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Bioremediator Formula for Cultivated Plants Grown on Post-Mining Soil

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Abstract. Research in the form of bioremediation through *Bacillus* sp. and VAM tests to degrade nickel and chromium needs to be carried out to obtain the best formula for plant growth. Thus, this research has the following objectives: (1) to obtain the best treatment of soil heavy metal bioremediator (2) to reuse post-mining land into productive land for cultivated plants. The research method will be divided into two activities (1) provision of bioremediator formula and (2) testing of bioremediator formulation on cultivated plants. Based on the study's results, 150 ml *Bacillus*/plant (A3) has the highest effectiveness in reducing chromium by 0.922% in the soil. While the best formula for reducing nickel is 75 grams of VAM (A6) with a reduction capacity of 0.924%. All treatments on sorghum samples on each chromium and nickel produced values <1, this shows that sorghum only absorbs heavy metals and does not accumulate much of all chromium and nickel heavy metals. Parameters of plant height, number of leaves, wet weight, and dry weight with the DMRT at the α = 5% level showed a very significant effect on the treatment of VAM (A6) 75 grams. *Bacillus* sp and VAM can be used as a bioremediator agent for cultivated plants planted on post-mining soil.

Keywords: bacillus; bioremediatory; post- mining soil; sorghum; VAM

INTRODUCTION

Southeast Sulawesi is the largest nickel mining province in Indonesia, with an area of 198,624.66 hectares for 138 nickel mining companies (Badan Pusat Statistik, 2022). In addition to bringing prosperity, mining also has a negative impact on human life due to environmental changes from mining interventions or activities that affect the balance of society. The mining industry has several impacts on Pomalaa, namely, the sea waters look murky in Hakatutobu and Tambea villages. In Hakatutobu, the water is even dark brown in colour. The position of these two villages is surrounded by nickel mining industry activities that dredge the land in the hilly areas around Pomalaa and shipping activities at the docks along the coast (Kadir et al., 2020). Forest destruction due to mining activities caused catastrophic floods in Southeast Sulawesi in 2019. Forests were damaged due to land conversion to plantations and mining (Syahri, Baharuddin, Fachruddin, & Yani, 2019; Nuryanto et al., 2023). Post-mining land reclamation at the largest nickel company in Pomalaa, Southeast Sulawesi, is carried out by revegetation. Preliminary research conducted by the proponent on revegetated nickel post-mining soil showed that the heavy metal content of nickel (Ni) reached 11103.74/kg and chromium (Cr) reached 4030.17 mg/kg, which is far above the threshold (Syahri et al., 2019). The threshold value (NAB) of heavy metals in sediment or soil for nickel is 0.31 µg/g, and for chromium is 0.5 µg/g (Badan Standarisasi Nasional, 2004). Potassium, calcium, iron, copper, and manganese deficits also occur in revegetated post-mining land sites in Pomalaa, Southeast Sulawesi (Widiatmaka et al., 2010). The presence of heavy metals in the soil can degrade soil function for plant growth. Revegetation is not known to be an effective method in rehabilitating post-mining land with heavy metal contaminants and low soil fertility status. Other remediation techniques are needed for environmental sustainability. Bioremediation is a technique of utilizing living organisms to overcome environmental problems such as soil and water pollution.

Bioremediation (in situ bioremediation) with the addition of microorganisms (exogenous microorganisms) is called bioaugmentation and with the addition of nutrients and electron acceptors to the growth

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environment of microorganisms to stimulate their growth is called biostimulation (Sheehan, 1997). Biological agents used for bioremediation to clean up contaminated areas are called Bioremediators. Bacteria are essential to bioremediation because they produce a range of enzymes that help break down contaminants. Certain bacteria produce oxygenases, which initiate the oxidation reactions that degrade hydrocarbons, including PAHs (Wróbel et al., 2023). Bacterial biofilms improve the efficiency of pollutant decomposition. Because biofilms protect bacteria, they can multiply and efficiently degrade contaminants under unfavorable conditions (Schommer et al., 2023). Enhancing the activity of native microorganisms by the provision of co-substrates, electron acceptors, or essential nutrients is the aim of biostimulation. This sustainable technique is based on the natural metabolic ability of the microorganisms at the contaminated location (De Jesus & Alkendi, 2023; Siyum et al., 2022). By boosting microbial activity using organic materials like compost and vascular Arbuscular Mycorrhiza (VAM), pollutants can be broken down naturally without the use of synthetic chemicals, which makes the remediation process more sustainable and eco-friendlier (Wróbel et al., 2023). Bioremediation appears as a ray of hope at the nexus of scientific advancement and environmental care (Tribedi et al., 2018).

