INDICATIVE ASSESSMENT OF Ni PHYTOMINING VIABILITY IN SERPENTINITE OUTCROP AT GINIGALPELESSA

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Degree of Master of Philosophy

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Thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy in Earth Resources Engineering

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DECLARATION OF THE SUPERVISORS

The above candidate has carried out research for the MPhil thesis under my supervision.

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Abstract

With the recent recognition of Ni as a critical metal, it is challenging to find alternative Ni resources and new extraction techniques to secure a stable supply of Ni. In this context, Ni phytomining has attracted widespread attention as an eco-friendly mining approach, which was especially developed to extract metal from low-grade metal resources. In phytomining, hyperaccumulator plants are used to recover Ni from Ni-rich low-grade soils such as serpentine. Ginigalpelessa is one of the largest serpentinite deposits in Sri Lanka, where the geology and serpentine toxicity are well-documented. However, limited approaches have been taken to study the potential of Ni phytomining in soil. Therefore, 31 locations were sampled and collected soil and rock to assess the Ni enrichment in the serpentine soil. The native plants were analyzed to identify hyperaccumulators for phytomining experiments. Though the total Ni grade in soil varied from 0.4-1.7 wt%, the low bioavailable fraction (1-4 wt%) makes it challenging to implement phytomining in the deposit. Hyperaccumulation assessment of native plants recognized *Apluda mutica* (*A. mutica*) as the best plant species for phytomining. During phytomining trials, the selected Ni accumulator species showed a strong negative correlation between hyperaccumulation and increasing soil treatments. *Crotalaria verrucosa* and *A. mutica* produced the highest Ni-rich bio-ores. The leaching assays were carried out with open burnt and incinerated bio-ores of *A. mutica* under different pulp densities (100 g/L and 200 g/L) and H_2SO_4 concentrations (1 mol/L and 5 mol/L). The highest leaching efficiency was observed as 59% in open burnt samples (under 100 g/L; 5 mol/L H_2SO_4). The high Ni-enriched locations (>1.5 wt%) in the deposit need to be assessed further for direct Ni mining while the remaining area can be developed for in-situ phytomining. The hyperaccumulators identified in the study can be used for soil remediation from Ni and Co-contaminated soils.

Keywords: Ni enrichment, Ni hyperaccumulators, bioconcentration factor, leaching efficiency

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LIST OF ABBREVIATIONS

Abbreviations	Description
EDTA	Ethylenediaminetetraacetic Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
REE	Rare Earth Elements
USGS	United State Geological Society
XRD	X-Ray Difraction

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

Global warming results from fossil fuel burning accelerate the transition of energy systems to clean energy (Suman et al., 2021; Ilankoon et al., 2022). During the heated debate at the Paris Agreement in 2015, it was highlighted as an alternative solution for fossil fuel burning to mitigate climate change (IFPEN, 2021). In this context, nickel (Ni) plays a key role in lithium-ion battery production which is used to power electronic vehicles and store renewable energy, such as solar and wind power (Kotal et al., 2022; Yang et al., 2023). Ni is a hard and ductile transition metal with atomic number 28, exhibiting a diverse array of chemical properties (USGS, 2023). As a transition metal, Ni provides high energy capacity, along with high conductivity and energy density, which improves the quality of the lithium-ion battery performance (Nuhu et al., 2023). Due to the increasing demand for Ni in clean energy transition and its wider variety of applications across the metal and mineral industries, Ni was recently recognized as a critical metal by the United States Geological Survey (USGS) (USGS, 2023). Currently, the Ni price in the global market is relatively high ($16,500 per ton) compared to other economically important metals like iron (Fe), aluminum (Al), copper (Cu), and zinc (Zn) ($132 per ton, $2,252 per ton, $8,553 per ton, and $8,490 per ton, respectively) (Marjolin, 2023).

The global Ni consumption is generally led by other Ni-based products, such as stainless steels, alloys, plating, and batteries. However, the increasing demand for batteries along with other Ni-based products has created a high demand for Ni for their production (Peters & Weil, 2016). Even though global Ni mining is carried out in high-grade deposits, the criticality of the steady Ni supply has urged the exploring of alternative sources of Ni in both primary and secondary phases, even in low-grade (Cerdeira-Pérez et al., 2019).

To date, many low-grade Ni resources, including ultramafic rock and soils, mine tailings, and galvanic sludge from industrial waste have exhibited the potential for Ni recovery (Tognacchini et al., 2020). However, conventional mining techniques sometimes would not be economically feasible to recover Ni from these low-grade Ni resources (Prior et al., 2012; Naidu et al., 2019). In addition, the conventional mining techniques are energy- and resource-intensive, thus requiring substantial site remediation at the end-of-life of the mining site and they already create huge environmental issues in high-grade ore mining (Jones et al., 2013; Ambaye et al., 2020). In this context, phytomining was introduced to the world as a plant-based eco-friendly mining approach, which is specially developed to recover metal from low-grade soils (Zhang et al., 2014; Li et al., 2020).

Ni phytomining uses hyperaccumulator plants as miners to recover Ni from mineralized soils or secondary deposits. These plants are often found naturally in Ni-enriched environments such as serpentine, which annually accumulate a sufficient quantity of Ni (more than 1000 mg/kg) in their upper ground biomass (Barbaroux et al., 2012; Tognacchini et al., 2020; Tripti et al., 2021). To date, more than 500 Ni hyperaccumulator species have been identified in Ni-rich serpentinite environments (Rosenkranz et al., 2019). Serpentine soils contain Ni grade in a range of >0.6 wt% which is considerably below the conventional mining grade (1.5-3.0 wt%), but sufficient to develop phytomining using Ni hyperaccumulator plants (Cerdeira-Pérez et al., 2019). Once harvested, these Ni hyperaccumulator plants are subjected to a series of pyrometallurgical and hydrometallurgical processes to extract commercially valuable pure Ni (Zhang et al., 2016) (Figure 1.1).

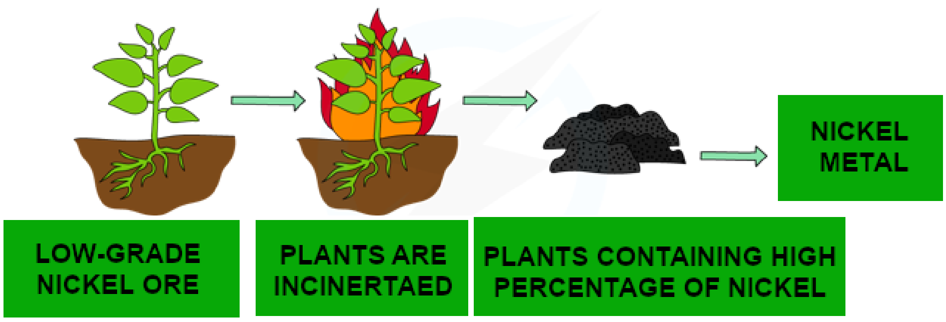

Figure 1.1 Simplified process of Ni phytomining and extraction from low-grade soils

In global phytomining studies, the ashes of *Alyssum murale* (*A. murale*) plant species grown in serpentine soil recover almost 12,000 mg/kg of Ni from leaching and produce Ni ammonium disulphate containing 13.2% of Ni (Barbaroux et al., 2012). Moreover, many studies have efficiently extracted and recovered a high concentration of Ni from different hyperaccumulator biomass emphasizing that Ni phytomining is a promising approach to contribute to the increasing demand (Bani et al., 2015b; Nkrumah et al., 2019; Li et al., 2020). Since these potential hyperaccumulator species like *A. murale* are found mostly under temperate climates and could be invasive when used in tropical regions, geobotanists are exploring native and indigenous Ni hyperaccumulators to implement Ni phytomining in tropical serpentinite regions (Galey et al., 2017; Reeves et al., 2018). The current trends in phytomining have shifted towards agromining, where high-biomass crops are grown in metal-rich fields to uptake a particular metal or group of metals (van der Ent et al., 2015; Li et al., 2020). These practices promote the value of the under-valued lands like serpentine and open up opportunities to gain economic benefits. Therefore, field preparation, enhancing the soil conditions, weed control, introducing proper harvesting methods, and optimizing the recovery of the metal from leaching experiments need to be focused on simultaneously to implement a successful phytomining or agromining approach in the serpentine soil (Nkrumah et al., 2021).

1.2 Research Problem

With the increasing demand for Ni in clean energy transition technology, Ni phytomining is currently implemented in low-grade serpentine soils where the Ni grade is >0.6 wt% (Norgate & Jahanshahi, 2010). In this context, Ginigalpelessa is one of the largest serpentinite bodies in Sri Lanka, which is enriched with high concentrations of Ni in the soil. Even though geobotanical studies have identified three Ni hyperaccumulators native to Ginigalpelessa and its surrounding serpentinite bodies, there have been no significant attempts to recover Ni using phytomining (Rajakaruna & Baker, 2004). In addition, the local communities have been experiencing health issues due to serpentine toxicity (health issues caused by high concentrations of Ni, chromium (Cr), and cobalt (Co)), and need to implement proper remediation measures to remove these heavy metals from soil (Vithanage et al., 2019; Dushyantha et al., 2021). The lack of studies on extracting Ni from the local serpentine soil and converting its toxicity into economic benefits requires proper experiments in both field and laboratory scales. Therefore, detailed studies using native and introduced hyperaccumulators are necessary to identify the feasibility of the phytomining method for extracting and recovering Ni from the Ginigalpelessa serpentine soil.

1.3 Objectives

1.3.1 Main objective

- To develop a sustainable phytomining technique to extract Ni and other valuable metals like Co and Cr from the serpentine soil in Ginigalpelessa using the best hyperaccumulator plant species available in Sri Lanka.

1.3.2 Specific Objectives

- To assess the Ni and other valuable metal hyperaccumulation among indigenous hyperaccumulators for phytomining at Ginigalpelessa serpentinite outcrop.
- To evaluate the influence of soil enrichment practices like fertilizers of selected hyperaccumulator plants on their phytomining efficiency.
- To leach out Ni and other valuable elements from bio-ore using lixiviants and assess the leaching parameters to recover Ni as a sustainable metal extraction method.

1.4 Scope and significance of the research

Ginigalpelessa serpentine soil was previously under-valued and disregarded for agricultural purposes, due to the poor nutrient content and the high Ni, Cr, Co, magnesium (Mg), and manganous (Mn) toxicity. The growing demand for Ni in the global market and the emergence of recovering Ni from low-grade Ni resources has given significant insight into promoting these underrated lands. Furthermore, successful field experiments with phytomining on a global scale have proved the capability of recovering Ni from serpentine soil. Even though this approach is new to

Sri Lanka, it is necessary to investigate the potential of Ni phytomining to support the country’s current economic situation. Meanwhile, this approach would reduce the toxicity effects in serpentine soil, and enhance the soil quality for agricultural purposes. Therefore, it is necessary to determine the phytomining potential in local serpentine soil. In addition, the study aims to assess the Ni and other metal enrichment in Ginigalpelessa serpentine soil by analyzing the mineralogy and metal concentrations in soil and rocks in terms of the economic potential of lateralized nickeliferous soils. This study would be the groundwork to establish Ni phytomining in local serpentine soils in Sri Lanka.

CHAPTER 2: LITERATURE REVIEW

2.1 The story of Nickel

Nickel (Ni) is known as the fifth most common element on Earth which occurs extensively in Earth's core and crust. The concentration of Ni in the earth's crust is 80 ppm whereas the earth's core is composed of 10% of Ni. Moreover, it is found in more than 100 minerals as a major constituent along with iron (Harasim & Filipek, 2015). Ni naturally occurs in soil, rock, and water and accumulates in plants as an micro component. Due to the transition properties of Ni, it has been employed in many industrial and commercial applications (Nickle Institute, 2016).

2.1.1 Applications of Ni

According to the recent publications of the United States Geological Survey (USGS), Ni has been recognized as a critical metal due to increased applications in a diverse range of end-user sectors, including engineering, transportation, architecture, building & construction, and especially in the clean energy industry (Nickel Institute, 2022; USGS, 2023). The unique physical and mechanical properties of this metal, such as formability, ductility, resistance to corrosion, and malleability have resulted in its use for the aforementioned wide spectrum of industrial applications (Figure 2.1). In addition, products containing Ni are usually recyclable, durable, and efficient in energy use (Nickle Institute, 2016). At present, approximately two-thirds of Ni mined in the world is used in producing stainless steel. In addition, Ni is widely used in producing non-ferrous alloys and alloy steels for specialized industrial, military, and aerospace applications. This metal has been used in fabricating turbine blades and discs for jet engines and electrical turbines used in power generation. Around 11% of Ni is used in producing electronic batteries and 8% is used for casting and plating (Figure 2.2). Moreover, it is used in the preparation of commercial chemicals like nickel carbonate, ($NiCO_3$), nickel chloride ($NiCl_2$), nickel oxide (NiO), and nickel sulfate ($NiSO_4$). Therefore, Ni is often used as a catalyst along with its alloys for hydrogenated reactions. However, among all of these applications, the value of Ni has increased with the emerging of clean energy transition technologies where Ni is used as a cathode material for batteries.

Field of Applications	Properties	Ni content (wt%)	
Stainless steel	• Good formability and ductility • High weldability • Good toughness • High melting point ~ 1453°C • Boiling point ~ 2913°C • Corrosion resistance • Chemically unreactive	*Type 304	8 - 10
		*Type 316	10 - 14
Cathode material of rechargeable batteries	• Recyclable • High energy density • High energy storage capacity	Lithium Nickel Cobalt Aluminum (NCA)	80
		Lithium Nickel Manganese Cobalt (NMC)	33
Alloys	• High temperature scaling • Shape memory • Low expansion • Good thermal conductivity 97.5 $Wm^{-1}K^{-1}$ • Corrosion resistance to CO_2 and H_2S • High melting point ~ 1453°C	Cu-Ni	2 - 45
		Ni-Cr-Fe	35 - 60
		Ni-Cr-Si	70 - 80
		Ni-Cr-Al	35 - 95
Plating	• Corrosion and wear resistant • Bright and attractive finish	Zn-Ni coating	12 – 15

Figure 2. 1 Properties and applications of Ni (Sources: Kamerud et al., 2013; Bai & Bai, 2018; Nickel Institute, 2022).

Note: *Type 304 and *Type 316 are the most common types of stainless steel produced in the metal industry.

2.2 Applications of Ni in clean energy technologies

Clean energy technologies emerged as a primary solution for global warming during the last decade to mitigate climate change, especially after the heated debate at the Paris Agreement on climate change (IFPEN, 2021). Therefore, it is necessary to increase the utilization of renewable energy and electric mobility to achieve climate neutrality while reducing the usage of fossil fuels as mitigation of climate change has become one of the main global environmental concerns in the coming century. It also contributes to achieve SDGs such as affordable and clean energy (Goal 7), industry, innovation, and infrastructure (Goal 9), and particularly climate action (Goal 13) (United Nations, 2016).

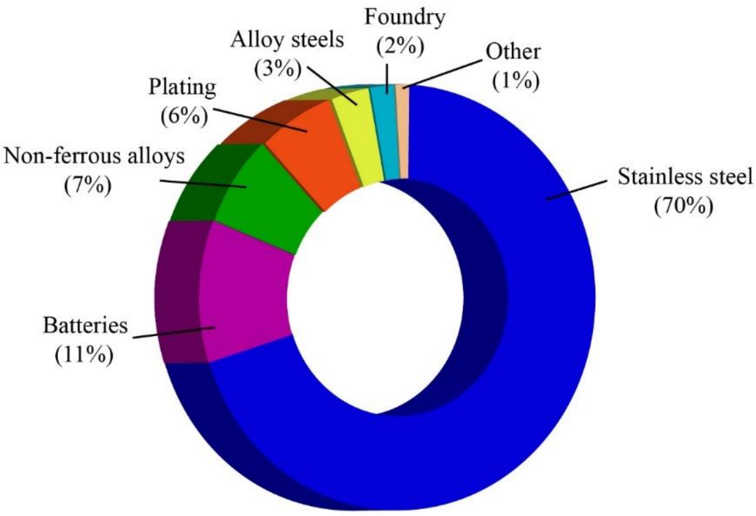

Figure 2. 2 Applications of nickel in the metal and mineral industry in 2021

Source: (Nickel Institute, 2022).

In this context, the metal industry has a great responsibility to support the zero carbon emission economy and, Ni with its unique properties plays a key role in the energy transition from fossil fuel to clean energy (Chordia et al., 2021; Nickel Institute, 2022). Significantly, the importance of Ni has been raised especially in the production of lithium-ion batteries for electrical vehicles.

2.2.1 Lithium-ion batteries

Ni has been used in the battery industry for a long time, particularly in the production of nickel-cadmium (NiCd) and rechargeable batteries (nickel metal hydride). During the mid-1990s, Lithium-ion batteries were developed with the inspiration of rechargeable batteries, and they were initially used for camcorders. The greater energy storage capacity of these batteries and the low manufacturing cost makes them beneficial in the power and energy sector (Gaines et al., 2011; Väyrynen & Salminen, 2012; Nickel Institute, 2022).

Among different lithium-ion batteries in the world, Nickel Manganese Cobalt and Nickel Cobalt Aluminum highly rely on Ni (33 wt% and 80 wt% of Ni, respectively). To date, Lithium-ion batteries have been incorporated into electrical vehicles and the demand for these batteries has increased over recent years due to the improved battery capacity that enables traveling over long distances. The use of Ni in these batteries enhances their energy density, which reduces the battery size. Furthermore, these Ni-containing batteries can be recharged and reused (Nickel Institute, 2022). Although electric vehicles account only for a small market share according to current estimations, their demand will rapidly increase in the future in global automobile stock with the clean energy transition.

The emerging of renewable energy, such as solar and wind for power generation has increased the need for energy storage. In this context, Li-ion batteries have become a dominant technology where the high storage capacity can be deployed in storing such energy resources and released when necessary (Peters & Weil, 2016). To date, countries in Asia, Europe, and the United States have invested billions in renewable energy and the global suppliers of Li-ion batteries have developed their production (Nickel Institute, 2022).

2.3 Ni production, consumption, and demand

2.3.1 Ni production

Over the last few decades, global Ni production has undergone significant fluctuations due to various factors such as economic instability, pandemic events (e.g. recent Covid-19 pandemic), and decreasing demand for Ni in the metal industry (Apostolikas et al., 2009; Fiscor, 2022). Since the 1980s, the global mining and production of Ni were highly dominated by Russia and Canada, and this has significantly changed over the last two decades. According to the estimations of the

USGS, the global market for Ni is currently dominated by Southeast Asian countries like Indonesia (36%) and the Philippines (13%) (USGS, 2023) (Figure 2.3).

The use of Ni in the metal industry varies as class 1 and class 2, according to its degree of purity. Class 1 has 99.98% Ni which is used in producing cathodes, briquettes, granules, and powders. Pure Ni is mostly extracted from sulfide ores and 55% of global productions are dependent on this category. Currently, class 1 Ni is highly used for the manufacturing of electronic batteries. Class 2 Ni products mainly comprise ferronickel and Ni pig iron. Nevertheless the purity, still a higher percentage of Ni is used from the class 2 category for producing stainless steels, as it contains a high amount of iron (Campagnol et al., 2017; IFPEN, 2021).

Ni extraction and processing have also undergone similar changes compared to the mining of raw materials. During the 1990s, Ni smelting and refining was dominated by Russia, Canada, and many European countries. However, over the last two decades, their influence on Ni recovery has gradually declined with the rise of Ni production in Asian countries. Moreover, in some countries, such as Finland, Greece, South Africa, Colombia, and Madagascar, Ni production has been reduced below the expected levels of USGS Ni commodity data, especially from 2020-2023 (USGS, 2023).

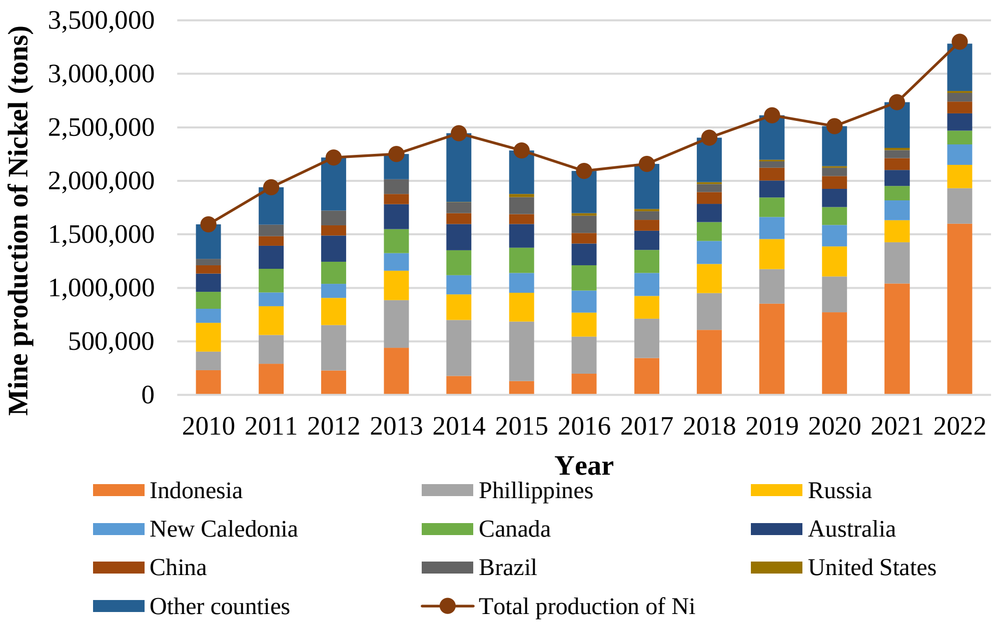

Figure 2. 3 Global Ni production from (2010-2022)

Source: (USGS, 2023)

2.3.2 Ni consumption and demand

Due to the rising Ni production in the Asian region, Ni consumption has also undergone a few alterations. In 2017, nearly 75% of Ni was consumed by Asian countries while Europe accounted for only 16%. During the 1990s, the demand for Ni was almost similar in both Europe (38%) and Asia (37%). To date, China has been the world's leading Ni-consuming country which owns 31.2% of Ni products in the global market followed by Indonesia and Japan (IFPEN, 2021). Furthermore, the extraordinary demand for Ni in China and their dominance in stainless steel production (owned 52% market share by 2015) is the reason for the rapid transformation of Ni consumption in the world over the past 20 years (Reck & Rotter, 2012).

With the introduction of clean energy transition technologies in the 21st century, the significance of Ni-bearing batteries increased, which in turn accelerates the demand for materials and metals required in battery production (Peters & Weil, 2016). In addition, the evolvement of modern electric vehicles encourages automakers to develop alternative battery chemistries to enhance efficiency in energy storage (Barkenbus, 2020). For example, Ni consumption in the cathode of lithium-ion batteries is currently 8% and expected to rise by 80% in the future. Furthermore, existing Ni-based batteries lead slightly ahead of iron-based batteries in the global market due to their high energy densities (Casey, 2021; Stover, 2022). Therefore, it is predicted that global demand for Ni will increase significantly up to 41% in 2040 with the expected elevation in battery use (Alvial-Hein et al., 2021; Jordan, 2022) (Figure 2.4). Moreover, these estimations claim that Ni demand for electronic batteries will grow further compared to the stainless steel industry, where the estimated Ni requirement increases only by 5% per year (Casey, 2021). In this regard, the growing demand for Ni for electronic batteries will make huge pressure on the global supply chain for the sustainability of the future metal industry.

