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# Effects of Endomycorrhizal Inoculation on the Growth of *Jatropha curcas* L., *Acacia mangium* Willd., and *Casuarina equisetifolia* J. R. & G. Forst Seedlings in Nickel-Mined Soil in Southern Palawan, Philippines

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## ABSTRACT

The influence of vesicular-arbuscular-mycorrhizal fungi (VAMF) on the growth and survival of *Jatropha curcas, Acacia mangium* and *Casuarina equisetifolia* in the abandoned mined areas with 1.01% nickel concentration (161 times greater than the normal nickel concentration for natural soils) was examined at the experimental area of the Western Philippines University (WPU), Aborlan, Palawan for 8 months. The study aimed to determine rehabilitation strategies in the abandoned mined sites in the province. Specifically, the objectives of the study were to: (1) Evaluate the growth performance of *J. curcas, A. mangium* and *C. equisetifolia* seedlings in the fertile and nickel-contaminated soils with VAM inoculation; (2) Determine the phytoremediation potential of three species in the nickel-mined soil. The height, diameter, biomass yield, root volume, root-shoot ratio, were used as parameters to determine the growth and nutrient pattern uptake of the species. Nickel content of plant tissues was used to determine phytoremediation potential of the species.

In the nickel mined soils, *Acacia mangium* (70.19 cms.) obtained significantly better height performance than *Casuarina equisetifolia* (56.12 cms.) and *Jatropha curcas* (24.67 cms.). The *Acacia mangium* also showed no significant difference on biomass yield in the nickel mined soil (22.63g/plant) and fertile soil (29.21g/plant); and root volume at 14.20 $cms.^3$ and 16.79 $cms.^3$ respectively. The non-significant variations on biomass yield and root volume on both soils indicate that *Acacia mangium* had performed even in the nickel contaminated soil.

VAMF-inoculated seedlings of *Casuarina equisetifolia* were significantly taller with a mean of 2.14 cms. as compared to the non-inoculated seedlings with 0.52 cm. *Casuarina equisetifolia* also registered larger stem diameter by 260% over the non-inoculated seedlings. VAMF influence was evident on the nickel uptake of *Acacia mangium* which accumulated statistically higher amount of nickel content at

12.22 mg/kg. of its dry weight as against the 7.26 mg/kg from the non-inoculated seedlings.

Based on the results, the growth and survival of *Acacia mangium* and *Casuarina equisetifolia* in the nickel contaminated soil manifest tolerance to nickel toxicity and therefore potential as rehabilitation species in the abandoned nickel-mined sites in Palawan.

*Keywords:* *Mycorrhiza, inoculation, phytoremediation, mine sites, Casuarina equisetifolia, Acacia mangium, Jatropha curcas,*

## INTRODUCTION

Mining in the province of Palawan has been operational for almost four decades now. At present, there are four existing nickel-mining concessions in the province. The oldest is the Rio Tuba Nickel Mining Corporation (RTNMC) which started nickel mining in 1969 covering an area of 5,265 hectares of land while the Platinum Group Mining Companies located in Narra, Palawan, and the Berong Nickel Corporation in Quezon, Palawan, started their operations in 2000 and 2006, respectively. The open-pit mining operation involves the flattening of the mountain tops and creating huge craters which is considered destructive because of the use of chemicals to separate the huge volumes of ground rock from the ore. After extraction, the mined areas are abandoned and left with most of the soils under extreme acidity and generally low of microbial populations. The continuous mining activity in the province had resulted to soil degradation and soil infertility.

With the passage of Republic Act No. 7942 known as the Philippine Mining Act of 1995, the government required the rehabilitation of these abandoned mined sites through reforestation. However, due to the infertility coupled with extreme acidity of soils, reforestation species in these abandoned mined areas have low survival rates and poor growth.

The search for tolerant species that can adapt to the harsh conditions is a feasible strategy to achieve success in the reforestation of the abandoned nickel mined sites. The introduction of mycorrhizal fungi could also help in the enhancement of growth of the tree species that will be used to rehabilitate the abandoned mined sites. Mycorrhiza is a symbiotic association that forms between the roots of most plant species and fungi. This symbiotic association enhances the ability of plants to take phosphorus and sometimes other nutrients that are generally immobile and present in low concentrations in the soil solution.

This study was designed to determine the growth performance of the three species in the nickel-mined soils under Palawan condition. It was designed to evaluate the efficacy of these three species in the abandoned nickel mined sites.

## Objectives

Generally, the study was undertaken to determine rehabilitation strategies of the abandoned nickel-mined areas in the province of Palawan. Specifically, the study was conducted to evaluate the growth performance and survival of *Jatropha curcas, Acacia mangium* and *Casuarina equisetifolia* seedlings in the fertile and nickel-mined soils with VAM inoculation and determine phytoremediation potentials of these species in the nickel-mined sites.