Isolation, screening, identification and biochemical tests which include 3% KOH test, H_2O_2, oxidation-fermentation (OF), endospores, IAA, GA_3, phosphate (P) solubilization, nitrogen (N) fixation and bacterial reduction power test on nickel and chromium in post-nickel mine soil have been carried out in preliminary research and obtained *Bacillus* sp bacteria as potential bacteria to become bioaugmentation agents with the ability to improve soil fertility and reduce nickel metal by 0. 080 ppm at a media concentration of 700 ppm and reduced chromium metal by 0.055 ppm at a media concentration of 700 ppm. The use of VAM with the hyperaccumulator plant *Vetiveria zizanioides* in post-mining soils has also been carried out in advanced research and obtained that VAM are a potential combination for post-mining land biostimulation. *Bacillus* sp. is a type of bacteria that has been identified from the previous research of the proposer where based on a literature review (Baskaran & Sathiavelu, 2022) it is said that *Bacillus* sp. can reduce the amount of metals such as lead, cadmium, mercury, chromium, arsenic, or nickel in the environment. The use of *Bacillus* strains immobilized by biochar on contaminated soil can help plants grow by reducing toxins in the soil and preventing the accumulation of metals in plants and increasing microbial and enzyme activity in the soil (Purba et al., 2020; Patil & Desai, 2024). *Bacillus* sp is a potential bioaugmentation agent for post-mining land. Further research that has been carried out by the proposer is a test of VAM as a biostimulation agent on the hyperaccumulator plant Vetiveria zizanioides and it was obtained that Vetiveria zizanioides can absorb 38.49 ppm of nickel and 64.63 ppm of chromium in soil with VAM concentration of 20% (w / w). VAM has the potential to increase the ability and effectiveness of metal absorption by expanding the root absorption area of hyperaccumulator plants (*Vetiveria zizanioides*).

This research is included in the study of green economy which is a national research priority that emphasises the concept of maintaining sustainable natural resources and the environment. Two stages of previous research have been carried out as a problem-solving approach for post-mining land remediation, further research on the use of *Bacillus* sp. and VAM as bioremediator agents on nickel and chromium contaminated land needs to be carried out to see the extent to which post-mining land can be reused for agricultural cultivation.

METHODS

Implementation of research

This research was conducted from August - November 2024 in Kolaka, Southeast Sulawesi. This research is a laboratory and field experiment consisting of two main series of activities, namely:

1. Provision of bioremediator formulation
2. Testing of bioremediator formulation on cultivated plants

This research method

1. Provision of bioremediator formulation
   a. *Bacillus* sp. bacteria are rejuvenated on *nutrient agar* (NA) medium using the scratch method in a petri dish and incubated for 2 x 24 hours. Bacterial propagation consists of pre-culture, namely 1 colony of bacteria from pure culture transferred into 10 ml of *nutrient broth* (NB) medium in a culture bottle and incubated on a shaker at 150 rpm for 24 hours and main-culture, namely 1 ml of suspension from the pre-culture transferred into 25 ml of sterile coconut water in a culture bottle and incubated in the same way for 3 × 24 hours. Coconut water can be a medium for the growth and proliferation of microbes (Onifade & Jeff-Agboola, 2003). Coconut water enriched with *Bacillus substilis* affects plant height, plant dry weight, root wet weight, and root dry weight (Sari et al., 2021).

   b. Rejuvenation and propagation of VAM is carried out by trapping on corn plants planted until the vegetative period of the plant. The soil media used are compost and post-mining soil in a ratio of 1:1. Trapping and propagation of Vesicular Mycorrhizal Arbuscula (VMA) was conducted on maize plants for 90 days of cultivation, then Absolute Frequency (FM) and Relative Frequency (FR) were calculated. FM and FR calculations were carried out as preliminary data on mycorrhizal abundance in the study. Frequency is the number of occurrences of each species encountered from the entire capture. FM is something that shows the number of times a certain mycorrhizal spore is found in the soil of each observation expressed absolutely. Meanwhile, FR is something that shows the frequency of the presence of a type of mycorrhizal spores in the soil and can describe the distribution of this type of mycorrhizal spores (Aji et al., 2021).

