biodiversity and describe species richness and evenness (Zambounis *et al.,* 2019). The rarefaction curve reaches its peak in each type of land. It indicates that the sequencing depth is sufficient to detect all genera in each sample and helps describe bacterial diversity. The more diverse samples were RT and HN, while the less diverse was TF (**Figure 5A**). The rank abundance curve is the curve that shows the environment with high diversity, namely the shallow curve and long tail. High diversity was shown in the RT group. Species abundance curves at four locations show a log series shape (**Figure 5B**). The species abundance curve model can see whether an area is disturbed (with a log series form) or not (with a standard log form). The primary disturbances are caused by human activities, especially tin mining (RG, RT, and TF) and using forest wood (HN).

Species accumulation boxplots can display species diversity as the sample increases, assess the adequacy of sample size, and predict species richness. The boxplot of species accumulation begins to reach asymptotic values when the sample size reaches 12. It indicates that the sample size selected for sequencing was sufficient, and the sampling effort was sufficient to identify the most abundant genus. Thus, the study site was well represented by this sample (**Figure 5C**). On a Venn diagram, each circle represents one sample or group. Values in the overlapping section represent general OTUs, and others represent specific OTUs in each sample. Based on the diagram, it was found that the areas that represent general OTUs are unique and overlap with the 1962 value, while the OTUs with a specific value of 564 in HN, 799 specifics in RG, 945 specifics in RT, and 621 specifics in TF. The highest specific OTU value was in RT, and the lowest was in HN (**Figure 5D**).

The top ten species in the different taxonomic ranks were selected to form a histogram of the relative abundance distribution. Regarding the relative abundance distribution, the highest known families are Xanthobacteraceae in HN, Acidhothermaceae in RG and RT, and JG30-KF-AS9 in TF. *Acidothermus* was the highest genus in HN, RG, RT, and JG30-KF-AS9 in TF (**Figure 6**).

The top 100 genera were selected, and an evolutionary tree was drawn using a sequence of aligned representations. Different branch colors represent different phyla. The relative abundance of each genus in each group is shown outside the circle, and different colors represent different groups. Three ranks of the genus with relative abundance from highest to lowest in each group, namely: *Acidothermus, Bradyrhizobium,* and *Conexibacter* in the HN group; *Acidothermus, Bradyrhizobium,* and *Conexibacter/Candidatus-Xiphinematobacter,* in RG group; *Acidothermus, 1921-2* and *Subgroup 13*in RT group; *B12-WMSP1, Sinomonas,* and *Acidothermus* in TF group (**Figure 7**).

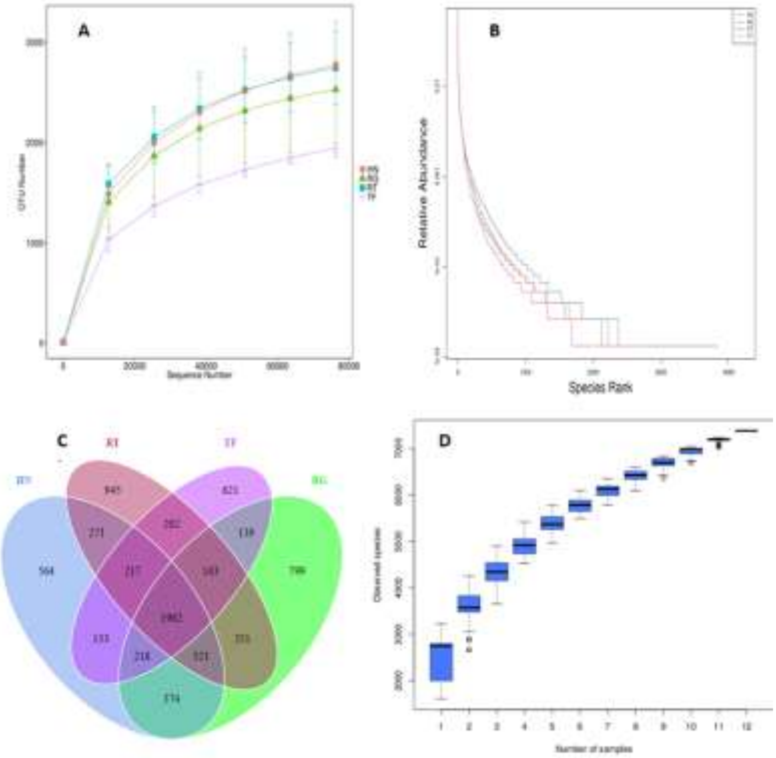

Figure 5. Rarefaction curve (A) and rank abundance curve (B), species accumulation boxplot (C), and Venn diagram (D) in four different fields. Notes: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), and TF (active tin mine).