## Significance of the Study

One of the serious concerns of the people of Palawan is the effect of mining activities in the province. Heavy metals such as nickel are toxic environmental pollutants especially when their concentrations exceed the critical level (Cadiz *et al* 2009). Sadly, more mining claims were subsequently applied for in Palawan. In 2010, mining applications totaled to 429, covering an area of 700,000 hectares or about half of the 1,489,655 total land area of the Province (PCSD, 2010). In 1995, the guiding principles emphasized by the government were the pursuance of responsible mining and rehabilitation of abandoned mined sites to safeguard the ecological integrity of areas affected by mining. However, vast mining areas lie unsightly to the public as they have remained abandoned for decades without any rehabilitation efforts made. Dubbed as the country's last ecological frontier because of its high diversity, Palawan is one of the provinces that have large areas of abandoned mined sites. These areas were left out after several years of mining operations leaving behind toxic waste materials, overburdened areas that are stony, rocky and acidic, open pits and mine tailings. With these, there is the dire need to look for possible strategies to rehabilitate these areas. Hence, this study.

# MATERIALS AND METHODS

## Duration and Scope of the Study

Seedlings of the three species *Jatropha curcas, Acacia mangium* and *Casuarina equisetifolia* were grown in fertile (garden soil) and nickel-mined soil to evaluate their growth performance. The study included the assessment of mycorrhizal inoculation on growth performance of the three species. The study was a pot experiment using two soil types undertaken for a period of eight months from July 2008 to February 2009.

## Description of the Experimental Site

The experiment was undertaken at the experimental area of the Western Philippines University (WPU), Aborlan, Palawan. The climatic condition during the whole duration of the study was taken from the PAGASA Experimental Station in Palawan from July 2008 to February 2009. No rainfall observed during the months of July and September with low rainfall observed on the month of August with an average precipitation of 3.88 mm. High precipitations were observed on the fourth and fifth month of the study with 36.00 and 10.20 mm, respectively but precipitation decreased thereafter until the termination of the study with mean ranging from 0.40 to 4.40 mm.

Figure 1. The experimental area.

## Collection and Preparation of Materials

### *Description of the Nickel-Mined Site*

The nickel-contaminated soils used in the study came from the abandoned nickel-mined site in Narra, in southern Palawan (Figure 2). The area remains unattended since August 2000. Although patches of shrubs, grasses, ferns and trees flourished in the surrounding area, significant portion of the exposed contaminated soils remains unvegetated. Mined-out areas consist of the open pits which are left behind after the mining operation. These areas are highly deficient in organic matter, nitrogen, phosphorus and potassium while excessive in heavy metals.


Figure 2. An abandoned nickel-mined site, Narra, Palawan.

*Planting Materials and Source of Inoculum*

The three experimental plants used were: *Jatropha curcas, Acacia mangium,* and *Casuarina equisetifolia.* The vesicular-arbuscular-mycorrhizal (VAM) fungi inoculants of *Glomus tunicatum* obtained from BIOTECH, UPLB was used to inoculate the two types of soils. The inoculum consisted of infected root segments; hyphae, spores, and sand were added to some pots, at the rate of one table spoon per pot (about 10 g).

*Experimental Design, Treatments and Lay-out*

The experiment was set up as a 3 x 2 x 2 factorial design consisting of three plant species (*Jatropha curcas, Acacia mangium, Casuarina equisetifolia*), two soil types (garden and nickel-mined soil) and mycorrhizal fungus (VAM) with a non-inoculated control which were arranged in a Randomized Complete Block Design (RCBD) with four replicates per treatment combinations giving a total of 48 experimental units for the whole experiment.

*Transplanting and Mycovam Inoculation*

*Jatropha curcas*, *Acacia mangium* and *Casuarina equisetifolia* seedlings were placed in black polyethylene plastic bags containing about 4 kilogram of sterilized soils. Inoculation was achieved by mixing 10 grams of the inoculum 1 cm below the roots to ensure better infection. There were no other nutrients added to the soil throughout the entire duration of the experiment.

*Nursery Management*

All seedlings were hardened off for one month with minimal watering as needed. After hardening, seedlings were transferred to an open area to simulate field conditions. Other than weeding, no maintenance activities undertaken after the seedlings were transferred to the experimental site.

*Growth Monitoring /Measurement of Parameters at Harvest*

Baseline data on the initial diameter and height of the 3 experimental plants were recorded prior to field experiment which started in July 2008 until February 2009. The monthly incremental height and diameter growth were subjected to statistical analysis. Other parameters to assess the growth and survival of the three species under various treatments and replications were: biomass, root volume and shoot-root ratio.

## RESULTS AND DISCUSSION

### Properties of Soils Used in the Study

*Physico-chemical Properties*

The result of the analysis showed that soils used in the study were 4.0% sand, 47% silt and 49% clay in the abandoned nickel-mined soil. The conditions of the abandoned mined soils proved inhospitable for plants i.e., low moisture holding capacity and low nutrient levels. The low N, P and K in the nickel-mined soil (Table 1) were attributed to the open pit cast method that involves scraping of topsoil and excavation of the ground to extract the nickel. The open-pit mining method clears the vegetation which covers the deposits, inevitably exposing the soil and permanently changing the landscape of the area.

