Acaulospora sp: Can it help the growth of Canavalia ensiformis in heavy metal contaminated environment?

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Abstract. Environmental biophysical damage which conducted by miners who do not have or do not care about environmental impact analysis cause of increased heavy metal pollution. A study to see the ability of Acaulospora sp in helping the growth of Canavalia ensiformis in a land contaminated with heavy metals, arranged according to a randomized block design. The results showed that indigenous Acaulospora sp was able to help the growth of Canavalia ensiformis and tolerant of environment that was contaminated with heavy metals, so that it can be recommended as an environmentally friendly biological technology tool with a relatively low cost and safe in the process of rehabilitating an environment that is contaminated with heavy metals to improve environmental health. This research is possible to be developed by collaborating Acaulospora sp with genus indigenous mycorrhizae or other microorganisms to increase the productivity of phytorhizoremediation plants in binding heavy metals.

1. Introduction
In addition to having a positive impact, the mining industry also has a negative impact [1] in the form of biophysical damage to the environment that is very large and worrying [2], especially for miners who do not have or do not care about environmental impact analysis [3]. Several research results have been reported that due to mining causes environmental damage in the form of; soil compaction due to heavy equipment activity [4], damage to soil structure due to excavation [5], landslides due to shrinkage [6], reduced population of soil organisms due to habitat destruction [7], and increased heavy metal pollution [8].

Syam [9] explains that characteristics of soils on nickel postmining land in Sorowako by that landfill and compaction in land reconstruction activities, causes damage to the structure, porosity, and bulk density as physical characteristics of the soil which are important for plant growth. Soil conditions due to compaction cause poor water system (water infiltration and percolation) [4] and aeration which can directly bring negative impacts on the function and development of roots [10]. Plant roots cannot develop properly and its function as a nutrient absorption tool will be disrupted [11].

Canavalia ensiformis L (C. ensiformis) is one of the leguminous plants that is able to grow at high concentrations of heavy metals so that it can be used as a phytoaccumulator [12,13]. Some
Research results show that C. ensiformis is proven to be a good accumulator plant which is tolerant of lead metal (Pb) [14], Cadmiun (Cd) [15], Copper (Cu) dan Zing (Zn) [14]. Acaulospora sp is one of the genus arbuscular mycorrhiza that can symbiosis with plant roots [16]. Some research results show that Acaulospora sp can increase plant growth in environments contaminated with heavy metals Mn [17], Fe [18], Cr [19], Ni [20]. The results of this study provide an idea to examine Acaulospora sp. isolated from different environments to assist the growth of C. ensiformis in environments that are contaminated with heavy metals.

2. Methodology
This research is a quantitative study carried out in a heavy metal contaminated environment, Soroawako, Indonesia. The experimental design used was a Randomized Block Design with the treatment of Acaulospora sp, namely: Without Acaulospora sp. (control), Indigenous Acaulospora sp, which has been isolated from the nickel post-mining environment, Sorowako [21] and Exogenous Acaulospora sp which have been isolated from the sugar cane plantation environment, Takalar [22]. The observed variable is:

2.1. Number of roots infected (%) was calculated by modifying the formula given by Deguchi [23]

Colonization rate (%) = (Area of Acaulospora sp/Area of root) x100.

2.2. Growth analysis of C. ensiformis was calculated by modifying the formula given by Yano [24].

2.2.1. Net Assimilation Rate

\[
\text{NAR} = \left( \frac{W_2 - W_1}{T_2 - T_1} \right) \times \left( \frac{\ln LD_{\text{tot}2} - \ln LD_{\text{tot}1}}{LD_{\text{tot}2} - LD_{\text{tot}1}} \right) \text{ (g m}^{-2} \text{ day}^{-1})
\]

where:
\(W\) = Dry weight of plant (g),
\(LD_{\text{tot}}\) = Total of leaf area (m\(^2\))
\(T\) = Time (day)

2.2.2. Relative Growth Rate

\[
\text{RGR} = \left( \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \right) \text{ (g g}^{-1} \text{ day}^{-1})
\]

where:
\(W\) = Dry weight of plant (g);
\(T\) = Time (day).

