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DIVERSITY AND NICKEL ACCUMULATION SCREENING OF TREES IN SECONDARY FOREST OF DE LA SALLE UNIVERSITY MANILA- LAGUNA CAMPUS IN BIÑAN, LAGUNA, PHILIPPINES

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ABSTRACT

This paper reported the preliminary assessment of the existing vegetation in the secondary forest of De La Salle University Manila - Laguna Campus in Biñan, Laguna, Philippines, not only for conservation purposes but also to determine their potential for nickel hyperaccumulation. A vegetation analysis was conducted in a 1 hectare plot established inside the secondary-growth forest and all tree species with a diameter of equal to or above 5 cm were identified. Their corresponding height and diameter at breast height were likewise recorded. It was found that *Vitex parviflora* (Molave) and *Alstonia macrophylla* (Batino) were the two most dominant species in the area with Importance Values of 23.214% and 19.643%, respectively. Based from the computed species–area curve, the suitable sampling size appropriate for the secondary forest of DLSU-Laguna Campus is only 800 m^2 which means that beyond this sampling size, no additional new species may be encountered. To identify potential nickel hyperaccumulators, all tree species within the 1 hectare plot were identified and subjected to the preliminary nickel screening using the dimethylglyoxime (DMG) kit. Out of the forty-four (44) tree species

screened, only *Streblus asper* (Kalios) turned positive to the DMG test as evidenced by the appearance of magenta red stains produced by using the DMG kit. However, quantitative nickel analysis with the use of atomic absorption spectrophotometry (AAS) showed that the concentration of nickel in both leaves and stem of *S. asper* range only between 0.45-1.53 ppm, which is significantly below the threshold level of 1,000 ppm, hence, it cannot be considered as nickel hyperaccumulator and the results of this study warrants further investigation to further understand its mechanism of heavy metal uptake in its tissues.

Keywords: Vegetation, Secondary forest, De La Salle University Manila, nickel hyperaccumulation

INTRODUCTION

Forests are considered as the most important terrestrial ecosystems, accounting for majority of the plant biomass of the Earth **[1]**. Since forests are important biodiversity hotspots, the knowledge of the characteristics of the different tree species growing in these forests is essential to the management and conservation of their biodiversity **[2]**. However, environmental pollutants such as heavy metals can affect the biodiversity of these forests, wherein the mining of heavy metals like nickel generates pollution in soils, requiring deforestation in various mining sites, some of which are biodiversity hotspots, such as Indonesia and New Caledonia **[3]**.

In the search for feasible ways of rehabilitating environments contaminated with heavy metals, phytoremediation has sparked the interest of numerous researchers for its potential in the treatment of metal-polluted soil, water resources, and sediments **[4]**, and is being developed as a possible way of rehabilitating metal-contaminated environments worldwide **[5]**. Phytoremediation is possible through hyperaccumulator plants, which are able of tolerating and absorbing heavy metals such as copper, manganese, nickel, and zinc, isolating them into vacuoles, typically in large epidermal storage cells **[6]**, wherein increased expression of specific transport proteins in different plant tissues from metal absorption in the shoots up to the leaf epidermis storage sites moderate this process (hyperaccumulation). The term hyperaccumulator was coined by Roger D. Reeves as part of the title of a paper concerning the exceptional nickel accumulating ability of *Sebertia acuminate* **[7]**.

Heavy metals, such as nickel, are significant environmental contaminants despite their economic importance **[8]**. Nickel is a very

important element for its industrial uses such as the manufacture of stainless steel, superalloys, and rechargeable batteries **[9]**. However, nickel can contaminate the environment in a variety of ways, such as waste from equipment of metallurgy factories, irrigation of fields using metal-contaminated water, contamination of soil through sewage residue, transport of heavy metals from mines, and metal-contaminated organic and mineral fertilizers and pesticides **[10]**. Additionally, high nickel pollution in the soil can lead to different toxicity symptoms in plants that cannot tolerate high amounts of the metal, with symptoms such as necrotic lesions and loss of color around leaf veins **[11]**. However, since there are now over 300 taxa of nickel hyperaccumulators currently known **[12]**, identification of these and newer species in possible nickel-contaminated environments may aid in reducing high nickel concentrations in affected areas.