Table 1. Initial properties of the two soil types used in the experiment

| PROPERTY | GARDEN SOIL | NICKEL-MINEDSOIL SOIL |
|---|---|---|
| **Physical** | | |
| Particle size distribution | | |
| Sand (%) | 47 | 4.0 |
| Silt (%) | 32 | 47 |
| Clay (%) | 21 | 49 |
| Texture | Clay loam | Clay |
| **Chemical** | | |
| pH | 7.5 | 6.0 |
| Total N (%) | 0.15 | 0.03 |
| Available P (ppm) | 93.0 | 7.20 |
| Exchangeable K ($cmol^+/kg$ | 3.79 | 0.02 |
| Nickel (%) | | 1.01 |
| OM (%) | 3.57 | 0.93 |
| **Biological** | | |
| Spore count (per 100 g soil) | 95 | 2 |

*Organic Matter Content and Soil pH*

The two soils used in the study differed in their organic matter content as these were obtained from two different sites which were exposed to different environmental conditions. The organic matter of the fertile soil is considered very high at 3.57%; while very low at 0.93% in the abandoned nickel-mined soil when compared with the ideal soil organic matter of 3.0% (Betteridge, 2003).

*Nickel Content*

The soil obtained from the abandoned nickel-mined site contained about 1.01% or equivalent to 10,100 ppm which is 161 times greater than the normal concentration of natural soils which is set at 62.76 ppm. The fact that the nickel content of the soil used in this experiment is higher than the normal concentration, there is high probability to obtain retarded growth and low biomass production in the seedlings of *J. curcas, A. mangium* and *C. equisetifolia.*

## Effect of Treatments on the Growth Performance of the Three Species

*Plant Height*

Highly significant variations on height growth were observed from the effect of species and soil type, while VAM inoculation became significant only for *C. equisetifolia* on the last two months of the experiment. The interaction of VAM x species and VAM x soil x species were statistically insignificant. Using height as a parameter of growth and survival, it indicates that *Acacia mangium* and *Casuarina equisetifolia* are capable to survive and have tolerance to nickel contaminated soil. The inherent advantage i.e. N-fixing ability of the two species may have an effect on its performance since nitrogen is critical in stimulating growth and development as well as the better uptake of other nutrients (Brady, 1996; Galiana, *et al* 1996).

Table 2. Comparison of cumulative monthly height increment (cm) of three species as affected by soil type for 8 months

| SOIL TREATMENT | HEIGHT GROWTH (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov | Dec | Jan | Feb |
| *Jatropha curcas* | | | | | | | | |
| Garden Soil | 3.79 a | 2.57 a | 2.65 a | 3.32 a | 0.18 | 0.22 | 0.36 | 0.69 a |
| Nickel-mined Soil | 0.87 b | 0.08 b | 0.12 b | 0.03 b | 0.01 | 0.03 | 0.01 | 0.03 b |
| LSD test | ** | ** | ** | ** | ns | ns | ns | * |
| *Acacia mangium* | | | | | | | | |
| Garden Soil | 4.39 a | 4.68 a | 5.31 a | 6.81 a | 4.35 a | 0.82 | 0.88 | 0.20 |
| Nickel-mined Soil | 2.32 b | 2.30 b | 3.71 b | 2.80 b | 1.84 b | 0.68 | 0.92 | 0.30 |
| LSD test | ** | ** | ** | ** | ** | ns | ns | ns |
| *Casuarina equisetifolia* | | | | | | | | |
| Garden Soil | 5.07 a | 7.62 a | 3.47 a | 6.35 a | 3.01 a | 0.73 | 0.77 a | 1.29 |
| Nickel-mined Soil | 2.15 b | 2.13 b | 1.17 b | 2.39 b | 0.86 b | 0.78 | 0.20 b | 1.36 |
| LSD test | ** | ** | ** | ** | ** | ns | ** | ns |

Under each growing period, means followed by different letters within a soil treatment under a species are significantly different at the 5% probability level by the LSD test.
** = significant at 1% level, * = significant at 5% level, ns = not significant

In the nickel-mined soil, *A. mangium* showed no significant difference on the height increment of seedlings on both soil types from the sixth until eight months of the study which indicate that the species in the nickel contaminated soil were at par with those in the garden soil. The performance of *A. mangium* relative to height growth indicated that it can be a candidate species in the abandoned mined sites as successful species are those which are able to adapt to unfavorable growth conditions.

*Casuarina equisetifolia* showed the same trend with *A. mangium* in the nickel contaminated soil condition suggesting that the species was able to cope over time with the edaphic condition. The height response of the two species showed that both have the ability to survive, adapt and grow normally even if grown under unsuitable site conditions. These two species by far shows that *A. mangium* and *C. equisetifolia* have survival mechanisms to thrive in a nickel mined soil which is one important criterion used for selecting plants for phytoremediation of abandoned mined sites.

