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Bioaccumulation of nickel by five wild plant species on nickel-contaminated soil

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Abstract: A number of plant species have adapted well in the soil conditions of the mining area and were capable to accumulate nickel in the aerial part of plants. The differences of tolerance and bioaccumulation on Ni contaminated soil on five plant species obtained from Ni post-mining land were in vestigated in pot experiment. The results showed that Sarcotheca celebica had a high tolerance (root tolerance index of 128.45% and shoot tolerance index of 219.78%) and its capability to accumulate Ni in shoot (Translocation Factor value 8.67) was higher than that in the root. Tephrosia sp., Mimosa pigra and Celtis occidentalis were tolerance species that accumulated more Ni in the roots than in the shoots. Melastoma malabathricum was able to accumulate Ni the shoot in limited quantities.

Keywords: *nickel post-mining area, phytoremediation, native plant species*

I. INTRODUCTION

Nickel mining activities, through stripping of overburden to extract nickel ore and disposal of tailings, provide obvious sources of metal contamination. Soil contaminated with heavy metals is a very important environmental problem and it has been attracting considerable attention in recent years [1]. Nickel is often mobile in plants, and accumulates readily in plant leaves and seeds [2], thus, having a high potential to enter the food chain. Therefore, the accumulation of nickel by plants is related to its toxicity, which may have possible implications with respect to humans and animals through the food chain. Nickel toxicity may be the cause of a number of biological and physiological processes in plants. Wilting and leaf necrosis have been described as typical visible symptoms of Ni^{2+} toxicity [3]. Nickel concentration in uncontaminated soils ranges from 5 to 50 mg kg^{-1}, and that in the plants ranges from 0.4 to 3 mg kg^{-1} [4].

The idea of using plants to extract and remove metals from soil came from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate a high concentration of metals in their foliage [5]. Some plants that can accumulate extremely high levels of certain metals of more than 1000 mg kg^{-1} of dry weight are usually called hyperaccumulators [5][6]. These plants have been traditionally used as an indicator of mineral-rich sites in geological surveys as well as a bioindicator of contaminated soils during the monitoring of ecosystems. Recently they have attracted the attention of scientists due to their possible use in technology called phytoremediation [7].

Several shrubs and trees such as Melastoma sp., Crotalaria sp., Weinmannia fraxinea (D. Don) Miq., Dillenia serrate Thunb., Garcinia sp., Sarcotheca celebica Veldk., Colona scabra Burr. Merr. & Perry, Casuarina equisetifolia L., Vitex cofasus Rein., Calophyllum soulatri Burm.F., and Metrosidero spetiolata Kds. survive and reproduce on soils heavily contaminated with nickel [8]. A field survey conducted earlier revealed that Sarcotheca celebica Veldk. (Family Oxadilaceae) from three of nickel mine sites at Sorowako was capable to accumulate of 1039 mg Ni kg^{-1} dry weight [9]. Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent researches ([5] [10]. Ability to select plant species that are either resistant to or that can accumulate great amounts of heavy metals will certainly facilitate reclamation of contaminated area [11]. The objective of this study was to test the difference response of various plant species to nickel-contaminated soil obtained from nickel post-mining land of Sorowako, South Sulawesi to determine their tolerance and bioaccumulation on nickel-contaminated soil.

II. MATERIALS AND METHODS

Tephrosia sp, Melastoma malabathricum L., Mimosa pigra L., Celtis occidentalis L., and Sarcotheca celebica Veldk., were grown in pots containing nickel uncontaminated soil (control) or Ni-contaminated soil. Ten treatments (five plant species and two soils) were arranged in a randomized block design with three replicates. Each pot contained 5 kg of air-dried soil. The nickel-contaminated soil was obtained from Butoh, Sorowako (121°20'46.8'' E and 02°31'36.4'' S), and the uncontaminated soil (control) was collected from

Malino (119^o 34' 30" E dan 05^o 20' 41" S). Three composite soil samples were taken from each 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). Characteristics of the soils are presented in Table 1. The air-dried soils were sieved through a 2 mm sieve and homogenized before placing them in the pots.