While a number of studies have been conducted in the Philippines, none have been conducted in the secondary forest of De La Salle University Manila - Laguna Campus (DLSU-STC), Biñan, Laguna. This research assessed the tree diversity of the area and preliminary screened the nickel accumulation potential as a reference the species conservation and for future studies on nickel-accumulating plants and phytoremediation.

MATERIALS AND METHODS

Study site

The study was conducted in a one-hectare plot located in the secondary forest located inside the De La Salle University Manila-Laguna Campus located at Barangay Biñan, Biñan City, Laguna (14°15'39.6"N 121°02'25.9"E) (Figure 1). The secondary growth forest is located west of Laguna Boulevard, and lies south-east of the Carmona River. The Laguna Techno Park is located nearby at the east of DLSUManila-Laguna Campus with Integrated Micro-Electronics Inc. at the northeast. Barangay Biñan is surrounded by two nearby Barangays, namely Malamig, located at the east, and Timbao, which lies northeast. Among the reasons for selecting DLSU Manila-Laguna Campus include: (1) accessibility of the area, (2) no existing studies done yet to map the existing vegetation in the area and (3) to search for possible nickel accumulators in the area.

Vegetation analysis of the secondary forest

In order to conduct a vegetation analysis of the secondary forest of DLSU Manila-Laguna Campus, a 1 hectare plot was established over the site (Figure 1). Transect line was established from the southwest

corner of the plot, to the northeast corner, after which ten 10 m x 10 m quadrats were established. Each quadrat was plotted 5m from the transect line, and each quadrat was spaced from each other by 10 m. All trees with diameter at breast height (DBH) of 5cm and above were identified, described, photographed, and further verified using available literature. Among the parameters measures during the vegetation analysis include DBH, tree height, and crown diameter. The height was determined using a laser range finder (LRF). To determine the most important plant species in the area, the frequency, density, and importance value of each species were determined. Species of plants with the highest Importance Value (IV) were recorded as these are the plants that are most likely to affect/influence the growth of their surrounding vegetation. Species-area curve was likewise determined to establish the optimum number of plots needed to represent the secondary forest.

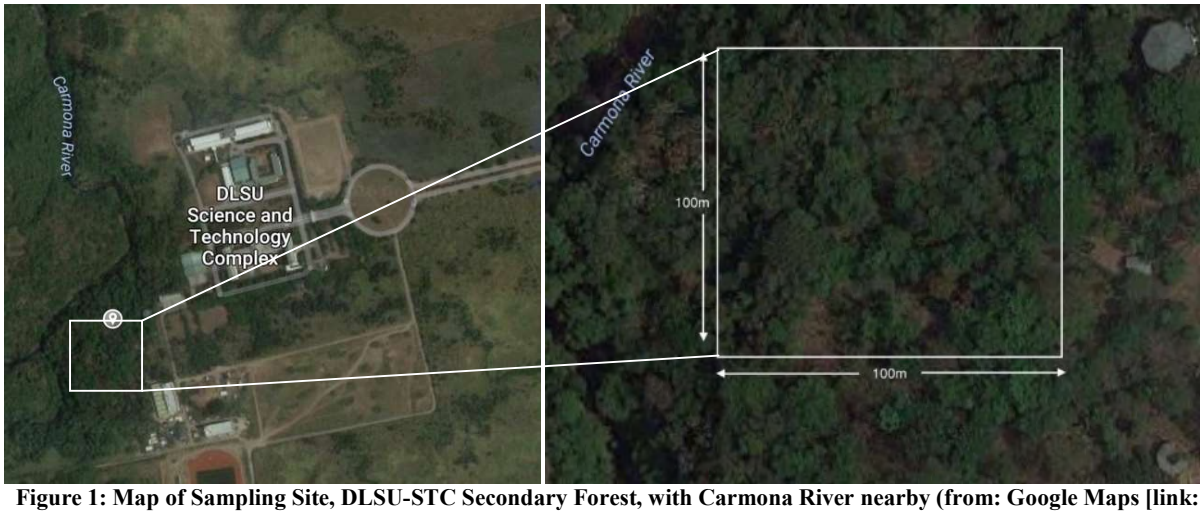

Figure 1: Map of Sampling Site, DLSU-STC Secondary Forest, with Carmona River nearby (from: Google Maps [link: https://www.google.com.ph/maps/@14.2619202,121.0408497,17z])