2. Bioremediator formulation test

The formulation test of superior bacteria *Bacillus* sp and VAM for controlling heavy metals Ni and Cr in sorghum cultivation consists of:

   a. Planting preparation. The plant chosen as a cultivated plant is sorghum. Sorghum is a cereal plant that is a local food source that substitutes rice. Sorghum is planted with a distance between polyethylene bags of 65 x 20 cm. The planting medium used was post-nickel mining soil from the nickel mining industry in Pomalaa, which was sampled in the previous preliminary study. There is no treatment of basic fertilizer in the preparation of the planting medium to see the single effect of the formulation/treatment. polyethylene bags are arranged according to the grouping.

   b. The introduction of the *Bacillus* sp and VAM formulas to cultivated plants is carried out 3 times, namely at 10 days after planting, 30 days after planting and 45 days after planting.

   c. Maintenance and harvesting
   The formulation test of superior bacteria *Bacillus* sp and VAM for the

reduction of heavy metals nickel and chromium on cultivated plants consists of the stages of planting preparation and planting on cultivated plants.

a. Planting preparation.

Planting preparation begins with taking planting media from post-mining soil in Kolaka Regency.

b. Planting in cultivated plants

Randomised Group Design (RAK) is a design used to control homogeneity. Which is used as a form of homogeneity control in a particular environment. Therefore, in this research, the source of variation in the group randomised design is the treatment. There was single factor pattern, consisting of 7 (seven) treatments, namely:

(A0) = Control
(A1) = 50 ml *bacillus*/plant
(A2) = 100 ml *bacillus*/plant
(A3) = 150 ml *bacillus*/plant
(A4) = 25 grams of VAM
(A5) = 50 grams of VAM
(A6) = 75 grams of VAM

This study has 7 treatments, 3 replications and 4 units in replications so that there are 84 polyethylene bags.

Research parameters

1. Post-mining soil was analyzed before and after bioremediator treatment. Initial soil nutrient tests were conducted on % C, % N, C/N, % BO, total P (P_2O_5 HCl), available P (Bray II pmm), available K (K-dd m.e /100g), total K (K_2O_5 HCl), pH (H_2O).
2. Reduction in metal content (%) of soil Determination of the percentage of heavy metals nickel and chromium by calculating the reduction power (DR) according to the equation: DR= C(a) – C(b) / C(a) x 100%; where C(a) is the initial Ni/Cr concentration (ppm) and C(b) is the final Ni/Cr concentration (ppm) and DR is the reducing power. The reduction of heavy metals nickel and chromium was carried out by Atomic Absorption Spectrophotometry (AAS) at the Laboratory of planting, crops, fertilizers, and water. Agricultural instrument standardisation implementation centre, South Sulawesi.
3. Bioconcentration factor (BCF) according to the equation: BCF Ni/Cr = heavy metal (Ni/Cr) plant / heavy metal (Ni/Cr) soil, where if BCF>1=high capability
4. Plant parameters were conducted during the growth and production phase of the plant. Growth observations included plant height, number of leaves, wet weight and dry weight. Observation data were tested using Analysis of variances (ANOVA) to determine if the treatment had a significant effect, and then the data were tested using the Duncan Multiple Range Test (DMRT) at the α level = 5%. Correlation analysis was conducted to find the relationship between the variables observed in the study.

RESULTS AND DISCUSSION

Provision of bioremediator formulation

Rejuvenation and propagation of VAM

The calculation of FM and FR is carried out as initial data on the abundance of mycorrhizae in the study. FM and FR are calculated after calculating the number of spores in the trapping. The results of calculating the number of spores in ten polyethylene bags of corn plants planted on post-mining land can be seen in Table 1.

In Table 1 above, it can be seen that the presence of the genus VAM is different in each replication. The most commonly found mycorrhizal genus is the *Glomus* sp. where this type is almost in every replication, in contrast to the types *Gigaspora* sp and

Acaulospora sp. These two types are rarely found because their numbers are small even after being trapped with corn plants. In this ex-mining soil sample, the largest number of spores was found in replication 4, with a total of 25 spores, while the smallest number was found in replication 3, with a total of 16 spores. Glomus is the type of mycorrhiza that is most widely distributed in the soil and can also be found in post-mining soil.