Other attributes that may have influenced on the height performance of *A. mangium* and *C. equisetifolia* were related to their somewhat distinct morphological features i.e., typically possess reduced leaf size in the nickel-mined soil relative to their counterparts on fertile soil; and root systems that are often more developed. These mechanisms i.e. develop tolerance to toxic levels of nickel, and the ability to adapt to edaphic restrictions, such as low nutrients and drought conditions are perhaps the defining characters which make these species candidates in the rehabilitation of the abandoned mined sites.

In this study, there was an inconsistency on the growth performance of *J. curcas* as there was no significant height variation during its fifth to seventh months (November and January) but on the eight month (February) a significant difference on height of seedlings in both soil types was observed showing a poor growth response in the nickel-contaminated soil. This indicates that the seedlings were not able to cope and tolerate the heavy metallic condition of the mined soil. Obviously, the toxic level of nickel, low NPK and low organic matter content of the nickel-mined soil had resulted to the inferior performance of the species relative to their counterparts in the fertile soil. The abandoned nickel mined-out areas were totally devoid of topsoil which contains the physical, nutritional and living microorganisms which plants co-exist with. Thus, it is expected that most plant exposed to abnormal conditions i.e., total rocky mineral media, intense light and heat, droughty and lacking in available nutrients for normal plant growth would end up as a general failure.

VAM inoculation showed no significant effect on the height increments of *J. curcas* and *A. mangium* (Table 3) as shown by the comparable height growth from the first until the eight month of the experiment. However, the VAM-inoculated seedlings of *C. equisetifolia* were significantly higher than the uninoculated seedlings during the seventh and eighth month (January-February) with a mean of 2.14 cm showing superior growth improvements compared to the uninoculated seedlings with 0.52 cm. The insignificant effect of VAM in the early five month measurement period (July – December) in the case of *C. equisetifolia* could be due to the "lag phase" where the influence of mycorrhizal inoculation is not yet manifested to the host plant (Brandon and Shelton, 1993; cited by Dhungana *et al*., 2008). Norland (1993) reported that it may take three to four months or even longer for fungal inoculum to germinate and infect a root as spores. This is because in some cases, inoculum remains ungerminated for an extensive period of time due to unfavorable environmental conditions such as insufficient availability of sporophores and lack of spores (Sturges, 2004).

Table 3. Comparison of cumulative monthly height increments (cm) of three species as affected by VAM inoculation for 8 months

| INOCULATION TREATMENTS | HEIGHT GROWTH (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov | Dec | Jan | Feb |
| *Jatropha curcas* | | | | | | | | |
| Control | 2.66 | 1.61 | 1.25 | 1.53 | 0.09 | 0.14 | 0.11 | 0.31 |
| With inoculation | 2.00 | 1.05 | 1.52 | 1.81 | 0.10 | 0.11 | 0.26 | 0.41 |
| LSD test | ns | ns | ns | ns | ns | ns | ns | ns |
| *Acacia mangium* | | | | | | | | |
| Control | 3.40 | 3.77 | 4.53 | 4.45 | 2.90 | 0.66 | 0.83 | 0.13 |
| With inoculation | 3.30 | 3.21 | 4.49 | 4.44 | 3.29 | 0.83 | 0.97 | 0.37 |
| LSD test | ns | ns | ns | ns | ns | ns | ns | ns |
| *Casuarina equisetifolia* | | | | | | | | |
| Control | 3.53 | 4.36 | 2.37 | 4.09 | 1.87 | 0.76 | 1.60 | 0.52 [b] |
| With inoculation | 3.69 | 5.39 | 2.27 | 4.65 | 2.00 | 0.75 | 1.11 | 2.14 [a] |
| LSD test | ns | ns | ns | ns | ns | ns | ** | ** |

Under each growing period, means followed by different letters within a VAM treatment under a species are significantly different at the 5% probability level by the LSD test.
** = significant at 1% level, ns = not significant

*Stem Diameter*

The adaptation of *J. curcas* to nickel-mined sites was not realized until the eight month period as shown by the significantly smaller stems of seedlings in the nickel-mined soil. The physical and chemical condition of the nickel mined-soil including the high amount of nickel produces the cumulative effect to which the seedlings of *J. curcas* were not able to overcome. In the case of *A. mangium* and *C. equisetifolia,* a comparable growth increments in the stem diameter of seedlings in the garden and nickel-mined soils were observed during the sixth to eight (December to February) month for *A. mangium* and seventh and eighth month

(January –February) for *C. equisetifolia* which implied adaptation of both species to nickel-mined condition.

