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Determination of Macronutrients and Trace Elements in Potential Nickel Hyperaccumulators from Ultramafic Areas in Sabah using Atomic Spectroscopic Analysis

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ABSTRACT

Hyperaccumulators species are widely distributed on ultramafic lands where it is capable to accumulate extremely high concentration of metalloids such as Ni, Co and Mn in the above-ground parts without showing any signs of toxicity. However, related information on identified hyperaccumulators from Sabah such as elemental distribution is insufficient due to lack of fundamental studies. The aim of this study is to contemplate hypernickelophores as tropical metal crop candidate for sustainable metal farming and environmental cleanup through evaluation of macronutrients and trace elements such as Na, Ca, K, Ni, Co and Mn in identified hyperaccumulators using Atomic Absorption Spectrophotometer (AAS). 8 species of previously identified hyperaccumulating plants (*Psychotria sarmentosa, Glochidion* sp. 'bambangan', *Rinorea, bengalensis, Rinorea javanica, Actephila alenbakeri, Walsura pinnata, Xylosma luzonensis* and *Mischocarpus sundaicus* were collected from serpentinite area in Kinabalu Park for this study. 4 hypernickelophores which are *G.* sp. 'bambangan', *R. bengalensis, R. javanica* and *P. sarmentosa* were found with the accumulation of Ni as much as 10 784 mg/kg, 13 196 mg/kg, 13 780 mg/kg and 17 085 mg/kg, respectively. These findings suggest that these species hold promise for phytoremediation and phytoextraction in Malaysia, particularly in regions with ultramafic soils. Therefore, further study on *G.* sp. 'bambangan' should be carried out as it is an undescribed taxon which is endemic to Sabah. Apart from that, it is crucial to determine the most suitable agronomic practices for the hypernickelophore so the metal bioavailabilty can be increased. Therefore, it can be utilized in the application of metal farming which is a novel mineral extraction technology.

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INTRODUCTION

Nickel hyperaccumulators are defined as plants that accumulate more than 1000 mg/kg of nickel (Ni) in their above-ground biomass, with those exceeding 10,000 mg/kg classified as hypernickelophores [1], [2]. These plants possess three primary traits which are high capacity for metal uptake from the soil, efficient root-to-shoot translocation of metal ions and effective detoxification and sequestration of trace elements in their tissues [3]. The physiological mechanisms that enable these processes include the release of root exudates containing Ni-chelators which enhance Ni uptake and translocation to the leaves [4], [5]. Thus, the concentrations of Ni can be significantly higher in leaves compared to other plant parts. Currently, the study of hyperaccumulating plants as new resources in the development of novel plant-based technologies for the treatment of polluted sites and extraction of rare metal is extensively carried out in Sabah where the study has been proven to be successful in USA, Europe and China since 1980s [6]. However, the accurate number of identified hyperaccumulators in Sabah and related information such as elemental distribution is not known due to lack of fundamental studies regarding hyperaccumulators in Sabah, Malaysia.

Atomic spectroscopic analysis played a pivotal role in the discovery and characterization of hyperaccumulator plants in 1900’s. The analysis helps to enhance our understanding of metal accumulation in flora fundamentally. Initially, methods such as Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) were employed to identify plants with high metal concentrations. The discovery of hyperaccumulators in Sabah, Malaysia started in 1900’s when Brooks and Wither (1977) found Rinorea bengalensis in the family of Violaceae can accumulate Ni up to 17,500 g/g (dry weight) using AAS. Apart from that, another study conducted by Proctor et al. (1989) found that Shorea tenuiramulosa in the family of Dipterocarpaceae which is located at Mount Silam can accumulate Ni up to 1.0 mg/g in its leaf dry matter prior to AAS analysis on the plant samples collected from the site. Following the discovery, it can be suggested that ultramafic areas in Sabah host interesting plants. This study investigates various Ni hyperaccumulators in Sabah, concentrating on an unidentified taxon referred as *G.* sp. 'bambangan' which, can enhance the understanding of Ni accumulation mechanisms and increase the global database of hyperaccumulator plants, thereby emphasising the significance of exploration in ultramafic regions.

Hyperaccumulators plant species is beneficial for soil remediation and hitherto, Chaney introduced the concept of phytomining in which hyperaccumulators are used to accumulate metals from soil into the plant shoots so they can be harvested and transformed to bio-ores. His discovery was followed by Morel in 2000’s nearly three decades later, where he proposed the term agromining so the scope of technology can be broadened to the entire soil-plant-ore agrosystems [9]. In the late 2010s, pot trials for two identified Ni hyperaccumulators from Sabah which are *Phyllanthus rufuschaneyi* and *Rinorea bengalensis* had been carried out to test the agronomic systems of potential tropical metal crops [6], [47]. Field trials for *P. rufuschaneyi* was carried out a year later and the findings showed that it is a promising metal crop to be developed in Sabah [10].