2.3. Dry weight of plant (g) calculated by weighing the dry weight per sample plant then divided by the plants number of sample

3. Results and Discussion
The results analysis of variance showed that treatment of Acaulospora sp. no significant effect to percentage of C. ensiformis roots infected. However, the based on average value of percentage of C. ensiformis roots infected by Acaulospora sp. showed that Acaulospora sp. treatment able to produce roots infected by hyphae and / or vesicles, there is a tendency for percentage of C. ensiformis roots infected by indigenous Acaulospora sp. 95.24% higher than C. ensiformis without Acaulospora sp,
whereas exogenous *Acaulospora* sp. was only able to produce of roots infected 52.38% higher than *C. ensiformis* without *Acaulospora* sp. (Table 1).

### Table 1. Average of observed variables at *C. ensiformis* with *Acaulospora* sp treatment in heavy metal contaminated environments.

| Treatments               | Roots infected (%) | NAR g.m⁻².day⁻¹ (x,xx.10⁻⁴) | RGR g.g⁻².day⁻¹ | DWP g |
|--------------------------|--------------------|-----------------------------|-----------------|-------|
| No *Acaulospora* sp      | 42ações             | 3,8ações                     | 0,046ações      | 7,39ações |
| Indigenous *Acaulospora* sp | 82ações             | 7,4ações                     | 0,048ações      | 8,01ações |
| Exogenous *Acaulospora* sp | 64ações             | 4,3ações                     | 0,042ações      | 5,83ações |

Note: NAR: Net Assimilation Rate, RGR: Relative Growth Rate, DWP: Dry weight of plant. The number followed by same symbols (a, b, c) shows no difference between treatments based on the Duncan test in 5% level e

The percentage of *C. ensiformis* roots infected on treatment without *Acaulospora* sp (control) is very small, the possibility of *C. ensiformis* roots infected occurs naturally by *Acaulospora* sp contained in the planting media. While indigenous *Acaulospora* sp can infect more *C. ensiformis* roots, because of the possibility of indigenous *Acaulospora* sp. has adapted on environments contained high of heavy metals concentrations, so that it can help plants in the process of growth and carry out its function as a biological agent. Indigenous *Acaulospora* sp. also alleged to have experienced the stage of adaptation at the level of domestication, the stage where the process of adaptation of organisms can adjust to their environment to complete their life cycle [25–27].

Begum [28] and Chen [29] suggested that indigenous mycorrhiza has a high potential to form extensive infections because indigenous mycorrhiza has a higher tolerance to environmental conditions with high stress. Furthermore, Berruti [30] suggested that the use of mycorrhiza from polluted locations that have tolerated metal toxicity and are able to adapt well, can be developed as a source of inoculants [31].

### Table 2. Overburden soil properties in heavy metal contaminated environment, Sorowako.

| Physical properties | Number | Chemical properties | Number |
|---------------------|--------|---------------------|--------|
| Texture             | Clay loam | pH (H₂O) | 5.62 |
| Sand (%)            | 32     | BC (%)             | 69     |
| Silt (%)            | 35     | C-organic (%)      | 1,88   |
| Clay (%)            | 33     | Ni (ppm)           | 14,200 |
|                     |        | Fe (ppm)           | 691,400|
|                     |        | Si (ppm)           | 172,600|
|                     |        | Ca (ppm)           | 2,26   |
|                     |        | Mg (ppm)           | 3,96   |
|                     |        | K (ppm)            | 0,27   |
|                     |        | Na (ppm)           | 0,12   |

*Canavalia ensiformis* with the treatment of exogenous *Acaulospora* sp. was also found part of the infected root, but it was suspected that the infection was carried out by *Acaulospora* sp. wich contained on planting media, while exogenous *Acaulospora* sp. is still trying to obtain energy from the host plant (*C. ensiformis*) to undergo one of the stages of adaptation on a new environment that contains a heavy metal (Table 2), i.e. acclimatization stage, the stage in which the organism tries to be able to maintain life in a new place by changing its physiological and / or morphological ability to adapt with a new environment [32,33].