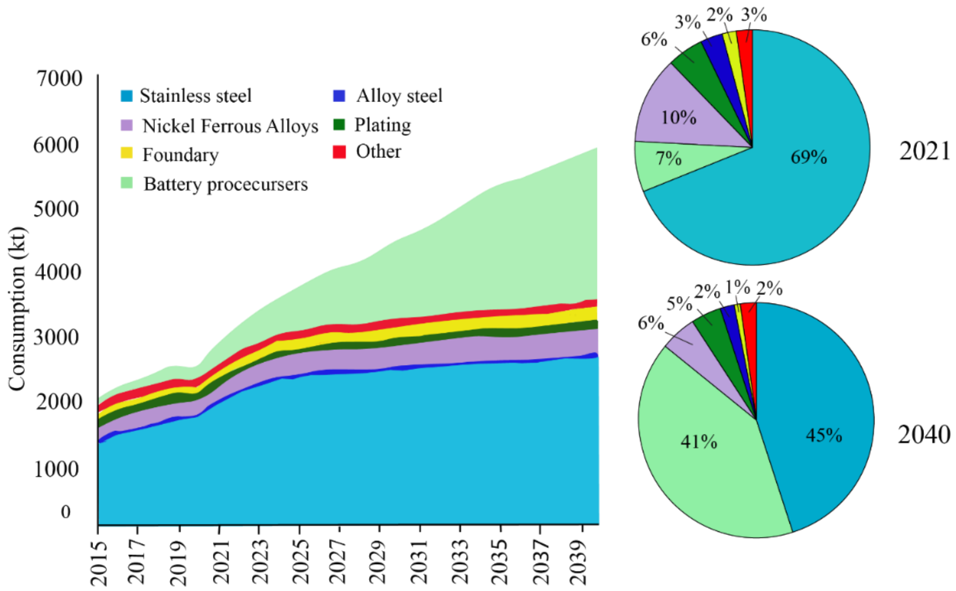

Figure 2. 4 Predicted demand for Ni by 2040 (Mitchell & Pickens, 2022).

2.4 Ni occurrences and their global distribution

2.4.1 Global distribution of Ni occurrences

Being one of the most common elements, Ni can be found in approximately 627 Ni-bearing deposits worldwide, accounting for 300 million tons of Ni. They are mostly disseminated in the world's leading Ni-producing countries such as Indonesia (22.4%), Australia (21.3%), Brazil (17%), Russia (7.3%), Cuba (5.9%), and the Philippines (5.1%) (Mudd & Jowitt, 2022; Nickel Institute, 2022) (Figure 2.5). Primarily, laterite (60%) and Ni-sulfide (40%) deposits are considered as the major high-grade sources of Ni (USGS, 2023). Laterite deposits are commonly found in Southeast Asia, Australia, and South America, whereas Ni-sulfide deposits mainly occur in Russia, Canada, Australia, and Africa. Moreover, there are a few other Ni-bearing mineral deposits including hydrothermal-related and magmatic Ti-V-Fe oxide deposits where the Ni concentration is relatively low (Mudd & Jowitt, 2014; Subasinghe et al., 2022). In addition to terrestrial Ni resources, several other sources have also been identified in the deep sea with a significant content of Ni. However, deep-sea mining for Ni is still not implemented in the world (BGS, 2008; Petersen et al., 2016).

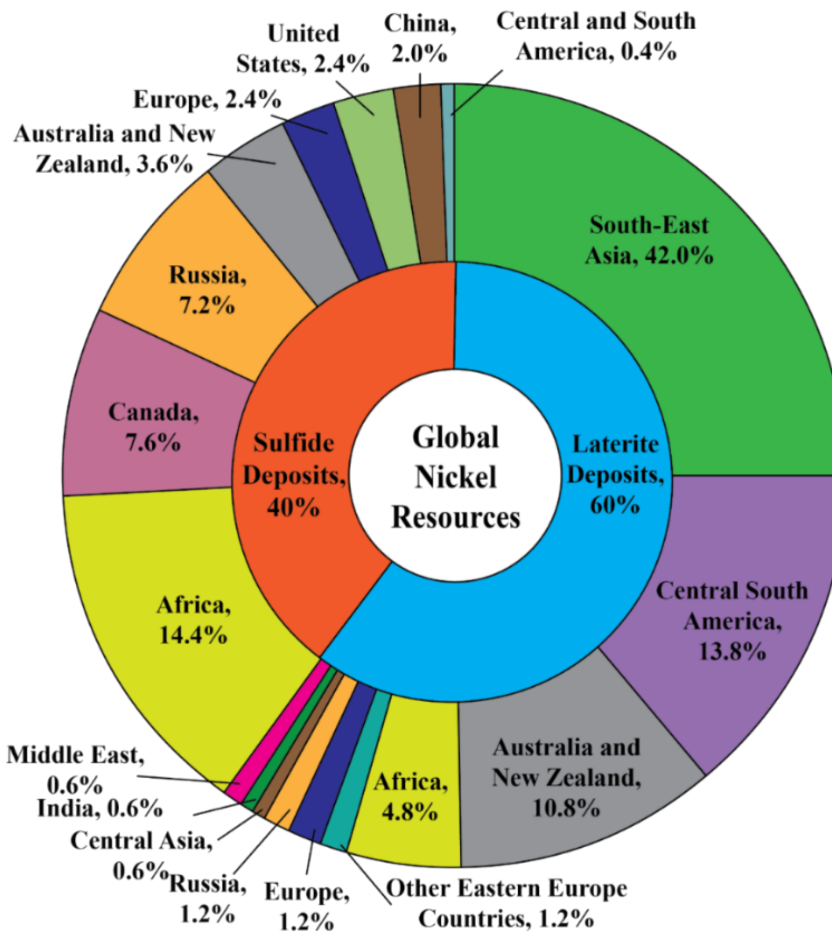

Figure 2. 5 Global distribution of Ni resources based on deposit types

Source: (IFPEN, 2021)

2.4.2 Ni-bearing minerals

Ni is a transition element that is naturally abundant in both ferrous and non-ferrous (sulfide) environments. As a result of the intensive weathering of ultramafic rock, the iron-rich laterite deposits contain Ni-bearing ore minerals, such as nickeliferous limonite [(Fe, Ni) O(OH)], smectite (Ni-bearing clay silicates, such as nontronite and Fe-montmorillonite), and garnierite (hydrous Mg-Ni-silicates). Furthermore, the Ni-sulfide deposits found in volcanic and plutonic settings contain pentlandite [$(Ni, Fe)_9S_8$] as the principal ore mineral of Ni (Wells et al., 2009; USGS, 2023). Ni can also be found to a minor degree in violarite (Ni_2FeS_4) and pyrrhotite

(Fe_7S_8). To date, about 173 Ni-bearing minerals have been identified in different geographical regions in the world with different modes of occurrence (Table 2.1).

Table 2. 1 The economically important Ni-bearing minerals and their mode of occurrence in the world

Mineral name	**Mineral Group**	**Formula**	**The most common modes of occurrences**
Pentlandite	Sulfide	$(Fe, Ni)_9S_8$	In mafic intrusions or remobilized phase after metamorphism
Pyrrhotite (formed by intergrown with pentlandite)	Sulfide	$Fe_{1-x}S_x$	In mafic intrusions
Garnierite	Hydrous nickel silicate (Serpentine)	$(NiMg)_3Si_2O_5(OH)_4$	In laterites related to ultramafic rocks
Nickeliferous limonite	Hydroxide	$(Fe, Ni)O(OH)$	In laterites related to ultramafic rocks
Millerite	Sulfide	NiS	Mafic intrusion where metasomatism has remobilized Ni and S from pentlandite; also from metamorphism of olivine.
Niccolite	Nickel arsenide	NiAs	Hydrothermal replacement of pentlandite in mafic intrusions or metasomatism of low-Sulphur mafic rocks. In seafloor manganese nodules.
Nickeliferous goethite	Hydrated oxide	$(Fe, Ni)O(OH)$	In laterites related to ultramafic rocks
Siegenite	Sulfide	$(Ni, Co)_3S_4$	Hydrothermal veins

Source: (BGS, 2008)

2.5 Major Ni deposits in the world

2.5.1 Ni laterite deposits

The origin of laterite deposits is associated with the prolonged weathering conditions in Mg-rich ultramafic rocks. Most of the Ni laterites are formed from ophiolitic ultramafic rocks, such as peridotites, harzburgites, and dunites, while few unique deposits formed from komatiite rocks or olivine-rich serpentinite rocks (Kamenetsky et al., 2016). These rocks contain primarily 0.2-0.4 wt% of Ni (Elias, 2002). Lateritization generally involves the breaking of major minerals, the leaching of mobile elements, the enrichment of immobile elements, and the formation of new and stable minerals under weathering conditions (Gleeson et al., 2003). The weathered materials formed during the lateritization then overlay as successive layers on the parent ultramafic rock which is known as the laterite profile (Elias, 2002; Putzolu et al., 2020; Ito et al., 2021). As illustrated in Figure 2.6a, the saprolite layer is highly enriched with Ni ranging from 1.5-3 wt%. The Ni laterite deposits are mainly found in the tropical belt, where warm and humid climates accelerate the weathering conditions (Butt & Cluzel, 2013).

There are mainly three types of Ni-bearing laterite deposits, (i) hydrous Mg-silicate laterites with Ni-rich minerals namely, serpentine, chlorite, sepiolite, garnierite, and talc, (ii) clay silicate laterites with nontronite and saponite smectites, and (iii) oxide laterites where Ni is substituted or adsorbed onto goethite. Among them, hydrous Mg-silicate laterites are the most dominant Ni-bearing deposits, which also host the highest Ni grades (1.8-2.5 wt%) (Golightly, 2010). According to the present database, approximately 224 in-ground laterite deposits with an overall 178.1 million tons of Ni have been identified and documented in the world (Mudd & Jowitt, 2022).

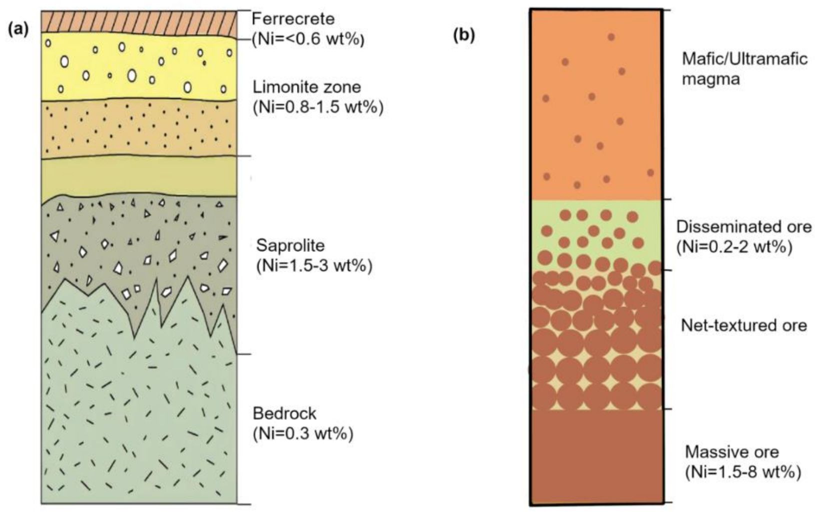

Figure 2. 6 (a) Schematic cross-section of the laterite profile developed on the ultramafic rock; (b) Vertical gradation of a Ni-sulfide deposit

Source: (Foose et al., 1986; Ashcroft, 2019)

Generally, laterite deposits are rich in aluminum, iron, and magnesium, along with high Ni concentrations (Villanova-de-Benavent et al., 2017). To date, Ni mining is carried out in high-grade laterite deposits, such as Sorowako in Indonesia, Goro in New Caledonia, and Murrin Murrin in Australia, where the Ni concentration varies from 1-1.5% (Wang, 2016). In addition to Ni, these deposits host other critical metals, such as rare earth elements (REEs), platinum-group elements (PGE), Sc and Co which are economically important in the metal and mineral industry (Aiglsperger et al., 2019; Batapola et al., 2020).

2.5.2 Magmatic Ni-sulfide deposits

Magmatic Ni-sulfide deposits are derived from the segregation of immiscible sulfide liquids originating from mafic or ultramafic silicate magmas (Barnes et al., 2017). During the formation, immiscible sulfide droplets settle through the low-dense silica magma (Figure 2.6b) (Foose et al., 1986). Typical Ni-sulfide deposits are mainly associated with ultramafic or mafic igneous rocks, which may vary according to their geological settings. In this context, magmatic Ni-sulfide deposits are subdivided as (i) Archean komatiite, (ii) Proterozoic komatiite, (iii) flood basalt-related, (iv) layered intrusion, (v) Alaskan-type deposit, (vi) PGE-discordant intrusion, (vii) Magmatic feeder, (viii) intrusion-related and (ix) meteorite impact melt-related (Hoatson et al., 2006; Naldrett, 2010). However, all these deposits are commonly called magmatic sulfide deposits since they result from similar genesis (Foose et al., 1986).

Ni-bearing sulfide ores are mostly found in association with copper-bearing ores. They contain approximately 10 wt% of Ni with other major elements, such as Cu, Fe, and S (2 wt%, 40 wt%, and 30 wt%, respectively) (Wang et al., 2022). Moreover, magmatic sulfide deposits are often considered as represent sources for PGE along with Ni and Cu, and they are split as sulfur-rich Ni-Cu-PGE deposits and sulfur-poor PGE-dominated deposits. To date, 253 in-ground magmatic sulfide deposits have been explored in the world with a total of 118 million tons of Ni (Mudd & Jowitt, 2022). Out of them, the most commercially important Ni-bearing deposits are found in association with the Archean komatiites rocks, such as Raglan in Canada (Hoatson et al., 2006).

2.6 Secondary sources of Ni

The expected surging demand for Ni and the shortage of Ni in high-grade Ni ores have encouraged metal mining industries to explore previously untapped Ni deposits throughout the world. In this context, low-grade Ni resources and economically unattractive deposits have been identified to determine as potential alternative sources of Ni. Hence, in the future, Ni mining industries expect to utilize both primary and secondary sources to recover Ni as a sustainable solution (Meshram et al., 2019; Mudd & Jowitt, 2022).

Until the 21st century, only high-grade ores of Ni were mined, whereas mining of low-grade ores was considered economically impractical. In general, Ni-ores should contain at least 3% (30,000 mg/kg) of Ni to mine economically using traditional mining techniques (Novo et al., 2017). However, at present, even ores enriched in 0.1% of Ni, which is presented in industrial residues and mine waste, are being used for mining due to the depletion of high-grade ores. Furthermore, field trials have ensured that the recovery of Ni from low-grade sources, such as mine tailings, galvanic sludge, electronic waste of rechargeable batteries from industrial wastes, and ultramafic soils is possible mainly due to the accelerating demand for Ni (Tognacchini et al., 2020).

2.6.1 Mine tailings

Conventional mining operations in the world daily generate substantial quantities of tailings after recovering their primary ore minerals or metals (Sirkeci et al., 2006; Dushyantha et al., 2023). Even though they are regarded as the uneconomic fraction of the ore, releasing such tailings into the environment may cause adverse effects on humans and the ecosystem since they may contain harmful trace metals and production chemicals (Pakhomova et al., 2021). According to recent estimations, the global metal mines annually generate 282.5 billion tons of waste with valuable metals like Cu, Au, Fe, Pb/Zn, and Ni, where they contribute to the global tailing by 46 wt%, 21 wt%, 9 wt%, 3 wt%, and 2 wt%, respectively (LePan, 2021). With the increasing demand for valuable metals and sustainable waste management in the mining industry, it is necessary to develop feasible flowsheets to recover metals from mine tailings as an alternative low-grade source for metals (Peek et al., 2011).

Limnotic laterites contain low concentrations of Ni (1-1.6 wt%) and high concentrations of Fe (van der Ent et al., 2013). Therefore, a high concentration of sulfuric acid is required to leach out the Ni in the hydrometallurgical process. In this regard, Ni-containing limonitic laterite ores are often refined using a hydrometallurgical process with high pressure and temperature (autoclaved at 200-300˚C temperature) to reduce acid consumption (Norgate & Jahanshahi, 2010). Table 2.2 indicates example studies of the Ni recovery from mine tailings.

2.6.2 Galvanic sludge

Galvanic sludge is an effluent formed during the wastewater treatment process of Ni/Cr plating industries (Vilarinho et al., 2012). It is a polymetallic sludge which contains valuable metals like Ni, Cu, and Zn (Silva et al., 2005a). Due to the high concentration of these heavy metals, releasing galvanic waste into the environment creates severe ecological impacts. Thus, they are usually disposed of in landfills to overcome the issue (Silva et al., 2005a; Tognacchini et al., 2020). In recent years with the rising metal demand, economically important metals, such as Ni, Cu, and Zn in galvanic sludge are being recovered using hydrometallurgical techniques, most commonly with sulfuric acid leaching method (Silva et al., 2005b; Vilarinho et al., 2012; Tognacchini et al., 2020). Furthermore, countries like Portugal where Ni/Cr plating plants are well-functioned, have been already studying the recovery of Ni from this sulfuric leachate via solvent extraction method (Table 2.2).

Even though galvanic sludge is rich in Ni, most of the existing nickel recovery processes are not economically feasible due to high capital costs and energy consumption. Therefore, present studies are focused on environmentally friendly and plant-based metal recovery methods, such as phytomining. In this context, a phytomining study revealed that the *Odontarrhena chalcidica* plant was capable of accumulating more than 1 wt% of Ni from its shoot biomass (>1000 mg/kg of Ni), when they grew in galvanic sludge mixed soil (Tognacchini et al., 2020).

2.6.3 Electronic waste of batteries

Due to the high demand and consumption of battery-based products, nearly 500,000 tons of lithium-ion battery waste are generated annually (Baum et al., 2022). In order to manage the environmental regulations for the safe disposal of electronic waste, electric vehicle batteries are recycled in many parts of the world, especially in countries like China, South Korea, the United States, Belgium, Australia, and Germany (Espinosa et al., 2004; Joulié et al., 2014; Ian Tiseo, 2022). Moreover, as an important component in lithium-ion battery cathodes, studies have estimated that these batteries contain higher concentrations of Ni than in primary ore bodies, and therefore, the battery can be recycled up to 100 wt% (Wang et al., 2022).

The recovery of Ni from waste batteries is conducted through hydrometallurgical or pyrometallurgical processes. In the pyrometallurgical process, metal oxides in waste batteries are reduced under high temperatures. Even though this method is simple, it requires high energy consumption and recovers a low amount of Ni compared to the hydrometallurgical process. Therefore, the hydrometallurgical process using different inorganic acids (H_2SO_4, HNO_3, and HCl) and organic acids is considered more efficient for Ni leaching and recovery (Wang et al., 2022) (see Table 2.2). Espinosa et al. (2004) found that recycling Ni-Cd battery plates could recover more than 2 kg of Ni from each plate. Furthermore, some Ni-metal hydride batteries (Ni-NM) contain other valuable metals, such as Co, Mn, Zn and REEs (mostly Ce, La, Nd and Pr), from which 100 wt% Co and <99 wt% of total REE could be leached along with a 96 wt% of Ni (Wang et al., 2022).

Table 2. 2 Ni recovery potentials from secondary ores

Secondary source	**Source type**	**Recovery percentage (wt%)**	**Recovery technique**	**Reference**
1. Mine tailings	Nickeliferous pyrrhotite tailing	75	Oxidizing iron pyrite at a modified temperature and recovering the Ni from the hydrometallurgical process	Duffy et al. 2015
	Limnotic laterites	70	Hydrometallurgical process with sulfuric acid under high temperature and pressure (autoclaved at 200-300˚C temperature)	Zhuang et al. 2006
	Magmatic Ni-Cu sulfide tailing	91.5	Hydrometallurgical process with nitric acid as the leaching reagent	Xie et al. 2005
	Magmatic Ni sulfide tailing	92-95	Hydrometallurgical process using Purolite S930 resin as chelating agent	Kuz'Min and Kuz'Min 2014
2. Industrial waste	Galvanic sludge	63	Solvent extraction at pH 6 using di-(2-ethylhexyl)-phosphoric acid (D2EHPA)	Silva et al. 2005a
		98	Hydrometallurgical process by precipitating nickel hydroxide	Vilarinho et al. 2012

	Electronic batteries	70	Hydrometallurgical process using HCl as the leaching reagent	Joulié et al. 2014
		99.87	Hydrometallurgical process using Malic acid as the leaching reagent	Meng et al., 2018)
		96	Hydrometallurgical process using HCl as the leaching reagent	Wang et al., 2022

2.6.4 Serpentine soil

Global serpentinite regions occupy 1% of the terrestrial landscape, including the Mediterranean, and Eurasian regions in addition to Asia-pacific regions (Galey et al., 2017). Even though the area of the serpentine region is small, they are widely distributed in tropical (Brazil, Malaysia, Indonesia, New Caledonia, and Oman) and temperate regions (Europe, Northern USA, Turkey, and Australia) (Hseu et al., 2018; Nascimento et al., 2022). Furthermore, it has been recognized that the majority of serpentinite deposits are disseminated between large tectonic plates of the upper mantle (Guillot et al., 2015). Geochemical studies conducted in serpentinite environments to date have revealed the significant features of serpentinite minerals based on different geographical settings (Kumarathilaka et al., 2014).

Serpentinite rocks originated mostly in oceanic lithosphere and mantle rocks in subduction zones (Wakabayashi, 2017). The formation of serpentinites is generally known as serpentinization, which occurs as a result of low-temperature (300–600°C) metamorphic alteration and hydrous alternation in igneous or metamorphic rocks (Wickramasinghe et al., 2016). During this chemical process, major minerals, such as magnesium (Mg)- or iron (Fe)-rich olivine ($(Mg, Fe)_2SiO_4$) and pyroxene ($(Mg, Fe)_2Si_2O_6$ or $Ca (Mg, Fe) Si_2O_6$) are converted into secondary minerals, such as serpentine (lizardite, antigorite, and chrysotile), magnetite, carbonatite, talc, and brucite by altering their chemical, hydraulic, magnetic, mechanical, and seismic characteristics (Farough et al., 2016). Mafic minerals in serpentinite rocks are rich in

Mg and Fe along with high concentrations of valuable metals like Ni, Cr, and Co (Vithanage et al., 2019).

The principal composition of the three main serpentine minerals (lizardite, antigorite, and chrysotile) is $Mg_3Si_2O_5(OH)_4$ where the morphology of the three minerals changes with the structure (Howie, 2005). At low temperatures, diopside-free peridotite forms chrysotile, brucite, and talc. At about 200°C, antigorite is formed replacing chrysotile which is the only stable serpentine mineral at 300°C (Hoinkes et al., 2005). The naturally occurring serpentine minerals contain a variation of SiO_2, MgO, and H_2O, and a minor propotion of Al_2O_3, Fe_2O_3, MnO, FeO, CaO, NiO, and Cr_2O_3. These minerals exhibt substitution of Si^{4+} cations by Fe^{3+}, Al^{3+}, or Cr^{3+} and Mg^{2+} by Ca^{2+}, Mn^{2+}, Fe^{2+} or Ni^{2+} (Hseu et al., 2018). Peridotite containing a certain amount of CaO forms diopside which is also a stable form of serpentine (Hoinkes et al., 2005).

