



Taxonomic survey of nickel accumulating plants in a mining site of Manicani Island, Guiuan, Eastern Samar, Philippines

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Abstract

A taxonomic survey of the nickel accumulating plants found in Manicani Island, Eastern Samar, Philippines is conducted to assess the phytoremediation potentials of indigenous vascular plant species found in the area. Dimethylglyoxime (DMG) kit field test was conducted to screen the encountered vascular plant species on site for nickel content in their aboveground tissues. Atomic absorption spectrophotometry (AAS) was done both on the soil and leaves of the collected plants to determine their respective nickel contents. *Vitex parviflora* A.Juss. together with other fourteen species from thirteen genera and twelve families were classified as hemi-accumulators (nickel content between 100-999 µg/g in dry matter). Fourteen species were classified as nickel non-accumulators, ten species still need further identification measures to confirm its species identity and *Ficus pseudopalma* which is endemic to the Philippines is found in the island. A careful morphological examination combined with molecular identification protocols are recommended to know the identities of the unknown plant species.

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Introduction

Heavy metal accumulators are plants with the rare ability to extract given metals and metalloids, have the capacity to accumulate them in normally toxic shoot tissue concentrations without any evidence of physiological stress (Baker and Brooks, 1989; Baker *et al.*, 2000). This marvel has been observed in less than 0.2% of all angiosperms, commonly manifesting as exceptionally high foliar concentrations ($>1000 \mu\text{g/g}$ dry weight) of one of these elements in the leaf dry matter (Pollard and Baker, 1997). Commonly, these plant species known as metal accumulators are predominantly herbaceous and generally occur on substrates high in content of these accumulated elements. Host soil environments are commonly serpentine, characterized with high levels of heavy metals and magnesium, usually depleted in plant macronutrients, but surprisingly supports highly specialized floras (Brooks, 1998). As of present, there are still many metalliferous parts of the tropics in which no plant collections have been undertaken, thus resulting to very limited analytical work on this area. As presented by Proctor (2003) and Reeves (2003), these sites include parts of the Philippines and Indonesia. In same paper, it has been estimated that the Philippine ultramafics (referring to the geological formations containing high Mg/Fe ratios)

make up around 5% of the country's land area. With the prior knowledge that these land areas support large assemblages of extreme nickel hyperaccumulators and nickel accumulators, the researcher conducted this study. In this paper, the researchers collected and taxonomically identified the species found in metal rich soils of Manicani Island, and conducted a field semi-quantitative screening for nickel accumulation (among the plants encountered) on site. The abovementioned field screening test was adapted from the works of Baker *et al.* (1992) and Fernando *et al.* (2014). The field screening involved thoroughly washing of the leaf samples with distilled water, crushing these in a mortar and pestle, and then testing with filter paper previously soaked in 1% of the nickel specific colorimetric reagent, dimethylglyoxime, dissolved in 95% ethanol. The formation of pink or purplish red color indicated exceptionally high (above $1,000 \mu\text{g g}^{-1}$) concentration of Ni in the dry plant matter.

Materials and methods

Collection site

Manicani Island is located in the municipality of Guiuan, province of Eastern Samar, and is situated $11^{\circ} 0' 5''$ North, $125^{\circ} 39' 8''$ East with a total land area of 11.66 km^2 (Figure 1).