Two factor related to adaptation of mycorrhizal, namely a metal immobilization process that occurs in the rhizosphere cause a gradual decrease in concentration of heavy metals [34], and second factor namely the gradual change in the structure of community over profile of phospholipid fatty acids which affect of organisms more tolerant [35]. Although heavy metals can cause changes in the microbial community, but microorganisms are more resistant to heavy metals [36]. Xie [37] suggested that the
The evolution of tolerance to heavy metals can take place quickly and some mycorrhizal strains can tolerate within one or two years.

The results of analysis of variance showed that *Acaulospora* sp. treatment had no significant effect on the net assimilation rate (NAR) and relative growth rate (RGR) of *C. ensiformis* (Table 1). *Canavalia ensiformis* treated with indigenous *Acaulospora* sp. treatment has a higher assimilation production (NAR) of 94.74% with LTR of 4.35% compared to control treatments. Allegedly, besides *C. ensiformis* which given indigenous *Acaulospora* sp. does not experience heavy metal stress, especially at the beginning of plant growth at the age of 21 days after planting (DAP). As a result of indigenous *Acaulospora* sp. which has been adapt and associated with plant roots causes high heavy metal concentrations can be inhibited by reducing the rate of transport of heavy metal to the top of plant.

Mycorrhizae not only increase the rate of nutrient transfer in plant roots [38], but also increase resistance to biotic and abiotic stresses [39]. In addition, mycorrhizae also help maintain plant growth stability in polluted conditions [40]. The mechanism of protection against heavy metals and toxic elements was given by mycorrhiza can be through the effect of filtration [41], the deactivated of chemically or accumulates heavy metal element into the hyphae [42].

Results of statistical analysis on the plant dry weight variable of *C. ensiformis* with *Acaulospora* sp. treatment had no significant effect at the 5% level. *C. ensiformis* with indigenous *Acaulospora* sp. treatment had a dry weight of 8.74% higher than those without *Acaulospora* sp (Table 1). It is thought that plants have more dominant vegetative growth, where nickel heavy metal concentration of high is no longer a limiting factor, but helps the absorption of Fe as an enzyme in photosynthesis [43], and essential factor in activating the urease enzyme, which is needed for nitrogen metabolism [44], so the positive impact of highly fertile vegetative growth causes high assimilate production which is manifested in the form of high plant dry weight.

4. Conclusion
Indigenous *Acaulospora* sp. able to help growth of *C. ensiformis* and has been tolerant in heavy metal contaminated environments, so that it can be developed as a source of inoculants to improve environmental health.

References
[1] Haddaway N R, Cooke S J, Lesser P, Macura B, Nilsson A E, Taylor J J and Raito K 2019 Evidence of the impacts of metal mining and the effectiveness of mining mitigation measures on social–ecological systems in Arctic and boreal regions: a systematic map protocol Environ. Evid. 8 9
[2] Aretz B, Doblhammer G and Janssen F 2019 Effects of changes in living environment on physical health: a prospective German cohort study of non-movers Eur. J. Public Health 29 1147–53
[3] Omotehinse A O and Ako B D 2019 The environmental implications of the exploration and exploitation of solid minerals in Nigeria with a special focus on Tin in Jos and Coal in Enugu J. Sustain. Min. 18 18–24
[4] Alaoui A, Rogger M, Peth S and Blöschl G 2018 Does soil compaction increase floods? A review J. Hydrol. 557 631–42
[5] Zhao L and Hou R 2019 Human causes of soil loss in rural karst environments: a case study of Guizhou, China Sci. Rep. 9 1–11
[6] Fell R 1994 Landslide risk assessment and acceptable risk Can. Geotech. J. 31 261–72
[7] Ali H, Khan E and Ilahi I 2019 Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation J. Chem. 2019
[8] Zwolak A, Sarzyńska M, Szpyrka E and Stawarczyk K 2019 Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: a Review Water, Air, Soil Pollut. 230 164
[9] Syam N, Wardiyati T, Maghfoer M D, Handayanto E, Ibrahim B and Muchdar A 2016 Effect of
accumulator plants on growth and nickel accumulation of soybean on metal-contaminated soil