The serpentine soil is formed due to the prolonged weathering conditions of serpentinite rocks, which contain a high concentration of Ni (average Ni concentrations in serpentine soil and rocks are 1700-10,000 mg/kg and 1500-4000 mg/kg, respectively) (Morrison et al., 2015; Dushyantha et al., 2021). Furthermore, serpentine soil typically contains Ni concentrations in the range of 0.1-0.6 wt%, which is lower than the required Ni ore grade (1.8 wt%) for conventional mining (Carpen & Giese, 2022; Biocyclopedia, 2023). However, the wide dissemination of serpentinite deposits in the world has identified serpentine soil as a potential candidate for Ni even if it has a low grade.

2.7 Recovery of Ni from serpentine soil

Due to the economical impracticability of utilizing conventional mining in serpentine soil, the abundance of hyper-tolerance Ni accumulating plants in serpentinite regions has influenced to develop plant-based mining technique, namely phytomining to recover Ni from serpentine soil (C. Li et al., 2020). Phytomining is an environmentally sound mining approach, particularly developed for the mining of low-grade metal ores. In the Ni phytomining method, Ni-accumulator or hyperaccumulator plants are cultivated in Ni-rich low-grade soils to accumulate specific metals in high concentrations. The bio-ore produced from the cultivated hyperaccumulator plants is then used to recover the Ni (Zhang et al., 2014). Moreover, studies have identified that the Ni-rich biomass ash, of which Ni concentration is higher than its natural abundance in serpentine soils. To date, more than 500 Ni hyperaccumulator species have been discovered globally through geobotanical studies, which can be employed in the phytomining approach (Reeves et al., 2018). These plants could accumulate 0.1 wt% (>1000 mg kg^{-1}) of Ni from their leaf dry weight and are known as hyperaccumulators for Ni (Baker et al., 2020). Therefore, the phytomining efficiency of Ni highly depends on the bioaccumulation levels of the selected plant species.

The importance of phytomining is that these Ni hyperaccumulators are able to accumulate Ni selectively from other metals such as Co and Cr in the soil, whereas conventional mining requires complex processing to separate metals (Sheoran et al., 2009). In addition, these hyperaccumulator plants are naturally adapted to the hyper-tolerance for the accumulated toxic metal ions and do not inhibit their plant metabolism or express any toxic symptoms. In addition, these plants are unique since they can survive against herbivory and under extreme weather conditions such as droughts (Boyd, 2004). The significance of hyperaccumulators is their ecological interference with non-hyperaccumulator plants is very low (Lange et al., 2018). Therefore, the Ni mining industry is currently progressing toward Ni-recovery from worldwide serpentine soils via phytomining technology (Nascimento et al., 2022).

2.8 Ni phytomining from serpentine soil

Many countries, including Malaysia, Indonesia, Philippines, Australia, Albania, USA, and Zambia are currently focusing on the commercial production of Ni using phytomining in serpentine soils. Figure 2.7 indicates the global serpentinite regions in the world where phytomining field trials have been carried out previously. Phytomining research in serpentine soils is often carried out in tropical settings where the Ni enrichment is relatively high due to intensive weathering (Rosenkranz et al., 2019). For example, phytomining is applied to recover residual Ni from the world’s largest serpentinite deposit in Sulawesi of Indonesia (15, 400 km^2) (Van der Ent et al., 2013). In addition, temperate countries like Albania and North America are practicing phytomining on a pilot scale in serpentinite deposits (Echevarria et al., 2015). Table 2.3 depicts the Ni phytomining studies in different serpentinite environments.

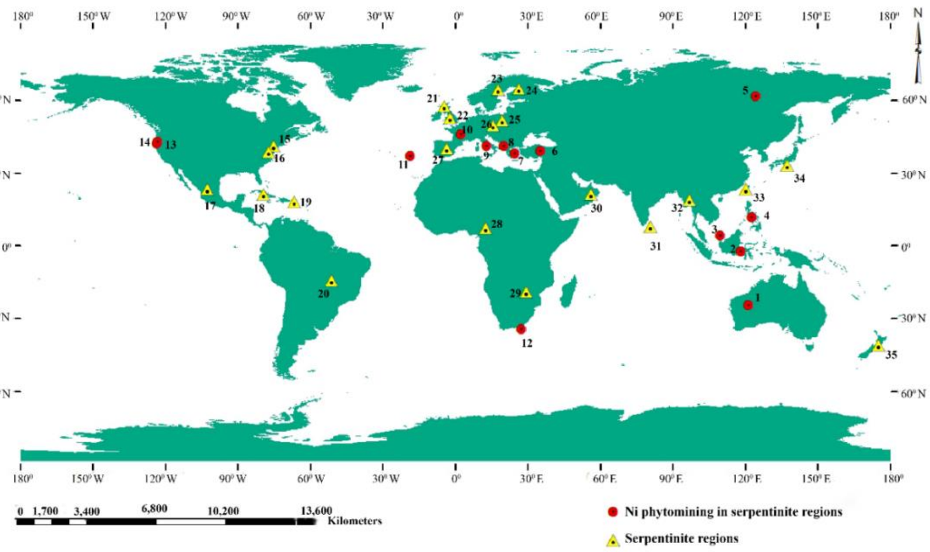

Figure 2. 7 Distribution of selected serpentinite regions and Ni phytomining studies conducted to date, in the world. (Note: Regions where Ni phytomining studies have been conducted: 1. Australia, 2. Indonesia, 3. Malaysia, 4. Philippines, 5. Siberia, 6. Turkey, 7. Greece, 8. Albania, 9. Italy, 10. France, 11. Portugal, 12. South Africa 13. Nickel Mountain, USA, 14. Cave Junction, USA. Other serpentinite regions: 15. New Foundland, USA, 16. Maryland, USA, 17. Mexico, 18. Cuba, 19. Puerto Rico, 20. Brazil, 21. Scotland, 22. England, 23. Norway, 24. Finland, 25. Poland, 26. Czech Republic, 27. Spain, 28. Cameroon, 29. Zimbabwe, 30. Oman, 31. Sri Lanka, 32. Myanmar, 33. Taiwan, 34. Japan and 35. New Zealand

Source: (Kumarathilaka et al. 2014)

2.8.1 Ni phytomining field trials in serpentine soils in the world

Successful field applications in Ni phytomining have already shown the economic potential of Ni recovery from serpentine soils. This method has been able to produce nearly 99% purity ammonium nickel sulfate hexahydrate salt (ANSH), which has a high market price (Zhang et al., 2016). In addition, Bani et al. (2015a) were able to gain a net revenue of nearly $ 1,116 ha^{-1} from the phytomining approach developed for the Albanian serpentine soil using *Alyssum murale.* Furthermore, the study observed that reasonable soil improvement strategies can effectively enhance the Ni hyperaccumulation of the plant where it can produce Ni up to 105 kg/ha. Similarly, another phytomining study used *A. murale* species where the plant bio-ore contained approximately 13.2 wt% of Ni (Barbaroux et al., 2012). Since serpentine soil has poor soil conditions and rarely supports agricultural practices, Ni phytomining can be an alternative solution for land use while restoring the nutrients in the soil and recreating the environment (Akinbile et al., 2021).

However, the maturity of the plants and the economic benefits of this method are the limitation factors of the method (Li et al., 2020). One of the biggest challenges of this method is that this method requires much time for plant growth and careful monitoring of the plants. In addition, the area used for the phytomining should be large and needs to be closer to a proper access road network for effective transportation of the biomass for the processing plant. The fluctuation of economic price is also a limiting factor for implementing phytomining due to the high capital cost spent for land, labor, and agronomic expenses (Akinbile et al., 2021).

Table 2. 3 Ni Hyperaccumulator plants and their accumulation levels with respect to the phytomining and phytoremediation studies in different serpentinite environments in the world.

Study area/Country	**Plant Species**	**Ni concentration in plant biomass (mg/kg)**	**Recovered Ni yield from dry biomass (kg/ha)**	**References**
Tuscany, Italy	*Alyssum bertolonii*	768	72	Robinson et al., 1997
Tuscany, Italy	*Alyssum bertolonii*	7,600	91.2	Anderson et al., 1999
Australia	*Alyssum bertolonii*	13,400	120.6	

	Berkheya coddii	17,000	374	Anderson et al., 1999; Harris et al., 2009
Italy	*Alyssum bertolonii*	7,000	63	Brooks et al., 2001
South Africa	*Berkheya coddii*	5,000	110	
South Africa	*Berkheya coddii*	55,000 ± 15,000	-	Mesjasz-Przybyłowicz et al., 2004
Greece and Albania	*Alyssum Sp.*	820	-	Zhang et al., 2014
	Bornmuellera thymphaea	1,390	-	
	Leptoplax emarginata	3,190	-	
Vosges Mountains, eastern France	*Leptoplax emarginata*	45.2 (DTPA extractable Ni)	-	Lucisine et al., 2014
	Noccaea tymphaea	42.6	-	
	Alyssum murale	44.6	-	
North-eastern Mediterranean region, Italy	*Alyssoides utriculata*	155.46±75.89	-	Roccotiello et al., 2015
Braganca, Portugal	*Alyssum serpyllifolium*	3,405	27.4	Morais et al., 2015
Morais, Portugal	*Alyssum serpyllifolium*	4,008	27.7	
Urals, Russia	*Alyssum obovatum*	6,008	-	Teptina & Paukov, 2015
	Alyssum tortuosum	1,789	-	
	Alyssum litvinovii	160	-	

	Noccaea thlaspidioides	741	-	
Harsin, Western Iran	*Alyssum bracteatum*	8,460	-	Ghaderian et al., 2015
Kizildag mountains, Turkey	*Noccaea camlikensis*	16,650	-	Aksoy et al., 2015
	Alyssum murale	12,570	-	
	Bornmuellera kiyakii	8,780	-	
Domosdovë, Albania	*Alyssum murale* (DOM 1)	7,100	77	Bani et al., 2015b
	(DOM 6)	8,900	41	
Pojskë, Albania	*Alyssum murale*	11,000	112	
Pojskë, Albania	*Alyssum murale*	167,800	-	Houzelot et al., 2017
Pogradec, Albania	*Alyssum murale (leaves)*	5,100	-	Guilpain et al., 2018
Sabah, Malaysia	*Rinorea bengalensis*	13,700	-	
Melide ultramafic complex, North-West Spain	*Alyssum murale*	4,200	4.0	Pardo et al., 2018
	Leptoplax emarginata	4,500	3.0	
Galicia, New Spain	*Bornmuellera emarginata*	6,174	2.9	Cerdeira-Pérez et al., 2019
	Noccaea caerulescens	3,627	1.9	
	Odontarrhena serpyllifolia	3,520	0.7	

	Odontharrena muralis	1,140	2.3	
Burgenland, Eastern Australia	*Odontarrhena chalcidica*	8,400-225,000	55	Rosenkranz et al., 2019
	Noccaea goesingensis	-	36	
Burgenland, Eastern Australia	*Bornmuellera tymphaea*	17,100	44.0	Hipfinger et al., 2022
	Bornmuellera emarginata	17,600	45.6	
	Odontarrhena chalcidica	16,000	94.3	
	Berkheya coddii	7,940	8.54	

2.9 Leaching experiments from Ni hyperaccumulator biomass

The success of nickel phytomining has been achieved through a series of plant harvesting, processing, leaching, and recovery using advanced mineral engineering techniques. The dry hyperaccumulator biomass is smelted to produce the Ni-rich ash (bio-ore) (Sheoran et al., 2009). The bio-ore is then subjected to acid leaching where inorganic solvents such as nitric (HNO_3), sulfuric (H_2SO_4), and hydrochloric (HCl) acids are used to dissolve the metal selectively (Barbaroux et al., 2009; Houzelot et al., 2018). Acid leaching from hyperaccumulator biomass is one of the critical stages of the Ni phytomining process.

Over the past years, efforts have been made to recover Ni from laboratory to pilot scale. Previous literature reveals that the ashing of Ni hyperaccumulator often produces 15-20% of Ni-rich bio-ore compared to burning and dry biomass, which reduces the amount of acid required in leaching (Guilpain et al., 2018). There are two possible paths to recover Ni from these ashes either from hydrometallurgical or pyrometallurgical processes (Barbaroux et al., 2009, 2011). Many studies have been carried out to determine the best leaching reagent and effective values of the parameters such as acid concentration (mol/L), agitation speed (rpm), solid/liquid (g/L), temperature (ºC), and time (min) to enhance the leaching kinetics (Barbaroux et al., 2011; Echevarria et al., 2015; Houzelot et al., 2018). All these parameters are considered critical in the leaching stage since the quality of the leachate and the cost of purification are highly dependent on them (Zhang et al., 2016). According to previous studies, sulfuric acid has been identified as the most effective leaching reagent to leach out the optimum concentration of Ni from the plant bio-ore to the leachate (Barbaroux et al., 2009; Guilpain et al., 2018).

To date, the most efficient leaching experiments were carried out with *A. murale* seeds where nearly 97% of Ni was leached out to the liquid phase (Barbaroux et al., 2009). According to the study, the optimum leaching level was observed with 15% seed (solid) concentration dissolved at 0.5 mol/L sulfuric acid for 120 min heated at 90 ºC. In addition, the extraction rate can be increased by exposing the remaining residuals to a washing series. Under the same temperature and time conditions, another study recovered 62% of Ni using 3 mol/L sulfuric acid from *A. murale* biomass. Alternatively, a study extracted Ni from *A. murale* biomass using ultra water leaching method and the extraction yield was nearly 90%. The leaching experiments were carried out for 4% and 8% pulp densities of plant biomass with ultrapure water at 20 ºC for 15 min (Guilpain et al., 2018). However, during the selective precipitation trials of Ni, it has been observed that the Ni precipitation directly affects the organic content in the leachate. The partially bound Ni with organic matter prevents the nickel hydroxide precipitation since organic matter coagulates and flocculates highly at high pH conditions (Barbaroux et al., 2011). Therefore, more detailed studies need to be carried out to improve the leaching and recovery of the optimum percentage of Ni from plant biomass to optimize the purity and efficiency of this method. Figure 2.8 illustrates a process flow diagram for the recovery of Ni from hyperaccumulator biomass.

2.10 Influencing factors for Ni phytomining

The phytomining of Ni highly depends on the bioaccumulation levels of the selected hyperaccumulator species. However, in order to enhance the metal accumulation levels in hyperaccumulator plant biomass, plant-associated factors (metal hyper-tolerance and accumulation levels for plants) and soil-associated factors (pH and soil fertilizers) should be optimized (Sheoran et al., 2009). Therefore, global phytomining studies have developed concerning the following factors.

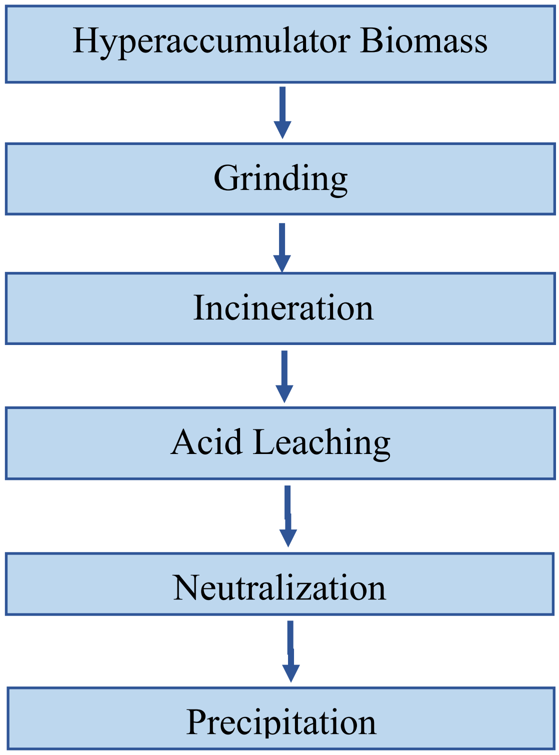

Figure 2. 8 A simplified flow diagram of a Ni recovery process from hyperaccumulator biomass

2.10.1 Plant-associated factors

2.10.1.1 Hyper-tolerance of plants

Naturally, hyperaccumulator plants have a hyper-tolerance for the accumulated toxic metal ions, which shall not inhibit their plant metabolism or express any symptoms. In addition, these plants are unique since they can survive against herbivory and under extreme weather conditions such as droughts (Boyd, 2004; Alhousari and Greger, 2018; Singh et al., 2020). Hyperaccumulators have significantly low ecological interference with non-hyperaccumulators (Boyd & Jaffré, 2001; Lange et al., 2018).

2.10.1.2 Accumulation levels of plants

Nearly 523 Ni-hyperaccumulator plants have been identified to date globally (Reeves et al., 2018), which can retain extremely high Ni concentrations in their plant biomass without showing any toxic symptoms (Van der Ent et al., 2013). Amongst these hyperaccumulators, several plants in the Brassicaceae family have gained the wide attention of researchers since the 1990s. The world's first Ni hyperaccumulator (*Sebertia accuminata*) was identified by Jaffré et al. (1976). A few decades later, Chaney et al. (1999) recovered 2.5 wt% of Ni accumulated in plant tissues of Brassicaceae plants, which can be used in Ni refinery, or smelting operations. A group of Turkish scientists observed a significant association between the Ni content in the Brassicaceae plant, and the phyto-availability in the soil to commercially develop phytomining in contaminated soils (Altinozlu et al., 2012). Most of these Ni hyperaccumulator plants are significantly restricted to ultramafic soils (Sheoran et al., 2010; Van der Ent et al., 2013; Reeves et al., 2018). *Odontharrena muralis* stated as *Alyssum murale* (*A. murale*) in previous literature (Guilpain et al., 2018), is known to be one of the most effective Brassicaceae plants for Ni phytomining since it can accumulate about 3% of Ni in its dry weight (Whiting et al., 2003). Similarly, field trials and pot culturing of the *A. bertolonii* plant revealed that there is a great potential for harvesting 9 t/ha of Ni yield in Italy (Bani et al., 2015a; Broadhurst & Chaney, 2016). However, according to recent studies, *Pycnandra acuminata* from New Caledonia is known to be the best Ni hyperaccumulator in the world in which, accumulating about 25.7 wt% of Ni in its green latex (Mesjasz-Przybylowicz et al., 2016).

A comprehensive phytomining study with *A. murale* observed relatively high concentrations of Ni in seeds (1.31 wt%) and flowers (1.60 wt%) of the plant compared to its stem (0.60 wt%) and roots (0.22 wt%). Moreover, the study developed a leaching method to recover 97 wt% of Ni from the seeds of *A. murale* (Barbaroux et al., 2011). Significantly, Ni accumulation in the wild *Alyssum* plant was 10,000 times higher compared to the *Zea mays* plant (2 mg/kg, and 20,000 mg/k, respectively) further proving the efficiency of the hyperaccumulator (Chaney et al., 2005). Moreover, Mesjasz-Przybyłowicz et al. (2004) reported very high Ni levels in soil and *Berkheya coddii* plant (18,000 mg/kg) in Africa.

2.10.2 Soil-associated factors

2.10.2.1 Soil pH level

Soil pH level significantly influences the solubility of trace metals, which ultimately affects their bioavailability in soil (Chaney et al., 2007; Sheoran et al., 2009). Soil acidity, one of the major edaphic characteristics, strongly influences plant growth and metal uptake in plants. As previous studies revealed, the solubility of divalent cations such as Zn, Cu, Co, and Ni increases with reducing pH (4 – 6), while higher acidic conditions (< 2) may reduce the Ni accumulation level (Chaney et al., 2007; Nkrumah et al., 2016). Furthermore, as Weng el al. (2004) reported, the addition of chemical fertilizers might reduce Ni accumulation rate. Therefore, many studies recommend maintaining a slightly acidic condition (pH 5 - 7) in the growth media to increase the metal uptake by plants during phytomining (Robinson et al., 1997; Chaney et al., 2007; Nkrumah et al., 2016).

2.10.2.2 Fertilizer

The success of phytomining is highly dependent on the amount of Ni yield harvested and recovered at a commercial scale. Therefore, the phytomining of Ni has been studied with different fertilizer amendments in previous literature. For example, the application of NPK fertilizer to the serpentine soil has significantly increased the accumulation (Control- 2500 mg/kg and fertilizer - 4200 mg/kg) of Ni in Berkheya coddii leaves (Robinson et al., 1997; Sheoran et al., 2009). In addition, a two-year study in Spain observed that Ni concentration in the harvested biomass of controlled fertilization (1st year-884 kg/ha and 2nd year-1625 kg/ha) was higher than non-fertilized serpentine soil (1st year-160 kg/ha and 2nd year-400 kg/ha1) (Saad et al., 2021). However, the application of inorganic (NPK) or organic (compost and cow manure) fertilizers significantly showed a lower impact on Ni accumulation in

Odontarrhena chalcidica shoots (Control - 11.6 g/kg, NPK - 11.6 g/kg, Cow manure - 12.5 g/kg and Organic matter -13.6 g/kg) (Hipfinger et al., 2022). Furthermore, Harasim and Filipek (2015) mentioned that bioavailable metals like Ni are more likely to bind with organic matter than absorbed by plants.

Phytomining studies recommend applying Ca into soils like serpentine annually, due to its low Ca/Mg ratio. In addition, for maintaining a sufficient pH level, $CaCO_3$ and $CaSO_4$ can be applied to the fields to improve the Ni accumulation and tolerance level of Ni hyperaccumulator plants (Chaney et al., 2007; Nkrumah et al., 2016). However, the evidence on the direct influence of Ca application to the Ni hyperaccumulator plants is still limited (Nkrumah et al., 2016).

2.10.2.3 Bioavailability of metals in soil

Metal bioavailability in the soil is considered as the ability of a metal to be dissolved and released from the soil matrix into the soil solution (pore water) in a form, that can be absorbed by plants (Sullivan & Gadd, 2019; Chen et al., 2023). Therefore, in phytomining and phytoremediation, researchers are concerned about the bioaccessible metal fraction in the soil. Generally, the bioavailability of metals depends on soil texture, organic matter, pH, and also the binding strength of the metal. Some metals such as Fe, Cd, Cu, Zn, and Pb tend to be bioavailable in acidic soils whereas Ca and Mg are bioavailable in neutral soil. Dissolved organic matter content in the soil solution also increases the bioavailability of metal (Kim et al., 2015). However, the complexation of metals with organic matter may reduce this phenomenon. The bioavailability and mobility of these metals in serpentine soils are controlled by five metal-bound fractions. They are defined as; (i) exchangeable; (ii) bound to organic matter; (iii) bound to Fe and Mn oxides; (iv) bound to carbonate in soil; and (v) residual)(Li & Thornton, 2001; Yusuf, 2007; Antić-Mladenović et al., 2011). In serpentine soils, Ni is highly bounded by Fe and Mn oxides, followed by organic matter and carbonates, respectively (Antić-Mladenović et al., 2011).

2.11 Implementing Ni phytomining in serpentine soils in Sri Lanka

2.11.1 Geological setting of Sri Lanka

The regional metamorphism and geology of Sri Lanka originated during the Precambrian era when Gondwana, the ancient supercontinent, split (Osanai et al., 2016). As this phenomenon happened, Sri Lanka was at a central position in this supercontinent, and thus it has a stronger geological correlation with Southern India, Madagascar, and Eastern Antarctica. Geologically, ~90% of Sri Lanka is underlain by Precambrian crystalline rocks, and the remains by Miocene limestone, a minor amount of Jurassic sedimentary rocks along with quaternary sediments (Cooray, 1994; Mathavan et al., 1999). Based on the geochemical characteristics and Nd model ages, the geological terrain of Sri Lanka into four crustal units, namely Highland Complex (HC), Vijayan Complex (VC), Wanni Complex (WC), and Kadugannawa Complex (KC) (Cooray, 1984; Mathavan et al., 1999; He et al., 2016).