Preliminary nickel screening

Following methods from Reeves et al. **[13]**, Reeves et al. **[14]**, and Bani et al. **[15]**, leaf samples from each plant identified were screened using the DMG kit, which is a piece of 4-5cm filter paper impregnated with dimethylglyoxime (J.T. Baker®) (Papers were soaked in 1 g DMG/100 ml ethanol solution, after which the papers were oven-dried at 30-40^{o}C until all ethanol has evaporated.) The DMG kit was sprayed with a small amount of water, after which individual crushed leaf samples were rubbed onto the kit to determine the presence of nickel. Three trials were conducted per species using 3 individual leaf samples to

confirm false positives or false negatives. Additionally, as stated by Reeves et al. **[14]** in their study on Ni hyperaccumulation in the Serpentine Flora of Cuba, most Ni hyperaccumulators will consistently show concentrations of nickel above 1000ppm, and that hyperaccumulation is defined on the basis of the behavior of 'at least one specimen growing in the area', wherein their study made use of one specimen per species, the amount of sampling used for this study is adequate for the objective of conducting a preliminary assessment. Leaf samples were crushed prior to rubbing in order to allow possible accumulated nickel in the leaf vacuoles to react with the dimethylglyoxime. If the filter paper impregnated with DMG exhibited a magenta-red color after rubbing, this indicated nickel concentrations of > 1000 ppm, which is the criterion for a tree species to be considered as a nickel hyperaccumulator **[13, 14, 15]**.

Quantitative screening for nickel hyperaccumulation potential of selected species

The leaf and stem samples that turn positive for the presence of nickel, were assessed quantitatively via Atomic Absorption Spectrophotometry (AAS). For each selected plant specimen, a corresponding soil sample was collected (at a depth of 10-20cm) and analyzed in order to correlate the amount of nickel present in the leaf tissues of each plant and the soil that they grow in. All quantitative analyses were done at UPLB-BIOTECH using the following procedure: leaf and stem samples were thoroughly washed in demineralized water to remove all foreign matter attached to the sample such as soil or sand which could interfere with the analysis. Samples were air-dried as quickly as possible, after which they were grinded, and stored in tightly-stoppered bottles; 1 g of the prepared sample was mixed with 10 drops of H_2O and about 3-4ml of nitric acid (HNO_3) (1+1) on a hot plate set at 100-120^oC, where excess HNO_3 was allowed to evaporate; the resulting ash was then placed in a 50-ml Erlenmeyer flask and filtered for nickel analysis using an atomic absorption spectrophotometer (AAS) with the following configuration: wavelength (232 nm), lamp current (4 mA), and slit width (0.2 nm). The soil samples were air-dried, wherein 1g of each sample was subjected to acid digestion in 10mL of 2M HCl for 20-25 minutes in a hot water bath. Digested samples were then filtered with Whatman® grade 4 qualitative filter paper and adjusted with 10mLdeionized water. Prepared samples were then analyzed using the same procedure used for leaf and stem samples. The Nickel Bioconcentration

Factor (BCF) for both the leaf and stem samples were computed using the following formula, BCF_{sample} = (Ni concentration of plant sample / Ni concentration of soil).

Statistical analysis

The densities, frequencies, and importance values of the all plant species in the study site were summarized. Nickel concentration values of leaf, stem, and soil samples were recorded as means ± standard deviation, while a single factor ANOVA test was used to test for significant differences between species that underwent quantitative nickel screening.