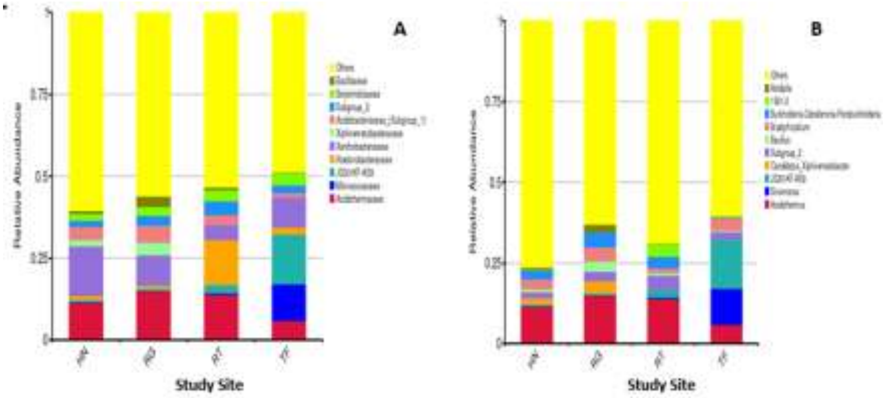

Figure 6. Relative abundance of species at family and genus levels in four different fields. Figures (A) and (B) have different types of bacteria. Notes: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine).

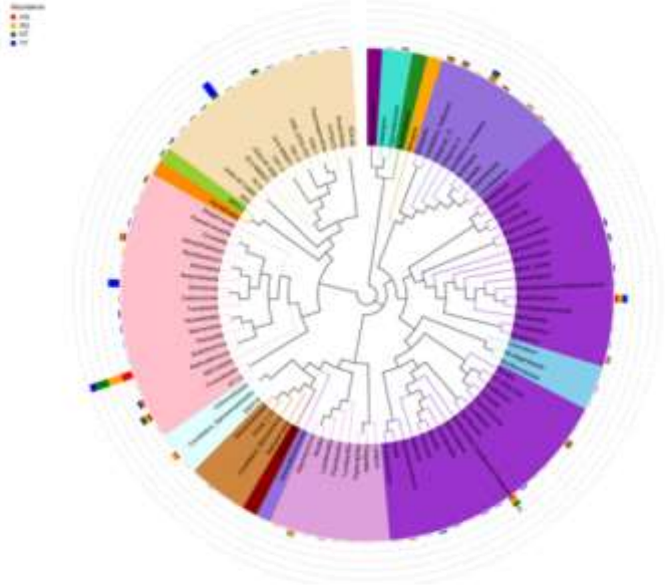

Figure 7. The evolutionary tree of the genus within the group on four different fields. Notes: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), and TF (active tin mine).

There were 23 species/genera of bacteria from six dominant bacterial phyla in all fields. Moreover, 17 species/genera of the same bacteria were found in the four fields. Based on the percentage in the overall taxon, tin mine revegetation land > 5 years has the highest percentage compared to the other three lands. *Bradyzobium* is the genus with the highest total percentage in all land types. *Sinomonas atrocyanea* is the bacterial species with the highest percentage in active tin mining areas. Some species are only found in certain areas, such as *Bacillus fordii*and*Bacillus lehensis* on tin mine revegetation land < 5 years, *Bacillus gibsonii* and bacterium enrichment on tin mine revegetation land > five years. From the 23 species/genera obtained, their potential was characterized based on several references. The potential consists of six categories: bacteria that play a role in the Carbon cycle, Nitrogen cycle, Phosphorus cycle, resistance to metals, resistance to heat, and production of siderophores. There are five species/genera whose potential has not been found in the literature (see **Table 2**).

Table 2. Species/genera of bacteria from phyla and their potential in four different fields.

No	***Phyla***	***Species/Genera of Bacteria***	**The percentage in the overall taxon (%)**				**Total**	**Potential**
			HN	**RG**	**RT**	**TF**		
1	Proteobacteria	*Bradyrhizobium*	3.270	4.344	1.581	3.964	13.159	P2,P4[aa]
2	Actinobacteria	*Sinomonas atrocyanea*	0.122	0.074	0.325	5.294	5.815	P3[a],P4[bb]
3	Verrucomicrobiota	*Candidatus Xiphinematobacter*	1.777	3.763	0.116	0.024	5.680	P1[s]
4	Chlorofexi	1921-2	0.181	0.117	3.836	0.310	4.444	-
5	Firmicutes	*Bacillus thuringiensis*	0.619	2.296	0.484	0.140	3.539	P3[a], P4[ff,gg,hh]
6	Proteobacteria	*Paraburkholderia* sp.	0.932	0.842	0.307	0.167	2.248	P1[z],P2[c], P3[x], P4[y,dd]
7	Actinobacteria	*Thermonosporaceae bacterium*	0.205	0.215	1.249	0.289	1.958	-
8	Proteobacteria	*Bukholderia* sp.	0.363	0.638	0.845	0.036	1.882	P3[d], P4[cc,dd], P6[dd]
9	Proteobacteria	*Paraburkholderia nodosa*	0.315	0.703	0.532	0.027	1.577	P3[e]
10	Proteobacteria	*Paraburkholderia phymatum*	0.118	0.642	0.129	0.012	0.901	P3[f]
11	Proteobacteria	*Burkholderia ubonensis*	0.068	0.097	0.202	0.001	0.368	P3[g]
12	Acidobacteriota	*Bacterium* Ellin7509	0.045	0.028	0.251	0.017	0.341	-
13	Firmicutes	*Bacillus aryabhattai*	0.025	0.113	0.052	0.033	0.223	P1[j], P2[h], P3[h], P4[i,bb]
14	Firmicutes	*Bacillus sporothermodurans*	0.012	0.049	0.042	0.003	0.106	P5[k]
15	Firmicutes	*Bacillus wuyishanensis*	0.004	0.033	0.005	0.000	0.042	-
16	Firmicutes	*Bacillus selenatarsenatis*	0.006	0.018	0.003	0.009	0.036	P2[l], P4[l]
17	Chlorofexi	*Chloroflexi bacterium*	0.001	0.005	0.012	0.003	0.021	P1[P], P2[4]
18	Firmicutes	*Bacillus thermolactis*	0.003	0.000	0.014	0.002	0.019	PI[r], P2[r]
19	Firmicutes	*Bacillus graminis*	0.003	0.010	0.002	0.003	0.018	PI[q], P2[q]
20	Firmicutes	*Bacillus fordii*	0.000	0.000	0.003	0.000	0.003	P1
21	Firmicutes	*Bacillus gibsonii*	0.000	0.002	0.000	0.000	0.002	P1[n], P2[u], P3[w], P4[t], P6[v]
22	Acidobacteriota	*Bacterium enrichment*	0.000	0.001	0.000	0.000	0.001	-
23	Firmicutes	*Bacillus lehensis*	0.000	0.000	0.001	0.000	0.001	P1
		Total	**8.069**	**13.990**	**9.991**	**10.334**	**42.384**	