Table 1. Soil physical and chemical characteristics of Butoh site (nickel-contaminated soil) and Malino site (nickel-uncontaminated soil) at Sorowako, South Sulawesi (values are mean ± standard error, n=3)

Soil Properties	Sites	
	Butoh	Malino
Total Ni (mg kg^{-1})	2464.71±270	50.50±3
Available Ni (mg kg^{-1})	4.74 ±270	>0.01
Clay (%)	62	70
Silt (%)	28	24
Sand (%)	10	6
pH (H_2O)	6.91	6.10
Organic matter content (%)	1.58	1.64
CEC (me100 g^{-1} soil)	23.52	22.53
N-Total (%)	0.14	0.15
P_2O_5 (mg kg^{-1})	11.13	11.24
Ca (me100 g^{-1}soil)	3.54	3.64
Mg (me100 g^{-1} soil)	2.84	2.45
Na (me100 g^{-1}soil)	0.32	0.47
K (me100 g^{-1}soil)	0.21	0.14

Seeds of Tephrosia, M. pigra and C. occidentalis, and seedlings of *M. malabathricum* and *S. celebica* seedling with uniform size (2-4 foliages) collected from the nickel post-mining lands were germinated in trays containing a mixture of sand and cow manure (1:1 by volume). After 2 weeks, the most vigorous seedlings were transferred to the pots (one plant by pot) to grow for 12 weeks. During the plant growth, water content of the growing medium was maintained to its field capacity by adding water daily.

Leaf area per plant was determined using a Portable Laser Leaf Area Meter, while chlorophyll was determined by Aceton/Arnon method before harvesting the plant. At harvest (12 weeks), plants were washed with tap water mixed with 3% HCl, and then rinsed two to three times with distilled water. The harvested plant shoot and root were dried at 65^{o}C for 24 h. For Ni analysis, 100 g of dried sample of each plant species were ground and digested in a mixture of 2 ml of HNO_3 (65%). This solution was then heated in an oven at 200^{o}C for about 14 hours until the sample dissolved completely. The extract was made up to 50 ml used to determine Ni concentration using Inductively-Coupled Plasma, Optical Emission Spectroscopy (ICP-OES). The soil samples were dried in an oven for 6 h at 105^{o}C. The dried soil samples were crushed and analyzed for Ni concentration using the similar procedure of the plant analysis. Data obtained were performed for analysis of variance (ANOVA) followed by Tukey HSD test to compare the means of treatments at $P < 0.05$. Tolerance index (TI) was calculated as biomass ratio of plants grown in nickel-contaminated soils to that grown on nickel-uncontaminated soil [12]. TI value of lower than 1 indicates a net decrease in biomass and suggests that the plant is, whereas that is equal to 1 indicates no difference relative to nickel-uncontaminated soil.

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 [13]. 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. 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 ([14]. 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 [15]. The expected value of TF> 1 if the outcome is phytoextraction, meaning that >100% metal roots that can be moved into the shoot.

III. RESULTS AND DISCUSSION

3.1. Plant growth

Nickel toxicity was evident in the form of the reduction of shoot, leaf area, chlorophyll, root dry weight and shoot dry weight (Table 2). The growth of the plants was highly affected with the existence of 4.74 mg Ni

kg^{-1}. Response of plants to Ni concentration depends on plant species. In general, leaf area, total chlorophyll root dry weight and shoot dry weight of all plant species tested decreased, except for S. celebica. The exposure of excess concentration of nickel significantly reduced shoot dry weight of Tephrosia sp., leaf area and shoot dry weight of C. occidentalis, chlorophyll content, root and shoot dry weight of M. pigra,. The most effected plant species was *M. malabathricum* (Table 2).

Inhibition of growth of plant height and number of leaves did not appear on S. celebica. The number of leaves of S. celebica grown 8 weeks on Ni-contaminated soil was even higher than that grown Ni-uncontaminated soil (Fig. 1). Toxicity symptoms of nickel were observed for other species tested, particularly *M. malabatricum.* The toxicity symptoms were observed in the form of inhibition of shoot development and the onset of leaf chlorosis and necrotic on M. malabatricum (Fig. 2).

Figure 1. Growth of 1) *Tephrosia* sp. (12 weeks), 2) *M. malabathricum* (4 weeks), 3) *M. pigra* (12 weeks), 4) *C. occidentalis* (12 weeks), and 5) *S. celebica* (12 weeks) on nickel uncontaminated and contaminated soils.

Figure 2. Toxicity symptom on *M. malabatricum* 1) 4 weeks, 2) 6 weeks, 3) 8 weeks, and 4) 10 weeks on Ni contaminated soil.