Agric. Agric. Sci. Procedia 9 13–9

[10] Abuarab M E, El-Mogy M M, Hassan A M, Abdeldaym E A, Abdelkader N H and BI El-Sawy M 2019 The effects of root aeration and different soil conditioners on the nutritional values, yield, and water productivity of potato in clay loam soil Agronomy 9 418

[11] Eleemike E E, Uzoh I M, Onwudiwe D C and Babalola O O 2019 The role of nanotechnology in the fortification of plant nutrients and improvement of crop production Appl. Sci. 9 499

[12] Hasan M, Uddin M, Ara-Sharmeen I, F Alharby H, Alzahrani Y, Hakeem K R and Zhang L 2019 Assisting Phytoremediation of Heavy Metals Using Chemical Amendments Plants 8 295

[13] Vendruscolo D, Santana N A, Souto K M, Ferreira P A A, Melo G W B de and Jacques R J S 2018 Differential behavior of the summer cover crops in the absorption and translocation of copper Ciência Rural 48

[14] da Silva M, de Andrade S A L and De- Campos A B 2018 Phytoremediation Potential of Jack Bean Plant for Multi- Element Contaminated Soils From Ribeira Valley, Brazil CLEAN–Soil, Air, Water 46 1700321

[15] Zancheta A C F, De Abreu C A, Zambrosi F C B, Erismann N de M and Lagôa A M M A 2015 Cadmium accumulation by jack-bean and sorghum in hydroponic culture Int. J. Phytoremediation 17 298–303

[16] Oh H-J and Lee S 2011 Landslide susceptibility mapping on Panaon Island, Philippines using a geographic information system Environ. Earth Sci. 62 935–51

[17] Adewole M B, Awotoye O O, Ohiembor M O and Salami A O 2010 Influence of mycorrhizal fungi on phytoremediating potential and yield of sunflower in Cd and Pb polluted soils J. Agric. Sci. 55 17–28

[18] Spruyt A, Buck M T, Mia A and Straker C J 2014 Arbuscular mycorrhiza (AM) status of rehabilitation plants of mine wastes in South Africa and determination of AM fungal diversity by analysis of the small subunit rRNA gene sequences South African J. Bot. 94 231–7

[19] Lopes Leal P, Varón-López M, Gonçalves de Oliveira Prado I, Valentim dos Santos J, Fonsêca Sousa Soares C R, Siqueira J O and de Souza Moreira F M 2016 Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation brazilian J. Microbiol. 47 853–62

[20] Akib M A, Mustari K, Kuswinanti T, Syaiful S A and Kumalawati Z 2019 Nickel (Ni) reduction in Sorowako post-mining soil through application of mycorrhiza Acaulospora sp. associated with Canavalia ensiformis L. J. Microb. Syst. Biotechnol. 1 30–7

[21] Akib M A, Mustari K, Kuswinanti T and Syaiful S A 2018 Abundance of arbuscular mychorrizal fungi in rehabilitation area of nickel post-mining land of Sorowako, South Sulawesi IOP Conference Series: Earth and Environmental Science vol 157 (IOP Publishing) p 12022

[22] Kumalawati Z, Musa Y, Amin N, Asrul L and Ridwan I 2014 Exploration of arbuscular mycorrhizal fungi from sugarcane rhizosphere in South Sulawesi Int J Sci Technol Res 3 201–3