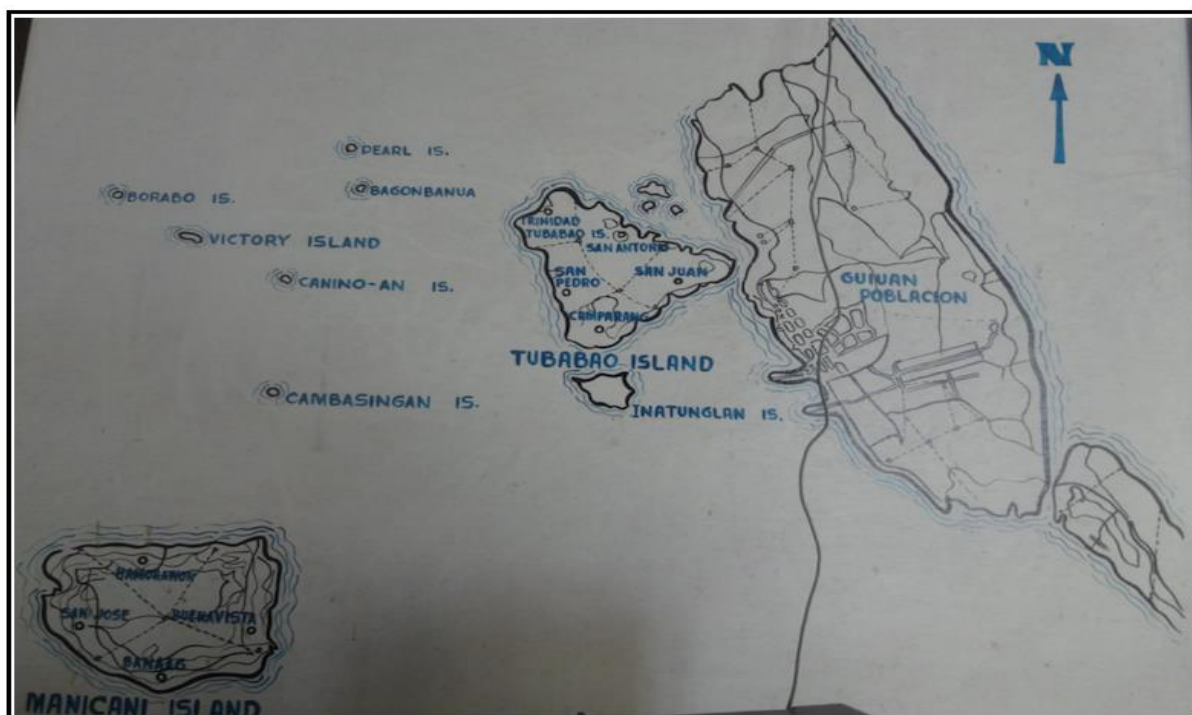


Fig. 1. The study area in Manicani Island, Guiuan, Eastern Samar.

The entire island of Manicani is divided into four fishing villages namely San Jose, Banaag, Hamorawon and Buenavista. The climate of the study area, as part of northeastern region of the Visayas, is classified as a Type II, with no dry season and a very pronounced maximum rain period from December to February (<https://www.weatheratlas.com/en/philippines/manicani-island>, accessed August 21, 2019; 10:05PM). This type of climate is described as an area with no single dry month. The minimum monthly rainfall occurs during the period from March to May. It is tropical with an average temperature of 28°C and 80% humidity.

In the island, it can be noted that there are two distinct vegetation or habitat types, the forest and the grassland. The forested areas are mostly characterized as secondary type, with observable regrowth of vegetation after the man-made disturbance brought by mining in the area.

The trees and plant species found in the area are closely spaced and contain lesser undergrowth. Likewise, grasslands are found on areas previously disturbed by mining operations. The plant diversity of the study site could be described through the most represented families which are Moraceae and Poaceae which are encountered anywhere. Among the notable species observed were *Saccharum spontaneum*, *Ardisia* sp., *Dicranopteris linearis*, *Pteridium aquilinum* and *Ipomoea pes-caprae*.

Plant Collection and DMG field screening test

Randomly selected individual plant species within the collection site was taken. There were four collection areas from the entire study site, each measuring a 10m x 10m plot. Two replicates per species were collected as voucher specimens and labeled with paper tags. Moreover, a field screening test on all encountered plant species in the location utilizing the DMG kit was undertaken by the researchers to screen which among the plant species in the area are potential accumulators of nickel in their aboveground tissues. Among all the plants in the study area, *V. parviflora* indicated a red color on the used DMG

paper (Figure 2) which led the researchers to choose this plant among others as test species to propagate and experiment in ensuing studies.

Collected Plant and Soil Samples

The plant materials were washed with distilled water, blot-dried and then oven-dried overnight at 60°C. Following the protocol of Baker *et al.* (2000), the samples were transferred to a pre-weighed crucible for dry ashing in a furnace at 200°C (1 h) then 500°C (4 h). Afterwards, the ash weights were taken after sufficient cooling. The plant samples were acid digested by dissolving in 5 mL HNO₃ and heated until the solution volume was reduced to 1 mL.

This step was repeated twice. After the volume has been reduced to 1 mL, the solution was allowed to cool down to room temperature. Forty mL of distilled water was added to each solution and then filtered into foil-covered polyethylene bottles. The pH of each filtrate was adjusted to pH 5 to 7. Next, the solutions were analyzed for nickel content through an atomic absorption spectrophotometer.