To realise this, it is crucial to determine the bioaccumulation factor (BAF) and translocation factor (TF) of a plant species in discovering potential Ni hyperaccumulators [11], [48]. The BAF measures how effectively a plant accumulates a particular metal from the soil into its tissues while TF measures the ability of a plant to transport the absorbed metal from the roots to the shoots. These factors would identify plant species with the best potential for environmental cleanup and metal recovery. Plants with high BAFs effectively remove Ni from soil, while those with high TFs ensure that the accumulated Ni can be harvested from the shoots. These factors provide comprehensive view of a plant’s ability to remediate contaminated sites and recover valuable metals which is essential for optimizing phytoremediation strategies and achieving sustainable environmental management [12]. The aim of this paper is to suggest hypernickelophores from ultramafic soils in Sabah as potential metal crops by determining the

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concentration of macronutrients and trace elements (Ca, Na, K, Ni, Co and Mn) in parts of hyperaccumulating plants as well as calculating BAF and TF of Ni in each species of Ni hyperaccumulators collected.

EXPERIMENTAL

Sample Collection

Plant samples of identified Ni hyperaccumulators which are *Psychotria sarmentosa*, *Glochidion* sp. 'bambangan', *Rinorea bengalensis*, *Rinorea javanica*, *Actephila alanbakeri*, *Walsura pinnata*, *Xylosma luzonensis*, and *Mischocarpus sundaicus* were collected from four ultramafic areas in Sabah: Pig Hill, Garas Hill, Sub-station Monggis, and Sub-station Serinsim. Prior to collection, a permit was obtained from Sabah Biodiversity Centre (License Reference Number: JKM/MBS.1000-2/2JLD. 13 (73)). The samples (leaves, stems, and roots) were placed in zip-locked bags to prevent decomposition and washed with tap water followed by distilled water to remove soil contamination. After drying with tissue paper, the plant parts were separated using stainless steel scissors which were thoroughly cleaned with distilled water and wiped with tissue paper between each sample to prevent cross-contamination, cut into small pieces, and dried in an oven at 70°C until a constant weight was achieved. Prior to the cutting of the plant samples, the scissors was cleaned using diluted methanol.

Soil samples were collected using an auger and hand shovel at depths ranging from 1 to 25 cm after removing debris. These samples were also placed in zip-locked bags for laboratory preparation. They were dried at 40°C until constant weight was reached, then ground with a pestle and mortar and passed through a 2 mm sieve. The prepared soil samples were labelled and stored in plastic containers before analysis using Atomic Absorption Spectrophotometer (AAS) [13].

Sample Digestion

The plant samples were digested using wet digestion method according to Uddin *et al*. (2016) where 0.5 g of ground samples were digested in a conical flask on a hot plate with the addition of 9 mL of freshly prepared acid mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in the ratio of 1:3. The choice of this acid ratio is based on the ability of HNO_3 to oxidize organic matter and dissolve metal ions, while HCl hydrochloric acid facilitates the formation of chloride complexes with trace elements in which enhancing their solubility and availability for analysis [15]. The 1:3 ratio has been commonly used in literature as it effectively digests plant materials without excessive losses of metals or excessive acid consumption [16], [17]. Upon digestion process was completed, the mixture were left to cool to room temperature, filtered into a 50 mL volumetric flask and diluted using distilled water up to the calibration mark.

The digestion method for soil samples was executed according to Asher *et al.* (2020). 1.0 g of the dried ground soil samples was weighed into a 250 mL conical flask with the addition of 20 mL of HNO_3, HCl and H_2SO_4 in the ratio of 5:1:1 and heated on a hot plate until completely digested. The H_2SO_4 in this mixture serves to enhance the breakdown of mineral components in the soil, particularly silicates, which are less soluble compared to organic materials [18]. HNO_3 and HCl work to oxidize and dissolve metals, and the 5:1:1 ratio is frequently used in soil digestion to ensure the complete dissolution of both organic and mineral phases [18]. This acid combination has been validated in standard methods and is considered effective for obtaining a broad range of elements from both organic and mineral components of soil [19].When the digestion process was completed, the samples were left to cool at room temperature before filtered into a 100 mL volumetric flask by using Whatman No. 42 filter paper. Distilled water was added up to the calibration mark and the samples were ready for the analysis of trace elements by using AAS.

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AAS Analysis

The digested samples were analysed using AAS Agilent 240 AA. The concentration of macronutrients (Ca, Na and K) and trace elements (Ni, Co and Mn) were determined by obtaining a corresponding standard calibration curve using ICP Multi-element Standard Solution IV. Blank samples was included in each run.

BAF and TF Calculation

Bioaccumulation factor (BAF) is the ratio of the concentration of trace elements in plants and in soil. BAF is calculated as an indicator of a plant's capacity to accumulate trace elements [20]. Equation 1 was used to calculate the BAF of Ni in each hyperaccumulator wherewhere, P_i is the concentration of a Ni in hyperaccumulator (mg/kg) and S_i is the concentration of the Ni in the soil where the hyperaccumulator grows (mg/kg).

$$BAF = \frac{P_i}{S_i} \quad (1)$$

$$TF = \frac{A_i}{B_i} \quad (2)$$

Translocation factor (TF) is the ratio of the concentration of trace elements in aboveground tissue to the concentration of trace elements in belowground tissue. [21]. Equation 2 was used calculate the TF of each Ni hyperaccumulator where A_i is the aboveground tissue concentration and B_i is the belowground tissue concentration.

