Perubahan Sifat Kimiawi dan Komunitas Bakteri Tanah pada Lahan Pasca Tambang Timah yang Direvegetasi di Pulau Bangka

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INTISARI

Kegiatan penambangan timah di Pulau Bangka menghasilkan limbah beberapa logam, seperti: Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Se, As, Fe, Mn, Cu, Zn, dan Al. Limbah tersebut mencemari biota, sumber air serta tanah. Salah satu upaya untuk menurunkan ketersediaan logam yaitu dengan pengolahan tanah secara biologi (bioremediasi) menggunakan bantuan bakteri tanah dan juga menggunakan tumbuhan revegetasi yang mampu mengakumulasi logam seperti akasia. Upaya perbaikan reklamasi tanah melalui perlakuan *below ground treatment* secara biologis berdasarkan hasil interaksi bakteri tanah dan tumbuhan akasia sangat perlu diketahui untuk mengukur keberhasilan reklamasi lahan pasca tambang timah di Pulau Bangka dalam upaya membantu pengembalian kondisi ekosistem ke ekosistem semula. Ada empat tujuan dalam penelitian, yaitu: mengungkapkan perubahan ekosistem, yang meliputi perubahan karakteristik fisika kimia tanah, kaitannya dengan kandungan logam di dalam tanah dan tumbuhan, keragaman bakteri di dalamnya; serta keragaman metabolomik larut air dalam tanah dan kandungan klorofil dan karotenoid daun, akibat keragaman umur upaya revegetasi pada ekosistem pasca penambangan timah di Pulau Bangka. Kebaharuan utama dari penelitian ini terletak pada kajian diversitas bakteri tanah dengan pendekatan metagenomik dan keragaman metabolomik tanah larut air dengan LC-MS/MS. Selain itu juga, ada kebaruan lainnya seperti kajian logam sebanyak 15 jenis di dalam tanah dan organ akasia serta kandungan karotenoid daun akasia. Penelitian dilakukan pada empat lahan berbeda di Pulau Bangka, yakni: tanah rizosfer lahan hutan (HN), lahan yang telah mengalami upaya revegetasi pasca penambangan timah lebih dari 5 tahun (RG) dan kurang dari 5 tahun (RT), serta tanah non-rizosfer tambang timah aktif (TF). Tiga titik sampling tanah diambil pada tanah yang melekat pada perakaran akasia, serta tanah non-rizosfer pada kedalaman 0-40 cm dengan bor tanah. Organ akasia yang diambil untuk analisis logam adalah bagian akar, batang dan daun. Identifikasi tumbuhan dilakukan di Herbarium Bangka Belitungense. Beberapa variabel yang diamati yaitu: karakter mikroklimat, karakter fisika kimia tanah (warna; tekstur; struktur; pH; C; N; C/N; P dan K potensial dan tersedia; kation dapat ditukar; kapasitas tukar kation [KTK]; kejenuhan basa [KB]; keasaman dapat tukar; kandungan logam (Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Fe, Mn, Cu, Zn, Al) di dalam tanah dan organ tumbuhan; karakteritik akumulasi logam (*translocation factor* [TF], *biocoeficient factor* [BCF], *bioaccumulation factor* [BAF]); diversitas bakteri tanah; keragaman metabolomik tanah larut air dan metabolomik tumbuhan (klorofil a, b, total klorofil dan karotenoid). Logam dianalisis dengan spektofotometri serapan atom. Diversitas bakteri diukur dengan metode metagenom 16S rRNA dengan NGS. Metabolomik tanah dianalisis dengan LC-Ms/Ms. Metabolomik tumbuhan diukur dengan spektrofotometer UV-Vis. Data dianalisis statistika *multivariate* dengan metode kluster dan PCA biplot, nalisis Korelasi Pearson dan *cannonical correlation analysis* (CCA). Analisis bioinformatika metagenome yang dianalisis adalah*:* keragaman Alfa (*Observed species, Goods coverage, Chao1, ACE, Shannon, Simpson Index)*, keragaman Beta (*Unifrac distance heatmap, PCA, PCoA*, dan *UPGMA*), NMDS, MRPP, Anosim, Adonis dan AMOVA, LefSE, T-test, MetaStat, serta CCA. Senyawa metabolit tanah diidentifikasi melalui website chemspider. Hasil analisis mikroklimat menunjukkan bahwa kelembaban udara dan kadar air tertinggi teramati di HN, sementara intensitas cahaya, radiasi matahari, tekanan udara dan kecepatan angin tertinggi teramati di TF. Kadar C, N, C/N, KTK, P potensial & tersedia, Ca, Mg paling rendah teramati di TF. Kegiatan reklamasi PT.Timah telah berhasil dilakukan pada RG karena ada kemiripan sifat tanah antara lahan tersebut dengan HN. Tingginya intensitas radiasi matahari, intensitas cahaya matahari, dan kecepatan angin di tambang timah aktif dapat berpengaruh sangat kuat terhadap kadar air tanah, suhu tanah dan kelembaban tanah. Kelembaban tanah memengaruhi tingkat pertumbuhan tumbuhan. Tinggi rendahnya seresah tumbuhan akan memengaruhi kandungan C, N, P, KTK, pH, Ca, Mg, K dan Na di dalam tanah. Logam tanah paling banyak yaitu: Sn, Mn, Pb, Zn, Mo. Serapan logam tertinggi diamati pada akar akasia. Akasia berpotensi sebagai fitoekstraktor untuk lima logam yaitu: Mn, Sn, Zn, Ag, dan Cu karena nilai TF, BCF, & BAF>1. Diversitas bakteri tanah tertinggi teramati di RT. Takson bakteri yang dominan, yaitu: filum Proteobacteria, kelas Alphaproteobacteria, ordo Rhizobiales, famili Xanthobacteraceae, dan genus Acidothermus. Ada 20 spesies bakteri tanah dari lima filum yang merajai. Beberapa spesies bakteri tanah berpotensi dalam siklus C, N, dan P. Beberapa spesies bakteri yang lainya tahan terhadap logam dan panas. Beberapa spesies lainnya dapat memproduksi siderofor. Komunitas bakteri di HN lebih mirip dengan RG. Hal ini erat kaitannya dengan kemiripan karakteristik fisika kimia tanah pada kedua lahan. Tidak semua senyawa kimia yang termasuk ke dalam senyawa metabolit tanah larut air. Senyawa kimia paling banyak ditemukan di RT. Senyawa kimia paling banyak ditemukan dari semua lahan daripada senyawa kimia lainnya yaitu senyawa memiliki nilai m/z 83,2771 dengan nama CoMg. Ada hal menarik yaitu ditemukannya senyawa organik halogen maupun senyawa anorganik halogen pada semua lahan dikarenakan kedekatan lokasi dengan daerah pantai yang masih banyak kandungan garam di dalam tanahnya. Senyawa kimia dengan atom C, H, dan O tertinggi berada pada lokasi bervegetasi dan cenderung termasuk senyawa metabolit tanah yang berasal dari aktivitas mikrob tanah. Senyawa tersebut kemungkinan berperan sebagai ligan yang dapat mengikat logam di dalam tanah, sehingga logam dapat masuk ke dalam tanaman. Kadar klorofil a paling tinggi di RG, klorofil b paling tinggi di HN, total klorofil paling tinggi di HN dan RG dan karotenoid paling tinggi di RG dan RT. Revegetasi mengurangi intensitas matahari dan mengubah sifat fisika kimia tanah, khususnya nutrien untuk bakteri tanah, yaitu C, N, P, K dan air. Hal tersebut akan meningkatkan keragaman bakteri tanah. Peningkatan keragaman bakteri tanah juga akan menyebabkan peningkatan pada keragaman metabolitnya. Keragaman metabolit yang meningkat juga ada kaitannya dengan keberadaan logam di lingkungannya yang merupakan wujud dari detoksifikasi logam sehingga logam dapat masuk ke dalam tumbuhan. Reaksi tumbuhan terhadap masuknya logam ke dalam organnya dapat dilihat dari kandungan klorofil dan karotenoid pada daun. Kondisi tersebut apabila terjadi suatu perputaran pada ekosistem, maka akan terlihat bahwa revegetasi berhasil menuju ke hutan. Hal ini ditunjukkan kemiripan antara sifat tanah dan keragaman bakteri di lahan revegetasi tambang timah > 5 tahun dengan hutan. Keragaman bakteri tanah di kedua lahan revegetasi cenderung meningkat dan ini menunjukkan bahwa proses revegetasi menunjukkan arah yang benar yakni kembali menuju hutan.