RESULTS AND DISCUSSION

Diversity of the secondary forest of DLSU Manila-Laguna

A belt transect line from the southwest to the northeast end of the sampling site was established, and in every ten meters a pair of quadrats (10m x 10m) was established, resulting in a total of ten quadrats spaced 5m away from the transect line established. A total of 19 tree species were identified in the vegetation analysis (Table 1). To determine the most importance species in the area, the Importance Values were calculated using the frequency and density data of each species identified. This in turn gives an overview of the secondary forest in terms of its most dominant species **[16]**. Results showed that *Vitex parviflora* was the most dominant species in the area with IV of 23.214% followed by *Alstonia macrophylla* at 19.643%. This implies that the two species are the ones that are most likely to influence the growth of the vegetation surrounding them.

The horizontal and vertical profiles of the 10 quadrats were established and are presented in Figure 2. It can be noticed that the trees per quadrat vary, as some quadrats contain 10 species, while quadrat 6 do not contain even single tree (profile not shown). This is due to the criterion of diameter at breast height (DBH) equal or above 5cm. As a secondary growth forest, the tallest tree only reached between 10-13m indicating the relatively young age of the trees in the stand. It was also found that there is no general trend in the dispersion of the different tree species in the area, as some quadrats have trees spread out from each other (Q1, Q9, Q10), while clumping of tree species can be observed in other quadrats (Q8). This shows that the forest is diverse in terms of the vertical and horizontal profile of its trees. Moreover, vertical and horizontal profiles show that the different trees in the forest vary in terms of height, which could be correlated to the diversity of tree species as seen in the species area curve (Figure 3). Also, they

indicated that most of the tree species are scattered throughout the forest resulting to some quadrats having numerous species while other quadrats contain few scattered or few cluttered trees. This could be due to the heterogeneity of the different quadrats from each other **[17]**, wherein not all specific quadrats are of the same conditions, and that other quadrats would have thicker vegetation than others.

In terms of vertical vegetation profiles, parameters such as tree height, and crown width can have an effect on surrounding plants species that are currently growing, and also other animal and insect species. Examples of these are that higher tree height in general can restrict the growth of surrounding vegetation as the amount of light these are able to absorb is limited **[18]**. However, the trees captured in the 9 quadrats are of moderate height, with the exception of some (Q5, Q7, Q8). In terms of horizontal profile, cluttered trees can affect the growth of surrounding vegetation due to competition, and can also hinder the entry of wind, and light especially when coupled with taller tree height **[19]**. Such is the case with Q1, Q8, Q9, and Q10.

In addition to this, the sole species that turned positive to the DMG test was not even captured in the 10 quadrats established hence its potential contribution to the diversity of the vegetation was not accounted. However, the potential of *Streblus asper* as a biological indicator for nickel pollution, if not its possible capability of nickel hyperaccumulation, should be considered.

Species-area curve of the secondary forest of DLSU-STC

The estimated minimum area needed to characterize the secondary forest of DLSU Manila-Laguna Campus in terms of the number of tree species was computed at 800 m^2, which means that having a sampling size larger than this will no longer add new species in the list (Figure 3). Additionally, this shows that the established 1,000 m^2 sampling size is an overestimate and may not be appropriate for the purpose of the study.

Related to the species-area curve is the concept of minimal area. The minimal area was determined from the graph at the 800 m^2 point, wherein the graph showed that only a few species were captured after this. This means that 800 m^2 is the ideal quadrat size to be used one desires to capture a majority of the tree species in the secondary forest. However, the minimal area could change in the span of years as there are a number of younger trees from various species scattered around the forest, and these were not captured by the vegetation analysis as these

trees did not meet the requirement of a 5 cm DBH in order to be recorded in the vegetation analysis. There are several factors that could have influenced the calculated species area curve of the secondary forest. One example of this is the clumping or clustering of captured species in the established quadrats. This could be due to restrictions to dispersal of the different species or due to heterogeneity of the habitats in the quadrats established **[17]**. Dispersal restrictions could have contributed to the clustering of different species in a single quadrat, which may have been the cause of the large quadrat size required to represent the area (800 m^2). On the other hand, heterogeneity of habitats could have contributed to the clustering itself, and the fact that only a few more species are added as the area increases.

