

Nickel (Ni) reduction in Sorowako post-mining soil through application of mycorrhiza *Acaulospora* sp. associated with *Canavalia ensiformis* L.

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Abstract

The nickel (Ni) content in a post-mining soil of Pomalaa mines reached 14,200 mg.kg⁻¹ and became a limiting factor in the plant growth process. A Ni reduction in the soil by using phyto-accumulator such as Jack bean (*Canavalia ensiformis* L.) can be improved by combining it with arbuscular mycorrhizal (AM) fungi. The purpose of this study was to determine the effect of the mycorrhizal fungus *Acaulospora* sp. on the efficiency of Ni reduction by *C. ensiformis*. This experiment was carried out by using a randomized block design with three different treatments, include: 1) *C. ensiformis* without *Acaulospora* sp. inoculation (negative control), 2) *C. ensiformis* inoculated with indigenous *Acaulospora* sp. and 3) *C. ensiformis* inoculated with non-indigenous *Acaulospora* sp. The study was conducted in the nursery that belongs to PT. Vale Indonesia Tbk., Sorowako, South Sulawesi, Indonesia. The results showed that highest nickel accumulation was found in the root inoculated with indigenous *Acaulospora* sp. (9500 mg.kg⁻¹), followed by stem (1400 mg.kg⁻¹), leaf and pod (1300 mg.kg⁻¹), seed (1200 mg.kg⁻¹), and flower (1100 mg.kg⁻¹). This study indicates that application of the indigenous *Acaulospora* sp. can improve *C. ensiformis* efficiency to reduce Ni content at Sorowako post-mining area.

Keywords: bioremediation, mycorrhiza, soil, Sulawesi, symbiosis

Introduction

The content of nickel in the Pomalaa post-mining area (Southeast Sulawesi) showing significant high concentration ranges from 265 to 272 ppm (Muhlis *et al.* 2015, Pasquet *et al.* 2016). The limit of Ni concentration that is toxic for plant growth is 100 ppm (w/w) (Widiatmaka *et al.* 2010); therefore, the post-mining area in the Pomalaa cannot be used for agricultural activities.

Canavalia ensiformis L. (Romeiro *et al.* 2007, Souza *et al.* 2013); *Phyllanthus serpentinus* S.Moore (Hidayat 2005, Chaney *et al.* 2007); *Alyssum bertolonii* Desv., *A. lesbiacum* Rech.f., *Berkheya coddii* Roessler, *Hybanthus floribundus* F.Muell., *Thlaspi*

goesingense Halácsy, *T. montanum* L., *Senecio coronatus* (Thunb.) Harv. (Hidayat 2005); *Ipomoea aquatica* Forssk., *Alternanthera sessilis* (L.) R.Br. ex DC., *Amaranthus viridis* L., *Basella alba* L., and *Lasia spinosa* (L.) Thwaites (Kananke *et al.* 2014); *Catharanthus roseus* (L.) G.Don (Subhashini & Swamy 2013); *Rinorea niccolifera* Fernando (Fernando *et al.* 2014), *Panicum maximum* Jacq. (Rahman 2015), *Glycine max* L., *Sarcotheca celebica* Veldkamp, *Melastoma malabathricum* L. (Netty *et al.* 2016) have been used as heavy metals phytoaccumulator in several studies. Our preliminary experiment showed that *C. ensiformis* was also capable of growing in the soil with high Ni content and accumulating the Ni in the root (unpublished data).

Arbuscular mycorrhizal (AM) is a fungal group that is commonly associated with plants and plays an essential role in the nutrients' absorption process by the plants from the soil. Various studies reported the effectiveness of the AM fungi in the synthetic fertilizer absorption (Pindi 2009, Monreal *et al.* 2010, Alizadeh 2012, Adavi & Tadayoun 2012), phosphate (P), nitrogen (N), potassium (K), zinc (Zn), and copper (Cu) (Wu *et al.* 2011, Rose *et al.* 2013, Nouri *et al.* 2014, Garcia & Zimmermann 2014). Application of the AM fungi in the field reduced the use of fertilizer up to 40% and increased plant growth by 46% compared to the synthetic fertilizer (Setiadi & Setiawan 2011). The effectiveness of the AM fungi application in post-mining land rehabilitation management programme was reported from Ni-mine (Husna *et al.* 2016), gold-mine (Prasetyo *et al.* 2010), and coal-mine (Margarettha 2010) areas. In this study, the indigenous AM fungi were used to reduce Ni content in the post-mining soil from Pomalaa mines, Southeast Sulawesi.