Kata kunci: Bangka, Logam, Metagenom, Metabolomik, Tambang Timah

Changes in Chemical Properties and Bacterial Communities of Soil in Revegetated Post-Tin Mining Land on Bangka Island

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ABSTRACT

Tin mining activities on Bangka Island produce waste from several metals, such as: Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Se, As, Fe, Mn, Cu, Zn, and Al. This waste pollutes biota, water sources and soil. One effort to reduce the availability of metals is by biological soil processing (bioremediation) using soil bacteria and also using revegetation plants that are able to accumulate metals such as acacia. Efforts to improve land reclamation through biological below ground treatment based on the results of the interaction of soil bacteria and acacia plants are very important to know to measure the success of post-tin mining land reclamation on Bangka Island in an effort to help restore ecosystem conditions to their original ecosystem. There are four objectives in this research, namely: to reveal changes in ecosystems, which include changes in the physical and chemical characteristics of the soil, its relation to the metal content in the soil and plants, the diversity of bacteria in it; as well as water-soluble metabolomic diversity in soil and plants (chlorophyll and carotenoid), due to the diversity of ages of revegetation efforts in post-tin mining ecosystems on Bangka Island. The main novelty of this research lies in studying soil bacterial diversity using a metagenomic approach and water-soluble soil metabolomic diversity using LC-MS/MS. Apart from that, there are other novelties such as the study of 15 types of metals in the soil and organs of acacia as well as the carotenoid content of acacia leaves..The research was conducted on four different lands on Bangka Island, namely: rhizospheric forest land (HN), land that has undergone post-tin mining revegetation efforts for more than 5 years (RG) and less than 5 years (RT), and non-rhizospheric soil of active tin mining (TF). Three soil sampling points were taken from soil attached to acacia roots, as well as non-rhizosphere soil at a depth of 0-40 cm with a soil drill.The metal content of the Acacia's roots, stems and leaves were analyzed. Plant identification were done in the Bangka Belitungense Herbarium. Some of the variables observed were: microclimatic characters, physical-chemical soil characteristics (color; texture; structure; pH; C; N; C/N; potential and available P and K; cations exchangeable; cation exchange capacity [CEC]; base saturation [KB]; exchangeable acidity; metals (Pb, Cd, Co, Cr, Ni, Mo, Ag, Sn, Fe, Mn, Cu, Zn, Al) contents in soil and plant organs; metal accumulation characteristics (translocation factor [TF], bioefficient factor [BCF], bioaccumulation factor [BAF]); diversity of soil bacteria; diversity of water-soluble soil metabolomics and plant metabolomics (chlorophyll a, b, total chlorophyll and carotenoids).