Table 4. Stem diameter increment of seedlings of three species grown in garden and nickel-mined soil for 8 months

| SOIL TREATMENT | DIAMETER GROWTH (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov | Dec | Jan | Feb |
| *Jatropha curcas* | | | | | | | | |
| Garden Soil | 0.89 [a] | 1.95 [a] | 1.94 [a] | 2.02 [a] | 1.22 [a] | 0.59 [a] | 0.06 [a] | 0.25 |
| Nickel-mined Soil | 0.55 [b] | 0.43 [b] | 0.31 [b] | 0.13 [b] | 0.02 [b] | 0.01 [b] | 0.00 [b] | 0.02 |
| LSD test | ** | ** | ** | ** | ** | ** | * | * |
| *Acacia mangium* | | | | | | | | |
| Garden Soil | 0.28 [a] | 0.37 [a] | 0.52 [a] | 0.64 [a] | 0.40 [a] | 0.20 [a] | 0.14 [a] | 0.02 |
| Nickel-mined Soil | 0.23 [a] | 0.29 [a] | 0.34 [a] | 0.15 [b] | 0.20 [b] | 0.18 [a] | 0.11 [a] | 0.02 |
| LSD test | Ns | ns | ns | ** | * | ns | ns | ns |
| *Casuarina equisetifolia* | | | | | | | | |
| Garden Soil | 0.63 [a] | 0.88 [a] | 1.10 [a] | 0.77 [a] | 0.87 [a] | 0.34 [a] | 0.12 [a] | 0.11 |
| Nickel-mined Soil | 0.42 [b] | 0.31 [b] | 0.36 [b] | 0.21 [b] | 0.14 [b] | 0.13 [b] | 0.13 [a] | 0.12 |
| LSD test | ** | ** | ** | ** | ** | * | ns | ns |

Under each growing period, means followed by different letters within a soil treatment under a species are significantly different at the 5% probability level by the LSD test.
** - significant at 1% level, * - significant at 5% level, ns- not significant

In this study, the influence of mycorrhizal inoculation was not evident on the diameter growth of *J. curcas* and *A. mangium*. Contrary to the response of these two species, *C. equisetifolia* inoculated with mycorrhiza obtained larger diameter growth which was evident on the later month of its growing period i.e., in January and February. The VAM-inoculated seedlings of *C. equisetifolia* registered larger increments in stem diameter by 260% over the non-inoculated seedlings indicating that VAM inoculation had played significant role on diameter growth of Casuarina (Table 5).

Table 5. Stem diameter increment of uninoculated and mycorrhizal inoculated seedlings of the three species grown for 8 months

| INOCULATION TREATMENTS | DIAMETER GROWTH (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov | Dec | Jan | Feb |
| *Jatropha curcas* | | | | | | | | |
| Uninoculated | 0.75 | 1.36 | 1.19 | 1.05 | 0.56 | 0.37 | 0.00 | 0.10 |
| With inoculation | 0.72 | 1.01 | 1.06 | 1.11 | 0.68 | 0.23 | 0.06 | 0.18 |
| LSD Test | ns | Ns | ns | ns | ns | ns | ns | Ns |
| *Acacia mangium* | | | | | | | | |
| Uninoculated | 0.27 | 0.31 | 0.48 | 0.41 | 0.30 | 0.21 | 0.11 | 0.01 |
| With inoculation | 0.23 | 0.35 | 0.37 | 0.38 | 0.31 | 0.17 | 0.14 | 0.02 |
| LSD Test | ns | ns | ns | ns | ns | ns | ns | Ns |
| *Casuarina equisetifolia* | | | | | | | | |
| Uninoculated | 0.54 | 0.61 | 0.71 | 0.52 | 0.45 | 0.27 | 0.06 [b] | 0.05 [b] |
| With inoculation | 0.52 | 0.58 | 0.75 | 0.46 | 0.56 | 0.20 | 0.18 [a] | 0.18 [a] |
| LSD Test | ns | Ns | ns | ns | ns | ns | * | * |

Under each growing period, means followed by different letters within a VAM treatment under a species are significantly different at the 5% probability level by the LSD test.
*-significant at 5% level, ns – not significant

*Biomass Yield*

Highly significant differences in biomass production were observed at the end of the measurement period due mainly to the differences of biomass allocation pattern at the species level and soil type. The effect of VAM inoculation including its interactions with the three species and soil type were statistically insignificant.

Table 6. Biomass yield (g/plant) of three species after 8 months of growing period as influenced by soil type

| SPECIES | SOIL TYPE | | |
|---|---|---|---|
| | GS | NMS | Difference |
| *Jatropha curcas* | 57.23 | 1.48 | 55.76 ** |
| *Acacia mangium* | 29.22 | 22.67 | 6.58 [ns] |
| *Casuarina equisetifolia* | 29.96 | 17.97 | 11.99 * |

** = significant at 1% level, * = significant at 5% level, ns = not significant

It is interesting to note that in spite of the soil condition, *A. mangium* in the nickel mined sites (22.63g/plant) showed no significant difference on biomass yield when compared with the garden soil (29.21g/plant) which indicated a comparable growth and nutrient uptake of *A. mangium* even to the high metallic condition of the mined sites. The poor condition of the soil did not stress the seedlings hence producing better biomass.