Data Analysis

The data from this study was analysed quantitatively using IBM SPSS Statisics 25 as the statistic software. The data obtained from AAS analysis was analysed using Shapiro-Wilk test to determine the p – value, whether it is normal ($p > 0.05$) or abnormal ($p < 0.05$). Data transformation was done for abnormal data. Data transformation was done by applying transformations such as log, square root or cube root. Transformations were employed to normalise the distribution of non-normally distributed data. The log, square root, or cube root transformations were selected according to the data distribution. Log transformations are commonly employed for data exhibiting a right-skewed distribution with an extensive range of values, as they reduce the size of huge numbers and stabilise variance. After transformation, the data was re-test for normality. The linear correlation was determined by using Pearson's Correlation or Spearman's Correlation based on the distribution of data. Pearson's Correlation was used for normally distributed data and Spearman's Correlation for data distributed abnormally.

RESULTS AND DISCUSSIONS

Elemental Concentration in Samples from Pig Hill

From Table 1, notable concentrations of Ni are observed in the leaves (10,784 mg/kg) and stems (1396 mg/kg) of *G*. sp. 'bambangan', indicating its hyperaccumulative property for Ni. Additionally, Ca, Na, K, Co, and Mn exhibit varying concentrations across different plant parts where the highest concentration can be observed in leaves (7808 mg/kg), roots (204 mg/kg), stems (3670 mg/kg), roots (29 mg/kg) and roots (142 mg/kg), respectively. Meanwhile for *P. sarmentosa,* only 63 mg/kg of Ni is observed in the leaves. High concentrations of Na, Ca and K are observed in the leaves (1507 mg/kg, 16181 mg/kg and 4184 mg/kg, respectively). The concentrations of Co and Mn also vary across plant parts with highest value can be observed in stems (63 mg/kg) and roots (315 mg/kg), respectively.

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The result shows that *G.* sp. 'bambangan' fulfill the criterion as a hypernickelophore where the accumulation of Ni in the aboveground tissue as much as 10784 mg/kg, however, *P. sarmentosa* did not show accumulation of Ni. This species has been chosen to be further studied in this study where it was analysed with Scanning Electron Microscope energy Dispersive X-Ray (SEM-EDX) to determine the distribution of Ni at the cell level as it is undescribed taxa endemic to Sabah. The same species of *G.* sp. 'bambangan' was found along rivers at lower montane forest in Kinabalu Park where it can accumulate Ni up to 17,600 mg/kg [22]. According to van der Ent *et al*. (2015), *P. sarmentosa* is a hypernickelophore when the samples of the species collected from lowlands in the secondary vegetation in Sabah which were Wuluh and Panataran Rivers in Kinabalu Park with accumulation of Ni up to 24,200 mg/kg. However, the accumulation of Ni in *P. sarmentosa* in this study is low not even exceeding the threshold level of a Ni hyperaccumulator which is only 63 mg/kg. Initially, a fieldwork was done at Pig Hill, Mesilau by the hyperaccumulators expert, Dr. van der Ent. The screening of hyperaccumulators was performed using DMG on *P. sarmentosa* leaf, however, it showed negative results where no colour changes was detected on the DMG paper. According to him, the tested leaf is not *P. sarmentosa* but another species of *Psychotria* which did not accumulate Ni in its above-ground parts. Therefore, the *P. sarmentosa* collected from Pig Hill in this study is another species of *Psychotria* which does not possess accumulation of Ni. Besides, the concentration of Ca is the highest in the leaves of both species which agrees with the study conducted by [23] as well as K, the concentration is also the highest in the leaves [24]. However, comparative studies showed that concentration of Na is the highest in the roots of hyperaccumulator but *P. sarmentosa* shows highest concentration of Na in the leaves [23].

Table 1. Elemental Concentration in Samples from Pig Hill

Species	Metals	Soil (mg/kg)	Leaf (mg/kg)	Stem (mg/kg)	Root (mg/kg)
Glochidion sp. 'bambangan'	Na	233±23	94±1	79±6	204±120
	Ca	4182±20	7808±353	2754±2188	4065±149
	K	473±42	2826±837	3670±32	660±131
	Ni	1194±258	10,784±6227	1396±885	301±179
	Co	333±44	28±13	28±42	29±10
	Mn	657±244	59±29	27±6	142±14
Psychotria sarmentosa	Na	536±317	1507±899	302±3	238±13
	Ca	4266±287	16,181±418	1877±1078	4644±1496
	K	581±204	4184±2300	2438±196	405±181
	Ni	1161±1263	63±31	41±22	104±22
	Co	299±59	9±11	63±21	6±18
	Mn	245±94	24±27	29±6	315±64

Elemental Concentration in Samples from Garas Hill

Table 2 shows the accumulations of Ni are extremely high in the leaves of *R. javanica* and *P. sarmentosa* which are 17,844 mg/kg and 17,085 mg/kg respectively. High concentration of Ni is also observed in the leaves of *A. alanbakeri* with value of 4540 mg/kg. *R. javanica, A. alanbakeri* and *P. sarmentosa* exhibits highest concentration of Na in the roots at 433 mg/kg, 1292 mg/kg and 1189 mg/kg, respectively. Ca concentrations are notable with highest concentration in the leaves of both *R. javanica* and *A. alanbakeri* with concentration of 17844 mg/kg and 12148 mg/kg, respectively. In contrast, the concentration of Ca is the highest in the roots of *P. sarmentosa* with value of 16502 mg/kg. Meanwhile for K, the concentration is also the highest in the leaves of *R. javanica* and *A. alanbakeri* (7928 mg/kg and 15534 mg/kg, respectively) but in stems for *P. sarmentosa* (2833 mg/kg). Both concentrations of Co and Mn in the parts

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of three species of Ni hyperaccumulators collected from Garas Hill are low in the range of 12 mg/kg to 360 mg/kg.

