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# Effect of Accumulator Plants on Growth and Nickel Accumulation of Soybean on Metal-Contaminated Soil

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**Abstract**

Environmental damage caused by heavy metals will be difficult to recover naturally and require remediation efforts. The use of plants to absorb and remove contaminants from soil organic and inorganic forms and convert them into non-toxic forms known as Phytoremediation. A number of soybean are grown in pot along with the accumulator plants (*Sarcotheca celebica* and *Melastoma malabathricum*) were investigated their Ni accumulation. The results were indicates that planting soybean either with *Melastoma* or *Sarcotheca* could increase biomass production and Ni accumulation that higher than soybean alone. The highest biomass production and Ni accumulation were obtained by growing *Melastoma* with single soybean in pot.


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*Keywords:*Phytoremediation; Accumulator spesies; Nickle accumulation

## 1. Introduction

During the last decades, Nickel (Ni) has become a serious concern as its concentration has reached up to 26,000 ppm in polluted soils (Alloway, 1995) i.e. 20–30 times higher than found in unpolluted areas. The toxicity of Ni in plants has become a world-wide problem threatening sustainable agriculture as well. Ni, in contrast to other toxic

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trace (heavy) metals like cadmium, lead, mercury, copper and chromium has received little attention from plant scientists due to its dual character and complex electronic chemistry which is a major hurdle in disclosing its toxicity mechanism in plants (Yusuf et al, 2011). The critical toxicity level of Ni is more than 10 mg $kg^{-1}$ dry mass (DM) in sensitive species (Kozlow, 2005), >50 mg $kg^{-1}$ DM in moderately tolerant species and >1,000 mg $kg^{-1}$ DM in Ni hyper accumulator plants such as Alyssum and Thalspi species (Yusuf et al., 2011).

The environmental stresses, such as heavy metals may restrict plant growth and photosynthetic rates. The toxic effects of higher concentration of Ni are observed at multiple levels, these include reduction in plant growth (Molaz, 2002), plant water relation and photosynthesis (Chen et al., 2009), inhibition of enzymatic activities as well as nitrogen metabolism (Gajewska et al., 2006), interference with the uptake of other essential metal ions (Chen et al., 2009) and reduced fruit yield and quality (Gajewska et al., 2006).

A number of plant species endemic to metalliferous soils were found to be able to accumulate metals to levels exceeding those considered as phytotoxic (Baker et al., 2000). Proper selection of plant species for phytoremediation plays an important role in the development of remediation methods (Salt et al., 2005). Specific constraints related to the soil, climates, context of application, among others are important factors to consider in the application of a specific plant species to a soil. Heavy metals can cause severe toxicity and may act as a powerful force for the evolution of tolerant plant populations. The search for indigenous plants, often better in terms of survival, growth and reproduction under metal-stressful field conditions may be an adequate approach to find plant species with metal resistance capabilities and even with the capacity to accumulate metals at very high levels (Netty et al., 2012; Netty et al., 2013).

In literature, many plants were used for phytoremediation of heavy metals from soil (Mojiri et al., 2013). However, the success of phytoextraction depends upon the identification of suitable plant species that tolerate and accumulate heavy metals and produce large amounts of biomass using established agricultural techniques (Mamdouh et al, 2014). The objectives of this study were to examine (1) the relationship between accumulator plants (*Melastoma malabathricum* and *Sarcotheca celebica*) and different number of soybeans per pot which grown together (intercrops); (2) the potential remediation of accumulator plants and soybeans through biomass production and accumulation of Ni on Ni contaminated soil.

## 2. Materials and Methods

The experiment was carried out at the screen house. The Ni-contaminated soil was obtained from post-mining sites in Sorowako, South Sulawesi (121°20’46.8” E and 02°31’36.4” S) in 2013. The air-dried soils were sieved through a 2 mm sieve and homogenized before placing them 20 kg for each pot. Three composite soil samples were taken from the site (0 to 20 cm depth) air dried, sieved, and analyzed for pH (H2O and KCl), organic C (Walkley and Black), N (Kjeldahl), P (Olsen), Ca, Mg, Na, K (1 N NH4OAc pH 7.0) and soil texture (hydrometer method).