On the other hand, surface soil samples taken from four marked sites in the study area were also collected. All the soil samples were air dried in the laboratory, before subjecting to further analyses. Soil samples were digested following a 3:1 concentration ratio (Hydrochloric acid: Nitric acid) then diluted appropriately for metal analyses of nickel content through an atomic absorption spectrophotometer. Table 1 presents the soil nickel content of the four selected sites in Manicani Island, Site A has a total soil nickel equal to 21,149 ppm, Site B has 21,140 ppm, Site C has 21,095 ppm and site D has 21,156 ppm respectively.

Results and discussion

A total of thirty five (35) species from thirty one (31) genera and twenty two (22) families were collected from the study site. As adapted from the work of van der Ent *et al.* (2012), Table 2 summarizes the plants found in the study area and classified into non-accumulators (plants with <100 µg/g nickel in dry

matter), hemi-accumulators (plants with 100-999 $\mu\text{g/g}$ nickel in dry matter) and hyperaccumulators (plants with $>1,000$ $\mu\text{g/g}$ ni in dry matter) based on the nickel content determined by AAS measurements.

Presented in Table 2 is the taxonomic list of the identified plant species found living in the nickeliferous environment of Manicani Island.

Table 1. Atomic Absorption Spectrophotometry (AAS) readings of the nickel content of soil samples taken from Manicani Island, Guian, Eastern Samar, Philippines.

Soil Samples	Nickel Content (ppm)
Site A	21149
Site B	21140
Site C	21095
Site D	21156

Results show that out of thirty five species, fifteen species belonging to fourteen genera and thirteen families were classified as hemi-accumulators, in which the nickel content was found to be between 100-999 $\mu\text{g/g}$ nickels in dry matter. Among the species found belonging to this classification are *Vitex parviflora*, *Canthium* sp., *Macaranga tanarius*,

Ficus pseudopalma, *Morinda citrifolia*, *Smilax* sp., *Passiflora foetida*, *Decaspermum* sp., *Alstonia* sp., *Ardisia* sp., *Ipomoea pes-caprae*, *Ficus* sp., *Melastoma malabathricum*, *Pteridium aquilinum* and an unknown species belonging to Rubiaceae family.

Table 2. Taxonomic list of identified non-nickel accumulators and hemi-accumulators plants (<100 $\mu\text{g/g}$ nickel in dry matter and 100-999 $\mu\text{g/g}$ nickel in dry matter, respectively) found in the metalliferous site of Manicani Island, Guian, Eastern Samar, Philippines.