Thus, both *R. javanica* and *P. sarmentosa* fulfill the criterion of hypernickelophore as the accumulation of Ni exceeds 10,000 mg/kg while for *A. alanbakeri,* it is a Ni hyperaccumulator. The accumulation of Ni in *R. javanica* collected near Kinabalu Park is lower from this study which is 9680 mg/kg [24]. Other than that, the accumulation of Ni in *A. alanbakeri* collected from Nalumad in study conducted by van der Ent *et al.* (2016) shows high accumulation of Ni which is 14,700 mg/kg but only 4540 mg/kg of Ni is observed in samples from Garas Hill in this study. In addition, co-accumulation is noticed in *A. alanbakeri* where it accumulates Co in leaves as much as 33 mg/kg which exceeds the new threshold limit identified by Purwadi *et al.* (2023), 32 mg/kg. The distribution of metals such as Ca, Na and K in Ni hyperaccumulators varies significantly among different plant tissues [27] which agrees with the findings from this study.

Plants have complex mechanisms for absorbing, translocating, and sequestering metals like Ni. The concentration of elements like Ni, Ca, Na, and K in different plant tissues is influenced by factors like tissue permeability, ion transporters, and detoxification processes [3], [28]. High concentrations of Ni in leaves may be due to higher expression of metal transporters like natural resistance-associated macrophage protein (NRAMP) and heavy metal ATPases (HMA), while roots may have lower concentrations due to plants using roots for uptake but preventing excessive translocation to the shoot [29]. Certain species may have evolved mechanisms to compartmentalize Ni in vacuoles or cell walls to reduce toxicity. The distribution of metals between plant tissues can be explained by differences in cellular structures, such as the epidermis and cuticle of leaves acting as barriers and root architecture and specialized structures like root hairs [30].

Table 2. Elemental Concentration in Samples from Garas Hill

Species	Metals	Soil (mg/kg)	Leaf (mg/kg)	Stem (mg/kg)	Root (mg/kg)
Rinorea javanica	Na	398±88	52±14	185±25	433±43
	Ca	5606±86	17,844±13,566	3681±1235	6660±3604
	K	63±48	7928±1546	2875±438	1798±279
	Ni	1101±1141	13,780±3702	1349±865	5027±1530
	Co	221±59	24±2	32±24	86±24
	Mn	606±142	56±24	25±7	83±52
Actephila alanbakeri	Na	470±96	235±15	261±12	1292±681
	Ca	4879±415	12,148±933	2141±1894	2706±1741
	K	413±379	15,534±702	4404±509	5711±1865
	Ni	1133±623	4540±569	662±575	222±165
	Co	149±67	33±19	22±13	11±8
	Mn	409±56	168±44	88±38	252±48
Psychotria sarmentosa	Na	260±19	116±10	303±17	1189±110
	Ca	5286±645	16,502±3032	3737±1006	17,283±1847
	K	649±82	1796±585	2833±348	1349±112
	Ni	2045±933	17,085±2247	42±8	2609±208
	Co	205±76	12±11	31±10	123±13
	Mn	219±73	293±151	20±4	360±11

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Elemental Concentration in Samples from Serinsim Sub-Station

In Table 3, that the accumulation of Ni in the leaves of *M. sundaicus* is the highest which is 2934 mg/kg followed by *X. luzonensis* with accumulation of Ni as much as 2896 mg/kg in the leaves. All species of Ni hyperaccumulator from Serinsim Sub-station show the highest concentration of Na in the leaf. Both *X. luzonensis* and *M. sundaicus* show highest concentration of Ca in the leaves with value of 15810 mg/kg and 16557 mg/kg, respectively but in roots for *W. pinnata* with value of 7923 mg/kg. Meanwhile for K, the highest concentration is observed in the leaves of *W. pinnata* and *X. luzonensis* (10196 mg/kg and 11032 mg/kg, respectively) but in the roots for *M. sundaicus* (4421 mg/kg). Apart from that, the ranges of concentration of trace elements such as Co and Mn in the samples collected from Serinsim Sub-station are low with value of 1 mg/kg to 188 mg/kg.

There are few potential reasons contributed to this phenomenon where identified Ni hyperaccumulator does not show significant Ni accumulation in ultramafic soils such as adaptation to soil conditions, variability of genetic, competition with other elements, stages of plant growth as well as pH and chemical properties of soil [28], [31], [32]. The identification of hyperaccumulator plants often involves studying their performance under specific conditions and not all identified hyperaccumulators may show the same response across diverse soil types. Thus, soil chemistry plays an important role in the phenomenon of Ni hyperaccumulation by plants, particularly in ultramafic soils. Several factors influence this complex interaction which includes soil pH, nutrient availability and the presence of competing elements.