Table 1. Data on the number of spores based on the VAM Genus

VAM genus	Trapping VAM									
	Test									
	1	2	3	4	5	6	7	8	9	10
	number of spores/100g soil									
Glomus sp.	21	15	15	21	21	21	18	19	16	20
Acaulospora sp.	-	1	1	4	1	1	-	1	1	-
Gigaspora sp.	2	1	-	-	1	-	-	-	1	2
Total spores	23	17	16	25	23	22	18	20	18	22

Table 2. FM and FR Values of VAM Genus

VAM genus	Trapping VAM	
	FM	FR
	---%---	
Glomus sp. 1	60	4.90
Glomus sp. 2	60	4.41
Glomus sp. 3	50	3.92
Glomus sp. 4	50	3.43
Glomus sp. 5	40	2.45
Glomus sp. 6	70	6.37
Glomus sp. 7	60	3.43
Glomus sp. 8	60	3.92
Glomus sp. 9	70	4.90
Glomus sp. 10	80	7.35
Glomus sp. 11	50	3.92
Glomus sp. 12	50	4.41
Glomus sp. 13	60	5.88
Glomus sp. 14	40	3.92
Glomus sp. 15	80	6.37
Glomus sp. 16	70	5.39
Glomus sp. 17	60	3.92
Glomus sp. 18	70	4.90
Glomus sp. 19	40	3.92
Glomus sp. 20	60	3.92
Acaulospora sp. 1	50	2.94
Acaulospora sp. 2	30	1.47
Gigaspora sp. 1	30	1.47
Gigaspora sp. 2	30	1.47
Gigaspora sp. 3	20	0.98

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The results of the identification of the diversity of VAM spore types in post-nickel mining soil found 3 mycorrhizal genera, namely *Glomus* sp, *Acaulospora* sp and *Gigaspora* sp. (Aswiani et al., 2024). Based on the results of calculating the number of spores obtained after trapping on corn plants, the FM (%) and FR (%) calculations were carried out for each genus VAM found. The results of the FM and FR calculations are shown in Table 2.

In Table 2 above, it can be seen that, in general, the level of genus presence in each replication (FM) and in a population (FR) is different. From the results of trapping, the highest FM value was obtained at 80%, which means that the level of VAM genus presence can be found almost in every replication and FR of 7.35%, which means that the presence of the genus in a population is the largest, in this case Glomus sp. 10. While the lowest FM value is 20%, in this case, *Gigaspora* sp. 3 with an FR value of 0.98%, which means that this genus is rarely found in each replication or in the population in trapping. Mycorrhizal colonization in post-coal mining land has different percentage values for each type of slope. The steeper the slope, the lower the colonization. Based on its frequency, Glomus sp. can be found on each type of slope, thus indicating that this type of AMF is able to form a good symbiosis with the plants found at the research location (Wisnubroto et al., 2023).

Bioremediator formulation test on cultivated plants

Plant parameters were conducted at the growth and production phase of the plant. Growth observations included plant height and number of leaves. Production parameters included wet weight and dry weight. Observation data were tested using Analysis of variances (ANOVA) to determine if the treatment had a significant effect, and then the data were tested using the Duncan Multiple Range Test (DMRT) at the α = 5% level. Correlation analysis was conducted to find the relationship between the variables observed in the study. Plant parameter data for height and number of leaves can be obtained from the beginning of growth until harvest, while wet weight and dry weight of the plant were obtained at harvest. Data on height and number of leaves parameters can be seen in Table 3 and Table 4 below.

Table 3. Height of sorghum plants

Treatment	Average	Duncan + Average
A0	28.08^a	28.08
A1	28.67^a	28.67
A2	32.75^a	32.75
A3	32.33^a	32.33
A4	37.25^a	37.25
A5	36^b	36.00
A6	36.75^b	

The wet seed weight per plant is one of the yield variables in sorghum plants. The wet seed weight is the fresh wet weight obtained at harvest. Based on the analysis of variance at the 5% level, there was a significant difference in the treatment. While in the treatment between bacillus and VMA. Fertilization treatment there was no significant difference. The results of the DMRT test at the 0.05 level showed the highest value in the A2 bacillus 100 ml treatment, namely 32.75a salt and

37.25agram VMA, significantly different from the treatment. This is thought to be because the fresh weight of the plant is influenced by genetics and the environment.