Note: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine), - (no reference yet), P1 (cycle C), P2 (N cycle), P3 (P cycle), P4 (resistance to metals), P5 (resistance to heat), P6 (production of siderophores), [a] (Raj *et al.*, 2014), [c] (Renata *et al.*, 2022), [d] (Gosh *et al.*, 2016), [e] (Agnol *et al.*, 2016), [f] (Bellés-Sancho *et al.*, 2021), [g] (Madhaiyan *et al.*, 2021), [h] (Deng *et al.*, 2022), [i] (Ramesh *et al.*,2014), [j] (Sari *et al.*, 2016b), [k] (Owusu-Darko *et al.*,2020), [l] (Yamamura *et al.*, 2003), [n] (Batool *et al.*, 2019), [p] (De Mandal *et al.*, 2017), [q](Bibi *et al.*, 2011), [r] (An Coorevits *et al.*, 2011), [s] (Bisker *et al.*, 2021), [t](Aminah & Nur, 2018), [u](Orhan, 2016), [v](Petrillo *et al.*, 2021), [w](Hayat *et al.*, 2013), [x](Kirui *et al.*, 2022), [y](Knuutinen *et al.*, 2019), [z](Wilhelm *et al.*,2021),[aa] (Jach *et al.*, 2022), [bb](Singh & Narzary *et al.*, 2021), [cc] (Zhang *et al.*, 2021), [dd] (Wang *et al.*, 2021), [ee](Damo *et al.*, 2020), [ff](Huang *et al.*, 2020),[gg](Oves *et al.*, 2012), [hh](Guerrero-Ceballos *et al.*,2021).

3.3. Alpha Diversity

The diversity of group communities in < 5 years and > 5 years of revegetation land was higher than in the forest and active tin mines. It is indicated by the group's high value of the Shannon and Simpson index. Community wealth in RT and GM were the same and had higher Chao1 and ACE values than HN and TF. The high value of Good-coverage in GE indicates that the depth of sequencing in the GE group is higher than the other three groups. The values of the Observed species, Chao1 and ACE, in the GM and RT groups were almost the same. The median was highest in the RT and the highest diversity in the GM group. It indicates that the community's wealth is almost the same between RT and GM. The sum of all branch lengths in the phylogenetic tree of all taxa was the highest in the HN group. Community diversity was highest in RT, while sequencing depth was in GM (**Figure 8**). In general, the alpha diversity in RT and then GM was higher than in HN and TF. This condition is also supported that the specific OTU data in RT (945) and RG (799) is greater than the specific OTU in TF (621) and HN (564). According to Liu *et al.*, (2017), the higher the alpha diversity, the higher the abundance of a sample.