The HC is located at the center of Sri Lanka, which comprises rocks metamorphosed under granulite facies, such as meta-igneous rocks (charnockitic gneisses, granitic gneisses, and granitoids) and meta-sedimentary rocks (meta-quartzites, marbles, and calc-silicates). The VC is dominant with granitoids, migmatitic and granitic gneisses, hornblende-biotite gneisses, and minor amphibolite scattered metasediments (quartzite, and calc-silicate rocks), metamorphosed under upper amphibolite facies. In the WC, there are both meta-sedimentary and meta-igneous rocks present which were formed due to metamorphism under upper amphibolite facies (Cooray, 1984, 1994; Kehelpannala, 1997; Mathavan et al., 1999; He et al., 2016).

Based on the gravity anomaly in between the boundary of HC and VC area, previous studies have proposed that there is a suture formed due to the thrusting of HC over VC. This incident is known to be the final amalgamation of the east and west Gondwana supercontinent (Munasinghe & Dissanayake, 1982; Cooray, 1994). In between this unique geological zone, studies have discovered six serpentinite deposits, namely Ussangoda, Indikolapelessa, Ginigalpelessa, Yodhaganawa, Katupotha, and Rupaha (Figure 2.9) (Weerasinghe & Iqbal, 2011; Kumara et al., 2015;).

2.11.2 Serpentinite deposits in Sri Lanka

To date, many studies have documented the nature of serpentinite deposits based on their soil geochemistry, biodiversity, and metal toxicity. Despite the general geological characteristics, studies have identified slight differences in the lithology of these serpentinite deposits due to the climatic zone and the geographical location that they pertain.

Ginigalpelessa serpentinite deposit is known to be the largest serpentinite body in Sri Lanka which extends to an area of 1.0 km^2, while Indikolapelessa is estimated to be 0.3 km^2 (Hewawasam et al., 2014). Since Ginigalpelessa and Indikolapelessa deposits are from the same geographical area (Udawalawe), the soil colour of these deposits is reddish brown. Geobotanical studies in both deposits have identified unique diversity of plants (Rajakaruna & Bohm, 2002). Furthermore, it has been observed that there is a clear demarcation between the serpentine and non-serpentine soil at the boundary of the Ginigalpelessa serpentinite deposit (Hewawasam et al., 2014).

Ussangoda serpentinite deposit is located on the Southern coast of Sri Lanka, which is conserved as a national park by the Department of Wildlife, Sri Lanka. The unique vegetation and geology have created many historical legends about the origin of the deposit. Geochemical analysis of Ussangoda has reported 2-3% of Ni in rock samples (Tennakone et al., 2007). Vithanage et al. (2014) stated that the soil color in this deposit is reddish than other outcrops due to the weathering of ferro-laterites, which releases an excessive amount of Fe and Cr. Furthermore, the above study claims that there is relatively higher electrical conductivity (EC) in Ussangoda soil than in other deposits due to the deposition of salt spray from the coastal zone.

Yodhaganawa's serpentinite body has also been declared as an ecologically sensitive area that is a part of Wasgamuwa National Park (Rajakaruna & Baker, 2004). This forested habitat near Yodhaganawa is the reason for the high organic carbon (391 mg/kg) in the soil. Furthermore, soil samples of this deposit have exhibited elevated levels of Ni and Cr (6,130 mg/kg and >10,000 mg/kg, respectively) (Vithanage et al., 2014). The geology and geochemistry of Rupaha and Katupotha are not as well-documented as in other deposits. However, Fernando et al. (2021) revealed that heavy metal concentrations in serpentinite rocks at the Rupaha deposit were very low compared to others.

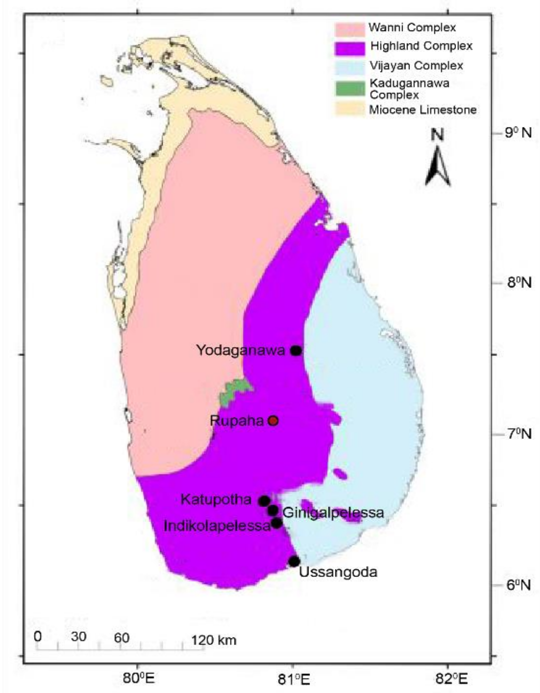

Figure 2. 9 Serpentinite deposits and lithotectonic boundaries in Sri Lanka (1) Ussangoda, (2) Indikolapelessa, (3) Ginigalpelessa, (4) Katupotha, (5) Rupaha, and (6) Yodhaganawa. Source: (Kumarathilaka et al., 2014)

2.11.3 Geochemistry of serpentine soil

The geochemistry of serpentine soil mostly depends on the mineralogy of the parent material and climatic conditions (Kumarathilaka et al., 2014). For example, the Mg abundance is high in tropical serpentine soil due to the fully completed hydrolysis process during soil formation, which is partially completed in the temperate region. Therefore, Mg has become prominent in the cation exchange sites of soil in serpentinite deposits of Sri Lanka, resulting in a low Ca/Mg ratio. In addition, macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K) are depleted in the serpentine soil compared to non-serpentine soils, due to the lack of organic matter (Vithanage et al., 2019).

Geochemical studies in the deposits have observed elevated concentrations of Ni, Cr, and Co in both rock and soil samples. Therefore, the distribution of these heavy metals in serpentine soil has been studied in both mineral scale and profile scale. In general, due to the weathering conditions heavy metals in serpentine soil are concentrated in the secondary phases and progressively enriched towards the surface (Kumarathilaka et al., 2014; Vithanage et al., 2014). Nevertheless, the pattern of transportation and enrichment of heavy metals is not consistent in the serpentine soil profile, due to the variation in weathering rates of different minerals present in the deposit (Vithanage et al., 2019). In this context, nickel in Udawalawe serpentinite deposits has shown a slight preference for the limnetic phase which lies between 10-36 m from the surface (Dissanayaka, 1982).

In comparison with other metals, Ni concentration in the serpentine soil emphasizes that Ni is the most bioavailable metal in the deposit (Vithanage et al., 2014; Morais et al., 2015). In addition, previous studies related to Udawalawe serpentinite bodies have highlighted a slight preference for Ni in the ferrous oxide (Fe_2O_3) phase, especially associated with the limonitic layer in the soil. The variation of Ni with manganese oxide (MnO) has also been defined by Dissanayake (1982), showing that nearly one-tenth of Ni is associated with MnO in the soil. Furthermore, Ni enrichment in the upper soil horizon of the deposit indicates that the metal-bound fraction of Ni varies from greatest to lowest as residual > Fe and Mn oxides > Organic matter bound > Exchangeable > Carbonate bound (Kumarathilaka et al., 2014). Since plants have adapted to these harsh conditions, the vegetation cover in serpentine soil is less diverse than in non-serpentine soil (Figure 2.10).

2.11.4 Climatic conditions and tropical weathering in serpentinite deposits

Sri Lankan rocks have been subjected to periodical weathering under tropical and subtropical climatic conditions (Jayawardena & Izawa, 1994). Due to its geographical location close to the equator (latitudes: 5° 55–9° 5’ N and longitudes: 79°

42’–81° 53’ E), Sri Lanka experiences equatorial climatic conditions. Therefore, the country receives sunlight throughout the year (Marambe et al., 2015). In addition, there are mainly four main monsoonal rain periods, such as Northeast monsoon (December-February), the first inter-monsoon (March-April), the Southwest monsoon (May-September), and the second inter-monsoon (October-November) (Esham & Garforth, 2013; Dananjaya, 2017). These different climatic conditions have divided the country into three climatic regions as wet zone, intermediate zone, and dry zone. In this context, serpentinite deposits in Sri Lanka are located in the dry and intermediate zones of the country where they receive minimum rainfall from the second inter-monsoon and northeast monsoon (Dushyantha et al., 2021).

2.11.5 Metal toxicity in serpentinite deposits

Globally, natural serpentine soil toxicity is higher in tropical regions compared to temporal ones (Van der Ent et al., 2015; Vithanage et al., 2019). It is documented that weathering rates of rocks are high in tropical countries with high rainfall and temperature (Galey et al., 2017). As a result, it accelerates the release of toxic heavy metals, such as Cr, Ni, Co, and Mn in ultramafic rocks to the overlying soil profile. Although the dry zone of Sri Lanka, where serpentinite bodies occur, experiences minimum rainfall, the extreme temperature conditions may have caused the high weathering rates evident in the aforementioned serpentinite deposits in Sri Lanka (Rajakaruna & Bohm, 2002; Rajapaksha et al., 2012; Kumarathilaka et al., 2014). Therefore, natural heavy metal occurrences in the overlaying soil profile of these serpentinite deposits have exceeded the permissible limits imposed by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) (e.g. Ni - 50 mg/kg, Cr - 100 mg/kg, and Co - 50 mg/kg) (Chiroma et al., 2014). This heavy metal toxicity in serpentine soil is known as “serpentine syndrome” (Oze et al., 2007; Vithanage et al., 2019), and increased levels of Ni, Cr, and Co concentration have been identified in the soils of Ginigalpelessa, Indikolapelessa, Ussangoda, and Yodhaganawa serpentinite deposits (Vithanage et al., 2014). Furthermore, based on the results of Fernando et al. (2021), heavy metals concentrations of rock samples in the Rupaha deposit are surprisingly very low when compared to the other serpentinite deposits (Ginigalpelessa – 2,356 mg/kg, Indikolapelessa – 3,022 mg/kg, Yodhaganawa – 2,724 mg/kg, Rupaha – 4 mg/kg and Ussangoda – 4,098 mg /kg).

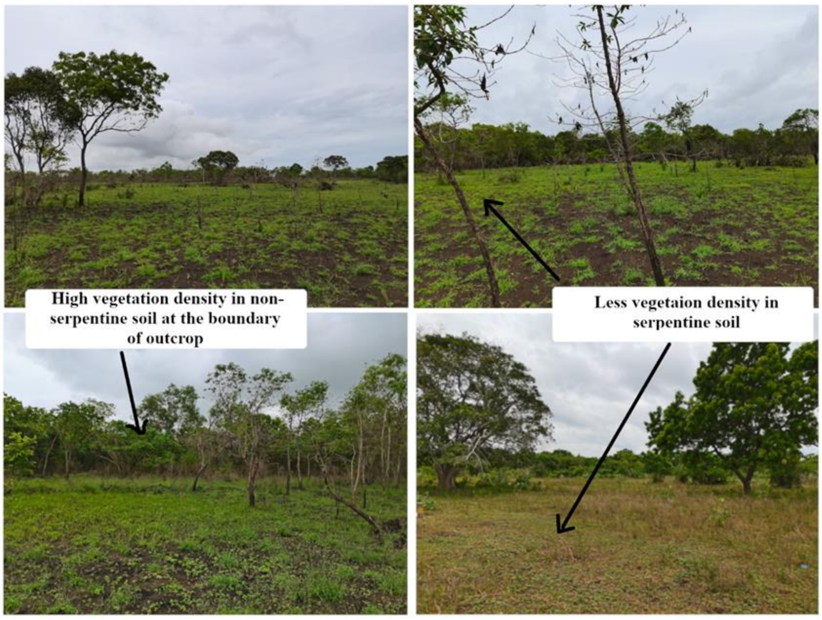

Figure 2. 10 Density variation between the vegetation cover of serpentine and non-serpentine soil.

Over the last few years, several studies have identified the contamination of heavy metals, such as Ni, Cr, and Co in the Ginigalpelessa serpentinite deposits and their impacts on the local ecosystem and human health around the Ginigalpelessa area. Furthermore, studies have claimed that nutrient deficiency (low N, P and K, Ca/Mg ratio) and the unusual enrichment of heavy metals in soil have inhibited plant growth (Degree of ecological risk: Cr - 50%, Ni - 43 % and Co - 7%), thus and severely affected on agricultural production (Dushyantha et al., 2021). Similarly, Rajakaruna and Baker (2004) reported that these unfavorable soil conditions have failed the attempt at sugar cane cultivation in the Ginigalpelessa serpentinite deposit, located at the boundary of the Sevanagala sugar plantation. Significantly, dermal, and oral exposure to the metal-enriched soil has caused non-carcinogenic health impacts (Skin redness and skin rashes) on residents in the Ginigalpelessa area, especially in children (Vithanage et al., 2019; Dushyantha et al., 2021). Heavy metal enrichments in different serpentinite deposits are indicated in Table 2.4. Additionally, groundwater contamination due to the serpentinite rock weathering has been identified in the vicinity of Udawalawa serpentinite bodies (Udagedara et al., 2014). Even though the practice of soil remediation with hyperaccumulator plants has been raised by several studies, this risk has been continuing for years without any proper solution (Rajakaruna et al., 2006; Tennakone et al., 2007; Neilson & Rajakaruna, 2012).

Table 2. 4 Heavy metal concentrations in serpentine soils in Sri Lanka (Modified by: Vithanage et al. 2014, 2019)

Serpentinite deposits	Metal concertation (mg/kg)			Reference
	Ni	Cr	Co	
Ginigalpelessa	6,649	32,472	359	Dushyantha et al. 2021
	5,945	9,948	219	Vithanage et al., 2014, 2019
Ussangoda	6,776	10,707	157	
Indikolapelessa	4,705	6,737	241	
Yodhaganawa	6,567	14,880	555	

2.11.6 Field trials for the removal of metal toxicity

Metal toxicity in serpentine soil should be removed to reduce the environmental and social impacts. However, being a developing country in South Asia, it is difficult to apply conventional and costly remediation methods, such as soil washing and soil removal in the serpentinite soils of Sri Lanka. Therefore, phytoremediation – the removal of heavy metals using plants has been identified as an attractive alternation for removing heavy metals from contaminated serpentine soil sites in Sri Lanka (Rajakaruna et al., 2006), which is an eco-friendly, low-cost, and widely accepted method (Hauptvogl et al., 2019). In addition to the removal of heavy metals from serpentine soils by different methods, a few other studies have found solutions by cultivating suitable crops in this soil using phytotoxicity reduction mechanisms, such as biochar. In this context, woody biochar was considered as an effective soil amendment practice to remove the bioavailability of Ni, Cr, and Mn from serpentine soil and improve the bacterial and fungal activity in the soil (Herath et al., 2015; Bandara et al., 2017).

Even though the studies have only focused on metal toxicity removal from the Sri Lankan serpentine soils, there is a potential for the mining of Ni from these serpentinite deposits as a low-grade metal source. In this regard, implementing Ni phytomining in local serpentine soils will be a green approach to soil remediation while contributing to the country's Gross Domestic Product (GDP). The metal toxicity levels in serpentinite deposits identified in previous studies have already exceeded the average limit necessary for Ni phytomining (Vithanage et al., 2014; Nkrumah et al., 2016; Tognacchini et al., 2020). In a study conducted by Tennakone et al. (2007), nearly 3% of pure metallic Ni was recovered from the Ussangoda serpentinite deposit using the electrolysis method. The same study showed a remarkable level of Ni in a few plants and herbs in the Ussangoda serpentinite deposit, which were sufficient for small-scale commercial metal mining and extraction. Therefore, it is important to identify the best Ni hyperaccumulators for the successful implementation of phytomining in the serpentinite deposits in Sri Lanka.

2.11.7 Native Ni hyperaccumulators in Sri Lankan serpentinite deposits

Sri Lanka harbors a diverse range of native plants in serpentinite outcrops where there are merely 107 species identified and well documented (Van der Ent et al., 2015; Galey et al., 2017). Among those plant species, *Vernonia zeylanica* is the only species found as endemic to Sri Lanka (Galey et al., 2017). During a floristic study in these serpentinite outcrops, Rajakaruna and Bohm (2002) were able to identify three native Ni hyperaccumulators namely, *Hybanthus enneaspermus, Evolvulus alsinoides,* and *Crotalaria biflora* which accumulate 1,709, 1,115 and 1,088 mg/kg of Ni, respectively (Figure 2.11). Among these three plants, *Hybanthus enneaspermus* and *Evolvulus alsinoides* have been found in serpentinite bodies in Queensland, Australia. Furthermore, the study revealed that certain plant species could accumulate high concentrations of Ni, even if they have not reached the hyperaccumulation levels (*Epaltes divaricate* - 733 mg/kg, *Aristida setacea* - 439 mg/kg, and *Fimbristylis falcata*

– 371 mg/kg) (Rajakaruna and Bohm, 2002; Rajakaruna et al., 2006). However, when referring to Kumara et al. (2015), Ni accumulation in *Morinda tinctoria* was intriguing. The plant samples collected from Ginigalpelessa denoted higher accumulation levels than the same plant collected from a different geographical location (mean values of *M. tinctoria* in serpentine and non-serpentine soils were 72.00 mg/kg and 5.34 mg/kg, respectively), proving that some plants in the nature can grow and reproduce in both metalliferous and non-metalliferous soils (facultative hyperaccumulators) (Pollard et al., 2014; Leguizamo et al., 2017). However, a recent study in five serpentinite outcrops (excluding Katupotha) of Sri Lanka revealed that Ni hyperaccumulators are absent in these deposits as the observed metal concentration in foliar is less than 1,000 mg/kg (Fernando et al., 2021). Still, it is more effective to use native species as Ni hyperaccumulators than to use introduced species, since introduced species may not adhere to the harsh environmental conditions (Leguizamo et al., 2017). In addition, scientists are more concerned about using introduced species since they may act invasively in the new environment (Che-Castaldo & Inouye, 2015; Wang et al., 2020). Therefore, carefully monitoring of non-native hyperaccumulator species is mandatory when introducing as metal crops in phytomining.

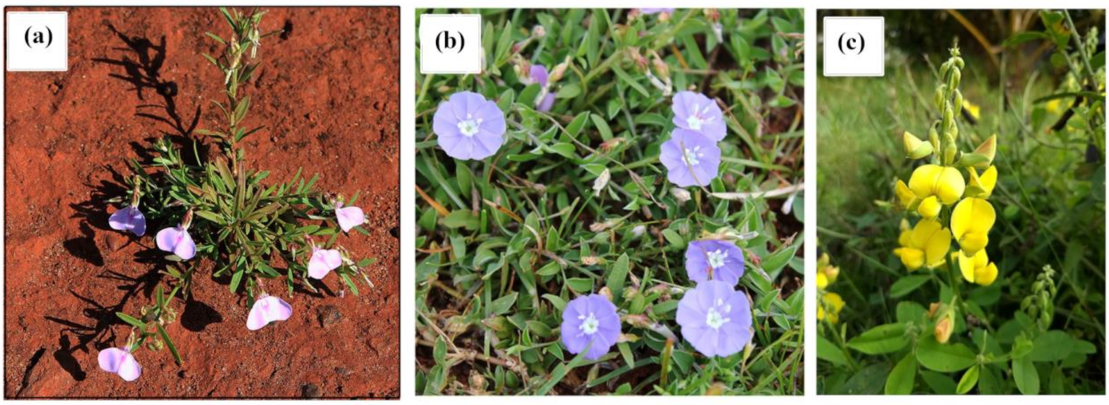

Figure 2. 11 Ni hyperaccumulator plants discovered from Ussangoda serpentinite outcrop, Sri Lanka. (a) *Evolvulus alsinoides*; (b) *Hybanthus enneaspermus*; (c) *Crotalaria biflora*.

Source: (Park, 2009; Fernando et al., 2021)

CHAPTER 3: METHODOLOGY

3.1 Study area

Ginigalpelessa serpentinite deposit is located in the Uva province of Sri Lanka, which extends to nearly 1.0 km^2 area (6°23′2.24″N, 80°53′18.94″E) (Figure 3.1a) (Hewawasam et al., 2014). It is one of the best-known serpentinite bodies in Sri Lanka where the petrology and geochemistry are well-documented (Dissanayake, 1982; Kumarathilaka et al., 2014). Geophysical studies in the Udawalawe region have revealed that the Ginigalpelessa serpentinite deposit is a deep-seated origin which lies along the sutured boundary of HC and VC (Dissanayake, 1982; Hewawasam et al., 2014). The deposit is composed of ultramafic serpentinite rocks and is surrounded by charnockites, hornblende-biotites, and biotite-hornblendes bearing gneisses (Figure 3.1b). These serpentinite rocks contain nearly 90% of Fe- and Mg-rich silicate minerals such as olivine and pyroxene (Kumarathilaka et al., 2014). According to the mineralogical studies conducted by Dissanayaka (1982), both Ginigalpelessa and Indikolapelessa serpentinite deposits are associated with four different types of serpentinite namely, (1) oolitic serpentinite; with oolitic silica and micaceous mineral (fuchsite and delessite), (2) fibrous serpentinite; composed of antigorite and silica with or without carbonates, (3) mesh-like serpentinite; with chrysotile and antigorite associated carbonatite and fuchsite or delessite, and (4) micaceous serpentinite; composed of fuchsite or delessite.

Figure 3.2a illustrates a cross-section of the serpentinite body at Ginigalpelessa. The topsoil layer of the deposit, particularly from the surface to 15cm depth, is enriched with 23.30 wt% of Fe_2O_3. Following this topsoil, there are remains of weathered serpentinite rocks. These weathered serpentinite rocks can be found up to 110.0 m depth from the surface, which often contains serpentinite boulders. Furthermore, the greenish colour in each weathered profile indicates the presence of nickeliferous laterites (Dissanayake, 1982).

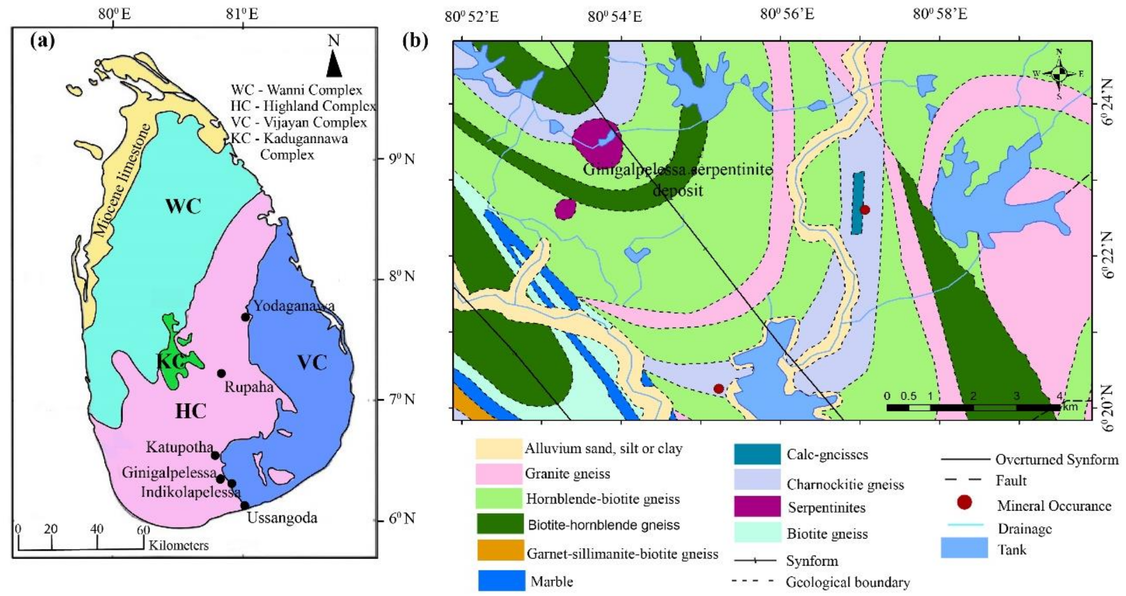

Figure 3. 1 (a) The general geological map of Sri Lankan showing the geographical location of the study area and the other serpentinite deposits of Sri Lanka (Source: (Vithanage et al., 2014)); (b) The simplified geological map of the Ginigalpelessa area (GSMB, 2001a).