Na is generally considered as a non-essential element for most plants, but it can play a role in osmotic regulation and nutrient transport [33]. In the context of Ni hyperaccumulators, Na may impact the plant's ability to cope with salinity stress and influence the overall ionic balance within plant tissues [34]. Some studies suggest that Na can compete with K for uptake, potentially affecting the accumulation of both elements in hyperaccumulators [35], [36]. Apart from that, Ca is crucial for various physiological processes in plants, including cell wall stability and signaling [37]. In Ni hyperaccumulators, Ca can influence the uptake and translocation of Ni. For instance, studies have shown that higher Ca concentrations can enhance the tolerance of plants to Ni toxicity by stabilizing cellular structures and mitigating oxidative stress caused by heavy metal accumulation [35], [38]

Elemental Concentration in Samples from Monggis Sub-station

From Table 4, it is shown that the concentration of Ni in the leaf of *R. bengalensis* is the highest which is 13196 mg/kg followed by *R. javanica, X. luzonensis* and *W. pinnata* with Ni concentration 5179 mg/kg, 3315 mg/kg and 1591 mg/kg, respectively. The concentration of Na is the highest in the root of all hyperaccumulators which are *W. pinnata, X. luzonensis, M. sundaicus, R. bengalensis* and *R. javanica* with the concentration of 554 mg/kg, 221 mg/kg, 736 mg/kg, 172 mg/kg and 998 mg/kg, respectively. The concentration of Ca is also the highest in the leaf of all hyperaccumulators in the range of 8858 mg/kg to 20,797 mg/kg. Lastly, the concentration of K is the highest in all leaf in the range of 5724 mg/kg to 18,990 mg/kg except for *M. sundaicus* which is in the stem with the value of 1724 mg/kg. On the other hand, the concentration of trace elements such as Co and Mn in all parts of Ni hyperaccumulators collected from Monggis Sub-station is low in the range of 6 mg/kg to 403 mg/kg. However, high concentration of Mn is observed in the roots of *R. bengalensis* with value of 1302 mg/kg. Most of the findings are in agreement with the past studies where concentration of Ca, Na and K is the highest in the leaf, root and stem, respectively (Neugebauer *et al*., 2022; Nkrumah *et al.,* 2018; van der Ent *et al.,* 2017; van der Ent *et al*., 2018)

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Table 3. Elemental Concentration in Samples from Serinsim Sub-station

Species	Metals	Soil (mg/kg)	Leaf (mg/kg)	Stem (mg/kg)	Root (mg/kg)
Walsura pinnata	Na	443±148	50±2	48±14	374±76
	Ca	5077±277	5625±1734	4193±5146	7923±2567
	K	130±42	10,196±4127	8607±4728	1167±108
	Ni	952±661	157±59	26±12	113±37
	Co	347±81	29±35	18±15	43±28
	Mn	290±93	14±9	11±4	99±13
Xylosma luzonensis	Na	333±82	105±13	92±7	292±53
	Ca	4963±457	15,810±654	1177±365	8845±4658
	K	162±87	11,032±840	4191±1087	3422±984
	Ni	972±205	2896±395	936±850	19±7
	Co	230±71	5±5	7±8	16±13
	Mn	236±106	98±20	30±19	188±11
Mischocarpus sundaicus	Na	206±30	79±13	43±9	404±36
	Ca	4873±278	16,557±62	8091±617	4488±702
	K	88±59	4144±4802	1048±125	4421±410
	Ni	1123±141	2934±3191	65±19	84±144
	Co	426±69	14±13	1±1	7±9
	Mn	506±244	24±21	7±2	171±4

R. bengalensis fulfil the criterion of a hypernickelophore where the accumulation of Ni exceed 10,000 mg/kg in the leaf but *M. sundaicus* only accumulates 663 mg/kg of Ni in its leaf which does not exceed the historical threshold level for Ni hyperaccumulating plants. However, referring to the new threshold value for Ni hyperaccumulators in study conducted by Purwadi *et al*. (2023) which is 280 mg/kg, indicating *M. sundaicus* as a Ni hyperaccumulator.Factors such as soil conditions, metal bioavailability, and soil heterogeneity could contribute to this failure. *M. sundaicus's* evolutionary history may have led it to adopt strategies for surviving in these soils without hyperaccumulating metals [40]. This could be due to tolerance of elevated Ni levels in roots or surrounding soil without translocating large amounts to leaves. Some plants, like *Noccaea caerulescens*, store metals in roots to reduce shoot toxicity [41]. The failure to meet hypernickelophore criteria could reflect a different ecological adaptation or a more conservative approach to metal uptake. Genetic constraints and environmental factors may also limit *M. sundaicus's* ability to express the high translocation ability required for Ni hyperaccumulation. Environmental factors such as competition from other plant species for resources could also reduce the available Ni for *M. sundaicus* to accumulate.

The results from this study emphasises the potential of some plant species as Ni hyperaccumulators, however numerous restrictions must be recognised. The sample size for certain species was comparatively limited, perhaps constraining the generalizability of the findings to larger populations or varying environmental conditions. *G.* sp. 'bambangan' is recognized as a potential hyperaccumulator; nevertheless, additional research is necessary to validate its efficacy across broader geographic regions and other ultramafic soil types. The study concentrated on a restricted number of sample locations, thus introducing biases that could influence data interpretation. Variability in soil conditions and climatic elements across various places may result in disparities in nickel uptake efficiency, necessitating more research to investigate the consistency of these findings in alternative sites.