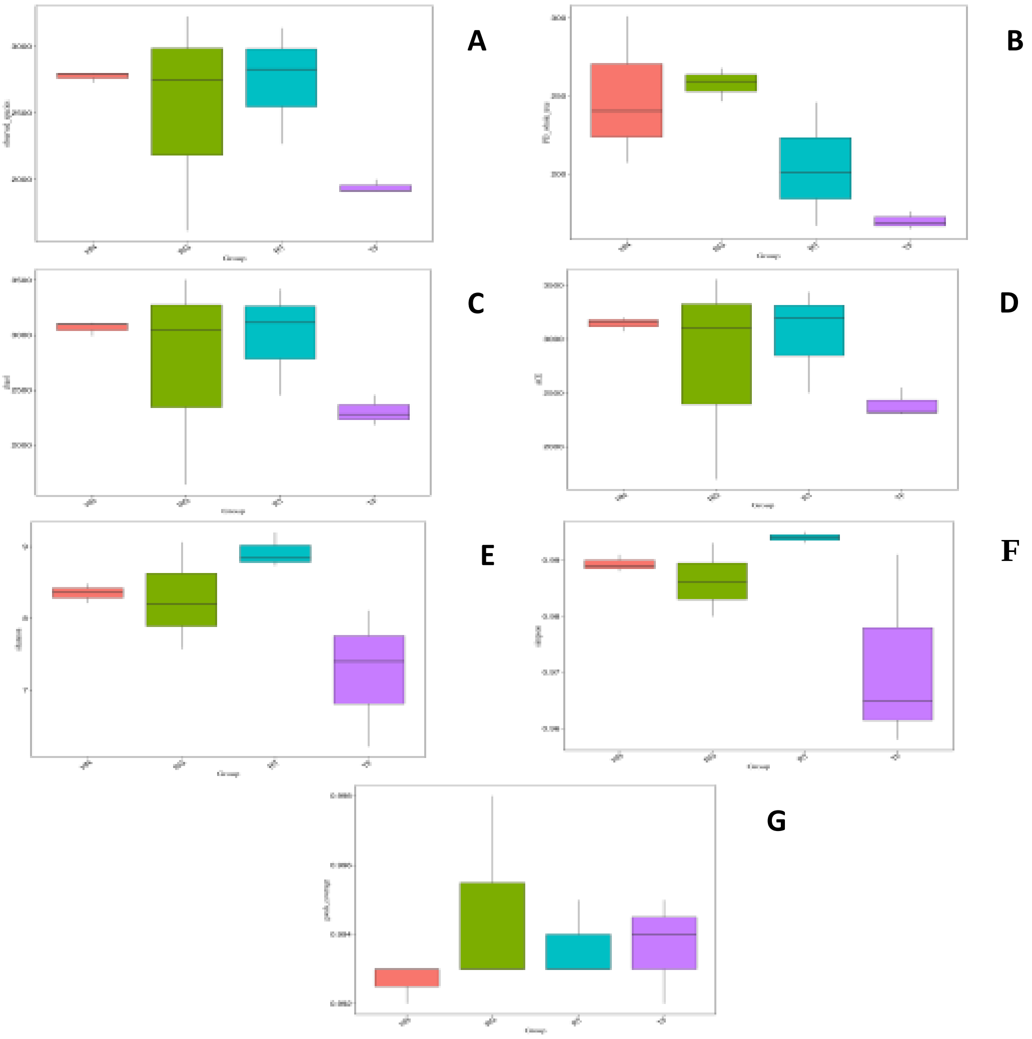

Figure 8. Multiple alpha diversity indices: (A) observed species, (B) phylogenetic diversity (PD) whole tree, (C) Chao1, (D) abundance-based coverage estimator (ACE), (E) Shannon index, (F) Simpson index, and (G) goods coverage. Remarks: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine).

3.4. Beta Diversity

3.4.1. Beta diversity heatmap

The inequality coefficient between the four groups was calculated based on the distance matrix of Weighted Unifrac and Unweighted Unifrac. The smaller the coefficient of inequality, the smaller the difference between the two samples. The RG group had the highest inequality with the TF group of 0.647 (Weighted Unifrac distance matrix) and 0.293 (Unweighted Unifrac distance matrix) and the second highest inequality with the RT group of 0.633 (Weighted Unifrac distance matrix) and 0.286 (Unweighted Unifrac distance matrix. The RG group had the lowest inequality with HN,0.411 (Unweighted Unifrac distance matrix) and 0.141 (Unweighted Unifrac distance matrix) (**Figure 9A**). The boxplot of Weighted Unifrac (**Figure 9B**) and Unweighted Unifrac (**Figure 9C**) shows that RT has a weighted inequality coefficient value. The highest Unifrac and RG have the highest Unweighted Unifrac coefficient of inequality compared to the other three groups, while HN has the lowest value based on these two inequality values. It indicates that the bacterial community in tin mining revegetation land > 5 years is more similar to a forest.