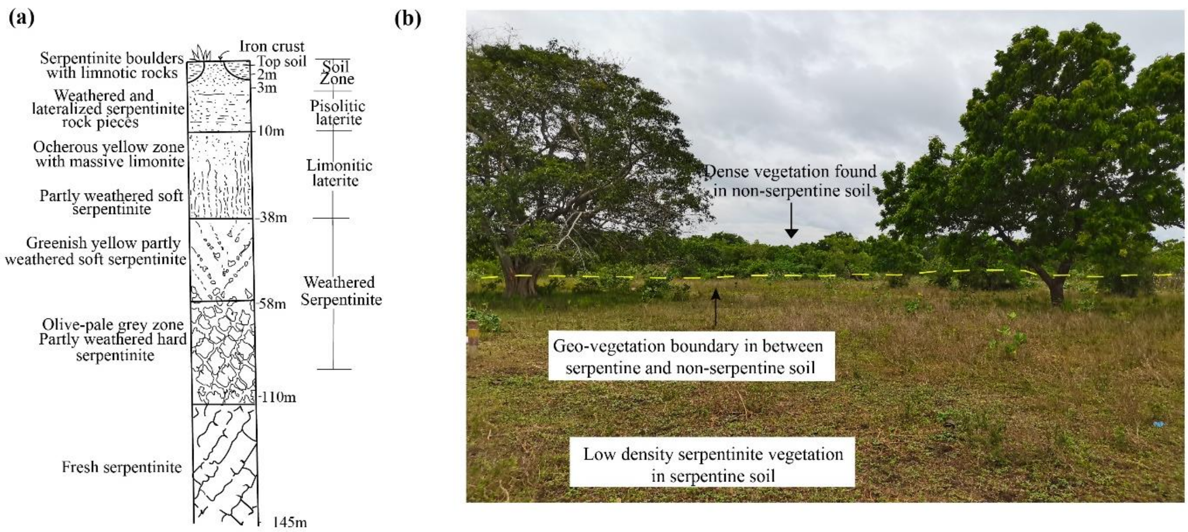

Figure 3. 2 (a) Generalized cross-section of the serpentinite body at Ginigalpelessa (Source: Dissanayake 1982); (b) Geo-vegetation boundary in between the serpentine and non-serpentine soils at Ginigalpelessa serpentinite deposit.

Owing to the topical climatic conditions in the area, the weathering rates are high in the serpentinite rocks resulting in elevated concentrations of Ni, Mn, Cr, and Co in the soil (Kumarathilaka et al., 2014; Dushyantha et al., 2021). The high metal toxicity and the low nutrient content in the soil have caused high species endemism in the deposit (Galey et al., 2017). In addition, the disturbances to the serpentine ecosystem from human interventions are clearly visible at the boundaries and some patches inside the deposit, where non-serpentine flora can be observed (Figure 3.2b).

3.2 Sample collection, preparation, and analysis

3.2.1 Soil and rock sample collection

A total of 31 soil and 25 rock samples (from the outcrops) were collected from the Ginigalpelessa serpentinite deposit covering 31 sampling locations (Fig. 3.3a). The grid sampling method was carried out where the soil samples were collected from each grid point of a 200.0 m × 200.0 m grid over the deposit. The soil samples were collected at 20.0 – 30.0 cm depth removing the topsoil (Figure 3.4). Furthermore, the rock samples were collected from the outcrops. In addition, ten soil samples were collected from outside the serpentinite deposit considering two cross-sectional lines (Figure 3.3b).

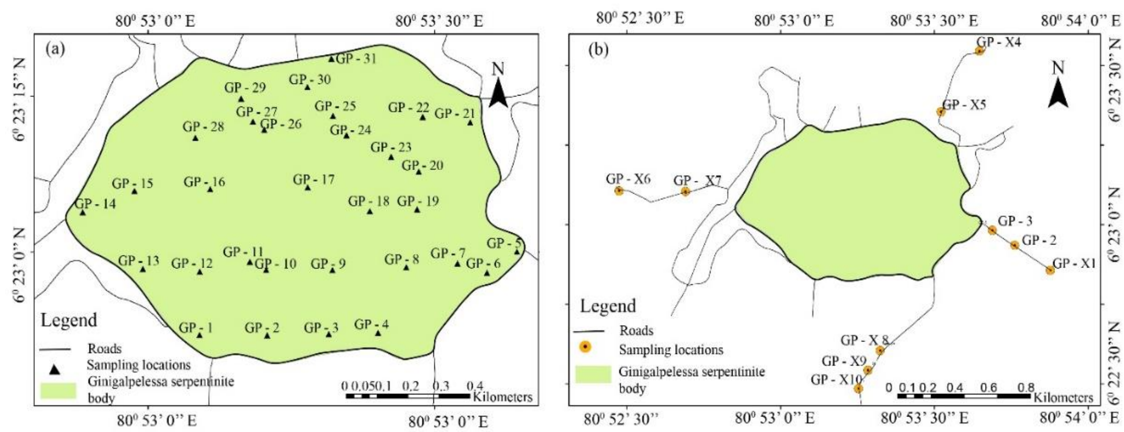

Figure 3. 3 The distribution of sampling locations (a) inside and (b) outside of the Ginigalpelessa serpentinite deposit.

Figure 3. 4 Field photographs of soil and rock sample collection in Ginigalpelessa deposit.

3.2.2 Soil and rock sample preparation to determine total metal concentration

All the soil samples were oven-dried at 105ºC for 48 h until they reached a constant weight. The serpentinite rocks samples were crushed first using the laboratory Jaw crusher to reduce the particle size (Figure 3.5a). Then both soil and rock samples were powdered using the laboratory Tema mill and sieved to obtain finely sized (< 63 µm) samples (Figure 3.5b and c). The processed rock and soil samples were stored to determine the metal and minerology present in Ginigalpelessa serpentinite deposits.

Figure 3. 5 (a) Crushing serpentinite rocks to reduce the particle size using laboratory jaw crusher; (b) Powdering rock/soil using laboratory Tema mill; (c) Sieving through 63 µm sieve to reduce the particle size.

Representative samples were prepared using coning and quartering method for the analysis. A 0.50 g of each prepared sample was digested using a mixture of HNO_3: HCl: H_2O_2 (1.0 mL: 3.0 mL: 1.0 mL) for 3 h at 120ºC. The digested samples were then filtered (through 0.45 µm syringe filters) and diluted 1000 times with deionized water (Figure 3.6). The samples were properly labelled and stored in the refrigerator.

Figure 3. 6 (a) Soil sample digestion with HNO_3, HCl, and H_2O_2; (b) Digested soil samples; (c) Sample analysis for Ni, Cr, and Co in serpentine soil and rock.

3.2.3 Mineralogy of soil and rock

X-ray diffraction method (XRD) was used to determine the mineralogy in Ginigalpelessa serpentine soils and rocks. This section mainly focused on identifying the mineralogical variation of the soil and rock samples particularly in the high, moderate, and low Ni concentrated areas in the deposit. The 5.00 g of each soil and rock samples (< 63 µm) were used for the XRD analysis considering 6 locations. The samples were analyzed employing a BRUKERD8 ADVANCE ECO X-ray diffractometer operating under standard conditions. The XRD patterns were collected between 2 theta values of (2.0-80.0) and at a scan speed 1.0º min^{-1}. A voltage of 40 kV X-ray diffractometer was applied with a current of 25 mA. The phase analysis and data refinement were performed utilizing the International Centre for Diffraction Database (ICDD). The XRD analysis was perfomed at the Department of Material Science & Engineering, University of Moratuwa, Sri Lanka.

3.2.4 Blank sample preparation for soil and rock samples

A blank sample was prepared using a mixture of HNO_3: HCl: H_2O_2 (1.0 mL: 3.0 mL: 1.0 mL) and digested at 120ºC for 3 h. The digested samples were diluted 1000 times using deionized water, filtered, and stored in the refrigerator.

3.2.5 Soil sample preparation to analyze the bioavailable metal fraction in soil

The bioavailability of a particular metal in the soil is generally defined as a proportion of the metal that is available for plant uptake from the total concentration (Zahid & Williams, 2016). The metal bioavailability was determined in selected ten soil samples using the EDTA extraction method. In this method, 2.00 g of dried soil (<2 mm) was added with 20.0 mL of 0.05 mol/L EDTA disodium salt at a ratio of 1:10 (m/V) liquid/solid. The solution was continuously stirred for 1 h and the leachate was filtered for the ICP-MS analysis (Feng et al., 2005; Wang et al., 2009).

3.2.6 Plant sample collection

To determine the relative abundance of plants, 3.0 m × 3.0 m grids were prepared in each sampling location (Figure 3.7). Then plant species and their species composition were recorded in each grid. The most dominant plant species from six families including, four tree species, one shrub species, and one grass species were selected for the hyperaccumulation analysis (Table 3.1). Shoot samples (aboveground biomass) and where possible, root samples (belowground biomass) of those species were collected to determine their metal accumulation levels.

Figure 3. 7 Field photographs of grid sampling and plant sample collection in Ginigalpelessa deposit.

Table 3. 1 The dominant plant species collected from the Ginigalpelessa serpentinite deposit.

Species No.	Scientific name	Family	Plant Habitat
1	*Pterospermum suberifolium*	Malvaceae	Tree
2	*Apluda mutica*	Poaceae	Grass
3	*Vitex pinnata*	Lamiaceae	Tree
4	*Morinda tinctoria*	Rubiaceae	Tree
5	*Tephrosia villosa*	Fabaceae	Herb
6	*Azadirachta indica*	Meliaceae	Tree
7	*Lantana camara*	Verbenaceae	Shrub
8	*Carissa spinarum*	Apocynaceae	Shrub

3.2.7 Plant sample preparation

All the plant samples collected from each grid were thoroughly washed using distilled water to remove the contaminated soil and oven-dried at 80 ºC for 48 h. The dried matter was manually crushed with a mortar and pestle and 0.50 g of each sample was incinerated at 550 ºC in muffle furnace to produce metal-enriched ash. The ashed samples were then digested using the mixture of HNO_3: HCl: H_2O_2 (1.0 mL: 3.0 mL: 1.0 mL) for 3 h at 120ºC. The digested samples were filtered and dissolved in deionized water by increasing the volume up to 25.0 ml. The samples were stored in the refrigerator and filtered again before ICP-MS analysis.

3.2.8 Blank sample preparation for plant analysis

The blank sample was prepared by digesting a mixture of 5.0 mL HNO_3 and 1.0 mL H_2O_2 for 3 h at 120ºC. The digested blank sample was added to a 25.0 mL volumetric flask and filled up to the mark with deionized water. The blank sample was also labeled separately and stored in the refrigerator.

3.2.9 Preparation of multi-elemental standard solution

A multi-elemental standard solution issued by Sigma-Aldrich, Germany was used for the preparation of standard solutions. The initial metal concentration of this stock solution is 100 ppm. Then, an intermediate solution of 250 ppb concentration was prepared from the stock solution. Using the intermediate solution, a range of standard solutions were prepared including 1 ppb, 5 ppb, 10 ppb, 25 ppb, and 50 ppb.

3.2.10 Metal analysis from ICP-MS

Metal concentrations in the prepared rock, soil, and plant samples were analyzed using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (ICapQ-Thermo Fisher, Bremen, Germany). Firstly, the prepared standard series was introduced to the instrument. Based on the accuracy of the standard curve, the blank samples, soil, rock, and plant samples were fed to the instrument and determined mainly the Ni, Cr, Co, Zn, Pb, and Cd concentrations in the samples. Three replicates of each sample were analyzed during the analysis. In order to maintain the quality of the analysis, certified international reference samples (San Joaquin NIST SRM 2709a from Sigma-Aldrich, Germany) were used. The ICP-MS analysis was performed at the analytical laboratory of the Department of Earth Resources Engineering, University of Moratuwa, Sri Lanka.

3.3 Phytomining pot trials in serpentine soil

3.3.1 Soil media preparation for pot experiments

The present study selected four hyperaccumulator species, namely *Brassica juncea* (Mustard greens), *Crotalaria verrucosa*, *Apluda mutica*, and *Imperata cylindrica* (Figure 3.8). These plants were selected according to their ecological significance or the abundance of the deposit. During the species diversity analysis, *C. verrucosa*, *A. mutica*, and *I. cylindrica* were identified in Gingalpelessa serpentinite deposit and among them, *A. mutica* was the most dominant species.

In addition, the *Crotalaria sp.* has been previously identified as a native hyperaccumulator species by Rajakaruna and Baker (2004). The *I. cylindrica* was selected since it can adapt to poor soil conditions and withstand and thrive in fire-based ecosystems (MacDonald et al, 2007; Sanford, 2011). In this study, *B. juncea* was selected since it is recognized as a multiple-metal accumulator species (Raj et al., 2020), which is not naturally abundant in the Ginigalpelessa deposit.

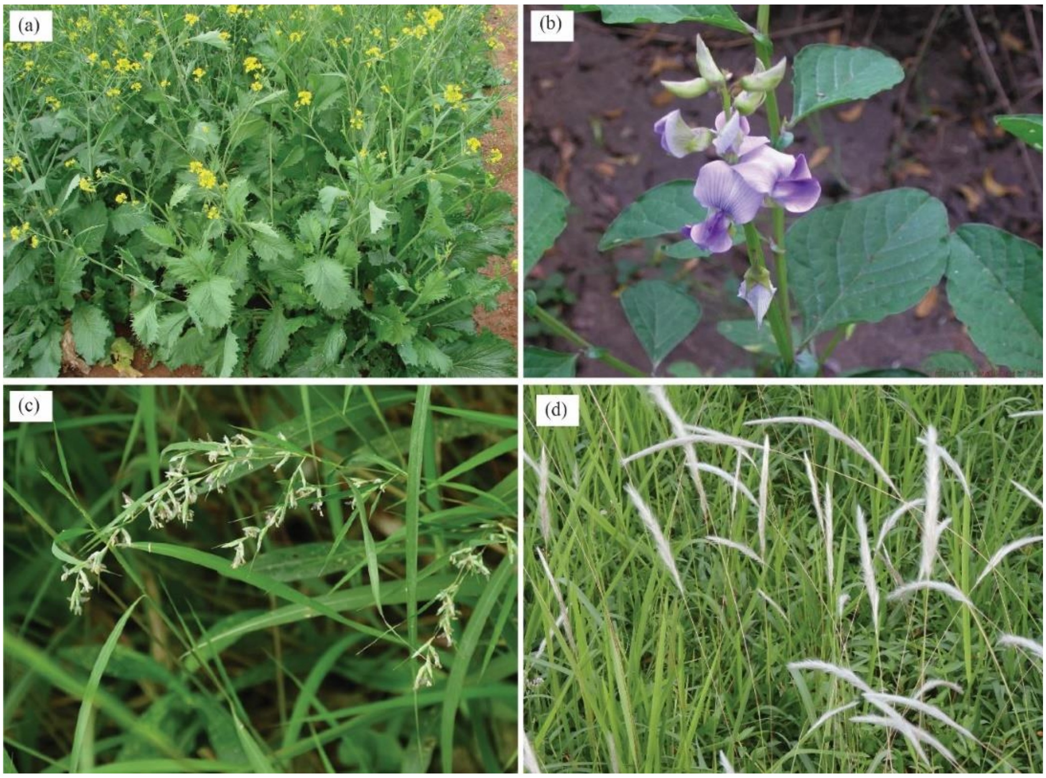

Figure 3. 8 Plant species selected for phytomining trials in serpentine soil. (a) *B. juncea*; (b) *C. verrucosa*; (c) *A. mutica*; (d) *I. cylindrica.*

The soil media was prepared by compositing soil from 10 sampling locations to prepare a homogeneous soil mixture. Then, the composite soil was mixed with 10 wt%, 20 wt%, and 30 wt% of organic fertilizer (compost) and inorganic fertilizer (NPK), separately in order to prepare different soil media (Figure 3.9). The compost fertilizer used in this study contains N = 1 wt%, P_2O_5 = 0.5 wt%, K_2O = 1 wt%, and pH 6.5 – 8.5. 2 kg of each soil mixture was filled into pots. The seeds of selected plant species were germinated in the different soil media and kept for three weeks until they started producing seedlings (Figure 3.10). The seedling ability of the plants was monitored. Then, the three-week-old seedlings were transplanted in the pots with the aforementioned soil media and kept in a greenhouse for six months. Three replicate pots were kept for each treatment separately and watered daily throughout the period. The plants were harvested when they reached the seed-producing stage (Figure 3.11).

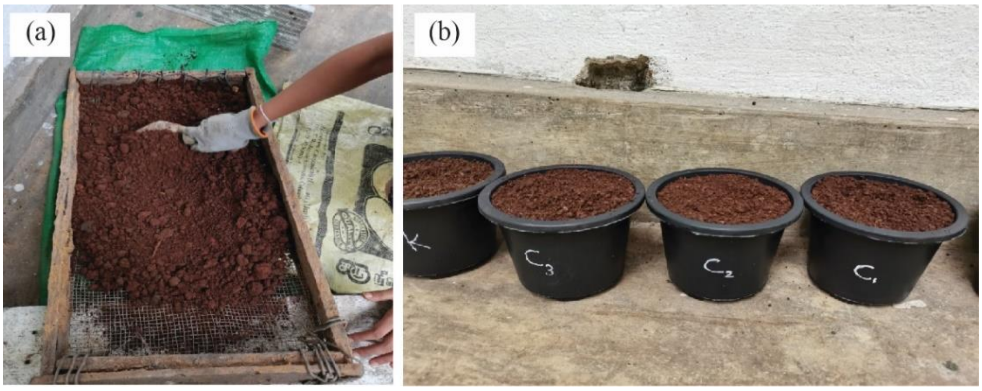

Figure 3. 9 Soil media preparation by mixing different ratios of compost and chemical fertilizers.

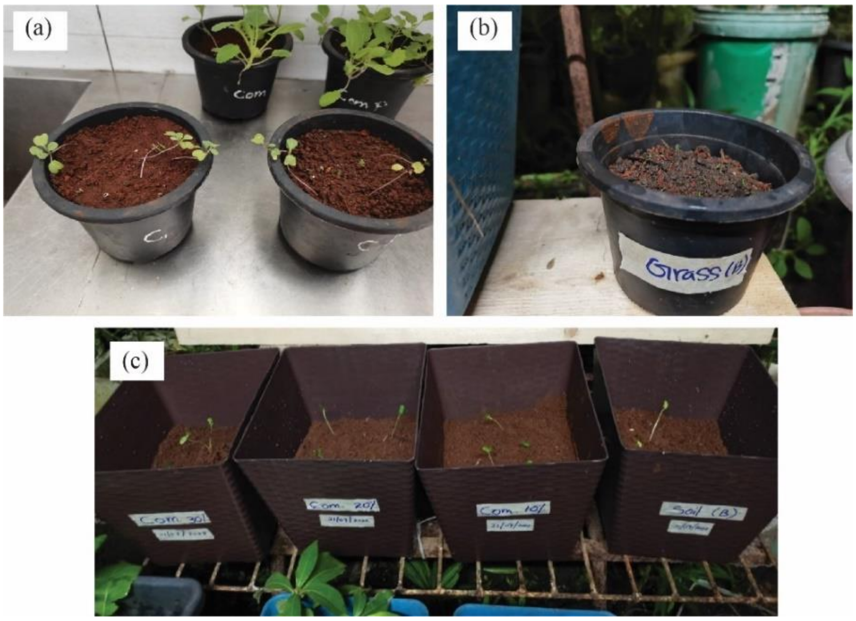

Figure 3. 10 Plants grown in soil media after 3 weeks. (a) *B. juncea*; (b) *I. cylindrica*; (c) *C. verrucosa*

Figure 3. 11 Plants at their harvesting stage (a) *B. juncea*; (b) *C. verrucosa*; (c) *I. cylindrica*; (d) *A. mutica*

3.3.2 Soil sample preparation for initial metal concentration analysis

In order to determine the initial Ni, Cr, and Co concentration in each soil medium, samples were prepared and digested using the soil sample preparation procedure mentioned in section 3.2.2. In addition, macro elements such as Ca, Mg, K, Fe, and Mn were analyzed using Atomic Adsorption Spectrometer (AAS) (iCE 3000 AA05121002v1.30) under the flame mode. Soil samples for AAS analysis were digested by adding the same mixture of acids used in section 3.2.2 and preparing a 25 mL solution in a volumetric flask using deionized water (stock solution). Then, 1 mL of the stock sample was diluted up to 100.0 mL to analyze them using AAS.

3.3.3 Phytomining plant samples preparation and analysis

The harvested plant samples were also processed and digested using the plant sample digestion method in section 3.2.7. Three replicates were analyzed for each species at different treatments using ICP-MS. A summary of plant sample processing is illustrated in Figure 3.11.

3.3.4 Nutrient analysis in soil treatments

The major nutrients such as phosphate (PO_4^{3-}), nitrate (NO_3^-), and potassium (K^+) concentrations in the soil treatments were analyzed to determine their influence on the hyperaccumulation of Ni and other metals. Therefore, soil samples of different treatments were analyzed.

3.3.4.1 Standard solution and standard curve phosphate analysis

The total phosphate concentrations in the samples were analyzed by colorimetric method. The chemical reagents were prepared using standard EPA methods. The vanadate-molybdate solution was prepared by mixing ammonium molybdate solution (solution A) and ammonium metavanadate (solution B). Solution A was prepared by dissolving 25.00 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in 300.0 mL distilled water. 1.25 g of ammonium metavanadate (NH_4VO_3) was mixed with 300.0 mL of distilled water and later, added with 330.0 mL of Conc. HCl in 1000.0 mL volumetric flask. Solution B was mixed with solution A at room temperature and topped up to 1000.0 mL solution. The solution was labelled as Vanadate-molybdate reagent and stored to prepare standard solutions. Approximately 1.00 g of KH_2PO_4 was heated at 120 ºC for an hour. Then 0.257 g of the KH_2PO_4 solid was measured and dissolved in a 1000.0 mL volumetric flask to prepare the standard solution. The standard series were prepared as 0.5 ppm, 1 ppm, 5 ppm, 7 ppm, 10 ppm, 12 ppm, and 15 ppm using the prepared solutions and analyzed the absorbance values from the spectrophotometer. The standard curve for the phosphate standard series was plotted against the absorbance.