In this study, VAM fungi did not significantly increase biomass yield which indicates that mycorrhizal fungi did not enhance the growth of the three species in as far as this parameter is concerned. The insignificant biomass production by the inoculated plants could be attributed to the incompatibility of the fungi to host roots and/or possibly due to the unfavorable condition of the soil, as mycorrhiza forms where host roots and compatible fungi are growing in close proximity and environmental conditions are favorable.

Table 7. Biomass yield (g/plant) of three species after 8 months of growing period as influenced by VAM inoculation

| SPECIES | BIOMASS YIELD (g/plant) | | |
|---|---|---|---|
| | -VAM | +VAM | Differences |
| *Jatropha curcas* | 29.78 | 28.93 | 0.85 $^{ns}$ |
| *Acacia mangium* | 22.36 | 29.49 | 7.13 $^{ns}$ |
| *Casuarina equisetifolia* | 23.61 | 24.36 | 0.70 $^{ns}$ |

ns = not significant

### *Volume of Roots*

Highly significant differences in root formation were observed at the end of the measurement period. These variations were due to the differences at the species level and soil type. Other treatments such as effects of VAM inoculation and its interactions with the three species and soil type were statistically insignificant (Table 8).

Table 8. Root volume ($cm^3$) of three species after 8 months of growing period as influenced by soil type

| SPECIES | ROOT VOLUME ($cm^3$) | | |
|---|---|---|---|
| | GS | NMS | Difference |
| *Jatropha curcas* | 61.64 | 0.79 | 60.85 ** |
| *Acacia mangium* | 16.79 | 14.20 | 2.59 $^{ns}$ |
| *Casuarina equisetifolia* | 24.75 | 14.35 | 10.40 ** |

** = significant at 1% level, ns = not significant

The root volume produced by the three species further explains the differences in biomass production as previously presented (Table 7). The *J. curcas* in the garden soil produced significantly higher root volume, however when considering the performance of this species in the mined soil the result showed that it had the poorest performance as a consequence of having shorter roots which reduced the uptake of nutrients and water. The lower root volume of *J. curcas* in the mined sites have also been the effect of nickel toxicity as manifested by having undeveloped roots and non-development of plant tissues which are the symptoms of high nickel concentrations in soil.

However, *A. mangium* grown in an adverse soil condition showed comparable root sizes with a non-significant variation at 16.79 $cm^3$ in the garden soil and 14.20 $cm^3$ in the nickel-mined soil. The data obtained in this study revealed that the performance of *A. mangium* in terms of root formation was not affected by the limited nutrients in the nickel-mined soil indicating metal tolerance and/or survival of species in a nickel-contaminated soil.

In this study, mycorrhizal inoculation did not influence the root formation of the *J. curcas* and *C. equisetifolia* as manifested by the similar root volume of inoculated and non-inoculated seedlings. However, the inoculated seedlings of *A. mangium* obtained significantly larger root volumes with 17.26 $cm^3$ as against the 13.76 $cm^3$ obtained from the non-inoculated plants indicating that VAM had enhanced nutrient uptake thus resulted to bigger root volume of *A. mangium*.

Table 9. Root volume ($cm^3$) of the three species after 8 months growing period as influenced by VA mycorrhiza inoculation

| SPECIES | ROOT VOLUME ($cm^3$) | | |
|---|---|---|---|
| | -VAM | +VAM | Difference |
| *Jatropha curcas* | 31.65 | 30.77 | 0.88 $^{ns}$ |
| *Acacia mangium* | 13.76 | 17.26 | 3.54 * |
| *Casuarina equisetifolia* | 19.36 | 19.78 | 0.45 $^{ns}$ |

* = significant at 5% level, ns = not significant

*Root-Shoot Ratio*

Result indicates that a highly significant difference in root-shoot ratio was observed at the eight measurement period on the three species due mainly to the differences at the species level and soil type. Other treatments such as the effects of VAM inoculation and its interactions with the three species and soil type were statistically insignificant.

Table 10. Shoot-root ratio of three species after 8 months of growing period as influenced by soil type

| SPECIES | SHOOT-ROOT RATIO | | |
|---|---|---|---|
| | GS | NMS | Difference |
| *Jatropha curcas* | 2.39 | 7.75 | 5.36 ** |
| *Acacia mangium* | 2.40 | 1.99 | 0.41 $^{ns}$ |
| *Casuarina equisetifolia* | 2.24 | 2.46 | 0.23 $^{ns}$ |

** = significant at 1% level, * = significant at 5% level, ns = not significant

*Acacia mangium* and *C. equisetifolia* appeared to be adapted in the nickel-mined soil as reflected by their respective RS ratio of 1.99 and 2.46 which is statistically insignificant with the RS in the garden soil. Seedlings with better root to shoot ratios have a higher survival percentage when planted in the field (Kung'u, 1995). The *J. curcas* with RS of 7.75 appeared to be non-adapted to the nickel-mined soil as it exceeded the average ideal ratio which ranges from 1 to 3 (Haase 2007; Chayed *et al*, 2009). The higher RS ratio in the nickel mined soil of *J. curcas* implied further that the seedlings were unhealthy and of poor quality indicating that it can easily be uprooted owing to their smaller root which was not in proportion to its shoot.