Preliminary nickel assessment of the secondary growth forest

The result of the complete enumeration of tree species within the established 2-hectare sampling plot resulted to the identification of 44 tree species belonging to 21 distinct families (Table 2). All the 44 species were tested for the presence of nickel by collecting their leaves and rubbing crushed leaf samples individually in the prepared DMG kit. Family Fabaceae had the most of the tree species in the area, with a total of 11 identified species. Out of the 44 species that were sampled, one species, *Streblus asper* (Common Name: Kalios) produced magenta red stains in the DMG kit which is indicative of the presence of nickel in its leaf tissues.

Streblus asper is a small tree belonging to family Moraceae, with oval shaped leaves approximately 3-4 inches long (Figure 4). This species is particularly popular in the Philippines and other tropical countries for its use in folk medicine. Its stems and leaves are traditionally used to treat dental disorders such as tooth decay **[20]**. The discovery of its possible nickel hyperaccumulation potential could possibly indicate its potential as nurse tree to other plants in the area that do not have the ability to absorb that considerable amount of heavy metal from the soil.

***Streblus asper*: potential nickel accumulator**

After the preliminary field screening for the presence of nickel utilizing the DMG kit, a quantitative analysis using atomic absorption spectrophotometry (AAS) was conducted. Results of the analysis of the samples (Table 3) showed that nickel concentrations were significantly lower than what was supposed to be indicated by the preliminary nickel assessment using the DMG kit. The nickel content of the leaf samples was only ~0.45

ppm, while the nickel content of the stem and soil samples were only ~1.53 ppm, and ~1.43 ppm, respectively. This indicates that the overall nickel concentrations of the stems and soil surrounding the species are relatively higher than in the leaves, but not above the threshold level. This is due to the fact that higher nickel concentrations were translocated to the stems and may have not reached the leaves.

Additionally, the calculated the Bioconcentration Factor (BCF) of the leaves indicated that the ratio of nickel concentration of the leaf and soil are <1, meaning that there is a higher concentration of nickel in the soil, whereas the BCF of the stem showed that there is a higher nickel concentration in the stems. This may mean that higher concentrations of nickel have been translocated from the roots of the plant to the stem and did not proceed into the leaves.

Table 1: Importance values of the different plant species found within the secondary growth forest of De La Salle University Manila - Laguna Campus

Tree Species	Relative Density	Relative Frequency	Importance Value (%)
Albizia procera	2.50	3.571	6.071
Alstonia macrophylla	12.50	7.143	19.643
Calophyllum inophyllum	2.50	3.571	6.071
Canarium ovatum	10.00	7.143	17.143
Cynometra ramiflora	2.50	3.571	6.071
Gardenia pseudopsidium	2.50	3.571	6.071
Gliricidia sepium	5.00	7.143	12.143
Jacaranda mimosifolia	2.50	3.571	6.071
Lagerstroemia speciosa	2.50	3.571	6.071
Leucaena leucocephala	2.50	3.571	6.071
Parkia javanica	5.00	3.571	12.143
Petersianthus quadrialatus	2.50	3.571	6.071
Pittosporum pentandrum	2.50	3.571	6.071
Polyscias nodosa	2.50	3.571	6.071
Pterocarpus indicus	10.00	7.143	17.143
Swietenia macrophylla	7.50	7.143	14.643
Terminalia microcarpa	5.00	7.143	12.143
Toona calantas	5.00	7.143	12.143
Vitex parviflora	12.5	10.714	23.214