Division	Order	Family	Scientific Name	Local Name (Bis.)	Ni content ($\mu\text{g/g}$ Ni in dry matter)	Operational Classification of Metal Uptake (Based on van der Ent <i>et al.</i> 2012)
Spermatophyta	Lamiales	Lamiaceae	<i>Vitex parviflora</i>	mhamorawon	973	hemi-accumulator
Pteridophyta	Polypodiales	Dennstaedtiaceae	<i>Pteridium aquilinum</i>	—	692	hemi-accumulator
Spermatophyta	Primulales	Primulaceae	<i>Ardisia</i> sp.	—	579	hemi-accumulator
Spermatophyta	Gentianales	Rubiaceae	<i>Canthium</i> sp.	—	562	hemi-accumulator
Spermatophyta	Gentianales	Rubiaceae	unidentified	—	452	hemi-accumulator
Spermatophyta	Rosales	Moraceae	<i>Ficus pseudopalma</i>	lubi-lubi	378	hemi-accumulator
Spermatophyta	Myrtales	Melastomataceae	<i>Melastoma malabathricum</i>	malatungaw	322	hemi-accumulator
Spermatophyta	Gentianales	Rubiaceae	<i>Morinda citrifolia</i>	bankuro	225	hemi-accumulator
Spermatophyta	Rosales	Moraceae	<i>Ficus</i> sp.	—	198	hemi-accumulator
Spermatophyta	Rosales	Moraceae	<i>Ficus pseudopalma</i>	lubi-lubi	198	hemi-accumulator
Spermatophyta	Gentianales	Apocynaceae	<i>Alstonia</i> sp.	—	190	hemi-accumulator
Spermatophyta	Malpighiales	Passifloraceae	<i>Passiflora foetida</i>	prutas-bagyo	187	hemi-accumulator
Spermatophyta	Solanales	Convolvulaceae	<i>Ipomoea pes-caprae</i>	Bagaswa	155	hemi-accumulator
Spermatophyta	Malpighiales	Euphorbiaceae	<i>Macaranga tanarius</i>	bilan	114	hemi-accumulator
Spermatophyta	Liliales	Smilacaceae	<i>Smilax</i> sp.	—	112	hemi-accumulator
Spermatophyta	Myrtales	Myrtaceae	<i>Decaspermum</i> sp.	—	110	hemi-accumulator
Spermatophyta	Fabales	Fabaceae	<i>Leucaena leucocephala</i>	Ipil-ipil	96	non-accumulator
Pteridophyta	Gleicheniales	Gleicheniaceae	<i>Dicranopteris linearis</i>	kilob	67	non-accumulator
Spermatophyta	Poales	Poaceae	<i>Saccharum spontaneum</i>	talahib	56	non-accumulator
Spermatophyta	Asterales	Goodeniaceae	<i>Scaevola micrantha</i>	—	53	non-accumulator
Spermatophyta	Asterales	Asteraceae	<i>Chromolaena odorata</i>	hagonoy	45	non-accumulator
Spermatophyta	Rosales	Moraceae	<i>Ficus septica</i>	hawili	33	non-accumulator
Spermatophyta	Poales	Flagellariaceae	<i>Flagellaria indica</i>	baling-uway	32	non-accumulator
Spermatophyta	Sapindales	Anacardiaceae	<i>Buchanania arborescens</i>	an-an	22	non-accumulator
Spermatophyta	Malvales	Malvaceae	<i>Commersonia bartramia</i>	anitap	20	non-accumulator
Spermatophyta	Fabales	Fabaceae	<i>Acacia auriculiformis</i>	auri	18	non-accumulator
Spermatophyta	Rosales	Moraceae	<i>Ficus tinctoria</i>	balete	17	non-accumulator
Spermatophyta	Unidentified	unidentified	Unidentified	—	13	non-accumulator
Spermatophyta	Pandanales	Pandanaceae	<i>Benstonea copelandii</i>	pandan	12	non-accumulator
Spermatophyta	Rosales	Moraceae	<i>Artocarpus</i> sp.	—	12	non-accumulator
Spermatophyta	Unidentified	Unidentified	Unidentified	—	N/A	not analyzed
Spermatophyta	Lamiales	Lamiaceae	<i>Premna odorata</i>	alagaw	N/A	not analyzed
Spermatophyta	Lamiales	Verbenaceae	<i>Stachytarpheta jamaicensis</i>	kandikandilaan	N/A	not analyzed
Spermatophyta	Lamiales	Verbenaceae	<i>Lantana camara</i>	koronitas	N/A	not analyzed

In a taxonomic survey in Sri Lanka serpentine soils, *Canthium puberulum* was found to accumulate 350 µg metal per gram dry leaf tissue (Rajakaruna and Baker, 2004). Although AAS readings were not successfully analyzed in this research, *Premna odorata* of the Verbenaceae, has been presented in the paper of Ocon *et al.* (2018), as one of the four species (from the 59 species identified in the study area of the nickel-rich Kinalablaban Delta, Cagdianao, Claver, Surigao del Norte, Philippines) which tested positive as potential nickel hyperaccumulator alongside *Ardisia elliptica*, *Phyllanthus securinegoides* and *Phyllanthus* sp.

In this taxonomic survey of nickel accumulating plants of Manicani Island, a certain species of *Ardisia* of the Primulaceae family has also been identified as one of the hemi-accumulators with nickel content of 579 ppm (above ground tissue). Significant levels of lead has also been detected in the tissues of *Smilax myosotiflora* (Ang and Kiyoshi, 2004). *Alstonia scholaris* has been identified as a possible biomonitor of lead and cadmium in the polluted environments of Pakistan (Shafiq *et al.* 2011).

In same study, the leaves of *Alstonia scholaris* has been found to contain significantly high levels of lead and cadmium (105 mg/kg lead and 30 mg/kg cadmium). *Pteridium aquilinum* is considered to be one of the plants most resistant to metals (Kubicka *et al.*, 2015). This fern meets the demands for a good bioindicator to improve environmental control. *P. aquilinum* has been identified as an arsenic hyperaccumulator (Chang, 2005; Olaifa and Omekam 2014) alongside another fern *Pteris vitatta*. *Melastoma malabathricum* has been known as among the plant species posing to be good metal accumulators (Ashraf *et al.*, 2011). *Decaspermum* sp. of the Myrtaceae family was seen to have the lowest value of nickel content in its leaf tissue (dry matter) among all identified hemi-accumulators with AAS reading of 110 µg/g nickel in dry matter. While *V. parviflora* belonging to the Lamiaceae family has the highest nickel measure at 973 µg/g nickel in dry matter. This AAS result confirms the DMG field

screening result, with *V. parviflora* presenting its good phytoremediation potential.