Materials and methods

This study was conducted in a nursery that belongs to PT. Vale Indonesia Tbk., Sorowako, South Sulawesi (latitude: 2°18' S, 121°3' E), at an altitude of ± 1388 m above sea level. Sorowako climate type is a tropical rainforest area (Köppen-Geiger climate classification) with an annual average temperature of 75.56° F and rainfall of 2019 mm.year⁻¹.

The experiment was carried out using randomized block design (RBD) with three treatments of *Acaulospora* sp. as follows: 1) *C. ensiformis* without *Acaulospora* sp. Inoculation (negative control), 2) *C. ensiformis* inoculated with indigenous *Acaulospora* sp., and 3) *C. ensiformis* inoculated with non-indigenous *Acaulospora* sp. Each treatment was conducted in three replicates. Each plant was inoculated with 22 spores of indigenous *Acaulospora* sp. that was isolated from plant rhizosphere growth in the post-mining area using wet sieving and decanting method (Gerdemann & Nicolson 1963) and a modification of Sucrose density gradient centrifugation method (Daniels & Skipper 1982). A non-indigenous *Acaulospora* sp. used in this study was isolated from the rhizosphere of sugarcane (Kumalawati *et al.* 2014).

Data of Ni concentration in the plant, Ni reduction rate, and Ni absorption efficiency were analysed using a descriptive statistic. The media used is overburden (OB) soil from RANTE mining unit. Soil samples were analysed in the Laboratory of Chemistry and Soil Fertility, Department Soil Science Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. Soil texture was determined by the hydrometer method, C-organic by Walkley and Black method, pH with Cation Exchange Capacity with 1 M NH₄OAc, base cation ammonium acetate was measured with an atomic absorption spectroscopy (AAS), base saturation was calculated with % BS = (bases cation/CEC) × 100% (table 1).

The soil was put into polybags until it reaches 3/4 of the polybags size. The poly bags contain seeds of *C. ensiformis* (1 seed.polybag⁻¹) amended with *Acaulospora* sp. spores. Two g.plant⁻¹ of synthetic fertilizer was applied to the plants after seven days. Watering was conducted every morning, and pest/disease control was conducted when diseases symptoms

appear. The concentration of Ni was measured in all plant organs (root, stem, leaf, flower, pod, and seed) after 30, 60, and 90 days after planting (DAP). The analysis was conducted in the laboratory of chemistry, Polytechnic of Ujung Pandang.

Results and discussion

The development of science in the field of phytoremediation is accelerating (Sheoran *et al.* 2010). Phytorhizoremediation, a specific form of phytoremediation involving symbiosis of plants and rhizospheric microbes, appears as one of the promising methods in reducing heavy metals contents in the contaminated soil (Kala 2014, Bisht *et al.* 2015). Among the rhizospheric microbes, AM fungi have been found by many researchers as a capable microbial symbiont in phytorhizoremediation due to their effectiveness in helping plants to survive against soil conditions that contain heavy metals as well as to absorb nutrients from the soil (Meier *et al.* 2012).