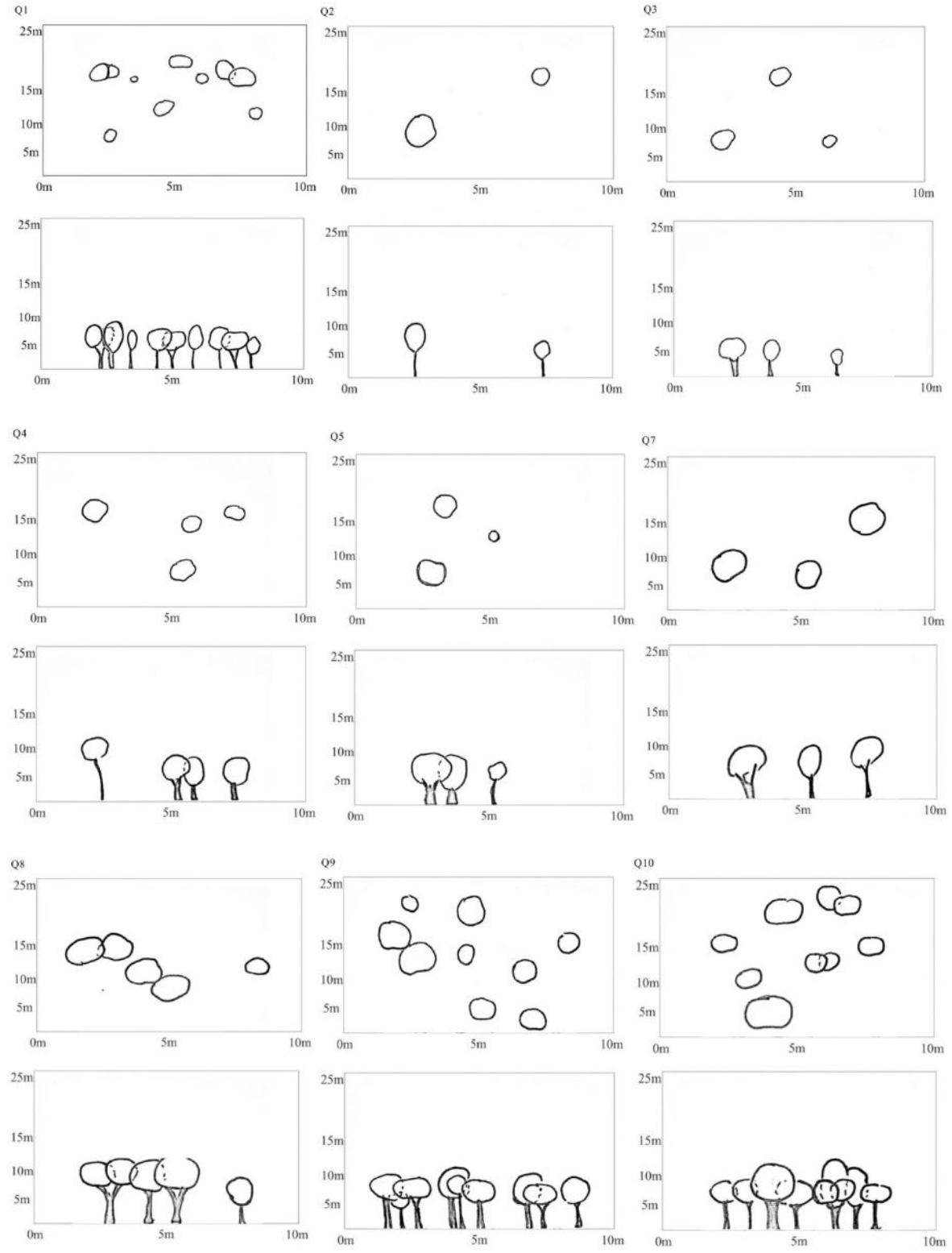

Figure 2: Horizontal and vertical profiles of the secondary forest of DLSU Manila-Laguna Campus