Fig. 2. *Vitex parviflora* A.Juss. shown as a potential nickel accumulator by a field test using filter paper soaked in 1% dimethylglyoxime (DMG) dissolved in 95% ethanol.

V. parviflora known for its number of folkloric medicinal applications including stomachache, post childbirth recovery, fever and wound healing (Ata *et al.*, 2009) has previously been identified in the paper of Fernando *et al.* (2013) as a nickel hemi-accumulator with leaves and stem tissues nickel content equivalent to 226 µg/g and 58 µg/g. In the same paper, the potential use of indigenous nickel plant accumulators such as *V. parviflora* was seen beneficial for small-scale mining in the Philippines. A fern species *P. aquilinum* belonging to the Dennstaedtiaceae family was abundantly found in the collection site and classified to be a hemi-accumulator species with nickel content equal to 692 µg/g. Apart from the phytoremediation potential of *P. aquilinum*, its medicinal application is also comparable to that of *V. parviflora*. Analyzed leaf extracts of *P. aquilinum* revealed some phytochemical constituents, antioxidant activity and antimicrobial property (Kardong *et al.*, 2013).

Its ethanol and petroleum ether extracts showed antimicrobial activity against *Bacillus subtilis*,

Streptococcus aureus, *Proteus vulgaris* and *Escherichia coli*. *Canthium* sp. found to be a hemiaccumulator as well with nickel content equivalent to 562 µg/g has been widely known for its unexplored traditional medicinal applications. In the paper of Sasmal *et al.* (2014), genus *Canthium* has been presented as one of the traditional medicinal plants in India which is used for treatment of various ailments. Different parts of plants (ie., leaves, bark, stem, fruits, root and even whole plant) have shown to have various pharmacological activities like antimicrobial, antioxidant, hepatoprotective, antimalarial, anti-diabetic, and anti-asthmatic activities. *Macaranga tanarius* (also a hemiaccumulator), has been presented to contain a wide spectrum of pharmacological activities including anti-cancer, anti-inflammatory, anti-oxidant, antimicrobial and anti-plasmodial activities (Magadula, 2014).



Fig. 3. Specimen of *Vitex parviflora* A.Juss. (Lamiaceae) shown to be a nickel hemi-accumulator.

In the same manner, categorized as a hemiaccumulator in this study, *Ardisia* sp. belongs to a genus found throughout the tropical and subtropical regions of the world known for its traditional medicinal uses like alleviation of liver cancer, swelling, rheumatism, earache, cough, fever, diarrhea, broken bones, dysmenorrhea, respiratory tract infections, traumatic injuries, inflammation, pain,

snake and insect bites, birth complications and blood circulation problems (de Mejia and Ramirez-Marez, 2011).

With these, it can be noted that the occurrence of these plant species with phytoremediation potentials could be hypothesized to be associated and highly related to plants with extracts that have medicinal activities. The researchers deduced that a mechanism in heavy metal tolerance and accumulation could be a significant and crucial explanation to this possible connection. However, further studies should be conducted to allow the full analysis of this hypothesis. From the 35 identified plant species found in Manicani Island, 14 species were classified as nickel non-accumulators, namely, *Buchanania arborescens*, *Chromolaena odorata*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Flagellaria indica*, *Dicranopteris linearis*, *Scaveola micrantha*, *Commersonia bartramia*, *Ficus tinctoria*, *Benstonea copelandii*, *Saccharum spontaneum*, *Ficus septica*, *Artocarpus* sp. and an unidentified species. Of these nickel non-accumulators, *Leucaena leucocephala* belonging to the Fabaceae family has the highest nickel level at 96 µg/g nickel. Regardless of being identified as a nickel non-accumulator in this study, Ssenku *et al.* (2017) presented *Leucaena leucocephala* to have numerous inherent characteristics that can be exploited to augment phytoremediation and lower the cost of regeneration. This species can survive in harsh environmental conditions with the exception of heavily frosted conditions and occurs in a wide range of ecological settings. The species is fast growing, capable of reaching maturity in 6 to 7 months to produce a vast amount of seeds that can germinate into numerous seedlings to carry on further remediation of the polluted site. It can produce large quantities of phytomass that can accumulate heavy metals and can repeatedly be harvested to regenerate a polluted area through phytoextraction. On the other hand, an *Artocarpus* sp. of the Moraceae has the lowest nickel content in its leaf tissue with only 12 µg/g nickel in dry matter. Contradictory to this result, *Priyantha* and