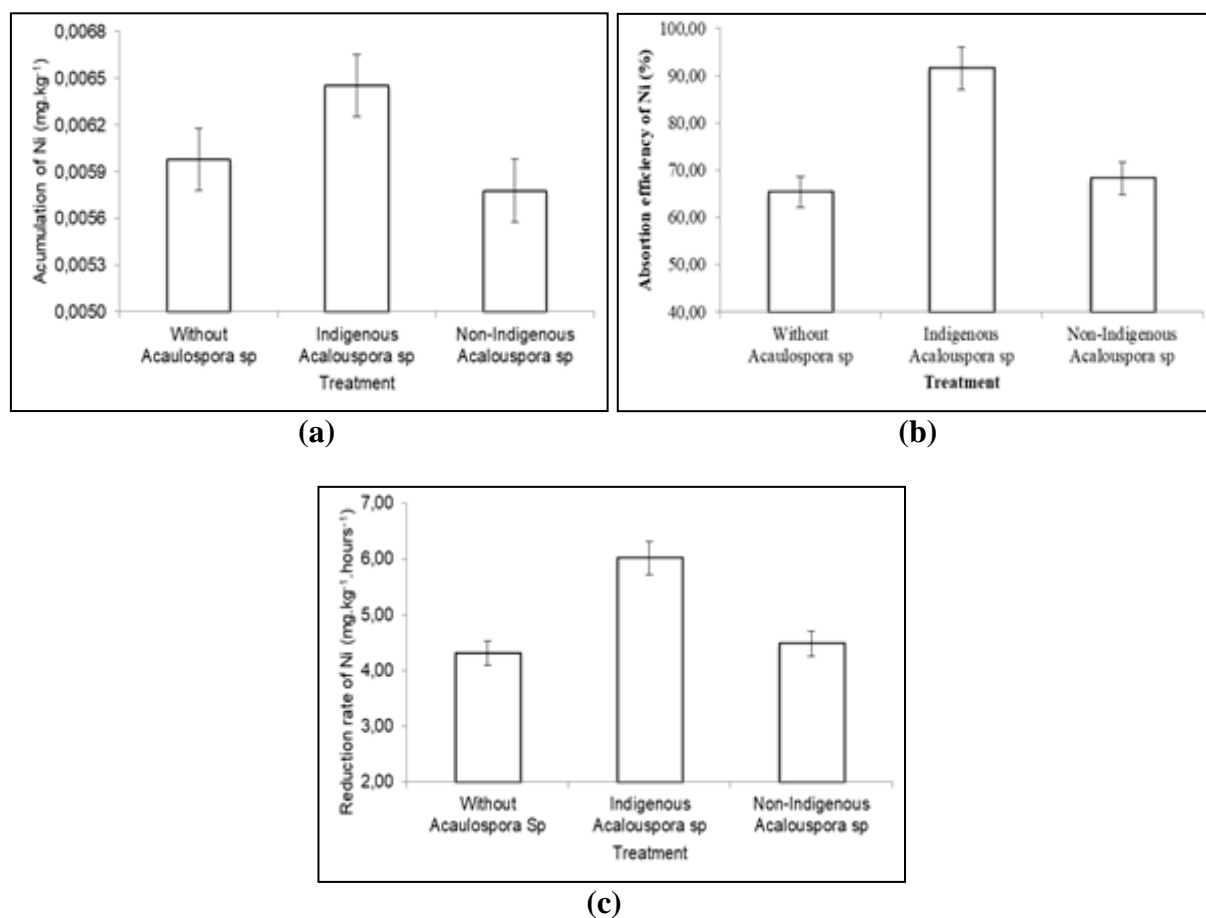


Figure 1. The measurement of Ni in *C. ensiformis* after treatments. (a) an accumulation of Ni, (b) a reduction rate of Ni, (c) an absorption efficiency of Ni.

The descriptive statistic shows that Ni accumulation increased about 8.33% in *C. ensiformis* inoculated with indigenous *Acaulospora* (Fig. 1a) with a reduction rate of Ni 40% higher than without *Acaulospora* sp. (Figure 1b). Inoculation of indigenous *Acaulospora* sp. on *C. ensiformis* also increasing Ni absorption efficiency up to 35.27% (Figure 1c), indicating that indigenous *Acaulospora* sp. capable in forming a mutualistic symbiosis with *C. ensiformis* as well as assisting the plant adaptation to high Ni content. Plant-AM symbiosis increases the surface area of roots to obtain nutrients from the soil through AM fungi, while *C. ensiformis* of able to provide glucose as a source of energy for the development of

mycorrhizal *Acaulospora* sp. (Smith & Read 2008, Hanafiah *et al.* 2015). The indigenous *Acaulospora* sp. isolated from the contaminated soils is generally more tolerant than those from uncontaminated soils. Therefore, screening of heavy metals-tolerant AM fungi from the polluted area is necessary for the discovery of the useful AM fungi to be used as plant symbiont in the recovery of contaminated soil (Maruthi *et al.* 2010).