The soil samples were dried in an oven for 6 h at 105$^{o}$C. The dried samples were crushed, and 100 mg dissolved in 2 ml of HNO3 (65%). This solution was heated in an oven at 200$^{o}$C for about 14 h, until the sample dissolved completely; the extract was made up to 50 ml, which was used to determine Ni concentrations (Netty et al., 2012). Triplicate of soil and plant samples were analyzed and their means with standard error (SE) are presented. Characteristics of the soils and Ni content are presented in Table 1.

Seedlings of *Melastoma malabathricum* and *Sarcotheca celebica* with uniform size (2-4 foliages) collected from the post-mining lands were planted on media adaptation for 2 weeks. The most vigorous seedlings were transferred to the pots to grow for 10 weeks. Soybean seeds var. Tanggamus were planted 3 to 5 seeds and a week later selected one, two or three plants per pot in accordance with the treatment. Plants were watered daily and fertilized urea, SP-36 and KCl respectively 50 kg $ha^{-1}$, 100 kg ha-1 and 100 kg $ha^{-1}$ were applied one week after planting.

Harvest crops were washed with tap water mixed with 3% HCl, and then rinsed twice with deionized water. The harvested plant stem, leaf and root were dried at 65$^{o}$C for 24 h. For Ni analysis, 100 g of dried sample of each parts of plant species were ground and digested in a mixture of 2 ml of $HNO_3$ (65%). This solution was heated in an oven at 200$^{o}$C for about 14 h until the sample dissolved completely (Netty et al., 2012). Extract was made up to 50 ml used to determine Total Ni and available Ni in soil samples by employ Inductively-Coupled Plasma, Optical Emission Spectroscopy (ICP-OES).

Data obtained were performed for analysis of variance (ANOVA) followed by Tukey's HSD test to compare the means of treatments at $P < 0.05$. The ability of plants to accumulate nickel was determined by Biological Concentration Factor (BCF) that was calculated as nickel concentration ratio of plant shoot to soil (Netty et al., 2013). BCF value of 1 to 10 indicates hyperaccumulator plant, BCF values of >0.1 to 1 indicates moderate accumulator plant, BCF value of 0.01 to 0.1 indicates low accumulator plant, and BCF value of <0.01 indicates non-accumulator plant (Netty et al., 2013). Translocation Factor (TF) that indicates the ability of plants in removing metals from the roots to the shoot was described as ratio of nickel in plant shoot to that in plant root (Salt et al., 1995). Metals accumulated by plants and more stored in the root indicated by TF value of less than 1. TF value of more than 1 indicates more translocation in the plant shoot. The expected value of TF >1 if the outcome is phytoextraction, meaning that >100% metal roots that can be moved into the shoot.

## 3. Results and Discussion

### *3.1. Properties for the Studied Soil*

Table 1: Some chemical and physical properties of the studied soil. The physical and chemical characteristics as well as the concentrations of available and total nickel of the soils under the study are presented in Table 1. These soils were classified as a type Oxisol. The available Ni and total Ni content of the collected soil samples were 11.73 mg $kg^{-1}$ (SE±1.21) and 9,083 mg $kg^{-1}$ (SE±270) respectively. The permissible limits of Ni in the soil are 100 mg $kg^{-1}$ as reported by Kabata and Pendias (2001).

Table 1. Chemical properties and the content of Nickel in soil

| Properties | Soil | Criteria |
|---|---|---|
| pH ($H_2O$) | 6.91 | Neutral |
| C-Organic (%) | 1.58 | Low |
| KTK (me100 $g^{-1}$ soil) | 23.52 | Medium |
| N-Total (%) | 0.14 | Medium |
| $P_2O_5$ (mg$kg^{-1}$) | 11.13 | Low |
| K (me100 $g^{-1}$ soil) | 0.21 | Low |
| Ca (me100 $g^{-1}$ soil) | 3.54 | Low |
| Mg (me100 $g^{-1}$ soil) | 2.84 | High |
| Ni-Available (mg $kg^{-1}$) | 11.73 (SE±1.21) | - |
| Ni-Total (mg $kg^{-1}$) | 9,083 (SE±270) | - |

### *3.2. Growth of Plants*

Soybean seedling growth normally without any symptom on Ni contaminated soil, either planted alone or intercrop with accumulator plants and showed rapid growth, especially at the age4 weeks to 8 weeks after planting. During the whole period of the experiment, soybeans grew well with no visible symptoms of Ni toxicity. The toxic symptoms generated by Ni include chlorosis and necrosis (Shaw et al., 2004), indicated only on Melastoma (Fig 1).