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Table 4. Elemental Concentration in Samples from Monggis Sub-station

Species	Metals	Soil (mg/kg)	Leaf (mg/kg)	Stem (mg/kg)	Root (mg/kg)
Walsura pinnata	Na	328±140	75±5	67±8	554±97
	Ca	3223±215	8858±1029	5269±1101	5439±980
	K	77±56	18,990±2132	12,934±2062	1168±59
	Ni	1356±144	1591±381	516±1	133±26
	Co	238±47	21±18	14±22	45±13
	Mn	56±21	119±16	10±5	90±8
Xylosma lusonensis	Na	137±53	83±14	62±8	221±25
	Ca	4515±1116	20,797±4397	2140±423	5139±2038
	K	137±76	17,438±221	6542±345	3249±877
	Ni	1448±138	3315±332	370±1	162±64
	Co	130±98	6±1	22±37	32±18
	Mn	1132±2	20±15	BLD	160±4
Mischocarpus sundaicus	Na	270±145	93±6	52±11	736±67
	Ca	3461±316	9129±213	7140±557	6805±2674
	K	51±29	992±272	1724±187	318±16
	Ni	1889±115	663±78	224±1	503±332
	Co	357±122	18±15	16±19	14±7
	Mn	184±52	16±7	8±4	201±10
Rinorea bengalensis	Na	114±25	80±9	51±11	172±38
	Ca	2495±1641	11,451±3986	6189±783	4493±276
	K	164±68	5724±841	3705±148	600±648
	Ni	2465±705	13,196±5045	6628±12	1142±910
	Co	937±805	21±28	44±42	149±35
	Mn	1335±564	182±6	10±4	1302±581
Rinorea javanica	Na	259±70	80±4	74±7	998±288
	Ca	5307±622	14921±491	6267±1764	6284±1742
	K	94±89	7140±243	2400±553	1418±89
	Ni	2450±405	5179±3142	4593±16	963±481
	Co	643±211	30±20	14±21	27±18
	Mn	1160±85	403±62	40±14	221±14

*BLD: below limit detection

Correlation between Accumulation of Nickel and Concentration of Macronutrients and Trace Elements

The correlation analysis reveals a moderate positive relationship between the log-transformed concentrations of Ca and Ni with r-value of 0.453 and a p-value of 0.04, indicating that as Ni concentration increases, Ca concentration also tends to rise. In contrast, the relationship between log-transformed concentrations of Ni and K shows a weak to moderate positive correlation (r-value = 0.266), but the p-value of 0.10 indicates that this correlation is not statistically significant at the 0.05 level. Lastly, the analysis of the relationship between Ni and Na reveals a weak negative correlation (r-value = -0.251) with a p-value of 0.123, suggesting that higher Ni concentrations might be associated with lower Na levels. However, since the p-value exceeds the significance threshold of 0.05, this correlation is also not statistically significant, indicating insufficient evidence to establish a meaningful relationship between Ni and Na concentrations in the dataset.

The correlation between Ni and Ca is statistically significant aligning with findings by van der Ent and Mulligan (2015) which noted a similar positive correlation in two Ni hyperaccumulator species which are *P*. cf. *securinegoides* and *R. bengalensis.* Consequently, there is insufficient evidence to claim a

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meaningful relationship between Ni and K concentrations in this dataset and van der Ent and Mulligan (2015) did not report on this correlation due to its lack of significance.