## Influence of Treatments on Phytoremediation Potentials of Three Species

Highly significant variations in nickel accumulation on the tissues of the three species were observed in the study. Both the species and VAM were seen to be highly responsible for these variations. Relative to the total dry matter yields of the three plant species, inoculation of VAM proved to be beneficial to *A. mangium* as the VAM inoculated seedlings accumulated higher amount of nickel at 12.22 mg/kg as against the 7.26 mg/kg from the uninoculated seedlings (Figure 3). Likewise, *J. curcas* without inoculation accumulated 4.34 mg/kg nickel as against the 0.56 mg/kg with inoculation which indicates that VAM did not play significant role for nickel uptake of the seedlings. Meanwhile, VAM inoculated and uninoculated seedlings of *C. equisetifolia* registered almost the same amount of nickel.

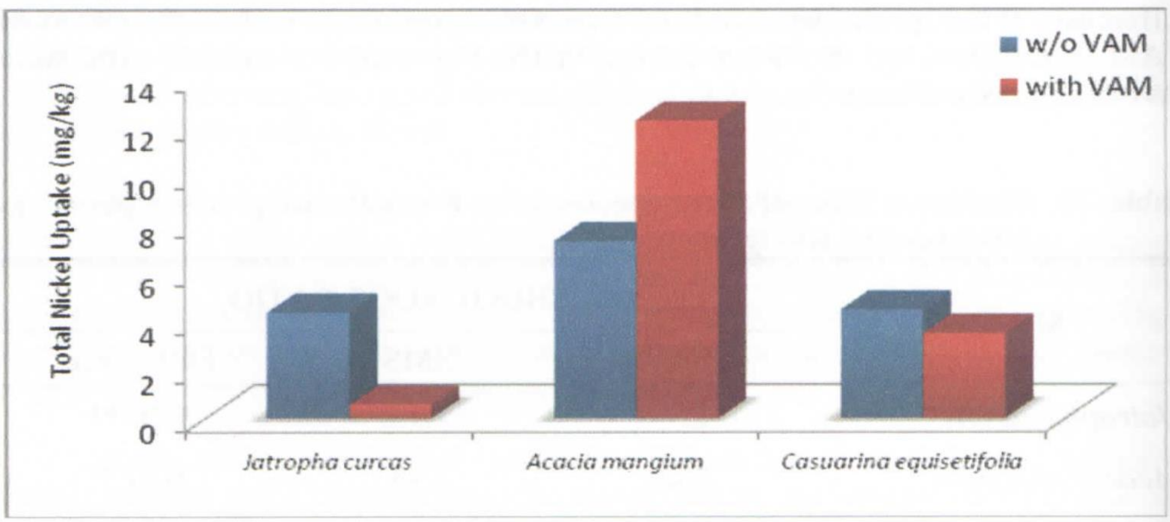


Figure 3. Total nickel uptake (mg/kg) of uninoculated and mycorrhizal inoculated 8-month old seedlings of three species

Based on the result, *A. mangium* grown in the nickel contaminated soil which accumulated the highest amount of nickel in their tissues, and yet survived and performed on most of the different growth paramaters investigated represent a potential species for heavy metal-remediation approach. Also, *C. equisetifolia* performance on mined soils on growth parameters i.e., diameter, height and biomass, registering nickel content at 3.49mg/kg and 4.44 mg/kg, with and without mycovam respectively, could also be a potential species for phytoremediation in the abandoned nickel-mined sites

The performance of the two species, the *A. mangium* and *C. equisetifolia* are of interest since only a small proportion of plant species on heavy metallic soil show capacity to withstand relatively high concentrations of heavy metals without toxic effects indicating that these plants show the potential for phytoextraction and filtering metals from water onto root systems or rhizofiltration (Schnoor, 1997). In this study, the better performance of these species i.e., their growth response to the elevated nickel content of the soil of about 10,100 ppm which is 161 times greater than the normal nickel concentration for natural soils is of interest. Reeves *et al.,* (2008) mentioned that a natural soil contains about 7-50 mg/kg nickel; plants on these soils generally contain nickel at levels of about 0.2-8 mg/kg. The nickel-mined soils used in this study had nickel at a level of 161 times greater, i.e., from about 700 to 5,000 mg/kg; and most of the plants that survive on these soils showed nickel concentrations about 10 times higher, i.e., from about 2 to 80 mg/kg.