Planting soybean with accumulator significantly affected the dry weight (DW) of leaf, stem and roots of all the studied plants. Dry weight of soybean and accumulators were in the order of leaf > stem > root (Fig 2a). There were increase of the leaf, stem and roots DW of soybean that were intercrop with Sarcotheca or Melastoma compared with all the plants studied that planted alone (Fig. 2a). The increasing of the DW of soybean and accumulators reaches more or less than 50 percent compared to the plants were planted alone. Planting accumulators with soybean (intercropping) indicates chemical soil conditions are better due to the positive interaction between accumulators and soybeans than just planting accumulators or soybean (Watanabe and Osaki, 2002). However, increasing number of soybeans planted in pot resulted in a decrease in DW of all plant studied. The more amount of soybeanin one pot decreased dry weight of plants produced, which occurs due to nutrient competition among the plants.


Figure 1. Growth performance of soybeans and accumulators in Ni contaminated soil. (1) Soybean seedlings; (2) intercrop *Sarcotheca* with 2 plants of soybean; (3) intercrop *Melastoma*with a soybean; (4) intercrop *Sarcotheca* with a soybean

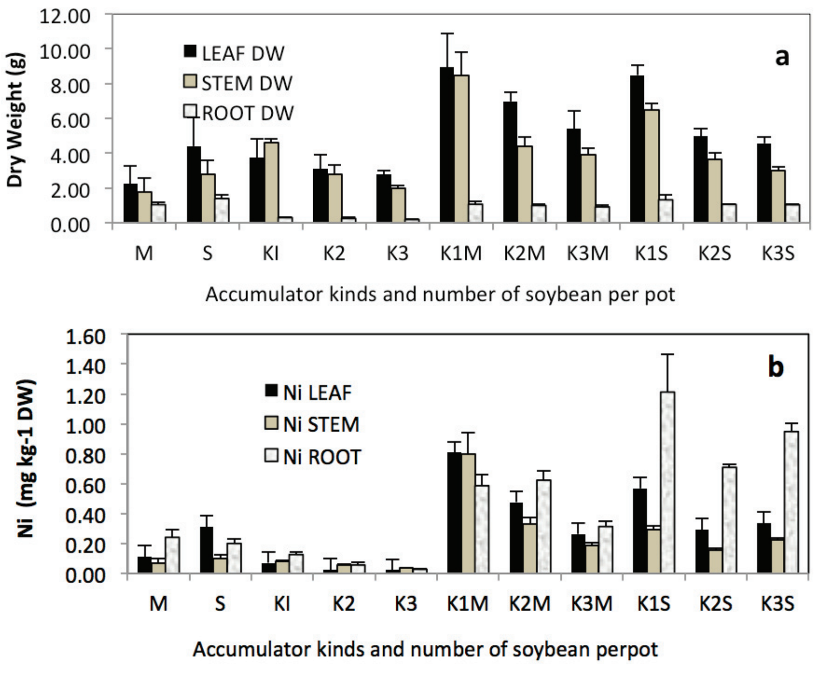


Figure 2.(a) DW of leaf, stem and root; (b) Ni accumulation of leaf, stem and root. M= *Melastoma;*S= *Sarcotheca*; K1= 1 soybean/ pot; K2= 2 soybeans /pot; K3= 3 soybeans/ pot. Vertical bars represent SE.

### *3.3. Nickel Accumulation of plants*

Figure 2b shows the effect of Ni accumulation in leafs, stems and roots of the studied plants. Nick el in soybean and accumulator tissues were in the order of root > leaf > stem (Fig 2b and Table 2). However, proportions of Ni among the tissues for different intercrop were different from each other. In comparison to among accumulator plant, Ni accumulation in stem and leaf (above ground tissues) of *Melastoma* were than Ni accumulation *Sarcotheca*. Ni in *Sarcotheca* was more in the roots than the other part of plant.

The highest Ni accumulation in roots were found in intercrop of 1 soybean with *Sarcotheca* was equal 1.21 mg kg$^{-1}$. In contrast, the highest Ni in stem and leaf obtained from intercrop of 1 soybean with *Melastoma* and significantly different from other intercrops, i.e. 0.80 mg kg$^{-1}$ and 0.81 mg kg$^{-1}$respectively (Table 2). Increase the amount of soybean in intercrop, decrease of Ni accumulation in plant tissues. The lowest of Ni accumulation in plant tissues (root, stem and leaf) were found in 1, 2 and 3 soybean (control).