Since roots were directly exposed to excess concentration of nickel, reduction in the root of M. malabatricum was strongly evident. The toxicity symptoms of nickel in *M. malabatricum* started with foliar chlorosis, particularly for older leaves. The chlorosis started at leaf margins and slowly led to the interveinal areas. Under acute nickel toxicity, necrotic spots may rapidly develop on leaf margins and later in the interveinal areas of leaves, and in severe cases plants may develop necrotic lesions on younger as well as older leaves and eventually entire leaf death [16].

High concentration of nickel has been shown to reduce growth of different vegetative parameters including plant height, and fresh and dry biomass production in a number of agricultural crops [16] [17]. The general signs associated with nickel toxicity in plants include reduced shoot and root growth decreased biomass production [18], Fe deficiency that induce chlorosis and can result in foliar necrosis [16]. For example, the high concentration of nickel inhibited production of new root hairs and deformations of existing ones in many plant species such as Betula papyrifera (paper birch) and Lonicera tatarica (honeysuckle) [16]. Toxicity to plant roots also occurs in barley at concentrations of 200 mM Ni, there has also been chlorosis, necrosis on leaves ('brown intervenal'), stunted plant growth, stunted roots, and reduced leaf area [6].

The impact of Ni toxicity on the physiology of plants depends on the type of plant species, growth stage, cultivation conditions, Ni concentration and exposure time in the soil [19. The toxic effects of higher concentration of Ni are observed at multiple levels, these include inhibition of mitotic activities [20], reduction in plant growth [21], plant water relation and photosynthesis [22], inhibition of enzymatic activities as well as nitrogen metabolism [23], interference with the uptake of other essential metal ions [22].

3.2. Nickel content in soil and plant

The content of available Ni in the soil was high (94.73 mg kg^{-1}) at planting time. After 12 weeks, the Ni content declined depending on plant species. The highest Ni content of the plant was observed for *S. celebica* (39.94 mg kg^{-1} dry matter) and the lowest was for *Tephrosia* (1.85 mg kg^{-1} dry matter). Nickel content in the plant or soil reflected the amount of nickel concentration in the dry weight of the plant or soil. Ni content in the

roots and shoot of the plants differed depending on plant species. In general, the content of Ni in the roots was higher than in the shoot, except for *M. malabathricum* and *S. celebica*. The highest of Ni content in the root was observed for *C. occidentalis* and was significantly different from the other species, while the lowest was observed for *Tephrosia* sp. The content of Ni in the shoot was not significantly different between species. The highest content of Ni was shown in *S. celebica* (0.18 mg kg^{-1} dry weight), and the lowest was for *C. occidentalis* (0.07 mg kg^{-1} dry weight) (Table 2). The highest soil Ni content was shown in *S. celebica.* Differences in soil Ni content was likely because of the presence of exudate compounds in the plants that were able to attract metals at the rhizosphere that increased the uptake of the plant. Furthermore, Ni around the roots would be more easily absorbed by plants that led to the increase of Ni accumulation in the shoot. Ni content of plant indicated strong and significant increase as Ni content of soil. Plant heavy metal content, which are indicators of actual plant heavy metals uptake, strongly and significantly increase as soil-heavy metal concentration increases [12]. This trend was found for each plant species studied. This is remarkable considering that some plants tolerate high tissue heavy metal content or concentration, with levels as high as 325 mg Ni dry $weight^{-1}$ for *Alysum corsicum* [24] and 125 000 mg Pb kg^{-1} 420 dry weight for *Raphanus sativus* [25].

3.3. Tolerance index, biological conversion factor, and translocation factor

The tolerance index (TI) of root and shoot ranged from 3.04 to 128.45 and 4.43 to 219.78, respectively (Table 2). The highest TI of root and shoot were observed in *S. celebica* that reached TI value of more than 100%. This indicated that this species was very tolerant to Ni-contaminated soil. The TI of root and shoot in *C. occidentalis* were also high almost 100%. On the contrary, *M. malabathricum* had the lowest TI values of root and shoot of less than 10%. This indicated that this species was very susceptible to excess content of nickel.
The bioconcentration factor (BCF) differed according to plant species (Table 2). The BCF values ranged from 0.01 to 0.07 indicating moderate to low accumulator [14]. The highest value of BCF was observed for *Tephrosia* sp.(0.07) and the lowest was for *S. celebica* (0.01). The Translocation Factor (TF) also differed in plant species ranging from 0.01 to 8.67. The highest value of TF that was observed for *S. celebica* (8.67) indicated that this species effectively transported nickel from roots to leaves, which is essential for phytoextraction of nickel. Several plant species can be used to phytoremediate mining and processing tailings and for revegetation of mining sites. Such species are biologically active plants and most are suitable for removal of heavy metal ions. An example of an effective plant species is *Brassica juncea*. This plant is capable of phytoaccumulating heavy metals from soil to a total content of 897 mg kg^{-1} such metals are mainly translocated to green leaves. *B. juncea* effectively transports lead from roots to leaves, which is essential for phytoextraction of lead [26].