At a low concentration, Ni and other heavy metals play a role as essential elements for plant metabolism and growth (Sarma *et al.* 2014, Shoukat *et al.* 2014). The Ni concentration that toxic to plants vary significantly from 25 to 246 $\mu\text{g}\cdot\text{g}^{-1}$ of a plant dry weight (Khan & Khan 2010, Bandopadhyay 2014), depending on the species of the plant and the type of cultivar. Figures 2a-b show that indigenous *Acaulospora* sp. was capable of increasing the average of the Ni accumulation in *C. ensiformis*, ranging from 1.40% to 2.54%, especially in the root. Sreekanth *et al.* (2013) reported that the toxicity of the Ni could lead to a chlorophyll content reduction and electron transport disruption in the plant.

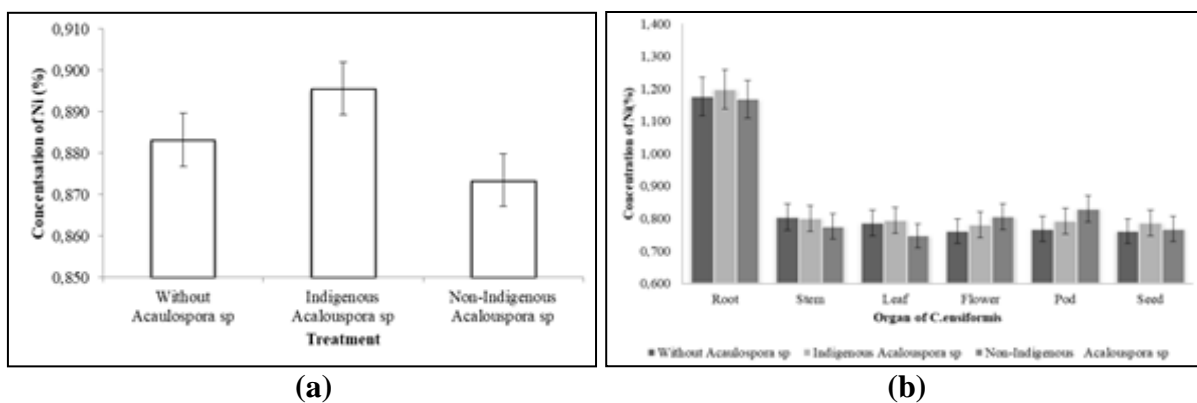


Figure 2. The concentration of Ni. (a) in *C. ensiformis*, (b) in different organs of *C. ensiformis* inoculated *Acaulospora* sp.

The primary mechanism adopted by the AM fungi to diminish the impact of heavy metals on the plants includes distributing metal into cortex cells (Turnau 1988), binding metal to cell wall or mycelium and immobilize inside vacuole or other organelle (Biro *et al.* 2010), releasing proteins of heat-shock and glutathione (Hildebrandt *et al.* 2007), precipitate or chelate metal in a soil matrix through glycoproteins or phosphate amine complex production in the mycelium (Andrade & Silveira 2008, Aloui *et al.* 2011), and reducing of metal strength by spur the growth of root and shoot (Mohammadi *et al.* 2011).

This study also found that time is also an essential factor in the Ni reduction by *C. ensiformis*-AM symbiosis application. The Ni concentration in the root of *C. ensiformis* inoculated with indigenous *Acaulospora* sp. at 30 days after planting (Figure 3a) was lower than those of colonization at 60 and 90 days after planting (Figures 3b-3c). It is possibly due to the symbiosis between *C. ensiformis*-AM fungus has not been well established at 30 days. Colonization of plant roots by AM fungi usually occurs about two weeks (14 days) after inoculation (Füzy *et al.* 2015). However, the AM colonization is affected by environmental temperature and P-contents in the soil (Heinemeyer & Fitter 2004).