A mass of 0.20 g from each powdered soil sample (<63 μm) were digested with 5.0 mL of Conc. HNO_3 in a 100.0 mL beaker and evaporated nearly to dryness. Then the digested samples were added with 50.0 mL of distilled water. The 10.0 mL of the dissolved samples were transferred into 100.0 mL volumetric flasks, mixed with 10.0 mL of Vanadate-molybdate reagent, and the total solution was topped up to 100.0 mL.

The filtered samples were used to analyze the absorbance value from the spectrophotometer. The phosphate concentrations of the samples were determined using the standard curve.

3.3.4.2 Total nitrate concentration in soil treatments

Nitrate concentrate in the soil samples were analyzed using portable nitrate-chloride meter. The dried soil samples (< 2 mm) were used to prepare the soil extract by mixing soil and water (1:5 ratio). Therefore, 5.00 g of soil sample was mixed with 25.0 mL of distilled water and shaken for about 5 min. The sample was allowed to settle and measured the nitrate concentration using the meter. This method was carried out for all the soil samples at pre-planting stage.

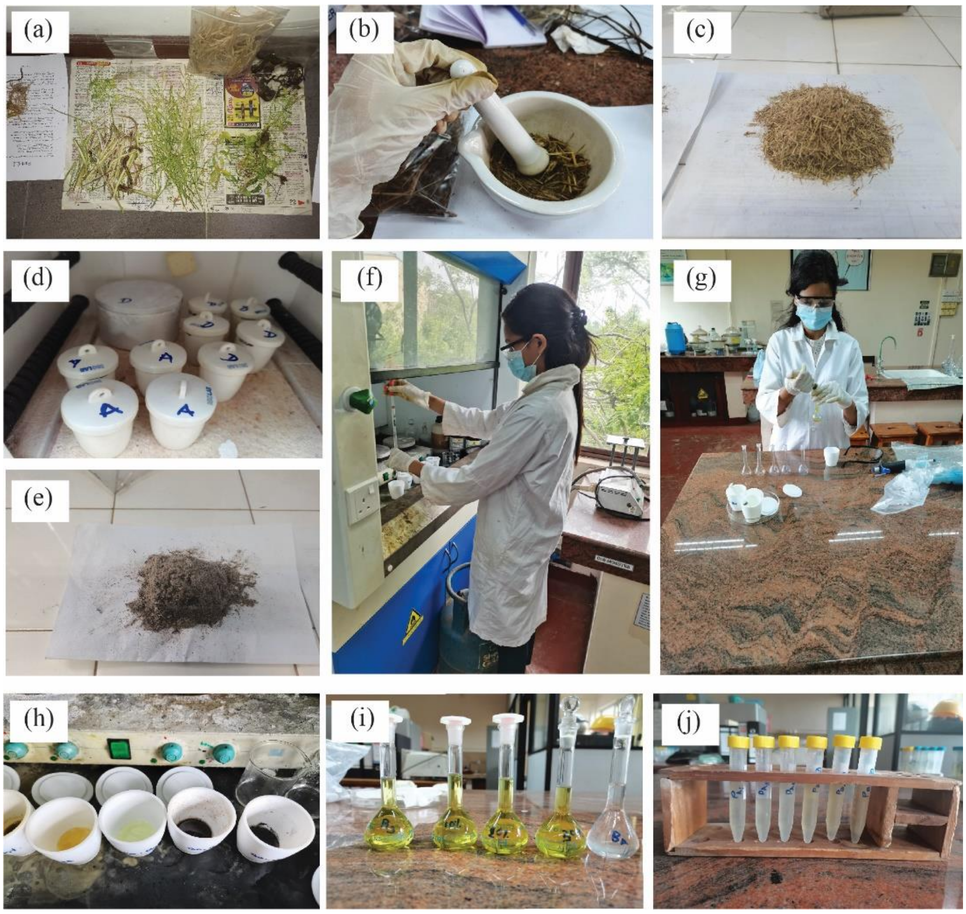

Figure 3. 12 Processing and digestion of phytomining plant samples. (a) Harvested plant biomass; (b) Grinding of dry biomass; (c) Grounded dry biomass; (d) Incineration of plant matter in muffle furnace; (e) Plant ash; (f) Digestion of dry

matter and ash plant samples; (g) Filtering the Digested solution; (h) Digested ash samples before filtering; (i) 25.0 mL solution prepared from Digested samples; (j) Diluted solutions for ICP-MS analysis.

3.4 Metal extraction and leaching from plant biomass

Ash samples of the *A. mutica* biomass were used for the leaching experiments. The samples were prepared from open air burning and incineration using the muffle furnace (see section 3.2.7) to determine the efficiency of the two ashing methods. The H_2SO_4 acid was used as the leaching reagent since it has been used in many Ni leaching experiments from plant biomass and shown effective results for commercial recovery of Ni (Barbaroux et al., 2011; 2012; Bani et al., 2015a). Different weights of ash samples 5.00 g and 10.00 g were measured from both ash types and they were mixed separately with 50.0 mL of the 1 mol/L and 5 mol/L sulfuric acid (H_2SO_4) concentrations. The leaching experiments were designed with two pulp densities (100 g/L and 200 g/L) and two sulfuric acid concentrations (1 mol/L and 5 mol/L) to identify the effective steps to recover Ni from *Apluda* ash. The leaching experiments were carried out in closed flasks to avoid the evaporation and solution loss.

The leaching experiments were performed at room temperature at 300 rpm speed for 2 h. During the leaching procedure, 2.0 mL of the leached solution was sampled at 30 min intervals to determine the solubilization of Ni and other metals. Those samples were filtered and diluted up to 10 mL for the elemental analysis using ICP-MS. All the methods were triplicated to maintain the quality of the analysis.

3.5 Calculations

3.5.1 Determination of spatial metal enrichment

The results of the ICP-MS analysis were used to develop the spatial distribution maps of Ni and other abundant metals. Inverse Distance Weighted (IDW) interpolation maps were developed for each metal, using ArcGIS 10.4 software.

3.5.2 Determination of bioconcentration factor and translocation factor

3.5.2.1 *Bioavailable ratio*

The bioavailable ratio was analyzed to determine metal availability for uptake by plants. The analysis is carried out by considering the bioavailable fraction of a certain metal and its total concentration in the soil. The values are calculated using Equation 1 (Zahid & Williams, 2016).

$$\text{Bioavailable ratio (\%)} = \frac{\text{bioavailable metal concentration in the soil (mg/kg)}}{\text{total metal concentration in soil (mg/kg)}} \qquad (1)$$

3.5.2.2 *Bioconcentration factor*

The bioconcentration factor (BCF) is an index that indicates the metal accumulation efficiency of a plant species from its surrounding environment. It can be used for plants grown in both soil and water media. Moreover, higher BCF values (> 1) are regarded as an indicator of efficient hyperaccumulation (Mishra & Pandey, 2019; Monei et al., 2021; Tabasi et al., 2018). Therefore, the BCF value for each plant was calculated using Equation 2 by considering the bioavailable soil metal concentration of the sampled area.

$$\text{BCF} = \frac{\text{metal concentration in the plant shoots (mg/kg)}}{\text{bioavailable metal concentration in soil (mg/kg)}} \qquad (2)$$

3.5.2.3 *Translocation factor*

The translocation factor (TC) reflects the efficiency of a plant species to translocate metals from root parts to aerial parts of a plant. When the TF value > 1, the plant is considered as an accumulator for the particular metal (Tabasi et al., 2018). The TF values of the metals in each plant were calculated using Equation 3.

$$\text{TF} = \frac{\text{metal concentration in the plant shoots (mg/kg)}}{\text{metal concentration in the plant roots (mg/kg)}} \qquad (3)$$

3.5.3 Calculation of Leaching Effciency

The leaching efficiency (α) of each trials was calulated using following equation.

$$\alpha = \frac{(C_1 * V * D) \times 100\%}{C_2 * M} \tag{4}$$

In this equation, C_1 is the metal concentration in the leachate and V is the acid volume used for the leaching assay. D is the dilution factor where C_2 is the total metals concentration in the sample (after aqua regia digestion) and M is the mass of the bio-ore with respect to the pulp density (Dushyantha et al., 2021).

CHAPTER 4: RESULTS AND DISCUSSION

In this chapter, results of Ni and other valuable metals considered in both soil and rock samples and metal accumulation levels of native species is discussed to identify the potentials zone for phytomining and the suitable native species. Moreover, results of phytomining pot trials are assessed and discussed by comparing the accumulation levels of selected four species to identify the most suitable plant to proceed with the Ni leaching experiments. The Ni leaching potential from the selected species has been evaluated using the Ni recovery percentage. Finally, the economic potential of Ni phytomining in serpentine is discussed by considering the recovery percentage from plant biomass at different soil treatment levels.

4.1 The metal concentration in serpentine soil and the selection of hyperaccumulator plants

4.1.1 The total metal distribution in serpentine soil and identification of high potential areas for phytomining

To determine the total concentration of metals in the serpentine soil, mainly six economically valuable metals including Ni, Cr, Co, zinc (Zn), lead (Pd), and (Cd) were considered. Table 4.1 present the total concentrations of the selected metals analyzed after aqua regia digestion. The serpentine soil samples exhibited enrichment of Ni, Cr, Co, and Zn throughout the deposit compared to the weathered serpentinite rock, whereas Pd and Cd showed a decline (Figure 4.1). In general, serpentinite environments display a high geochemical background of trace metals due to the local climatic conditions (humidity, temperature, and rainfall), weathering susceptibility of the host rock, and the edaphic factors of soil (pH, cation exchange capacity, redox potential, texture, organic matter content, amount of oxides, and carbonates (Caillaud, et al., 2009; Vithanage et al., 2014). The weathering process in serpentinite deposits results in the transporting of weathered parent materials (serpentinite rock) which concentrate in the secondary phase (serpentine soil). However, the distribution of such metals in the soil profile depends on their mobility and the bioavailability of the soil (Vithanage et al., 2019; Dushyantha et al., 2021).

The average concentration of total Ni was 8,731 mg/kg (ranging from 4,005 to 17,352 mg/kg) in the soil samples, and it was the highest, followed by 1,488 mg/kg of Cr (655 - 4,113 mg/kg), 487 mg/kg of Co (150 - 849 mg/kg), and 405 mg/kg of Zn (127 – 1,253 mg/kg). The concentrations of the above four metals in soil have exceeded the maximum allowable levels issued by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) for agricultural and industrial land use (Chiroma et., 2014) (Table 4.1). Moreover, the Ni, Cr, Co, and Zn concentrations in the Ginigalpelessa serpentine soil and rock were close to the values of previous literature (Ni = 4,000 – 20,000 mg/kg, Cr = 3,000 – 15,000 mg/kg, Co = 100 – 600 mg/kg, and Zn = 100 - 400 mg/kg) (Dissanayake, 1982; Vithanage et al., 2014; Dushyantha et al., 2021).

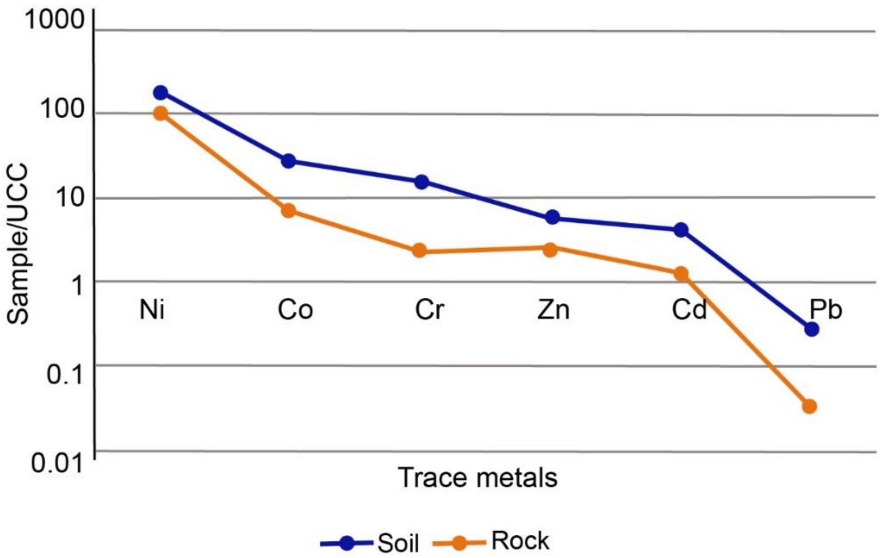

Figure 4. 1The relative enrichment of trace metals in serpentine soil and weathered serpentinite rocks compared to the Upper Continent Crust (UCC) (UCC values are from Rudnick et al. (2003)).

The spatial distribution of Ni, Cr, Co, and Zn in serpentine and non-serpentine soil is depicted in Figure 4.2. Here the Pb and Cd concentrations were not considered since the concentrations of those metals were below the detection limits in non-serpentine soil. As Figure 4.2 illustrates, the total concentrations of Ni, Cr, Co, and Zn within the serpentinite body were significantly higher than those outside the deposit. Furthermore, the enrichment of total Ni was extremely high compared to other metals,

Table 4. 1 The total metal concentration in serpentine soil and rock samples (mg/kg) in the Ginigalpelessa serpentinite deposit.

Metal	**Sample Type**	**Sampling locations**										
		1	2	3	4	5	6	7	8	9	10	11
Ni	Soil	7,764.40	4,048.98	8,495.04	6,597.86	10,332.06	9,769.37	10,634.51	8,436.74	8,198.25	8,186.17	10,381.03
	Rock	NA	NA	5071.83	NA	2,344.17	NA	4,915.47	4,355.43	2,575.68	7972.19	NA
Co	Soil	597.16	150.38	557.58	332.74	732.83	729.06	648.64	570.21	582.21	377.79	479.33
	Rock	NA	NA	142.00	NA	105.45	NA	163.32	104.94	99.12	171.08	NA
Cr	Soil	1037.20	1006.66	953.77	839.36	1,733.24	1,480.19	3,349.71	836.37	1,153.23	1,310.34	1,684.87
	Rock	NA	NA	65.13	NA	232.62	NA	50.81	164.81	171.71	521.11	NA
Zn	Soil	380.69	1,253.08	460.55	335.51	264.41	183.97	216.19	177.67	228.04	522.62	708.08
	Rock	NA	NA	203.94	NA	69.76	NA	96.10	93.49	77.04	341.55	NA
Cd	Soil	BDL	BDL	BDL	BDL	0.35	0.36	0.33	0.27	0.51	BDL	BDL
	Rock	NA	NA	NA	NA	0.24	NA	0.05	0.21	0.23	NA	NA
Pb	Soil	1.54	2.84	1.78	1.08	11.20	7.08	5.71	4.66	8.36	1.77	3.82
	Rock	NA	NA	BDL	NA	0.90	NA	5.23	BDL	0.36	0.03	NA

Metal	Sample Type	Sampling locations										
		12	13	14	15	16	17	18	19	20	21	22
Ni	Soil	10,217.43	9,327.16	10,793.44	17,351.69	8,451.08	8,627.61	9,199.85	5,812.04	6,674.19	5,576.74	10,538.50
	Rock	NA	6,896.10	4,935.29	17,417.07	5,480.28	1,864.68	2,555.80	2,991.07	NA	NA	3,699.68
Co	Soil	420.18	458.08	595.57	697.22	286.67	487.11	527.11	350.32	318.03	330.96	543.14
	Rock	NA	146.32	70.20	119.81	214.05	85.55	129.92	171.32	NA	NA	113.61
Cr	Soil	3,154.85	1,552.82	1,594.62	2,762.71	1,103.06	1,893.75	1,361.15	809.83	682.85	655.15	1,271.79
	Rock	NA	169.17	397.10	415.02	180.22	119.58	130.27	236.39	NA	NA	163.00
Zn	Soil	482.12	666.41	891.04	862.95	404.07	226.79	172.72	127.24	141.26	137.32	203.09
	Rock	NA	272.69	212.18	702.88	228.21	70.44	65.47	75.69	NA	NA	82.54
Cd	Soil	BDL	NA	BDL	BDL	BDL	0.41	0.56	0.25	0.37	0.30	0.41

	Rock	NA	NA	NA	NA	NA	0.01	0.05	0.04	NA	NA	0.11
Pb	Soil	1.06	3.14	3.91	2.62	0.62	8.94	4.72	2.75	3.79	7.64	8.81
	Rock	NA	BDL	BDL	3.91	BDL	0.01	0.39	0.69	NA	NA	0.12

Metal	**Sample Type**	**Sampling Locations**										
		23	24	25	26	27	28	29	30	31	Average	WHO/FAO
Ni	Soil	4,004.55	9,617.70	7,842.94	7,202.66	9,661.10	6,850.88	7,308.98	12,316.63	10,434.98	8,730.79 ± 2,501.35	50
	Rock	NA	6,431.40	1,691.97	1,806.76	3,619.66	4,969.09	3,403.23	8,233.41	NA	4,915 .73 ± 3,378.42	

Co	Soil	162.95	433.82	544.76	432.51	848.74	234.36	362.32	778.19	523.15	486.87 ± 171.86	50
	Rock	NA	145.63	71.72	89.22	109.42	117.46	100.44	149.05	NA	124.74 ± 36. 49	
Cr	Soil	798.46	1,456.84	1,031.17	938.65	1,283.72	906.57	871.62	4,112.53	2,496.76	1,487.86 ± 833.24	100
	Rock	NA	327.54	97.38	120.50	102.55	468.20	155.83	315.91	NA	219.28 ± 135.87	
Zn	Soil	470.03	540.29	162.76	215.56	230.89	285.99	448.46	232.10	908.24	404.52 ± 274.30	300
	Rock	NA	236.06	55.94	57.59	89.21	198.17	139.57	150.11	NA	167.55 ± 147.68	
Cd	Soil	BDL	BDL	0.26	0.51	0.54	BDL	BDL	0.44	0.40	0.39 ± 0.10	3

	Rock	NA	NA	0.15	0.02	0.18	NA	NA	0.11	NA	0.12 ± 0.09	
Pb	Soil	3.36	2.03	5.4	6.89	10.16	0.43	5.11	7.41	5.44	4.65 ± 2.91	100
	Rock	NA	BDL	0.03	0.11	0.07	BDL	BDL	1.82	NA	1.05 ±1.66	

NA- Not Available BDL- Below Detection Limit especially on the western (e.g. GP - 15) and northern (e.g. GP - 22) sides of the deposit. Despite some highly concentrated areas identified in the present study, the average Ni concentration was close to the values in the literature (Kumarathilaka et al., 2014, Dushyantha et al., 2021).

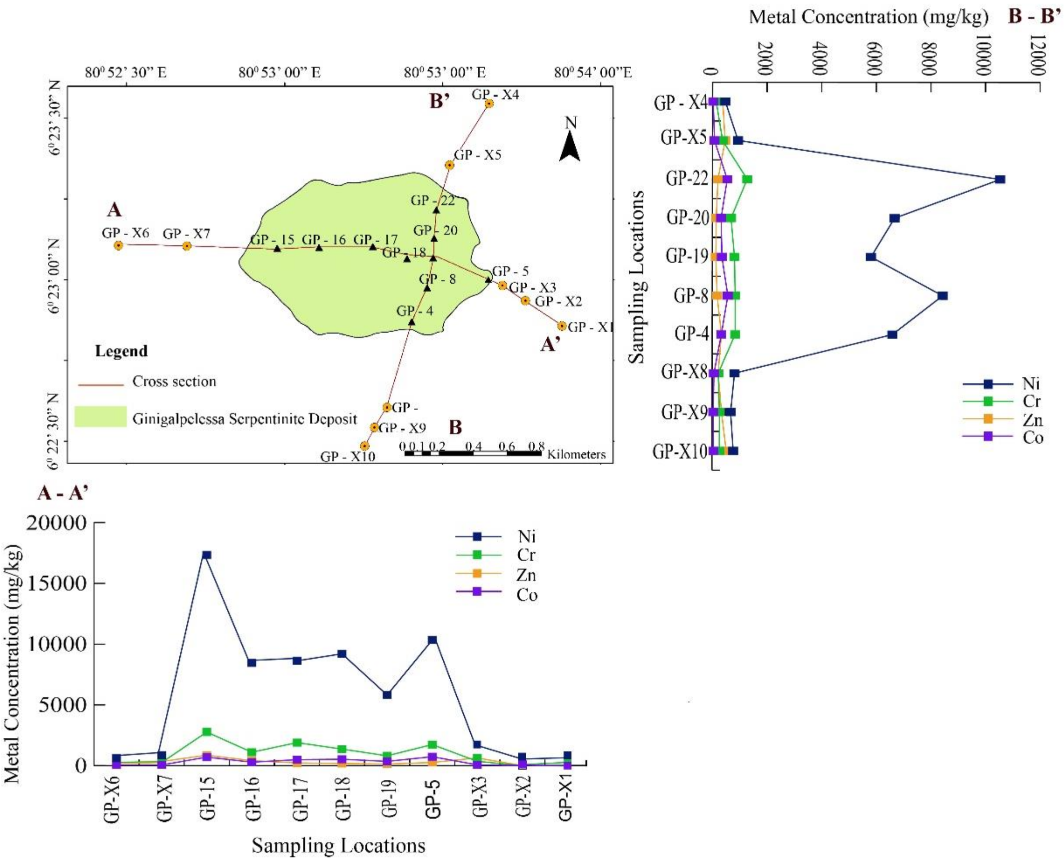

Figure 4. 2 The spatial distribution of Ni, Cr, Co, and Zn in serpentine and non-serpentine soil (2 graphs were drawn in line with sample location in A-A' and B-B' directions).

The spatial metal distribution of Ni, Cr, Co, and Zn within the deposit is illustrated in Figure 4.3. As Figure 4.3 indicates, most of the deposit has a greater potential for low-grade Ni mining since the average concentration is closer to the global phytomining grade of serpentine soils (0.6-1.2 wt%) (Norgate & Jahanshahi, 2010). Furthermore, the study identified some areas with high Cr (GP - 14 & 15, GP - 38), Co (GP - 14, 15, 28, & 27), and Zn (GP - 14, 15, & 2), even though their phytomining potential is relatively low. According to previous studies, the mineralogical composition of the serpentine soil and the high weathering effects may be the reason for the different Ni concentrations observed throughout the deposit (Dissanayake, 1982; Hewawasam et al., 2014; Kumarathilaka et al., 2014).