Each plant has the adaptability and tolerance to the environment. *C. occidentalis* was able to adapt to the conditions of Ni in soil indicated by well-developed roots to meet the nutrient needs of plants. Absorption of nutrients that does not have problems will lead to the process of photosynthesis to take place in the leaves goes smoothly because it is supported by a high leaf area. The effects of both of these appeared in the root and shoot dry weights, and TI values of root and shoot. However, considering to the ability of plants to absorb Ni, the highest Ni accumulation was in the root of *C. occidentalis*. Plants have a strategy to grow on contaminated soil. *C. occidentalis* plants including plants exlutor seems that plants have the ability to prevent metal from entering the aerial parts or maintain low or constant metal concentration over a broad range of metal concentration in soil, they mainly restrict metal in their roots [5]. *S.celebica* grown on elevated of Ni concentrations resulted in better growth of plant height, leaf area and total chlorophyll than that grown on uncontaminated soil. The better growth of root and shoot were reflected by the values of root and shoot TI of 128.45% and 219.78%, respectively, as well as the TF value that reached 8.67. The application of phytoremediation requires the ability phytoextraction particular plant genetics and physiology which can translocate and store a large amount of metal without any toxic symptoms [5]. Tolerance and accumulation ability of the Ni content of the agronomic measures that need to be pursued are *S.celebica* and *M..malabathricum* that can produce high biomass allowing their use in phytoremediation / phytoextraction method to be more effective and efficient. The translocation factors (TF) generally showes the movement of metal from soil to root and shoot, indicating the efficiency to accumulate the bioavailable metals from the system. TF gives an idea whether the native plant is an accumulator. High root to shoot translocation of heavy metals indicated that these plants have vital characteristics to be used in phytoextraction of these metals [13]. It is easy for plants species with TF> 1 to translocate metals from roots to shoots than which restrict those metals in their roots.

Table 2. Growth responses of five plants species on uncontaminated and Ni contaminated soil on 12 weeks age. Mean values followed by the same letter in the same parameter (row and column) are not statistically difference between species based on the Tukey HSD test ($p<0.05$)

Parameters	Soil conditions	Plant Species				
		Tephrosia sp.	*Melastoma malabatricum*	*Mimosa pigra*	*Celtis occidentalis*	*Sarcotheca celebica*
Leaf area (dm^2 $plant^{-1}$)	Uncontaminated	3.01 a	31.57 c	2.37 a	57.22 d	6.83 a
	Contaminated	1.10 a	1.95 a	1.39 a	30.68 c	13.95 b
Total Chlorophyll (mg g^{-1})	Uncontaminated	2.13 d	1.65 b	1.79 c	1.69 b	1.27 a
	Contaminated	1.95 d	0.69 a	1.71 a	1.40 b	2.03 d
Root DW (g $plant^{-1}$)	Uncontaminated	3.61 bc	7.31 d	9.23 d	9.19 d	0.47 a
	Contaminated	1.73 ab	0.22 a	4.85 c	8.60 d	0.60 a
Shoot DW (g $plant^{-1}$)	Uncontaminated	27.41 d	25.69 d	30.92 e	25.72 d	2.41 a
	Contaminated	8.75 b	1.14 a	10.31 b	20.45 c	5.30 b
TI (%)	Root	47.97	3.04	52.60	93.63	128.45
	Shoot	31.93	4.43	33.34	79.49	219.78
	Soil	1.85 a	2.56 b	2.95 b	2.59 b	13.31 c
Ni (mg kg^{-1})	Root	0.35 a	0.03 a	2.14 b	4.51 c	0.03 a
	Shoot	0.11 a	0.08 a	0.10 a	0.07 a	0.18 a
BCF		0.07	0.03	0.03	0.03	0.01
TF		0.32	3.31	0.05	0.01	8.67

IV. CONCLUSION

Sarcotheca celebica showed a higher tolerance to Ni and its capability to accumulate Ni in the shoot was higher than in the root. Tephrosia sp., Mimosa pigra and Celtis occidentalis were Ni tolerance species that accumulated more Ni in their roots and in their shoots. Melastoma malabathricum was able to accumulate Ni the shoot in limited quantities.

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