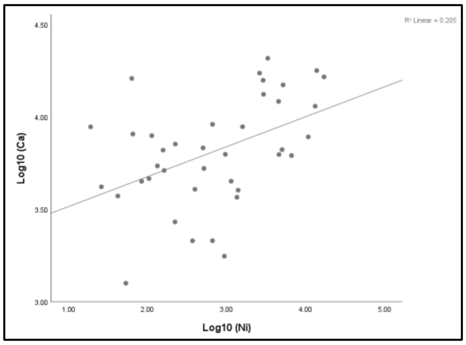

Figure 1 Correlation between Ni and Ca

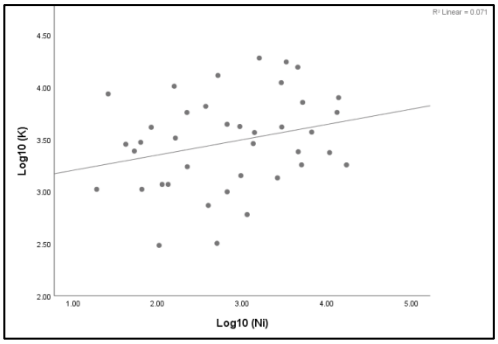

Figure 2 Correlation between Ni and K

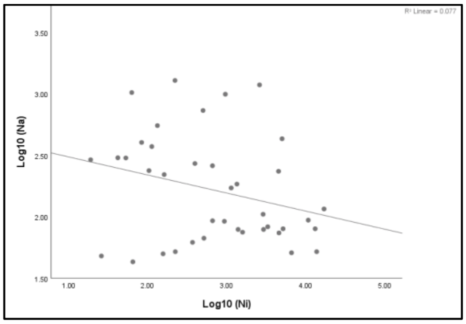

Figure 3 Correlation between Ni and Na

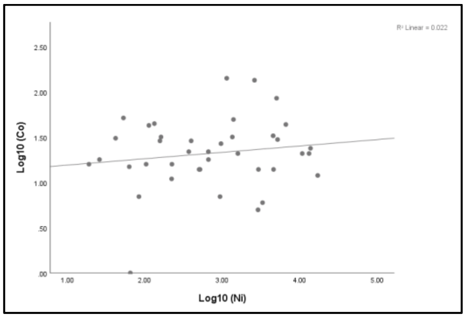

Figure 4 Correlation between Ni and Co

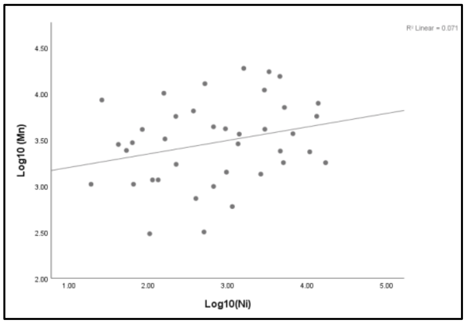

Figure 5 Correlation between Ni and Mn

Figure 4 shows a very weak positive relationship between the log-transformed concentrations of Ni and Co, with r-value of 0.073. The p-value of 0.659 indicates that this weak correlation is not statistically significant, suggesting there is no strong evidence for a meaningful relationship between Ni and Co in the dataset. Besides, Figure 5 illustrates the correlation between the log-transformed concentrations of Ni and Mn, showing r-value of 0.266 and p-value of 0.10. This indicates a weak positive relationship which suggests higher concentrations of Ni may be slightly associated with higher concentrations of Mn, but the relationship is not strong. The p-value of 0.10 indicates that this correlation is not statistically significant at the 0.05 level.

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The correlation of Ni and Co is consistent with the study by Mizuno and Kirihata (2016), which reported a positive correlation between Co and Ni with r-value of 0.299. In contrast, research by Jasmina *et al.* (2019) on *Noccaea kovatsii* from the Balkan Peninsula found a negative correlation between Ni and Co which was statistically significant (r-value = -0.01, p-value < 0.05). The differences in findings might be attributed to the differing climates, as Malaysia is located in a tropical climate while the Balkan Peninsula has a temperate climate. The correlation between Ni and Mn in this study aligns with Mizuno and Kirihata (2016) who also reported a positive correlation between Ni and Mn in plants from ultramafic areas in Sugashima Island, Japan with r-value of 0.042 and p-value of 0.722.

BAF and TF of Ni in Samples

Table 5 presents the Ni concentrations along with the TF and BAF for various plant species collected from different ultramafic areas in Sabah. Notable hyperaccumulators such as *G.* sp. 'bambangan', *R. javanica* and *R. bengalensis* exhibit significant Ni accumulation with high BAF values indicating their potential for effective Ni uptake. For example, *G.* sp. 'bambangan' has a BAF of 10.20 in its leaf, demonstrating its strong ability to accumulate Ni from the soil. Similarly, *R. javanica* shows an impressive BAF of 13.75, reflecting its efficiency in translocating and accumulating Ni in its aboveground tissues. These species can extract and store substantial amounts of Ni which makes them valuable for phytoremediation efforts in contaminated soils. In contrast, species like *W. pinnata* and *X. luzonensis* have lower TF and BAF values. This demonstrates the less effectiveness in Ni translocation and accumulation. For instance, *W. pinnata* has a BAF of only 0.19, suggesting minimal bioaccumulation relative to soil concentrations. This indicates that while these plants can take up Ni, they do not accumulate it effectively.

The accumulation of Ni in these plants could be influenced by several factors, including soil chemistry and composition, specific plant adaptations, and genetic variations [3], [44]. The chemical makeup of ultramafic soils affects the availability of Ni for uptake such as high soil pH or the presence of other metals can reduce Ni solubility and bioavailability to plants. In some cases, Ni may become less accessible due to the formation of insoluble complexes or interactions with other elements. The concentration of accessible Ni in soil can fluctuate based on soil pH, organic matter and clay content in which influence the uptake and accumulation of Ni by plants [45]. Ultramafic soils typically exhibit low fertility, which can hinder plant growth and may not consistently correspond with elevated Ni concentrations in plants if other factors, such as nutrient imbalance or metal toxicity, restrict uptake.

Additionally, different hyperaccumulator species have evolved various physiological mechanisms for Ni uptake and tolerance. A species that effectively accumulates Ni in one environment may not perform as well in another due to differences in these mechanisms. For example, the ability to regulate Ni transport proteins or detoxify it can vary based on genetic and environmental factors [3]. The adaptation of plants to ultramafic soils includes distinct physiological and biochemical characteristics that assist in reducing the harmful impacts of metals such as Ni. Plants may exhibit mechanisms like metallothionein production or the synthesis of organic acids that chelate metals and mitigate their toxicity [28]. Furthermore, species adapted to ultramafic soils may exhibit modified root-to-shoot ratios or may perform selective root absorption, enabling roots to avoid specific metals at elevated concentrations [37]. These adaptive qualities may influence the variability in BAF and TF values [46].