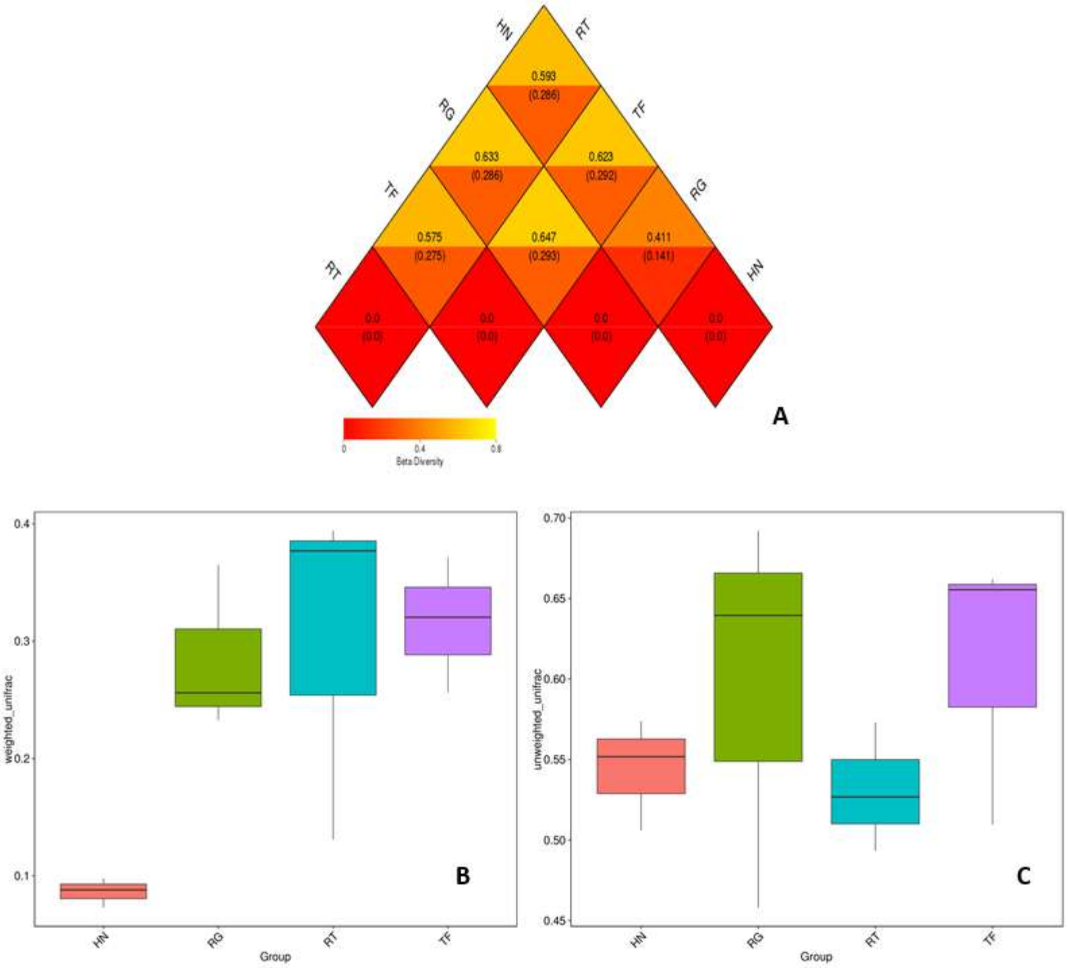

Figure 9. Heatmap of beta diversity in four groups based on the distance between Weighted Unifrac and Unweighted Unifrac: (A) distance matrix Weighted Unifrac and Unweighted Unifrac, (B) Boxplot Weighted Unifrac, and (C) Unweighted Unifrac boxplot. Notes: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine).

3.4.2. Principal coordinates analysis (PCoA), principal component analysis (PCA) and non-metric multidimensional scaling (NMDS), cluster analysis unweighted pair group method with arithmetic mean (UPGMA)

Beta diversity represents an explicit comparison of bacterial communities based on their composition. The beta diversity matrix assesses differences between bacterial communities.

To compare bacterial communities between each pair of community samples, a "distance" or inequality square matrix is calculated to reflect the dissimilarity between specific samples, such as the Unweighted Unifrac and the Weighted Unifrac distance.

The data in this distance matrix can be visualized using Principal Coordinate Analysis (PCoA), Principal Component Analysis (PCA), Non-Metric Multi-Dimensional Scaling (NMDS), and Unweighted Pair-group Method with Arithmetic Means (UPGMA).

Principal coordinate analysis (PCoA) is based on the distance matrix of Weighted Unifrac and Unweighted Unifrac to show the relationship between samples. The first axis on PCoA explains 35.33% (Weighted Unifrac distance matrix) and 20.82% (Unweighted Unifrac distance matrix) of data variability, and the second axis explains 24.81% (Weighted Unifrac distance matrix) and 14.88% (Weighted Unifrac distance matrix) Unweighted Unifrac distance).

The percentage on each axis indicates the value of the contribution to the discrepancy between samples. From the PCoA based on the Unifrac Weighted distance matrix, it is clear that the HN and RG groups are clustered and close together compared to the RT and TF groups. It indicates that the similarity of bacterial species composition is higher between HN and GM than between RT and TF.

The PCA and NMDS plots also show the same trend that the HN and RG groups are closer than the RT and TF groups. In the PCA graph, it can be stated that the more similar the community composition among the samples, the closer the data points are. Each data point in the NMDS graph represents a sample.

The distance between data points reflects the degree of variation. Samples belonging to the same group have the same color. The NMDS stress factor is 0.061, which means that the NMDS is considered reliable to some extent because the stress factor value is less than 0.2. The closer the sample is to the plot, the higher the similarity. Such conditions are also reflected in the results of the UPGMA cluster of the Weighted Unifrac and Unweighted Unifrac distance matrices. The bacterial phylum of the HN group was more similar to the RG, and the RT group was more similar to the TF (**Figure 10**).