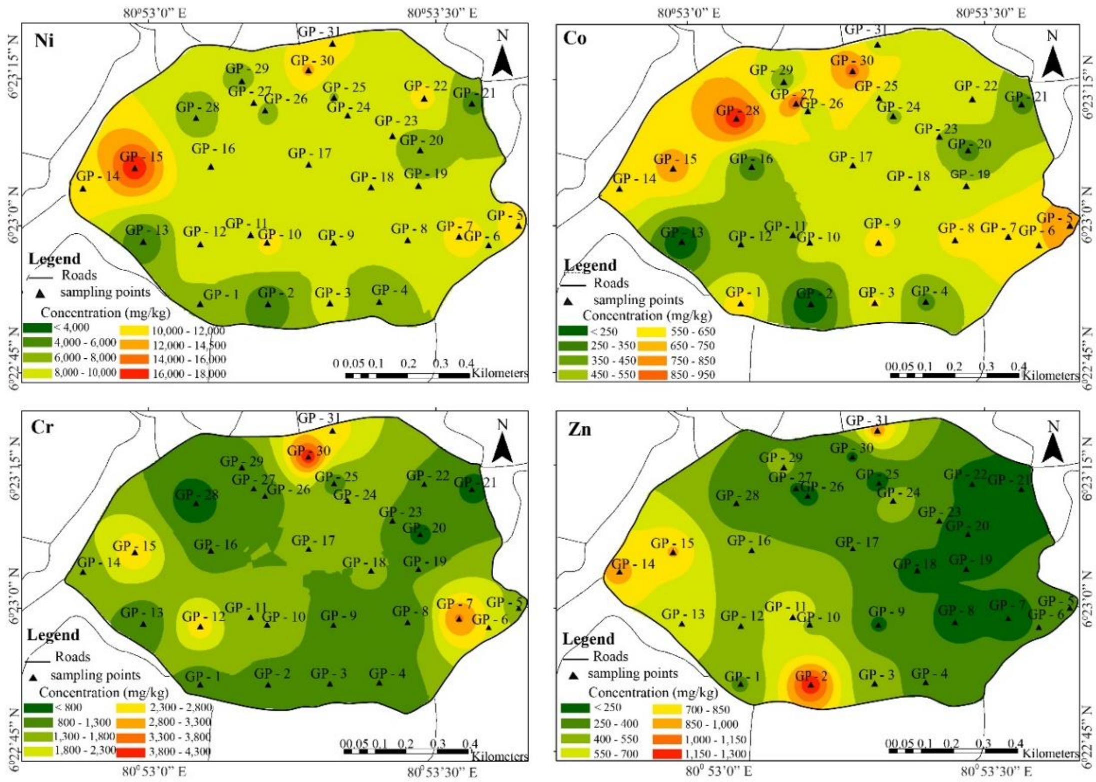

Figure 4. 3 Spatial distribution of a) Ni concentration, b) Co concentration, c) Cr concentration, and d) Zn concentration in the Ginigalpelessa serpentine soil.

4.1.2 Ni grade in Ginigalpelessa serpentine soil

The current study observed that areas with high concentrations of Ni in the serpentine soil are close to the Ni grade in high-grade Ni laterites in the world (e.g. GP 15). In addition, the areas enriched with > 10,000 mg/kg of Ni in the surface soil can be further explored vertically to determine the Ni availability in the underneath layers. According to previous studies, the enrichments of metals in the serpentine soil profiles are high in the upper layers, particularly from the surface to 30-100cm depth, compared to the bottom soil layers (Caillaud et al., 2009; Van der Ent et al., 2013a; Vithanage et al., 2019). Moreover, the potential for direct extraction of Ni from serpentine soil needs to be assessed further, particularly in those locations, while minimizing the environmental impact. However, the ecological significance of the serpentinite environments and the expensiveness and destruction of conventional mining practices implies phytomining is the best method to extract the metals from soil. In this context, the bioavailable concentration of Ni in the serpentine soil should be assessed to determine the potential of phytomining in Ginigalpelessa serpentine soil. Therefore, major oxide composition, mineralogy, bioavailable Ni concentration, and nutrient content (Morais et al., 2015) in the soil should be further studied to identify the factors affecting the enrichment of Ni in Ginigalpelessa serpentine soil.

4.1.3 Minerology in serpentine soil and rock

The XRD analysis revealed that antigorite ($(Mg, Fe)_3Si_2O_5(OH)_4$) is the dominant mineral in both soil and rock samples. It was similar to the findings of Vithanage et al. (2014). Along with antigorite, chrysotile ($Mg_3(Si_2O_5)(OH)_4$) and lizardite ($(Mg,Ni)_3(Si_2O_5)(OH)_4$) were also found in the rock samples. Those minerals were found in the serpentine soil indicating the impact of rock weathering. Figure 4.4 and Figure 4.5 show the XRD analysis of sample location 15, where the highest Ni content was detected. Antigorite and Crysotile minerals were found in both rock and soil samples. In addition, some samples indicated magnesite in both soil and rock samples. The results of the other soil and rock samples are attached in Apendix 1.

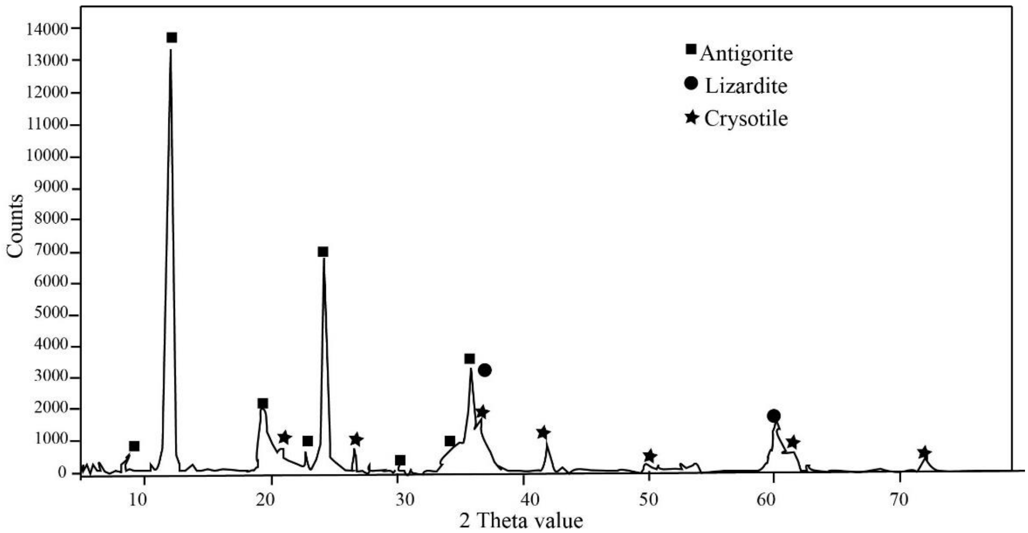

Figure 4. 4 Example for the XRD results of serpentinite rock samples in Ginigalpelessa serpentinite deposit

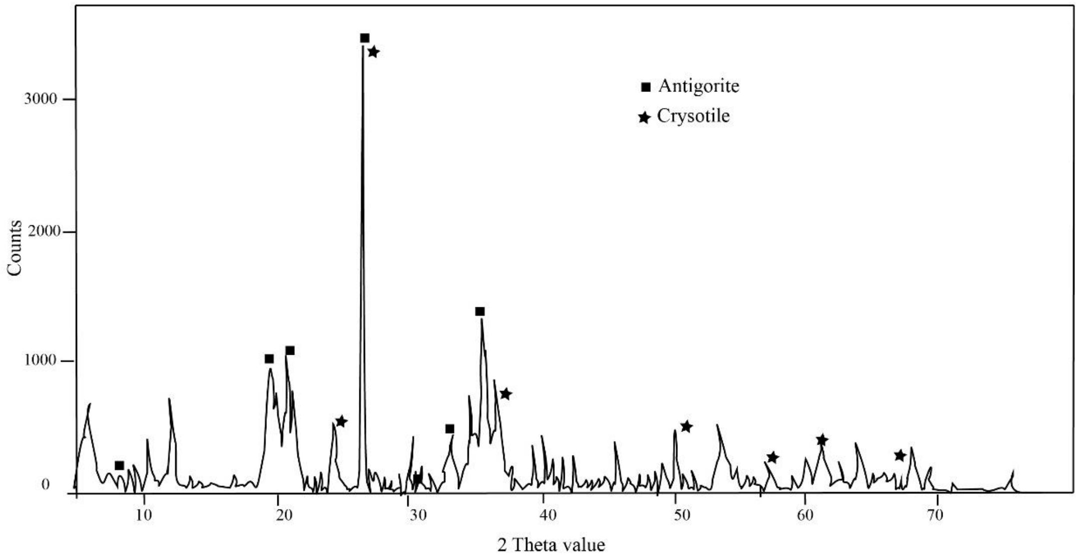

Figure 4. 5 Example for XRD results of serpentine soil samples in Ginigalpelessa serpentinite deposit

4.1.4 Dispersion of plants and metal accumulation

As reported by Rajakaruna and Bohm (2002); Galey et al. (2017); Fernando et al. (2021), the current study also observed low plant diversity within the deposit with a few tree species, shrubs, and grasses. Among them, *Apluda mutica* and *Morinda tinctoria* species were dominant on the deposit. In addition, *Vitex pinnata, Tephrosia purpurea, Azadirachta indica, Lantana camara***,** *Carissa spinarum*, and *Pterospermum suberifolium* were also abundant in most parts of the deposit.

The average metal accumulation levels of selected plant species are shown in Table 4.2 *A. mutica* accumulated high concentrations of Ni ranging from 1,150 mg/kg to 3,638 mg/kg in shoots and from 351 mg/kg to 1,446 mg/kg in roots, exceeding the hyperaccumulator threshold limits of Ni (1,000 mg/kg). These nominal threshold limits provide a practical guideline for the identification of hyperaccumulator species. Therefore, *A. mutica* species can be considered as a hyperaccumulator for Ni due to its ability to accumulate more than 1,000 mg/kg of Ni. Ni concentration of the other plant species considered in the study was lower than the threshold values (Table 4.2). Therefore, those plant species can be considered as excluder species that accumulate small concentrations of metal and survive in the contaminated soil (Mehes-Smith et al., 2013). The *M. tinctoria*, one of the dominant plant species observed on the deposit, accumulated 849 ± 119 mg/kg of Ni in its shoot biomass. Furthermore, the average Ni content of the *Morinda* plant measured in the current study was greater than those published by Dushyantha et al. (2021). However, all the plant species were able to tolerate and thrive under the toxicity effects of serpentine soil.

The Cr and Co accumulation levels in the plant samples were lower than the proposed threshold levels. Moreover, the accumulation of Zn by the plant species was below the detectable limit even though Zn concentration in serpentine soil is comparatively high. According to (Tabasi et al., 2018), the Zn hyperaccumulation process in plants is extremely rare as it often precipitates as sulfate in root parts of the plant, inhibiting its transportation in shoot parts.

4.1.5 Metal bioavailability in serpentine soil

EDTA treatment in soil extracted a range of 85.65 mg/kg to 370 mg/kg of Ni (1-4%) and 5.76 to 16.61 mg/kg of Co (1-3%) in Ginigalpelessa serpentine soil (Table 4.2). In this method, EDTA strongly bonds with Ni and Co in organic pools of the soil and extracts them into the solution phase (Wang et al., 2020). The present finding was compatible with the literature relevant to the study area, where both DTPA and $CaCl_2$ extractants have been used to analyze the bioavailability of Ni (Vithanage et al., 2014). Even though the total Cr concentration in the soil is high, the bioavailable ratio of Cr was very low. Therefore, the BCF and TF values were analyzed only for Ni and Co accumulations of the selected plant species.

4.1.6 BCF and TF values in plant samples

BCF and TF are important factors in screening hyperaccumulator plants for phytomining. Therefore, evaluating the BCF and TF of the plant would expedite determining hyperaccumulator species. According to the bioavailable Ni and Co concentrations in the soil, all the plant species showed high BCF values, except the BCF value of *P. suberifolium* species for Co accumulation. The *A. mutica* species which exceeded the hyperaccumulator threshold levels exhibited the highest BCF value for Ni (8.26). *Tephrosia villosa* also showed 7.03 BCF value for Ni, even though its hyperaccumulation level did not reach the threshold levels. Some plant species including *V. pinnata*, *A. indica*, and *L. camara* exhibited high BCF values for Co compared to Ni. However, none of those species exceeded the hyperaccumulator threshold levels for Co (Figure 4.6) (Table 4.2).

The arial parts of *A. mutica* species concentrated high amounts of Ni and Co compared to the roots part. Therefore, TF values of the *Apluda* sp. were greater than one (> 1) for Ni and Co (3.0 and 3.14, respectively) further proving that the plant species could be an hyperaccumulator for above metals. According to the literature, a high translocating factor is also a fundamental characteristic of a plant to become an effective hyperaccumulator (Brooks et al., 2001; Morais et al., 2015). However, further studies are required to confirm the hyperaccumulator ability of *A. mutica* species.

To become an ideal hyperaccumulator for phytomining, the plant species should fulfil the following criteria, such as the adaptability to tolerate metal toxicity whilst accumulating metals highly in shoot biomass, the ability to grow fast, repulsiveness to herbivory, widely dispersed in metal-rich soil, BCF and TF values > 1, and the ease to cultivate and harvest (Ha et al., 2011; Zhang et al., 2014). In this context, the hyperaccumulation ability of Ni in *A. mutica* species and the efficient bioaccumulation and translocation capacity from roots to shoots make it a potential candidate for phytomining. Therefore, *A. mutica* was selected for the phytomining trials from the initial metal accumulation experiments. Moreover, this grass species is native to South Indian region and has been identified in other serpentinite deposits (Rajakaruna & Bohm, 2002). According to the field observations, *Apluda* species is widely dispersed within the deposit showing a rapid growth (6-8 months). Moreover, the grass species grows up to 1 m in height with biomass ranging from 15,000-17,000 kg/ha.

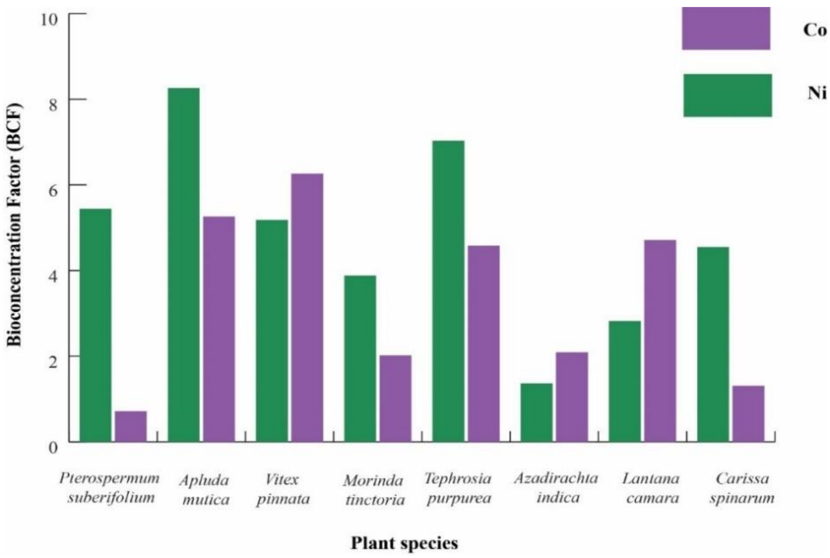

Figure 4. 6 BCF of Ni and Co in selected plant species collected from different sampling locations in the Ginigalpelessa serpentinite deposit.

4.2 Ni Phytomining potential in Ginigalpelessa serpentine soil

4.2.1 Macro element concentration in soil treatments

The major elements, such as N, P, K, Ca, Mg, Fe, and Mn which are necessary for plant growth were analyzed to determine their effect on phytomining. Table 4.3 represents the total macronutrient concentration in the different soil media. The nitrate content in natural serpentine soil in Ginigalpelessa varied from 875-1,015 mg/kg. In addition, the nitrate content in the fertilized-mixed serpentine soil media was also shown a similar range despite the slight increase of the nitrate with the elevated compost treatments. Phosphate concentration in the serpentine soil also increased from untreated soil to 10%, 20%, and 30% compost treatments (4,934 mg/kg, 5,757 mg/kg, 12,747 mg/kg, and 16,859 mg/kg, respectively). The major element concentrations in Ginigalpelessa serpentine soil such as phosphate, potassium, Fe, Mg, Mn, and Ca were close to the values of previous findings in the deposit (Vithanage et al., 2014, 2019; Dushyantha et al., 2021). As Vithanage et al. (2014) reported, average concentrations of major elements like phosphate and potassium in the natural soil were 1,000 mg/kg and 2,700 mg/kg. However, the slight deviations in the values may be attributed to the composition of the composite serpentine soil media and the average micro- and macro-element concentrations at different sampling locations.

Table 4. 2 Concentration of Ni and Co concentrations (mg/kg) in the biomass of selected plant species, hyperaccumulator threshold limits of Ni and Co, their bioavailability and the total concentrations in serpentine soil of Ginigalpelessa.

Plant species	**Sample type**	**Metal concentration (mg/kg)**						**Bioavailable ratio**		**BCF**		**TF**	
		Shoot and root		Total amount in soil		Bioavailable fraction in soil							
		Ni	Co	Ni	Co	Ni	Co	Ni	Co	Ni	Co	Ni	Co
Pterospermum suberifolium	Shoot	466.07	4.13	8627.61	487.11	85.65	5.76	1%	1%	5.44	0.72	-	-
Apluda mutica	Shoot	1577.84	66.71	9,237.16	628.26	369.65	14.97	4%	2%	8.26	5.26	-	-
	Root	701.60	38.03							-	-	3.06	3.14
Vitex pinnata	Shoot	443.93	36.09	8627.61	487.11	85.65	5.76	1%	1%	5.18	6.26	-	-
Morinda tinctoria	Shoot	741.65	25.57	10,793.44	595.57	190.95	12.68	2%	3%	3.88	2.02	-	-
Tephrosia villosa	Shoot	940.84	34.83	8,198.25	582.21	133.79	7.60	2%	1%	7.03	4.58	-	-
Azadirachta indica	Shoot	182.84	15.86	6,674.19	682.85	133.79	7.60	2%	1%	1.37	2.09	-	-

Lantana camara	Shoot	532.15	34.99	7,202.66	432.51	188.59	7.42	3%	2%	2.82	4.71	-	-
Carissa spinarum	Shoot	446.62	21.80	10,538.50	543.14	98.13	16.61	1%	3%	4.55	1.31	-	-
Hyperaccumulator threshold limits for plants		1000	300										

Note: Hyperaccumulator threshold limits values are taken from (Debnath et al. 2015; Reeves et al. 2018).

Analysis of Ca and Mg concentrations in soil treatment depicted that the Mg concentration in natural serpentine soil is extremely high compared to the Ca concentration showing a low Ca/Mg quotient (Untreated = 0.0013, Compost 10% = 0.0057, Compost 20% = 0.0028, and Compost 30% = 0.0035). However, the Ca concentration increased with fertilizer application from 0.01 (natural serpentine soil) to 0.03% (30% compost treatment). Other major elements, such as Fe and Mn content found in this soil were decreased with fertilizer application. Therefore, lower N, P, K, and Ca deficiency, Mn toxicity, and high Ni, Cr, and Co concentrations have caused poor productivity and stunted growth of the plant species (Salihaj & Bani, 2018).

4.2.2 The bioavailable concentrations of the metals

The size of the bioavailable metal reserve is critical in determining the yield and the phytomining efficiency of a particular plant (Chaney et al., 2021). Therefore, the bioavailable concentrations of Ni, Cr, and Co were assessed in each soil media as in Table 4.3. According to the analysis, soil Ni availability can vary with the soil treatments (whether organic or inorganic) and the mixing ratio of the compost. The present study identified that the bioavailable Ni and other metal concentrations are very low compared to the average total concentrations in the serpentine soil. Compared to the bioavailability of Ni and Co in the soil media, Cr showed lower concentrations, which triggers the poor uptake of Cr in plants. Successful phytomining field trials in low Ni bioavailable serpentine soils in Malaysia - 117 mg/kg (Bouman et al., 2018), Albania - 250-300 mg/kg (Barbaroux et al., 2012), Spain - 3.8-100 mg/kg (Cerdeira-Pérez et al., 2019), USA – 25-100 mg/kg (Chaney & Mahoney, 2014), and Turkey 10-150 mg/kg (ALTINÖZLÜ et al., 2012) assure the feasibility of Ni phytomining in Ginigalpelessa serpentinite deposit.

Table 4. 3 Macronutrient and bioavailable trace metal concentrations in the soil media

Metal/ Element	**Soil treatments**			
	10%	**20%**	**30%**	**Untreated**
Major nutrient concentration (mg/kg)				

Nitrate (N)	890	1,110	1,120	875
Phosphate (P)	15,756.58	25,493.42	33,717.11	9,868.42
Potassium (K)	383	803	1,283	3,748
Total major element concentration (%)				
Ca	0.011	0.012	0.013	0.03
Mg	7.98	2.16	4.45	8.37
Fe	16.66	9.38	10.33	17.36
Mn	0.51	0.53	0.59	0.57
Total trace element concentration (mg/kg)				
Ni	7162.09	6875.44	6513.93	8440.54
Cr	1553.66	1471.86	1376.73	1837.67
Co	763.66	773.54	800.95	1010.45
Bioavailable metal concentration (mg/kg)				
Ni	227.48	193.77	146.62	250.1
Cr	0.21	0.2	0.23	0.35
Co	16.46	3.75	3.15	23.66

4.2.3 Ni phytomining potential of the selected plant species

Among the selected metal accumulator species, *C. verrucosa* produced the highest Ni-rich ash from its biomass, particularly at 10% compost treatment (7,406±285 mg/kg) (Table 4.4). In this plant, the average Ni concentration in the 1 kg of ash was closer to the average total Ni concentration in the 1 kg of serpentine soil (~ 84%). Even though *B. juncea* was used as an introduced metal accumulator species it showed a good potential for Ni recovery, compared to the native species like *I. cylindrica*. However, the poor soil nutrients and the high Ni, Cr, and Co concentrations in the natural serpentine soil significantly affected the growth and survival of *B. juncea* in untreated soil. *A. mutica*, species also produced high Ni-rich ash in untreated and NPK-treated soils. In this regard, *C. verrucosa* and *A. mutica* species were recognized as the highest potential species for Ni phytomining from the present study. Along with Ni concentrations, this study identified that the accumulation of Cr and Co in plant species also altered when the addition of fertilizers.

4.2.4 Effects of soil amendments in phytomining

The analysis of each plant ash indicated that most of the plant species showed an inverse relationship with metal accumulation. For example, Ni accumulation in *B. juncea* was decreased when increasing the compost levels (Compost 10% = 2,657 mg/kg, Compost 20% = 1,552 mg/kg, and Compost 30% = 1,545 mg/kg) (Table 4.4). Similarly, Ni concentration in *I. cylindrica* ash samples was substantially decreased as 1,810 mg/kg > 1,547 mg/kg > 708 mg/kg with the increasing fertilizer content of 10% < 20% < 30%. However, Ni-rich *C. verrucosa* ash has shown an anomaly, especially at 20% compost treatment. The application of inorganic fertilizer has significantly improved the Ni hyperaccumulation in plants of all four species. In *A. mutica* species, plants grown in NPK fertilizer added media concentrated 5,798 mg/kg of Ni in the bio-ore whereas the untreated soil and 10% compost media concentrated only 3,867 mg/kg and 2,104 mg/kg, respectively. It is reported that the phosphorous fertilization remarkably affects the Ni yield in the plant biomass (Nkrumah et al., 2019).

Pearson coefficient analysis showed that there is a significantly strong negative correlation with Ni and Co concentrations in the ash samples of *B. juncea* species such as -0.8689 and -0.9862, respectively. However, the Cr content in the *B. juncea* plant ash depicted a weak correlation (-0.2507). *I. cylindrica* species also showed a strong negative correlation with the compost treatments (Ni = -0.7050, Cr = - 0.7447, and Co = -0.8270). Nevertheless, *C. verrucosa* species indicated weak negative correlations for Ni and Cr content in the plant ash when enhancing the nutrient levels (-0.4777 and -0.3405, respectively). The correlation values were calculated at a 99% confidence interval ($p < 0.005$).