Metals were analyzed by atomic absorption spectrophotometry. Bacterial diversity was access using metagenomic method of 16S rRNA analyzed using NGS. Water soluble soil metabolomics diversity was access using LC-MS/MS. Plant metabolomics were access using UV-Vis spectrophotometer. Data were analyzed using multivariate statistics using cluster methods and biplot PCA, Pearson correlation analysis and *cannonical correlation analysis* (CCA). The metagenome bioinformatics analysis analyzed were: Alpha diversity (Observed species, Goods coverage, Chao1, ACE, Shannon, Simpson Index), Beta diversity (Unifrac distance heatmap, PCA, PCoA, and UPGMA), NMDS, MRPP, Anosim, Adonis and AMOVA, LefSE, T-test, MetaStat, and CCA. Soil metabolites were identified through the chemspider website. The results of microclimate analysis showed that the highest humidity and water content were observed in HN, while the highest light intensity, solar radiation, air pressure and wind speed were observed in TF. The lowest levels of C, N, C/N, CEC, potential & available P, Ca, Mg were observed in TF. PT. Timah's reclamation activities have been successfully carried out on RG because as indicated by its similarity in soil properties with HN. The high intensity of solar radiation, sunlight intensity and wind speed in active tin mines can have a very strong effect on soil water content, soil temperature and soil moisture. Soil moisture affects plant growth rates. The level of plant litter will affect the content of C, N, P, CEC, pH, Ca, Mg, K and Na in the soil. The most abundant earth metals were found to be: Sn, Mn, Pb, Zn, and Mo. The highest metal uptake was found in acacia roots. Acacia has the potential as a phytoextractor for five metals namely: Mn, Sn, Zn, Ag, Cu because the value of TF, BCF, & BAF> 1. The highest diversity of soil bacteria was found at RT. The dominant bacterial taxa are: Proteobacteria phylum, Alphaproteobacteria class, Rhizobiales order, Xanthobacteraceae family, and Acidothermus genus. There are 20 species of soil bacteria from five predominant phyla. Some species of soil bacteria are potential in cycling C, N, and P. Several other species of bacteria are resistant to metals and heat. Several other species can produce siderophores. The bacterial community in HN is more similar to that of RG. This is closely related to the similarity of the physical and chemical characteristics of the soil in the two fields. Not all chemical compounds included in soil metabolite compounds are water soluble. Most chemical compounds found in RT. The most common chemical compound was found from all lands than any other chemical compound is the compound having an m/z value of 83,771 with the name CoMg. There is an interesting thing, namely the discovery of halogen organic compounds and halogen inorganic compounds in all lands due to the proximity of the location to coastal areas where there is still a lot of salt content in the soil. Chemical compounds with the highest C, H and O atoms are found in vegetated locations and tend to include soil metabolite compounds originating from soil microbial activity. These compounds may act as ligands that can bind metals in the soil, so that metals can enter plants. Metabolomic diversity is dominant in acacia leaves, namely: levels of chlorophyll a in RG, chlorophyll b in HN, total chlorophyll in

HN and RG and carotenoids in RG and RT. Revegetation reduces solar intensity and changes the physicochemical properties of soil, especially nutrients for soil bacteria, namely C, N, P, K and water. This will increase the diversity of soil bacteria. Increasing the diversity of soil bacteria will also cause an increase in the diversity of their metabolites. The increased diversity of metabolites is also related to the presence of metals in the environment, which is a form of metal detoxification so that metals can enter the plant. The reaction of plants to the entry of metals into their organs can be seen from the chlorophyll and carotenoid content in the leaves. In this condition, if a rotation occurs in the ecosystem, it will be seen that the revegetation has succeeded in leading to the forest. This is shown by the similarity between soil properties and bacterial diversity in tin mine revegetation land > 5 years and forests. The diversity of soil bacteria in both revegetation areas tends to increase and this shows that the revegetation process is showing the right direction, namely returning to the forest.

***Keywords**: Bangka, Metals, Metagenom, Metabolomics, Tin Mining*