The results of the PCA, PCoA, NMDS, and UPGMA plots showing the closeness between the HN and GM groups were strengthened by the results of the ANOSIM analysis. Based on the results of the ANOSIM analysis, it was found that the variation between groups was more significant than the variation within the group, but the difference was not statistically significant.

It is indicated by all R values between groups that are positive and more significant than zero, while all P values between groups are > 0.05. The highest variation between groups is HN-TF (R=1), then TF-RT (R=0.9259), and HN-RT (R=0.9259), while the lowest variation between groups was HN-RG (R=0.222).

3.5. Relation among soil chemistry, soil metals and diversity of soil bacteria in forests and revegetation areas of tin mines > 5 years and < 5 years, and active tin mines on Bangka Island

Several physio-chemical and metallic properties in the soil relate to the diversity of soil bacteria. Based on the Pearson correlation plot at the significance level of $p < 0.05$, it was found that the soil metal Mo had a significant relation with the total species/genera of soil bacteria (**Figure 11**).

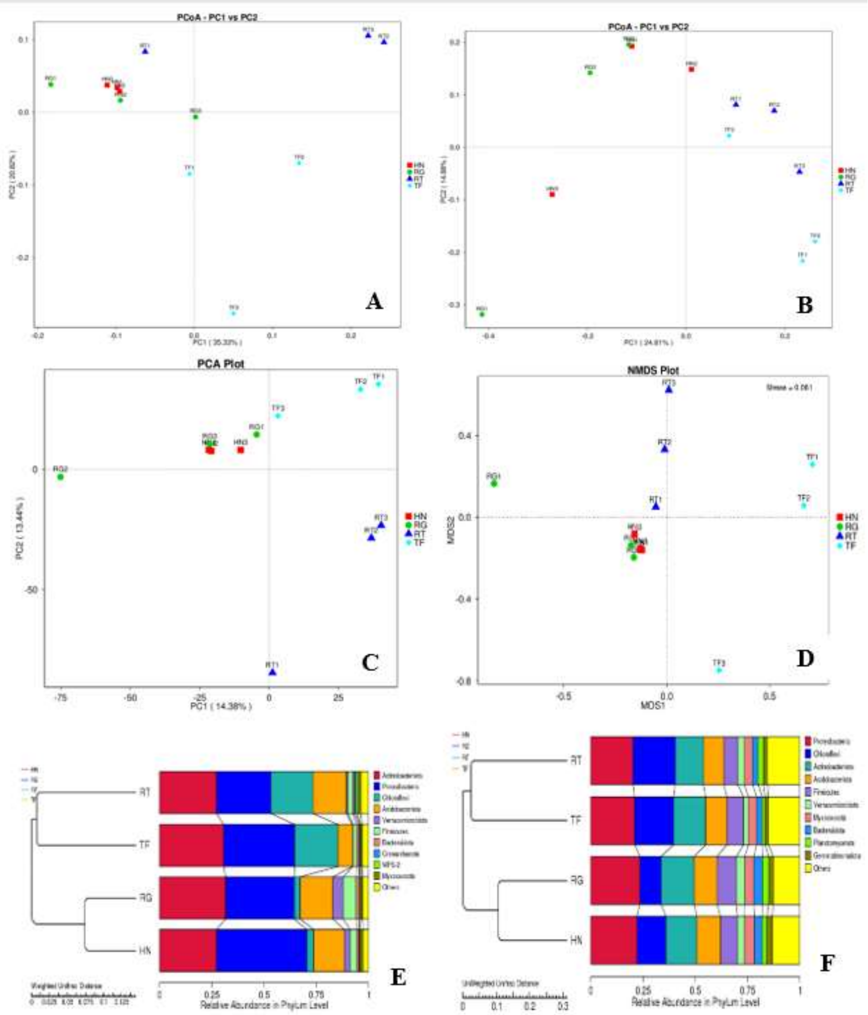

Figure 10. Beta diversity matrix: (A) PCoA Weighted Unifrac, (B) PCoA Unweighted Unifrac, (C) PCA, (D) NMDS, (E) UPGMA Weighted Unifrac, and (F) UPGMA Unweighted Unifrac. Notes: HN (forest), RG (tin mine revegetation land > 5 years), RT (tin mine revegetation land < 5 years), TF (active tin mine).

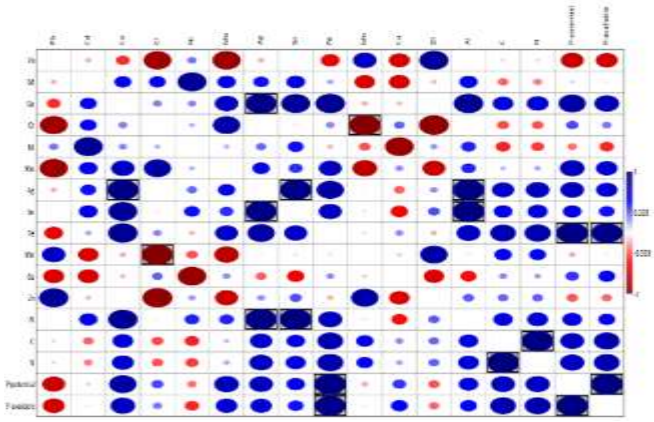

Figure 11. The Pearson Correlation plot among soil physio-chemistry, soil metals, and the total species/genera of bacteria with a significance of p<0.05. Remark: □ p<0.05.