Table 4. Number of leaves of sorghum plants

Treatment	Average	Duncan + Average
A0	28.08^a	30.60
A1	28.67^a	31.36
A2	32.33^b	35.09
A3	32.75^b	35.56
A4	36^c	38.84
A5	36.75^c	39.60
A6	37.25^c	

Dry weight and wet weight data of sorghum plants can be seen in Table 5 and Table 6.

Table 5. Wet weight of sorghum plants (Duncan test 5%)

Treatment	Average	Notation	Duncan
A0	28.08	a	44.50
A1	28.66	a	46.23
A2	32.75	a	50.75
A3	32.33	a	50.66
A4	37.25	a	55.79
A5	36.00	b	54.71
A6	36.75	b	

Table 6. Dry weight of sorghum plants (Duncan test 5%)

Treatment	Average	Notation	Duncan
A0	36.66	a	44.50
A1	57.50	a	46.23
A2	73.33	a	50.75
A3	88.33	a	50.66
A4	50.00	a	55.79
A5	54.16	b	54.71
A6	70.83	b	

Based on the data above, it is known parameters of plant height, number of leaves, wet weight, dry weight with the Duncan Multiple Range Test (DMRT) at the α = 5% level showed a very significant effect on the treatment of VAM (A6) 75 grams.

Research Parameters

1. Soil Nutrient Parameters

Soil nutrient parameters were conducted on % C, % N, C/N, % BO, available P (Bray II pmm), available K (K-dd m.e /100g) and pH (H_2O) and others. Soil sample analysis before bioremediation treatment can be seen in Table 7.

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Table 7. Soil analysis based on initial research soil samples

Soil parameter	Unit	Result	Criteria
soil texture	%	50	Sand
soil texture	%	42	Clay
soil texture	%	8	Dust
Salinity/DHL	dS/m	-	Very low
C	%	0.52	Low
N	%	0.19	Low
C/N		3	Very low
P_2O_5 (Ekstrak HCl 25%)	mg/100 gram	0	Very low
K_2O (Ekstrak HCl 25%)	mg/100 gram	20	Low
P_2O_5 (Olsen/Bray)	ppm	2	Very low
K_2O (Olsen/bray)	ppm	9	
Ekstrak KCl 1 N			
acidity	me/100 gram	0.00	Very low
soil aluminum saturation	me/100 gram	0.00	Very low
soil hydrogen saturation	me/100 gram	0.00	Very low
cation exchange rate			
Ca^{2+}	me/100 gram	0.41	Very low
Mg^{2+}	me/100 gram	0.34	Very low
K^{+}	me/100 gram	0.02	Very low
Na^{+}	me/100 gram	0.23	Low
Soil CTC	me/100 gram	8.82	Low
soil base saturation	%	11	Very low
soil reaction			
Soil pH (H_2O)	(1: 2,5)	7.35	neutral
Soil pH (KCl)	(1: 2,5)	6.52	slightly sour

Soil in post-mining areas dissolves high levels of heavy metals, thus inhibiting plant growth (Habibullah et al., 2021). High solubility of Al^{3+} binds P elements in the soil, making them unavailable to plants (Aurum et al., 2020). The presence of exchangeable aluminium buffers in the soil is a limiting factor for plant growth, such as inhibiting root growth and root extension from taking up soil nutrients (Febriyantiningrum et al., 2021). This affects the interaction of nutrient absorption by plants, such as suppressing the absorption of other essential nutrients (N, P, K, Ca, Mg, Zn, Fe) by plants (Sulistyani et al., 2023). Toxic elements can cause physiological deviations and biochemical processes during plant growth. In addition to the complete initial soil analysis, special initial soil analysis measurements were conducted to obtain data on the effectiveness of the bioremediator formula and the reduction of soil heavy metal levels. Based on the data in Table 7, it can be seen that the post-nickel mining land in Pomalaa has a low level of soil fertility, which can be seen from the low content of macronutrients, low soil cation exchange capacity, and low soil base saturation. Soil heavy metal content in soil samples was also measured. The content of heavy metals chromium, nickel and sulfur in soil samples for cultivation media can be seen in Table 8 below.

Table 8. Heavy metal analysis of post-mining soil as a growing medium for sorghum

No	Soil parameter	Unit	Result	Standard (US HHS Dept)
1	Chromium (Cr)	ppm	24572	20 ppm
2	Nickel (Ni)	ppm	39977	4-80 ppm
3	Sulfur (S)	ppm	158	

2. Soil analysis after *Bacillus* sp + VAM treatment

The content of heavy metals chromium, nickel, soil pH, pH (1: 2.5) H2O and pH (1: 2.5) KCl, nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) nutrients in post-mining soil of sorghum planting media after the introduction of the *Bacillus* sp and VAM formulas can be seen in table 9 below.