[23] Deguchi S, Matsuda Y, Takenaka C, Sugiura Y, Ozawa H and Ogata Y 2017 Proposal of a new method of colonization rate of arbuscular mycorrhizal fungi in the roots of Chengiopanax sciadophylloides Mycobiology 45 15–9

[24] Luo L and Zhang W 2014 A review on biological adaptation: with applications in engineering science Selforganizology 1 23–30

[25] McDonnell M J and Hahs A K 2015 Adaptation and adaptedness of organisms to urban environments Ann. Rev. Ecol. Evol. Syst. 46 261–80

[26] Keskin B and Köse E Ö 2015 Understanding adaptation and natural selection: Common misconceptions Int. J. Acad. Res. Educ. 1 53–63

[27] Begum N, Qin C, Ahanger M A, Raza S, Khan M I, Ashraf M, Ahmed N and Zhang L 2019
Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance Front. Plant Sci. 10 1068

[29] Wu Q, Leung J Y S, Geng X, Chen S, Huang X, Li H, Huang Z, Zhu L, Chen J and Lu Y 2015 Heavy metal contamination of soil and water in the vicinity of an abandoned e-waste recycling site: implications for dissemination of heavy metals Sci. Total Environ. 506 217–25

[30] Berruti A, Lumini E, Balestrini R and Bianciotto V 2016 Arbuscular mycorrhizal fungi as natural biofertilizers: let’s benefit from past successes Front. Microbiol. 6 1559

[31] Medina A and Azcón R 2010 Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions J. soil Sci. plant Nutr. 10 354–72

[32] Storz J F, Scott G R and Cheviron Z A 2010 Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates J. Exp. Biol. 213 4125–36

[33] Nievola C C, Carvalho C P, Carvalho V and Rodrigues E 2017 Rapid responses of plants to temperature changes Temperature 4 371–405

[34] Garg N and Aggarwal N 2012 Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of Cajanus cajan (L.) Millsp. genotypes grown in cadmium and lead contaminated soils Plant Growth Regul. 66 9–26

[35] Quideau S A, McIntosh A C S, Norris C E, Lloret E, Swallow M J B and Hannam K 2016 Extraction and analysis of microbial phospholipid fatty acids in soils JoVE (Journal Vis. Exp. e54360

[36] Chu D 2018 Effects of heavy metals on soil microbial community IOP Conference Series: Earth and environmental science vol 113 (IOP Publishing) p 12009

[37] Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L and Fu J 2016 Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation Front. Plant Sci. 7 755

[38] Wang W, Shi J, Xie Q, Jiang Y, Yu N and Wang E 2017 Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis Mol. Plant 10 1147–58

[39] Bahadur A, Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q, Pan J, Liu Y and Feng H 2019 Mechanistic Insights into Arbuscular Mycorrhizal Fungi-Mediated Drought Stress Tolerance in Plants Int. J. Mol. Sci. 20 4199

[40] Bi Y, Zhang Y and Zou H 2018 Plant growth and their root development after inoculation of arbuscular mycorrhizal fungi in coal mine subsidised areas Int. J. Coal Sci. Technol. 5 47–53

[41] Bano S A and Ashfaq D 2013 Role of mycorrhiza to reduce heavy metal stress Nat. Sci. 2013

[42] Abu-Elsaoud A M, Nafady N A and Abdel-Azeem A M 2017 Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat PLoS One 12

[43] Shahzad B, Tanveer M, Rehman A, Cheema S A, Fahad S, Rehman S and Sharma A 2018 Nickel: whether toxic or essential for plants and environment-A review Plant Physiol. Biochem. 132 641–51

[44] Singh A, Panting R J, Varma A, Saijo T, Waldran K J, Jong A, Ngamskulrungroj P, Chang Y C, Rutherford J C and Kwon-Chung K J 2013 Factors required for activation of urease as a virulence determinant in Cryptococcus neoformans MBio 4 e00220-13