Result of the analysis indicates further that *J. curcas* in this study is not a hyperaccumulator contrary to the claims made by Jamil *et al*., (2009) which reported that *J. curcas* is a hyperaccumulator as it has potential of establishing itself in a heavy metallic soil and can also accumulate many folds of heavy metals without attenuating its growth. A plant can be considered hyperaccumulator if it can survive and accumulate heavy metals at a level of more than 1,000mg/kg of its dry mass (Reeves, 2008). Many known hyperaccumulators, at least 400 species worldwide, (Proctor, 2003), belong to the family *Euphorbiacea* (where *J. curcas* also belongs) which include the largest number of nickel accumulators in the tropics (Sporne, 1969; Reeves, 2008). In a study by Berazain (1981) on the elemental composition of plant species in the tropics that include New Caledonia, Sabah and Palawan and Mindanao in the Philippines (Kersteri *et al*., 1979; Jaffre, 1980; Baker, 1992, cited by Reeves *et al*, 2008), he confirmed that many species of *Euphorbiaceae* are noted hyperaccumulators, with maximum nickel concentrations of 4,500 and 7,700 mg/kg dry matter. Proctor *et al*. (2003) listed only three nickel hyperaccumulators in the Philippines and these are: *Phyllanthus balgooyi* (Euphorbiaceae), *Brackenridgea palustris* (Ochnaceae), and *Walsura monophylla* (Meliaceae). The excessive nickel uptake by any plant is of interest, because of its potential for remediation of nickel-contaminated soils (phytoremediation) and for economical selective extraction of metal compounds by cropping hyperaccumulators (phytomining). Besides, a small proportion of plant species on mined soils, perhaps one to two percent of species worldwide show an extraordinarily high uptake of nickel from the soil over 1000 mg/kg (Reeves *et. al.,* 2008, Chayed *et. al.,* 2009).

## SUMMARY AND CONCLUSION

Results showed that the seedlings in the nickel mined soil of *A. mangium* and *C. equisetifolia* had the inherent advantage in terms of height growth over *J. curcas* throughout the duration of the study. The highest height growth measured was 70.19 cms in *A. mangium, C. equisetifolia* and *J. curcas* were 56.12 and 24.67 cms, respectively. In the nickel mined soil, *A. mangium* (22.63g/plant) showed no significant difference on biomass yield compared to the fertile soil (29.21g/plant) which indicated a better growth performance of *A. mangium* even to the high nickel content of the mined sites.

In both soils, *A. mangium* produced almost similar root volume as manifested by its non-significant variation at 16.79 $cm^3$ in the garden soil and 14.20 $cm^3$ in the nickel-mined soil. The non-significant variation of roots produced either in garden and nickel-mined soil by *A. mangium* proved that it had a survival means to adapt to the high nickel soil condition.

*Casuarina equisetifolia* and *A. mangium* obtained statistically similar root-shoot ratio in both soil types which indicates that *A. mangium* and *C. equisetifolia* appeared to be adapted in the nickel-mined soil as reflected by their respective RS ratio of 1.99 and 2.46 respectively, which is statistically insignificant with the RS in the fertile soil. VAM inoculation showed no significant effect on the height increments of *J. curcas* and *A. mangium* but VAM-inoculated seedlings of *C. equisetifolia* were significantly higher than the non-inoculated seedlings during the seventh and eighth month with a mean of 2.14 cms showing significantly better response compared to the non-inoculated seedlings with only 0.52 cm. Similarly, VAM inoculated *C. equisetifolia* obtained larger diameter growth which was evident on the seventh and eighth month of its growing period with larger increments in stem diameter by 260% over the non-inoculated seedlings. Meanwhile, VAM inoculated seedlings of *A. mangium* obtained significantly larger root volumes with 17.26 $cm^3$ as against the 13.76 $cm^3$ obtained from the uninoculated plants.

Finally, *A. mangium* and *C. equisetifolia* in this study showed tolerance to nickel toxicity and therefore potential species to rehabilitate the abandoned nickel mined sites in Palawan.

## RECOMMENDATION

Investigations involving a much longer growing period under field conditions is highly recommended in order to characterize the details of metal tolerance mechanisms, various aspects of the interactions between heavy metals and mycorrhizal fungi, including the effects of heavy metals on the occurrence of mycorrhizal fungi, heavy metal tolerance in these micro- organisms, and their effect on metal uptake and transfer to these plants.

The isolates of indigenous vesicular-arbuscular-mycorrhiza (VAM) fungi in the mined soils could also be explored as indigenous VAMF are probably adapted to local soil conditions as a result from long-term adjustment to soils with extreme properties and probably able to stimulate the growth of *J. curcas, A. mangium* and *C. equisetifolia* in the mined site. Isolation of indigenous and presumably stress-adapted VAMF is a potential biotechnological tool for inoculation of plants in highly disturbed ecosystems. Ecophysiological study of indigenous isolates and knowledge of their stress adaptation and tolerance mechanisms should be of great interest in regard to their potential use for mycorrhizal inoculation in the *J. curcas, A. mangium* and *C. equisetifolia*.

Finally, it is important to conduct further research to identify other fast growing with emphasis on native plants and metal accumulating species with high potential to speed up rehabilitation of the abandoned mined sites in the province.

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