Table 9. Soil analysis after *Bacillus* sp + VAM treatment

No	Sample	pH (1:2,5)		Organic Material			Cr	Ni
		H_2O	KCl	Nitrogen	Phosphorus	Potassium		
				%	Mg/100 gram	ppm	ppm	ppm
1	A1	7.77	6.93	0.28	4	12	2452	3910
2	A2	7.85	6.78	0.28	4	17	2082	3084
3	A3	7.94	6.82	0.29	3	9	1906	6330
4	A4	7.83	6.69	0.27	2	24	2778	4060
5	A5	7.80	6.74	0.32	3	22	2097	3068
6	A6	7.92	6.86	0.29	4	19	2298	3008

Table 9 shows soil analysis data after *Bacillus* sp and VAM treatment. It can be seen that there was an increase in soil nutrients after the Bacillus sp and VAM treatment when compared to the baseline soil nutrient data in Table 7. The Bacillus and VAM treatments also showed a reduction in heavy metal chromium and nickel in the soil. This is in line with research which states that Bacillus subtilis is able to remove chromium metal up to 95.19% with an initial concentration of 100 mg/L with an incubation time of 24 hours and an optimum pH of 7. The ability of bacteria to carry out extracellular detoxification mechanisms also occurs due to the interaction of chromium metal with hydroxyl groups on cellulose that lines the bacterial cell wall (Wróbel et al., 2023). *Bacillus* sp also plays a role in stimulating plant growth and is able to increase the availability of nutrients with its ability to fix nitrogen and dissolve phosphorus so that the availability of nutrients in the soil increases, which has implications for increasing the production and quality of the fruit produced (Handayani et al., 2019). Bacillus sp. has the potential for remediation of post-mining soil. This was in accordance with research which states that Bacillus subtilis has the potential to stabilize ex-gold mining soil (Sinambela et al., 2023). The content of heavy metals chromium, nickel, nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) nutrients in sorghum plants planted in post-mining soil media after the introduction of the *Bacillus* sp and VAM formulas can be seen in Table 10 below.

Table 10 shows plant analysis data after *Bacillus* sp and VAM treatment. Based on the data above, it can be seen that the highest nitrogen in plants is obtained in the A2 treatment, namely 1.52%, the highest phosphorus in plants is obtained in the A3 treatment, namely 0.17% and the highest potassium is obtained in the A4 treatment, namely 3.65%. For the highest chromium reduction, plants are obtained in the A2 treatment, namely 53.86 ppm and nickel in the A2 treatment, namely 64.99

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ppm. Sorghum can grow well on post-mining soil that has low soil fertility with *Bacillus* sp and VAM treatments. This is in line with research using oil palm empty fruit bunch biochar as a bio-ameliorant combined with mycorrhiza. The provision of mycorrhiza 0.4 g/kg of soil significantly increased root volume, root length and percentage of root infection and improved soil chemical properties in the parameters of phosphorus available to plants and decreased exchangeable aluminium in ex-nickel mining soil media (Angelita et al., 2020).

Table 10. Analysis of sorghum plants after *Bacillus* sp + VAM treatment

No	Sample	Organic Material			Cr	Ni	Method
		Nitrogen	Phosphorus	Potassium			
		%	%	%	ppm	ppm	
1	A0	1.36	0.10	2.78	35.22	30.20	Kjeldahl Spektrofotometri AAS
2	A1	1.37	0.11	3.58	24.11	15.06	
3	A2	1.54	0.14	3.24	53.86	64.99	
4	A3	1.40	0.17	3.55	28.92	25.81	
5	A4	1.31	0.13	3.65	33.23	29.26	
6	A5	1.40	0.12	3.46	36.53	39.33	
7	A6	1.42	0.15	3.41	31.51	30.01	

3. Reduction in metal content (%) of soil

Based on initial soil analysis data and soil analysis after the introduction of the *Bacillus* sp + VAM formula, the percentage of heavy metal reduction in the soil can be determined. The reduction power of heavy metals nickel and chromium in each *Bacillus* sp + VAM treatment can be seen in Table 11.