The amount of Ni accumulationin leaf and stem (shoot) is correlated ($R^2$= 0.877) with the amount of dry matter produced by shoots (Fig. 3). The greater amount of dry matter was derived from plant growth, the higher the number of Ni was accumulated in plant tissues. However, the greater the amount of DW was obtained from 1 soybean intercrop with *Melastoma* or *Sarcotheca*. Apparently, 1 soybean was optimal and do not had competition for grow than biomass production as compared to 2 or 3 soybean per pot.

Table 2. Dry Weight and Total Ni in part of the plants (mean ± SE)

| Treatments | Dry Weight (g) | | | Ni (mg kg$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Root | Stem | Leaf | Root | Stem | Leaf |
| M :*Melastoma* | 1.04 ±.16 b | 1.74±.84 a | 2.24±1.03a | 0.24±.05ab | 0,07±.03 a | 0,11±.05 ab |
| S: *Sarcotheca* | 1.39±.21 b | 2.80±.82 a | 4.40±1.64 ab | 0.20±.03 ab | 0.10±.03 ab | 0.31±.11 abc |
| K1: one soybean | 0.29±.03 a | 4.59±.22 ab | 3.74±1.08ab | 0.13±.02 a | 0.08±.01 a | 0.07±.02 a |
| K2: two soybean | 0.24±.07 a | 2.80±.51 a | 3.10±.81 ab | 0.06 ±.02a | 0.05±.01 a | 0.02±.01 a |
| K3: three soybean | 0.18±.02 a | 1.96±.19 a | 2.77±.22 a | 0.03±.01 a | 0.04±.00 a | 0.02±.00 a |
| K1M: 1 soybean+*Melastoma* | 1.06±.14 b | 8.48±1.34 c | 8.95±1.94 b | 0.59±.07 abcd | 0.80±.14 c | 0.81±.19 d |
| K2M: 2 soybean+*Melastoma* | 0.99±.09 b | 4.38±.56 ab | 6.98±.54 ab | 0.62±.06 bcd | 0.33±.04 b | 0.48±.04 bcd |
| K3M: 3 soybean+*Melastoma* | 0.91±.13 b | 3.89±.42 ab | 5.40±1.04 ab | 0.31±.04 abc | 0.18±.02 ab | 0.26±.05 abc |
| K1S: 1 soybean+*Sarcotheca* | 1.32±.30 b | 6.47±.41 bc | 8.48±.56 b | 1.21±.25 e | 0.29±.02 b | 0.57±.04 cd |
| K2S: 2 soybean+*Sarcotheca* | 1.05±.03 b | 3.64±.39 ab | 4.96±.42 ab | 0.71±.02 cd | 0.16±.02 ab | 0.29±.02 abc |
| K3S: 3 soybean+*Sarcotheca* | 1.03±.05 b | 2.97±.23 a | 4.56±.37 ab | 0.95±.06 de | 0.22±.02 ab | 0.34±.02 abc |

Value with the same letters and the same column were not significantly different from each other within treatments (one-way Anova Tukey's HSD test, p=0.05).

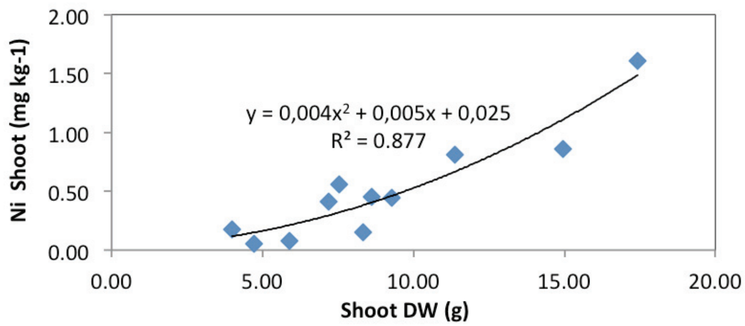


Figure3.Correlation between shoot dry weight and Ni accumulation in shoots of soybean and Accumulator (*Melastoma* and *Sorcotheca*).