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The relation between the two is powerful and inversely proportional. It means that the higher the Mo metal in the soil, the lower the total species/soil bacteria and vice versa. From the Pearson Correlation table, another robust and inverse relation was also obtained but not significant, such as the relation between the earth metal Cr and the total species/soil bacteria (**Table 3**). In addition, there is a powerful and directly proportional relation between Pb and total species/soil bacteria. This condition indicates that the higher the Pb, the higher the total species/soil bacteria. The physical and chemical properties of soil P (potential and available) are strongly and inversely related to the total species/soil bacteria. The higher the P in the soil, the lower the total bacteria/species in the soil.

The relationship between seventeen environmental factors (independent factors) and twenty-three species/genera of bacteria (bound factors) from four different fields can be studied by using *Canonical Correspondence Analysis* (CCA) (**Figure 12**). The high levels of Cd and Ni in active tin mines make *Sinomonas atrocyanea* mostly emerge from this location. Besides playing a role in the P cycle (Raj *et al.*, 2014), *Sinomonas atrocyanea* is also tolerant to Ni and Cr metals (Singh & Narzary 2021).

With the predominant Cu, Cr, and Mo metal content in the soil, several species of soil bacteria, such as *Bulkhorderia* sp., *Bacillus lehensis*, *B. fordii*, *B. thermolactis*, *Chloroflexi bacterium*, and *Thermonosporaceae bacterium* are most commonly found in tin mining revegetation land < 5 years. *Bacillus fordiis* and *Bacillus lehensis* play roles in the carbon cycle. *Bacillus thermolactis* also plays a role in the C and the N cycles (An Coorevits *et al.*, 2011). According to Shao *et al.* (2019), some of the species of *Bacillus* can accumulate Cr and Pb.

The other four bacterial species, namely: *Burkholderia ubonensis, Chloroflexi bacterium, Bukholderia* sp., and *Bacillus sporothermodurans,* in addition to emerging on this land, it has also begun to be found in tin mining revegetation land for > 5 years. This condition is also thought to have something to do with Cu metal, which levels are almost the same in the two fields. *Burkholderia ubonensis* and *Bukholderia* sp. play a role in the P cycle (Madhaiyan *et al.*, 2021; Gosh *et al.*, 2016). *Chloroflexi bacterium* plays a role in the C and N cycles (Yamamura *et al.*, 2003). Meanwhile, *Bacillus sporothermodurans*is resistant to a hot environment (Owusu-Darko *et al.*, 2020). According to Zhang *et al.* (2021) and Wang *et al.* (2021), besides producing siderophores, *Bukholderia* sp. is also tolerant of Cu, Cd, Fe, Zn, and Mg metals.

The diversity of soil bacteria in the forest is almost the same as the diversity of soil bacteria in the tin mining revegetation land > 5 years. It can also be seen in the similar physical and chemical conditions of soil metals, including levels of Carbon, Nitrogen, Phosphorus, and Fe in HN and RG. *Paraburkholderia* sp., *Bacillus* sp., *Candidatus-Xiphinematobacter*, and *Bacterium enrichment* were the dominant bacterial species in them. *Bacillus aryabhattai* plays a role in the C cycle (Sari *et al.*, 2016a), N, and P cycles (Deng *et al.*, 2022). Singh and Narzary (2021) also reported that*Bacillus aryabhattai* is tolerant to Ni, Cr, and as metals. *Candidatus-Xiphinematobacter* functions in the Carbon cycle (Bisker *et al.*, 2021). *Bacillus graminis* plays a role in the C and N cycles (Bibi *et al.*, 2011). Several other species are also involved in the P cycle: *Paraburkholderia* sp., *Paraburkholderia nodosa, Paraburkholderia phymatum, Bacillus thuringiensis* (Bellés-Sancho *et al.*, 2021; Agnol *et al.*, 2016; Raj *et al.*, 2014). *Bacillus thuringiensis* is also tolerant to Mn metal polluted environment (Huang *et al.*, 2020), Cd, Cu, Pb, Ni (Oves *et al.*, 2012), and Cr (Guerrero-Cebellos *et al.*, 2021). Therefore, several references mention the vital role of *Bacillus gibsonii,* namely in the C cycle (Batool *et al.*, 2019), N cycle (Orhan, 2016), P cycle (Hayat *et al.*, 2013), and produce siderophores (Petrillo *et al.*, 2021). The emergence of *Paraburkholderia* sp., which is the most found in the forest, is related to the levels of C, N, and P in the soil and soil metals, mainly Fe and Mn. It is also supported by the potential of *Paraburkholderia* sp. on the cycles of Carbon (Wilhelm *et al.*, 2021), Nitrogen (Renata *et al.*, 2022), and Phosphorus (Kirui *et al.*, 2022) and resistance to Ni metal polluted environment (Knuutinen *et al.*, 2019), Al and Fe (Damo *et al.*, 2022). *Bacillus selenatarsenatis* and *Bradyrhizobium,* besides being found in RG, it is also spread in HN, TF, and RT. This condition is also estimated to be influenced by the metal content distributed in the four fields: Sn, Al, Pb, Zn, Ag, and Co. According to Jach *et al.*, (2022), several species of the genus *Bradyrhizobium* play a role in the bioremediation of Pb, Cd, Cu, Zn, and As metals.