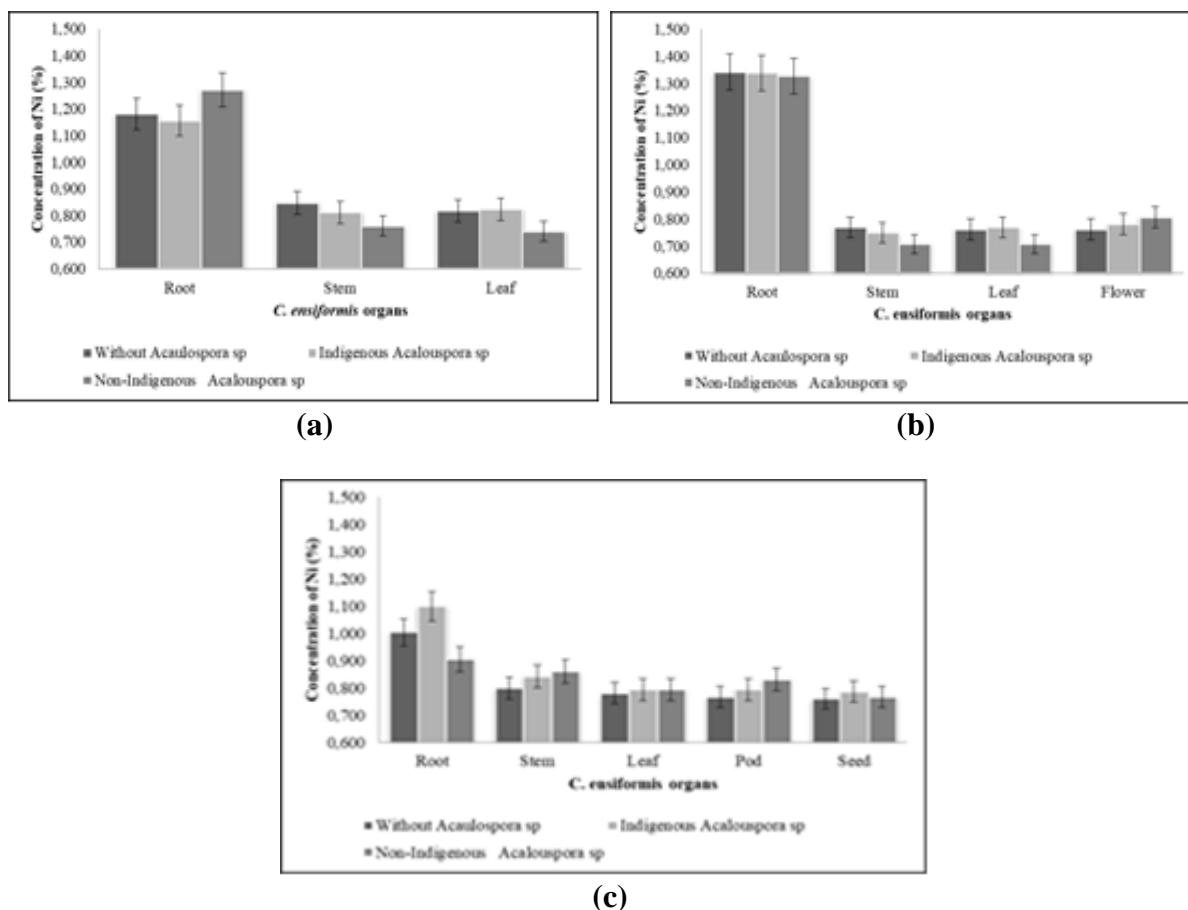


Figure 3. The concentration of Ni in different organs of *C. ensiformis* inoculated with *Acaulospora* sp. (a) 30 DAP, (b) 60 DAP, and (c) 90 DAP.

Conclusion

C. ensiformis inoculated with the indigenous mycorrhizal *Acaulospora* sp. was capable of increasing Ni accumulation by 8.33% with the Ni reduction rate in the soil about $6.02 \text{ mg.kg}^{-1} \cdot \text{hours}^{-1}$ and absorption efficiency of the Ni was 35.27%.

Conflict of Interest

The authors state no conflict of interest from this manuscript.

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