### *3.4. Bioconcentration Factor (BCF) and Transfer Factor (TF)*

Bioconcentration Factor (BCF) indicates ability of a plant to absorb a metal from soil. This study showed that only 1 soybean grown with Melastoma had BCF value is equal to 0.91. Increasing amount of soybean to be 2 or 3 soybean due to decreased of BCF value viz. 0.18 and 0.12 respectively. In contrast, intercropping 1, 2 or 3 soybean with *Sarcotheca* nearly unaffected to BCF value.

The transfer factor (TF) evaluates the ability of metal to transfer from root to shoot. Reviewing the soybean monoculture, the results showed that the more soybeans planted, the higher value of TF obtained namely 4.59, 7.99 and 13.43 respectively (Fig. b). BCF and TF have been used in evaluating phytoremediation efficiency of plants (Ghosh and Singh, 2005). The fact that soybean monoculture had large TF value but low Ni concentration indicated that, TF values must be evaluated in combination with BCF. The large TF value of 3 soybean grown and resulted from the small soybean root Ni accumulation.

For the purpose of phytoremediation, the most valuable species are those with largeB CF and large TF. In this study, the soybean intercrop with *Melastoma* showed a relatively large BCF value namely 0.9 and large TF viz.2.62,it appears to be the most valuable intercrop for enhancement of Ni removal from soil by soybean. This fact implies that exudates composition through intercrops could be a factor in Ni uptake by intercropped *Melastoma* with soybean or other legumes (Watanabe and Osaki, 2002). Ding et al. (2008) found that metal desorption from soil largely depended on the type oflow-molecular weight organic acids in soil solution. The exudates composition from intercrops, which may be different. The results suggest that intercropping might be a feasible practice in phytoremediation of soils for Ni,which is worthy of further investigation.

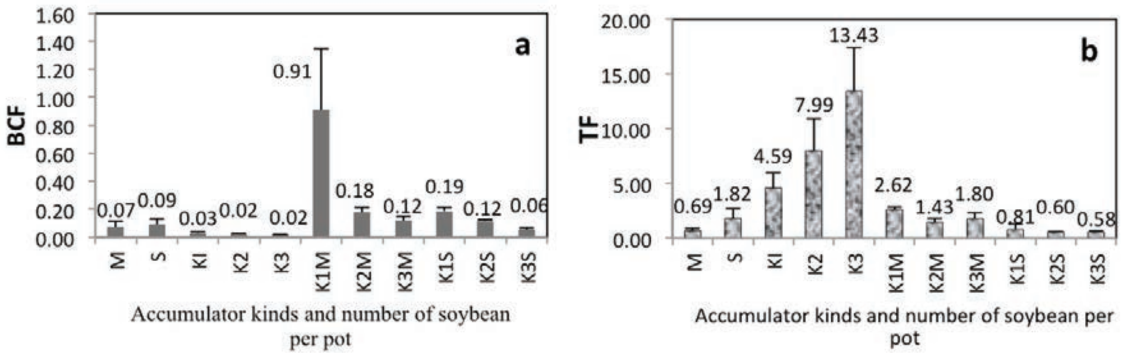


Figure 4. (a) BCF value and (b) TF value. M= *Melastoma*; S= *Sarcotheca*; K1= 1 soybean per pot; K2= 2 soybeans per pot; K3= 3 soybeans per pot.Vertical bars represent SE.

## 4. Conclusion

In general during the whole period of the experiment, soybeans grew well with no visible symptoms of Ni toxicity. The ability of soybean to tolerate the high levels of heavy metals in the soil is high but their ability for heavy metal accumulation is low in all plant tissues. Planting soybean more than one plant per pot causes a decreased in biomass production and Ni accumulation. Intercrop soybean with the accumulators either with *Melastoma* or *Sarcotheca* could increase biomass production and Ni accumulation which was higher than those in monoculture. The highest biomass production was obtained from 1 (one) soybean with *Melastoma* in stem and leaf ie 8.48 g and 8.95 g as well as Ni accumulation in stem and leaf ie 0.80 mg kg-1 DW and 0.81 mg kg-1 DW. Intercrop 1 (one) Soybean with *Sarcotheca* could increased biomass production and Ni accumulation is higher in root g kg-1 DW. The results suggest that intercropping might be a feasible practice in phytoremediation of soils for Ni, which is worthy of further investigation. For post- Ni mining land remediation should use *Sarcotheca* to remediate the soil layers, while *Melastoma* and Soybean to remediate a shallow layer of soil or near ground level.

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