Table 3. Pearson correlation among soil physical chemistry, soil metals, and the total species/genera of bacteria.

No	Variable	Bacteria	No	Variable	Bacteria	No	Variable	Bacteria
1	C	-0.14135	7	Co	-0.77332	13	Fe	-0.71084
2	N	-0.17468	8	Cr	-0.80940	14	Mn	0.74663
3	P-potential	-0.74047	9	Ni	-0.26110	15	Cu	-0.09888
4	P-available	-0.62874	10	Mo	**-0.99168**	16	Zn	0.62649
5	Pb	0.81089	11	Ag	-0.59494	17	Al	-0.52710
6	Cd	-0.65418	12	Sn	-0.50635			

Description: low/weak (0.200 - < 0.400); big enough/strong enough (0.400 - < 0.600); big/strong (0.600 - < 0.800); very large/very strong (0.8 - <1); sign (-): the relation is inverse.

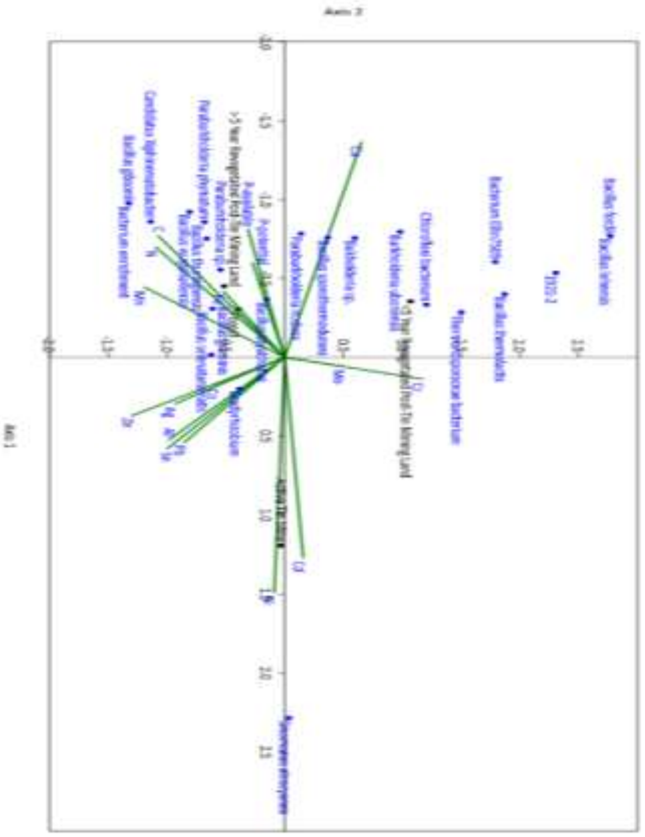

Figure 12. CCA between soil physico-chemical, soil metals and total species/genera of bacteria and the diversity of soil bacteria.

4. CONCLUSION

The reclamation carried out by PT Timah, Indonesia, is categorized as successful in tin mining revegetation of land > 5 years in Jelitik Village, Bangka, Indonesia. It is reflected in the physicochemical soil conditions of the tin mining revegetation land > 5 years, which almost resembles a forest, especially in the levels of organic C, N-total, P-potential, and P-available in the soil.

There are 23 species/genera of bacteria found in four different fields. Six potential species/genera of bacteria are grouped based on the soil's physical, chemical, and metallic properties, namely: role in C, N, and P cycles, resistance to metals, heat resistance, and production of siderophores. Diversity of soil bacteria in tin mining revegetation land < 5 years, then in tin mining revegetation land > five years higher than in forest and active tin mining.

There are 17 species/genera of soil bacteria that are equally distributed in the four fields, with *Bradyrhizobium* as the dominant genus in all fields.

The physicochemical properties, namely C, N, P, and Fe, as well as bacterial communities of the forest and the > 5 years revegetated post-tin mining land, similar to *Paraburkholderia* sp., *Bacillus* sp., *Candidatus-Xiphinematobacter*, and *Bacterium enrichment* were found to be the dominant bacterial species in them. The physicochemical properties of soil from < 5 years of revegetated post-tin mining land were dominated by Cu, Cr, and Mo. Dominant species in this soil were *Bulkhorderia* sp., *Bacillus lehensis*, *B. fordii*, *B. thermolactis*, *Chloroflexi bacterium*, and *Thermonosporaceae bacterium*. *Sinomonas atrocyanea* primarily emerged in active tin mining due to the high levels of Cd and Ni in the land.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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