Table 11. Reducing power of heavy metals nickel and chromium in the treatment

No	Sample	Chromium Reduction (DR)	Nickel Reduction (DR)
		(%)	(%)
1	A1	0.900	0.902
2	A2	0.915	0.922
3	A3	0.922	0.841
4	A4	0.886	0.898
5	A5	0.914	0.923
6	A6	0.906	0.924

Based on the data in Table 8 above, it is known that the treatment formula of 150 ml *bacillus*/plant (A3) has the highest effectiveness in reducing chromium heavy metals by 0,922% in the soil. While the best formula for reducing nickel is 75 grams of VAM (A6) with a reduction capacity of 0,924%. Based on this data, it can be seen that the higher the treatment dose, the higher the reduction capacity.

The bioconcentration factor (BCF) of plants is also calculated where if BCF> 1 = the plant's ability to absorb high. The bioconcentration factor (BCF) of sorghum plants can be seen in Table 12. The ability of plants to accumulate certain metals can be determined by calculating the bioconcentration factor (BCF) value. The bioconcentration factor is the ability of plants to collect certain heavy metals in response to the concentration of the metal in the substrate. Plants are divided into three categories including accumulators, excluders, and indicators. BCF values > 1

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are called accumulators, BCF < 1 are called excluders and approaching ones are called indicators (Ghosh, 2005).

Table 12. Bioconcentration factor (BCF) of sorghum plants

No	Sample	BCF Chromium	BCF Nickel
1	A1	0.009	0.003
2	A2	0.025	0.021
3	A3	0.015	0.010
4	A4	0.011	0.007
5	A5	0.017	0.013
6	A6	0.013	0.009

Bioconcentration factor (BCF) is the ratio of the concentration of a compound in an organism to the concentration in its substrate. The BCF value needs to be known to determine the bioaccumulation of heavy metals in plants due to contamination in the soil. A BCF value> 1 indicates that the plant can accumulate heavy metals, while a BCF value <1 indicates that the plant does not accumulate them and can only absorb heavy metals (Pratiwi & Ariesyady, 2014).

Hyperaccumulator plants are plants that are able to translocate pollutants or pollute metals to more parts of the plant. Hyperaccumulator plants are plants that can be used in the application of phytoremediation techniques where they have the ability to accumulate contaminants in the roots, stems and leaves. Plants can be said to be hyperaccumulators if there are characteristics including resistance to heavy metals, short life cycles, wide distribution and bioconcentration >1 (Mazumdar & Das, 2015). The BCF value from the calculation results that can be seen in Table 9 shows that all treatments on sorghum samples for each chromium and nickel metal produce values <1, this shows that sorghum only absorbs heavy metals and does not accumulate much of all chromium and nickel heavy metals so that the food crop is included in plants that are not hyperaccumulators but excluders. This is supported by research by Mafuyai, Ugbidye, & Ezekiel (2020), who also calculated the value of the vegetable bioconcentration factor on the soil and then obtained the results of the Pb metal bioconcentration factor in tomatoes 0.005, cayenne pepper 0.006 and spinach 0.001. The value of the Cd metal bioconcentration factor in tomatoes 0.036, cayenne pepper 0.067 and spinach 0.084. The results of the bioconcentration factor values of the two metals tend to be low and all vegetables have values <1 for the soil, so tomatoes, cayenne peppers, and spinach are not hyperaccumulator plants but excluders.

CONCLUSION

The treatment formula of 150 ml *bacillus*/plant (A3) has the highest effectiveness in reducing chromium heavy metals by 0.922% in the soil. While the best formula in reducing nickel is 75 grams of VAM (Vascular Arbuscular Mycorrhiza) (A6) with a reduction capacity of 0.924%. All treatments on sorghum samples on each chromium and nickel metal produced values <1; this shows that sorghum only absorbs heavy metals and does not accumulate much of all chromium and nickel heavy metals, so the food crop is included in the plant is not a hyperaccumulator but an excluder. Parameters of plant height, number of leaves, wet weight, and dry weight with the Duncan Multiple Range Test (DMRT) at the α = 5% level showed a very significant effect on the treatment of VAM (A6) 75 grams. *Bacillus* sp and VAM can be used as a bioremediator agent for cultivated plants planted on post-mining soil. Based on these data, further research needs to be done to prove whether the higher the treatment dose in line with the reduction capacity of the bioremediators.

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