Other than that, genetic diversity within hyperaccumulator species can lead to variability in their Ni accumulation capabilities where some individuals may be better adapted to specific soil conditions or possess enhanced accumulation traits. Plants have significant genetic diversity in their capacity to collect and transport metals. Populations of hyperaccumulators may exhibit variations in their metal uptake capability as a result of selective pressure over time, resulting in genetic divergence. Research on *Noccaea*

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caerulescens populations has shown that several genotypes preferentially collect greater metal concentrations in the shoots, whereas others exhibit higher accumulation in the roots, contingent upon their genetic composition [41]. This genetic variability is likely a contributing factor to the differences in BAF and TF values found both among species and within a single species across various sites.

Overall, these findings highlight the variability in Ni uptake and accumulation among different plant species. Selecting suitable hyperaccumulators is crucial for effective soil remediation and environmental management in ultramafic areas. The ability of certain species to significantly accumulate Ni could be harnessed for cleaning up contaminated soils and managing Ni pollution in these ecosystems.

Table 5. BAF and TF of Ni in Samples

	Species	**Ni (mg/kg)**				**TF**	**BAF**
		Soil	**Leaf**	**Stem**	**Root**		
Pig Hill	*Psychotria sarmentosa*	432	63	41	104	1	0.24
	Glochidion sp. 'bambangan	1194	10784	1396	301	40	10.20
Garas Hill	*Rinorea Javanica*	1101	13780	1349	5027	3	13.75
	Actephila alanbakeri	1133	4540	662	222	23	4.59
	Psychotria sarmentosa	2045	17085	42	2609	7	8.37
Serinsim Sub-station	*Walsura pinnata*	952	157	26	113	2	0.19
	Xylosma luzonensis	972	2896	936	19	203	3.94
	Mischocarpus sundaicus	1123	2934	65	84	36	2.67
Monggis Sub-station	*Walsura pinnata*	1356	1591	516	133	16	1.55
	Mischocarpus sundaicus	1889	663	224	503	2	0.47
	Rinorea bengalensis	2465	13196	6628	1142	17	8.04
	Rinorea javanica	2450	5179	4593	963	10	3.99
	Xylosma luzonensis	1448	3315	370	162	23	2.54

CONCLUSIONS

The concentration of macronutrients (Ca, K and Na) and trace elements (Ni, Co and Mn) were determined in 8 species of identified Ni hyperaccumulators which are *A. alanbakeri, X. luzonensis, M. sundaicus, W. pinnata, G.* sp. 'bambangan', *R. bengalensis, R. javanica and P. sarmentosa*. There are 4 hypernickelophores that have been discovered in this study which are *G.* sp. 'bambangan', *R. bengalensis, R. javanica and P. sarmentosa* with the accumulation of Ni as much as 10 784, 13 196, 13 780 and 17 085 mg/kg, respectively. Particularly noteworthy is the discovery of an undescribed taxon, *G.* sp. 'bambangan,' which is endemic to Sabah. The concentrations of Ni in these hypernickelophores, ranging from 10,784 mg/kg with TF = 40, BAF = 10.20 to an impressive 17,085 $\mu g g^{-1}$ with TF = 7, BAF = 8.37 in *P. sarmentosa*, underscore the potential of these plants for applications such as phytomining or phytoremediation. The results of this study possess significant significance for environmental management and economic growth in ultramafic regions of Malaysia. The identification of efficient Ni hyperaccumulators, including G. sp. 'bambangan' and R. javanica, may establish a basis for metal farming projects that focus on the growth of plants for metal extraction. Such projects could generate novel economic prospects, especially in rural or neglected areas where ultramafic soils are abundant. Moreover, incorporating these plants into regional environmental management strategies could alleviate soil contamination and promote sustainable land use practices. Local governments could mitigate the detrimental effects of Ni contamination by implementing frameworks for the phytoremediation of heavy metals, thereby fostering a more sustainable method for land reclamation and resource recovery.

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Further study on *G.* sp. 'bambangan' should be carried out as it is undescribed taxa endemic to Sabah. Field trials in ultramafic soils are needed to assess its effectiveness in real-world conditions. Genetic studies should be conducted to understand the mechanisms underlying Ni hyperaccumulation in *G.* sp. 'bambangan' and other species. Comparative studies with other hypernickelophores can provide insights into ecological adaptations and genetic factors contributing to hyperaccumulation. Large-scale use of *G.* sp. 'bambangan' in agromining or phytoremediation must be evaluated for environmental impact, including soil health and biodiversity. Long-term monitoring is necessary to prevent negative ecological consequences. In conclusion, further studies in both field and laboratory settings are crucial for unlocking *G.* sp. 'bambangan''s full potential for sustainable environmental management and resource recovery.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Nur Syafiqah Salim carried out the research, wrote and revised the article under supervision of ChM Dr Julenah Ag Nuddin assisted by Dr Anthony van der Ent. Encik Matsain Md Buang and Encik Sukaibin Sumail assisted samples collection at reserved forest under Sabah Parks. Prof. Guillaume Echevvaria gave the opportunity to collaborate with University of Lorraine, France in carrying out this study.

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