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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 bulkdensity 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 leguminoceae plants that is able to grow at high concentrations of heavy metals so that it can be used as a phytoaccumulator [12,13]. Some

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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*

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln LD_{tot2} - \ln LD_{tot1}}{LD_{tot2} - LD_{tot1}} \quad (g.m^{-2}.day^{-1})$$

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

2.2.2. *Relative Growth Rate*

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \quad (g.g^{-1}.day^{-1})$$

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 *Acalauspora* 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,

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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	RGR	DWP
	(%)	$g.m^{-2}.day^{-1}$ ($x,xx.10^{-4}$)	$g.g^{-2}.day^{-1}$	g
No *Acaulospora* sp	42^a	$3,8^a$	$0,046^a$	$7,39^a$
Indigenous *Acaulospora* sp	82^a	$7,4^a$	$0,048^a$	$8,01^a$
Exogenous *Acaulospora* sp	64^a	$4,3^a$	$0,042^a$	$5,83^a$

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 (H_2O)	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 wich 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 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 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* 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 has a wide adaptability, also because *C. ensiformis* wich 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 associat 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 of 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. the 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.

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