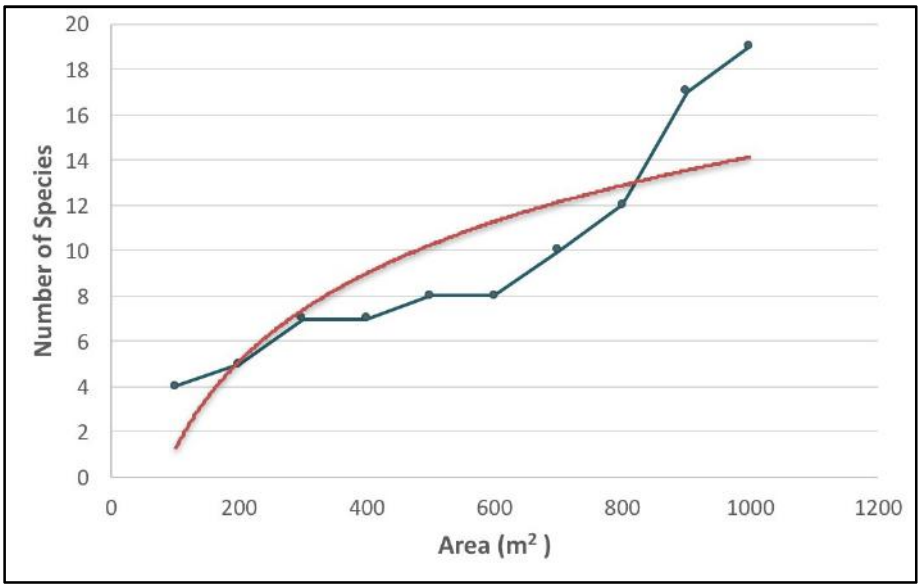

Figure 3: Species-area curve of the Secondary Forest of De La Salle University Manila – Laguna Campus.

Figure 4: Voucher specimen of *S. asper* collected from the secondary forest of DLSU Manila-Laguna Campus

Table 2: List of plant species identified within the secondary forest of De La Salle University Manila - Laguna Campus.

Family	Scientific Name	Common Name	Result of DMG Test
Apocynaceae	*Alstonia macrophylla*	Batino	negative
Apocynaceae	*Wrightia pubescens*	Lanete	negative
Anacardaeae	*Semecarpus cuneiformis*	Ligas	negative
Annonaceae	*Annona reticulata*	Anonas	negative
Araliaceae	*Polyscias nodosa*	Malapapaya	negative
Bignoniaceae	*Handroanthus chrysotrichus*	Golden Trumpet	negative
Bignoniaceae	*Jacaranda mimosifolia*	Jacaranda	negative
Bignoniaceae	*Spathodea campanulata*	African Tulip	negative
Burseraceae	*Canarium ovatum*	Pili	negative
Calophyllaceae	*Calophyllum inophyllum*	Bitaog	negative
Combretaceae	*Terminalia microcarpa*	Kalumpit	negative
Ebenaceae	*Diospyros blancoi*	Kamagong	negative
Euphorbiaceae	*Macaranga tanarius*	Binunga	negative
Fabaceae	*Albizia procera*	Akleng-parang	negative
Fabaceae	*Bauhinia monandra*	Alibangbang	negative
Fabaceae	*Cassia fistula*	Golden Shower	negative
Fabaceae	*Cynometra ramiflora*	Balitbitan	negative
Fabaceae	*Gliricidia sepium*	Madre de cacao	negative
Fabaceae	*Leucaena leucocephala*	Ipil-ipil	negative
Fabaceae	*Parkia javanica*	Cupang	negative
Fabaceae	*Pithecellobium dulce*	Camachile	negative
Fabaceae	*Pterocarpus indicus*	Narra	negative
Fabaceae	*Samanea saman*	Rain Tree	negative
Fabaceae	*Tamarindus indica*	Sampalok	negative
Lamiaceae	*Vitex parviflora*	Molave	negative
Lecythidaceae	*Petersianthus quadrialatus*	Toog	negative
Lythraceae	*Lagerstroemia speciosa*	Banaba	negative
Malvaceae	*Firmiana simplex*	Chinese parasol	negative
Malvaceae	*Hibiscus tiliaceus*	Balibago	negative
Meliaceae	*Azadirachta indica*	Neem	negative
Meliaceae	*Swietenia macrophylla*	Mahogany	negative
Meliaceae	*Toona calantas*	Kalantas	negative
Moraceae	*Atrocarpus blancoi*	Antipolo	negative
Moraceae	*Broussonetia luzonica*	Himbabao	negative
Moraceae	*Ficus nota*	Tibig	negative
Moraceae	*Ficus pseudopalma*	Lubi-lubi	negative
Moraceae	*Streblus asper*	Kalyos	positive
Myrtaceae	*Eucalyptus deglupta*	Bagras	negative
Myrtaceae	*Syzygium cumini*	Duhat	negative
Pittosporaceae	*Pittosporum pentandrum*	Mamalis	negative
Phyllantaceae	*Antidesma bunius*	Bignay	negative
Rubiaceae	*Gardenia pseudopsidium*	Malabayabas	negative
Urticaceae	*Leucosyke capitellata*	Isis	negative