Kotabewatta (2019) present the heavy metal remediation potential of *Artocarpus* species. Okolo *et al.* (2012) argued the worth of *Artocarpus altilis* in toxic metal removal from the environment. The peel of the edible fruit, *Artocarpus nobilis* (a plant endemic to Sri Lanka) showed remarkable adsorption capacity towards nickel metal ion. Removal efficiency of 50% was obtained in their study.



Fig. 4. Specimen of an unidentified plant species belonging to Rubiaceae.

Collected specimens of both categorically identified hemi-accumulators *V. parviflora* and an unidentified plant species belonging to Rubiaceae are illustrated in Figures 3 and 4. It is notable that among all plant samples processed, the above-mentioned species demonstrated a distinguished change in leaf color (green to dark purple). The unidentified species (see Figure 6), initially identified to belong to the Rubiaceae family has a promising potential for nickel accumulation with AAS value equal to 452 µg/g nickel content in dry matter.

In this taxonomic survey of nickel accumulating plants found in the mining site of Manicani Island, ten (10) of the total number of species surveyed still need final identification. Among these are *Canthium* sp. (Rubiaceae), *Smilax* sp. (Smilacaceae), *Decaspermum* sp. (Myrtaceae), *Alstonia* sp.

(Apocynaceae), *Ardisia* sp. (Primulaceae), *Artocarpus* sp. and *Ficus* sp. (both from Moraceae).



Fig. 5. Specimen of *Ficus pseudopalma* (Moraceae).

Consequently, the results of this study affirmed the choice and utilization of *V. parviflora* as test species propagated and utilized in other ensuing experiments on the characterization of plant features contributory to the plants' tolerance to nickel in the study area. In a developing nation like the Philippines, where current mining procedures with safeguards for environmental well-being are wanting, the findings of this study will have significant implications. The restoration and rehabilitation of degraded and abandoned mining sites, like in the case of Manicani Island, and their neighboring localities pose a consequential financial and technological challenge. Thus, phytoremediation presents a new method in cleaning up contaminated mining areas in the country where extravagant technology is unavailable, impractical and unaffordable. The identification and classification of the above-listed local plants with varying nickel accumulating potentials will contribute significantly on the restoration and conservation of mined areas. Heavy metals toxicity has unavoidable threat to the environment due to their increasing contamination and accumulation in the atmosphere which at the end is believed to pass through living beings by the route of the food chain. The removal of these heavy metals (e.g. toxic nickel from the

Manicani soils) can reduce health risks in mining sites and nearby communities. Henceforth, phytoremediation of metal contaminated environments present to be an efficient measure for soil amendment. The identification efforts brought about by taxonomic surveys such as this paper, will be very helpful phytoremediation agents.

Conclusions and Recommendations

A taxonomic inventory of the study site presents a total of thirty five (35) species from thirty one (31) genera and twenty two (22) families. Out of the thirty-five species, fifteen species belonging to fourteen genera and thirteen families were classified as hemi-accumulators. The DMG (dimethylgloxime) field test shows that *V. parviflora* among other nickel accumulating plants encountered in the study site demonstrated a purplish red change of color in the filter paper used for screening. This result reconciled with its Atomic Absorption Spectroscopy (AAS) readings equivalent to 973 ppm nickel accumulation in its aboveground tissues. In this study, ten (10) of the total number of species surveyed still need further identification measures to confirm its species identity. Among these, are *Canthium* sp. (Rubiaceae), *Smilax* sp. (Similacaceae), *Decaspermum* sp. (Myrtaceae), *Alstonia* sp. (Apocynaceae), *Ardisia* sp. (Primuliaceae), *Artocarpus* sp., *Ficus* sp. (both from Moraceae), an unknown nickel hemi-accumulator initially identified to belong to Rubiaciae family and two more unidentified species.

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