Table 3: Nickel concentrations of leaf, stem, and soil samples of *Streblus asper* and calculated nickel bioconcentration factors.

Sample	Nickel Concentration (ppm)	Bioconcentration Factor (BCF)
Leaves	0.45 ± 0.00	0.315
Stems	1.53 ± 0.21	1.070
Soil	1.43 ± 0.33	

It is possible that higher nickel concentrations may be found in leaves located further at the top of the tree, as nickel is more likely to be translocated to younger tissues **[21]**. This should be taken into consideration as the leaves of *S. asper* collected in the site where mostly matured and found in the lower part of tree since they were more accessible. Additionally, studies such as that of Jaffre et al. **[22]** concerning nickel uptake in the hyperaccumulators of New Caledonia note the abundance of chelators such as malate and citrate in the tissues of species such as *Psychotria gabriellae*. Taking this into consideration, there might have been a higher abundance of these chelators in the stems of *S. asper* than in the leaves.

Since the values of the quantitative nickel screening do not meet the threshold that the leaves supposedly met in the DMG testing, the reliability of the DMG kit as a screening for nickel is of interest. Several studies conducted in order to test the sensitivity and specificity of dimethylglyoxime, such as the study conducted by Thyssen et al. **[23]** who noted that the typical DMG kit has high specificity, but questionable sensitivity. Other studies stated that the use of DMG for screening nickel content in objects that are usually in direct contact with the skin, such as jewelry, in order to prevent skin disorders such as contact allergy dermatitis **[24]**. However, these studies also mentioned the increased sensitivity of dimethylglyoxime to elevated moisture and heat, which could be taken into consideration as the humidity and temperature of the secondary forest could have influenced the sensitivity of the prepared DMG kit to the relatively minute amount of nickel in the leaves of *S. asper.* Despite the aforementioned questionable reliability of using dimethylglyoxime in screening nickel concentrations >1000 ppm in leaves, most studies that focus on nickel hyperaccumulation still greatly utilize DMG kits in field tests, such as studies conducted by Reeves et al. **[25]** and Cecchi et al. **[26]**. Thus, provided that the DMG kit used in screening for nickel content was accurate and the results of the laboratory analysis confirmed the low nickel content in the tissues of *S. asper,* this only means that the said species cannot be considered as a hyperaccumulator. Additionally, due to the low nickel content of the soil, which is almost equal to that of the stem tissue, ratio of stem/soil, and leaf/soil nickel concentrations that can be useful to prove the hyperaccumulation potential of the species needs further investigation. A follow-up study of the potential of *S. asper* grown in

areas known for nickel contamination must be conducted in order to prove the ability of the tree to hyperaccumulate nickel into its leaves.

To further support the laboratory analysis done of selected plant tissues, a routine soil analysis was also done using composite sample collected in the study site. Results show that the soil is only slightly acidic-neutral (pH 6.4), with a significant amount of organic matter (4.35%), phosphorous (74 ppm), and potassium (2.43 me/100 kg) concentrations. A high percentage of organic matter in the soil may likely affect the behavior of the heavy metals present in the soil, such as nickel, wherein these heavy metals can form complexes with the dissolved organic matter (Weng et al. 2002). These, in turn, can influence the uptake of these heavy metals by plant roots.

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