The present study observed that compost treatment has not affected the bioavailability of Ni and other metals despite their mixing ratio with the serpentine soil. As Broadhurst and Chaney (2016) reported, the addition of compost significantly can affect the Ni yield and the growth of the *A. murale* biomass. However, organic fertilizers sometimes can reduce the extractable Ni concentration but ultimately increase the Ni yield by increasing the biomass (Álvarez-López et al., 2016). It describes the uncertainty of high Ni yield in the ash samples of *C. verrucosa* species at 30% compost treatment compared to the 20% treatment. Moreover, the compost treatments can improve the soil structures, porosity, soil microbial activity, water holding capacity, and organic matter which promote the plants' growth (Álvarez-López et al., 2016).

4.2.5 Toxicity removal using the hyperaccumulator species

Phytomining relatively removes a large amount of heavy metals from the soil while obtaining economic benefits from the valuable metals (Vithanage et al., 2019; Chaney et al., 2021). According to Dushyantha et al. (2021), the people who live close to the deposit and the surrounding ecosystem have been experiencing the toxicity effects of Ni, Cr, and Co enriched in the serpentine soil. Dermal and oral exposure to the Ni- and Cr-enriched soil has caused non-carcinogenic health impacts such as skin redness and skin rashes in residents of the Ginigalpelessa area. According to the study, nearly 54% of the affected people are children below age of 15. In addition, the study has claimed that nutrient deficiency (low N, P and K, Ca/Mg ratio) and the unusual enrichment of metals in soil have inhibited plant growth (Degree of ecological risk: Cr - 50%, Ni - 43 % and Co - 7%), thus and severely affect on agricultural production (Dushyantha et al., 2021). Similarly, Rajakaruna and Baker (2004) reported that these unfavorable soil conditions had failed the attempt at sugar cane cultivation in the Ginigalpelessa serpentinite deposit, located at the boundary of the Sevanagala sugar plantation.

In the present study, *A. mutica* species removed 53% of Ni and 76% of Co from the serpentine soil media. In addition, *B. juncea*, *C. verrucosa*, and *I. cylindrica* species also removed a significant amount of the metals at different fertilizer treatment levels (Table 4.4). In this context, continuous trials of Ni phytomining using these native hyperaccumulator species will remove the total toxicity effects from the serpentine soil. Since the weathering of serpentinite rock continuously releases metals like Ni into the soil and contaminates it over time, phytomining will be a sustainable approach in the long run to reduce the toxicity and recover the metals (Lesovaya et al., 2012; Sinha et al., 2021). However, the low bioavailability of Cr compared to the other two metals may restrict the Cr toxicity removal using these plants.

Table 4. 4 Average metal concentration in *B. juncea*, *C. verrucosa*, *A. mutica*, and *I. cylindrica* plants and the total metal concentration at the initial and final stage in serpentine soil media.

Plant	**Compost treatments (%)**	**Average metal concentration in the plant ash (mg/kg)**			**Initial metal concentration in soil media (mg/kg)**			**Metal concentration in the soil media after harvesting (mg/kg)**			**Metal removal percentage from soil (%)**		
		Ni	**Cr**	**Co**	**Ni**	**Cr**	**Co**	**Ni**	**Cr**	**Co**	**Ni**	**Cr**	**Co**
Brassica juncea	10	2657.03 ± 232.03	814.01 ± 117.80	153.84 ± 14.20	7162.09	1553.66	763.66	6285.03	1396.51	389.34	12	10	49
	20	1551.98 ± 119.74	285.14 ± 81.94	106.30 ± 8.31	6875.44	1471.86	773.54	5654.86	1150.14	377.92	18	22	51
	30	1544.53 ± 82.08	676.41 ± 120.21	80.17 ± 5.54	6513.93	1376.73	800.95	5194.30	1306.74	324.01	20	5	60

	NPK	2510.10 ± 70.53	828.29 ± 60.03	174.05 ± 6.25	8278.57	1795.48	980.77	6451.66	1497.34	404.21	22	17	59
	Untreated	NA	NA	NA	8440.54	1837.67	1010.45	NA	NA	NA	NA	NA	NA
Crotalaria verrucosa	10	7406.17 ± 285.35	1362.94 ± 53.45	107.35 ± 9.66	7162.09	1553.66	763.66	4865.87	1238.42	292.80	32	20	62
	20	2897.92 ± 38.84	598.00 ± 32.89	96.43 ± 2.42	6875.44	1471.86	773.54	4882.44	1197.28	309.48	29	19	60
	30	6188.33 ± 53.11	338.68 ± 17.08	28.80 ± 2.74	6513.93	1376.73	800.95	4696.08	970.88	295.73	28	29	63
	NPK	6649.36 ± 85.38	710.50 ± 28.35	136.98 ± 6.26	8278.57	1795.48	980.77	5682.78	1373.87	394.76	31	23	60
	Untreated	7278.61 ± 106.16	485.45 ± 78.25	108.98 ± 17.63	8440.54	1837.67	1010.45	5994.12	1506.67	365.76	29	18	64

Apluda mutica	10	2104.52 ± 56.61	323.23 ± 69.17	119.20 ± 3.17	7162.09	1553.66	763.66	6004.74	985.67	110.89	16	37	85
	20	NA	NA	NA	6875.44	1471.86	773.54	NA	NA	NA	NA	NA	NA
	30	NA	NA	NA	6513.93	1376.73	800.95	NA	NA	NA	NA	NA	NA
	NPK	5798.31 ± 26.72	860.62 ± 105.62	221.26 ± 29.36	8278.57	1795.48	980.77	3906.52	1104.66	234.55	53	38	76
	Untreated	3866.66 ± 39.10	2003.77 ± 390.65	181.87 ± 12.53	8440.54	1837.67	1010.45	4437.31	679.21	255.98	47	63	75
Imperata cylindrica	10	1809.80 ± 232.03	1417.96 ± 117.80	128.59 ± 14.20	7162.09	1553.66	763.66	3952.76	634.96	219.01	45	59	71
	20	1547.06 ± 119.74	846.03 ± 81.94	106.31 ± 8.31	6875.44	1471.86	773.54	4297.67	808.97	266.07	37	45	66

	30	708.48 ± 82.08	493.12 ± 120.21	44.19 ± 5.54	6513.93	1376.73	800.95	4633.19	670.33	277.88	29	51	65
	NPK	1830.54 ± 70.53	1398.33 ± 60.03	115.69 ± 6.25	8278.57	1795.48	980.77	4367.74	641.91	251.59	47	64	74
	Untreated	1483.76 ± 132.48	1045.12 ± 77.78	117.31 ± 4.27	8440.54	1837.67	1010.45	3807.65	677.78	281.12	55	63	72

NA- Data not available

4.3 Determination of metal recovery potential from bio-ore

The Ni-rich bio-ore of *A. mutica* species was used for the extraction of Ni from serpentine soil since the grass species is dominant at any sampling location in Ginigalpelessa. The leaching experiments were carried out as a preliminary study to assess the leaching efficiency of Ni from *A. mutica* bio-ore. In this regard, two bio-ore types of *A. mutica* biomass were assessed, such as bio-ores of open-air burning and incineration. Table 4.5 indicates the Ni concentration in the leachate and the leaching efficiency of Ni from the *A. mutica* ash samples. In the present study, the highest Ni concentration in the leachate (649 mg/kg) was exhibited under 100 g/L pulp density and 5 mol/L H_2SO_4 acid concentration. The lowest leaching efficiency was observed in incinerated ash samples which ranged from 7-8% under 1 mol/L H_2SO_4 acid concentration and 200 g/L pulp density.

The present study observed no significant changes in the Ni concentration in both ash samples (e.g. results of open burnt and incinerated ash samples under 5 mol/L H_2SO_4 acid concentration and 100 g/L pulp density) (Table 4.5). However, the highest leaching efficiency was calculated in the open burnt ash samples due to its low total Ni concentration (1,098±102 mg/kg) compared to the Ni concentration in the incinerated ash sample (2,100±167 mg/kg). Even though, the combustion temperature changes the elemental composition of the plant biomass and releases more organically-bound Ni, the overall leaching efficiency, cost-effectiveness, and simplicity in processing encourage to use open-air burning method over incineration (Vaughan et al., 2017). The study further analyzed the effect of acid concentration and pulp density on leaching efficiency from leaching assays. As in previous literature, parameters like agitation speed and temperature show miner effects on leaching efficiency, thus the present study kept those parameters constant during leaching experiments (Barbaroux et al., 2009; Dushyantha et al., 2021).

Table 4. 5 Summery of the leaching efficiency analysis of the *A. mutica* bio-ore respect to acid concentration and pulp density

Type of Ash sample	H_2SO_4 acid concentration (mol/L)	Pulp density (g/L)	Time (min)	Ni concentration in the leachate (mg/kg)	Leaching efficiency
Open burnt	1	100	30	369.60	34
			60	429.81	39
			90	493.63	45
			120	467.86	43
		200	30	184.71	17
			60	192.03	17
			90	218.05	20
			120	225.77	21
	5	100	30	513.55	47
			60	533.02	49
			90	626.05	57
			120	649.15	59
		200	30	342.84	31
			60	346.52	32
			90	388.84	35
			120	348.99	32
Incinerated	1	100	30	139.82	7
			60	146.90	7
			90	198.78	9
			120	194.52	9
		200	30	140.83	7

			60	158.54	8
			90	166.75	8
			120	155.29	7
	5	100	30	616.82	32
			60	601.77	29
			90	568.68	27
			120	532.16	25
		200	30	136.65	7
			60	159.01	8
			90	186.38	9
			120	188.38	9

4.3.1 Effect of pulp density and acid concentration

The effect of solid concentrations on leaching efficiency was studied for both bio-ore types (ash samples of *A. mutica*) with 100 g/L and 200 g/L pulp densities. The analysis was carried out for 2 h at room temperature with 5 mol/L and 1 mol/L H_2SO_4 concentrations under an agitation speed of 300 rpm.

A. mutica bio-ore samples from open burning and incineration were leached under the same conditions to determine the most efficient ashing method to develop a proper leaching method. As in Figure 4.7 a high recovery potential was observed at 100 g/L pulp density (45%), which is lower than the leaching efficiency of high lixiviant concentration (5 mol/L) (see Figures 4.7 and 4.8). Alternatively, Ni-rich 200 g/L pulp density depicted lower leaching efficiency under the same acid concentration and agitation speed indicating that the acid strength is insufficient to leach out the entire Ni content from the bio-ore. The high solid content in the leachate can form a surface barrier or an ash film causing the higher mass transfer resistances, which hinder the reaction between liquid and solid (Dushyantha et al., 2021).

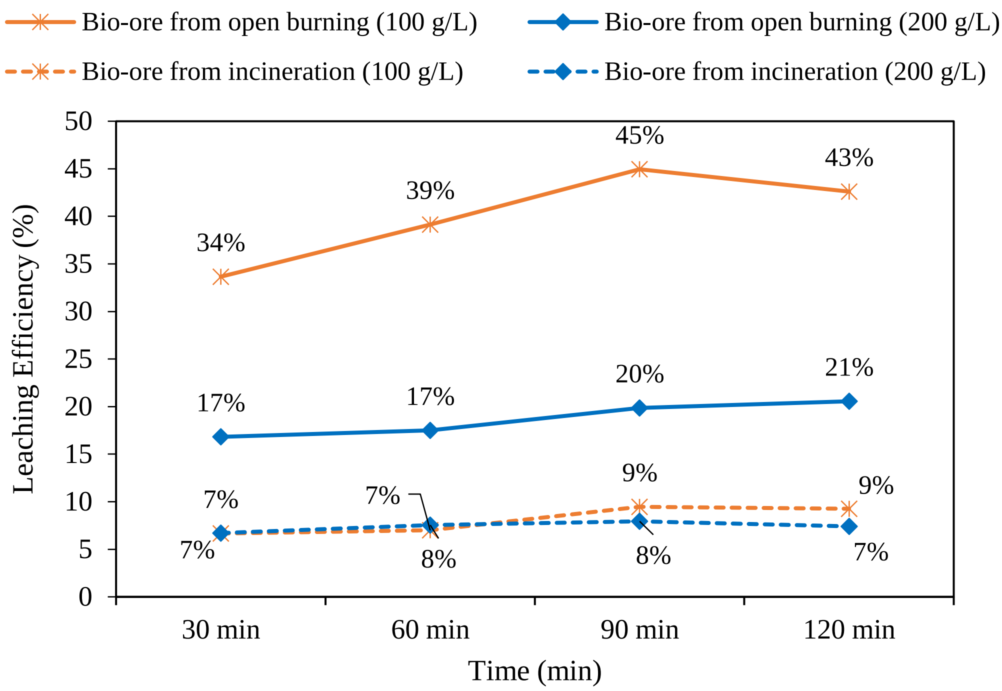

Figure 4. 7 Effect of pulp density on leaching efficiency of *A. mutica* bio-ore in open burning and incincenration under 1 mol/L H_2SO_4 concentration and 300 rpm at room temperature

Figure 4.8 also illustrates the effect of pulp density on leaching efficiency at 5 mol/L H_2SO_4 concentration. According to Figure 4.8, the maximum leaching efficiency (59%) was observed in the open burnt ash where the leaching conditions were 100 g/L pulp density, 5 mol/L H_2SO_4 concentration, and 300 rpm. Relatively, a lower leaching efficiency was observed at 200 g/L pulp density compared to 100 g/L. Therefore, under the experimented conditions, 100 g/L can be considered as the best pulp density for Ni leaching. However, further studies are required to optimize the leaching efficiency of Ni using different pulp densities to develop a proper leaching procedure for the *A. mutica* bio-ore. Moreover, previous studies have identified that lower pulp densities are generally not economically viable and require assessing the overall tank leaching operations when determining the adequate pulp density.

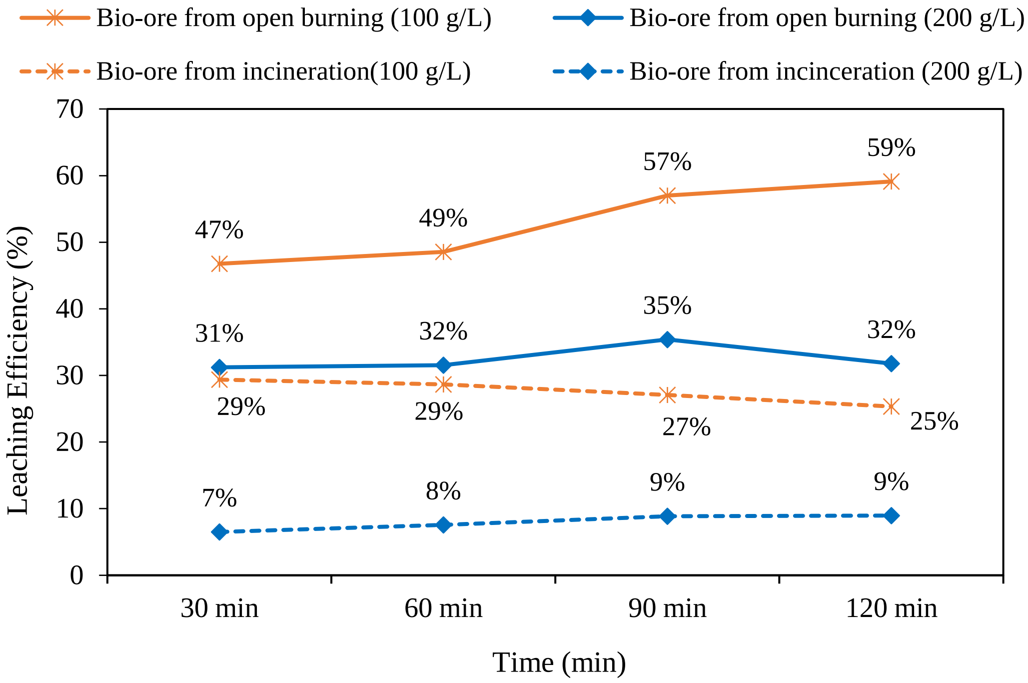

Figure 4. 8 Effect of pulp density on leaching efficiency with *A. mutica* bio-ore in open air burning and incincenration under 5 mol/L H_2SO_4 concentration and 300 rpm at room temperature

4.3.2 Effect of acid concentration

The effect of H_2SO_4 acid concentration (1 mol/L and 5 mol/L) was also investigated for the two pulp densities (100 g/L and 200 g/L) at 300 rpm, room temperature for 2 h. As shown in Figure 4. 9, after the 2 h leaching period, the leaching efficiency of Ni slightly increased from 43% to 59% when increasing H_2SO_4 acid concentration from 1 mol/L to 5 mol/L. The open burnt ash samples exhibited high leaching efficiency at 5 mol/L H_2SO_4 concentration compared to incinerated ash samples. Furthermore, the leaching efficiency of 200 g/L showed a slight increment when increasing the H_2SO_4 acid concentration (Figure 4.10). As discussed in section 4.3.1, the leaching potential of the above two lixiviant concentrations may be insufficient to leach out the maximum Ni concentration from 200 g/L pulp density.

Therefore, it was observed that the lixiviant concentration directly affects the leaching efficiency and more experiments needed to be carried out to determine the optimum level of pulp density at low acid concentrations (Barbaroux et al., 2009; Dushyantha et al., 2021). In the present study, 1 mol/L concentration of H_2SO_4 acid can also be considered for further studies, since the environmental effects caused from high concentration of the lixiviant (5 mol/L) and the cost contribute on the overall efficiency of leaching (Dushyantha et al., 2021; Vieceli et al., 2021).

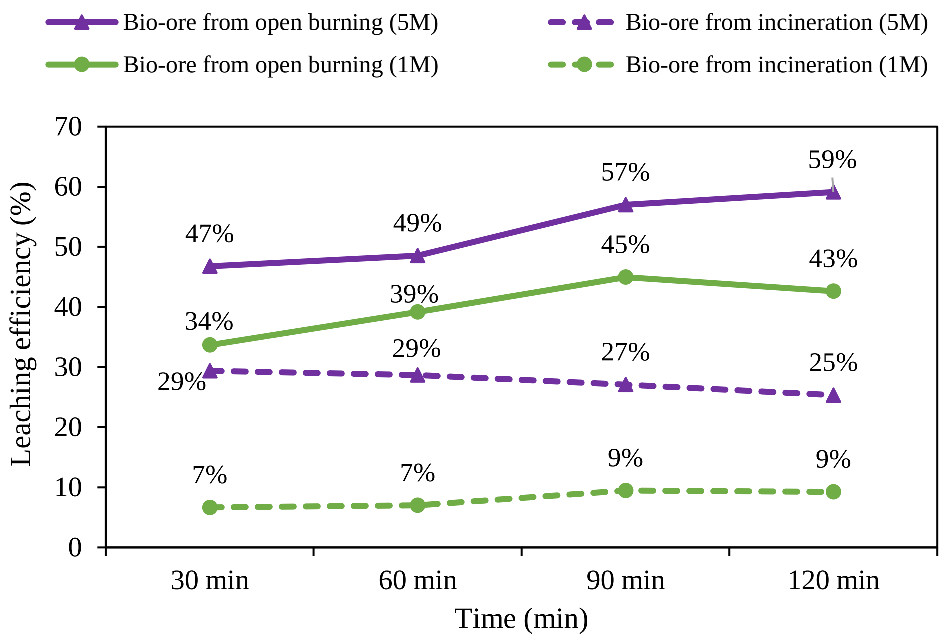

Figure 4. 9 Effect of H_2SO_4 concentration on leaching efficiency of *A. mutica* bio-ore under 100 g/L and 300 rpm at room temperature.

The leaching efficiency of Ni from plant bio-ore needs to be widely experimented when comes to the recovery stage. As in the present study, acid concentration and pulp density were dependent on each other showing both factors simultaneously affect the leaching efficiency. If the strength of the leaching reagent is insufficient to leach out the Ni concentrated in the bio-ore, leaching efficiency may decrease when increasing the pulp density (Barbaroux et al., 2011; Dushyantha et al., 2022). Therefore, further studies are necessary to identify the most suitable conditions for the optimum recovery of Ni from *A. mutica* bio-ore at a commercial scale.

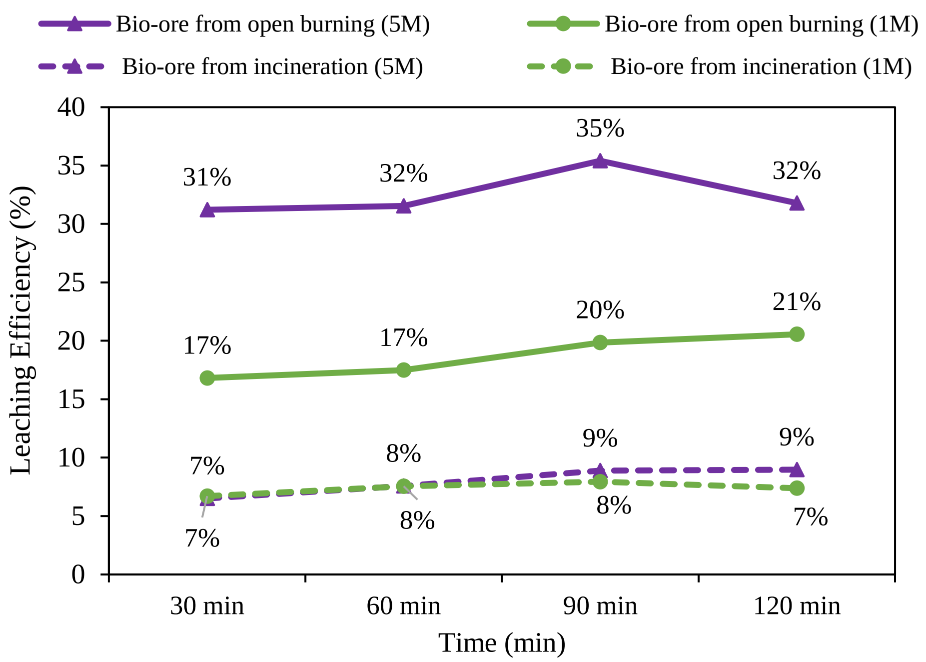

Figure 4. 10 Effect of H_2SO_4 concentration on leaching efficiency of *A. mutica* bio-ore under 200 g/L and 300 rpm at room temperature.

4.3.3 Optimization of the leaching efficiency

According to global phytomining studies, Ni recovery percentage in hyperaccumulators like *A. murale* and *Phyllanthus rufuschaneyi* are >90% when using H_2SO_4 acid as the leaching reagent (Barbaroux et al., 2011; Bouman et al., 2018). Even though *A. mutica* species accumulates a considerable concentration of Ni as a hyperaccumulator, the leaching efficiency of the present study was poor during leaching experiments. This low leaching efficiency can be attributed to many reasons such as the precipitation of calcium sulfate ($CaSO_4$) when the addition of H_2SO_4 as a leaching reagent or the formation of aluminum silicate ($Al_2O_3.2SiO_2$) in the leachate (Jally et al., 2021). Plants like *A. mutica* in the Poaceae family accumulate Si as a supporting structure and stand against herbivory (de Melo et al., 2010; Wang et al., 2019). When incinerated the biomass, the SiO_2 remains insoluble in water. Therefore, alkaline leaching with NaOH solution should be carried out prior to acid leaching to remove the aluminum and inhibit the formation of aluminum silicate glass matrix (Barbaroux et al., 2012; Jally et al., 2021). In addition, studies suggest washing the ash samples first to remove the Ca^{2+} ions, since the presence of Ca^{2+} may cause the formation of a white colour precipitate of $CaSO_4$ (Barbaroux et al., 2012). The shortcomings of the present leaching process can be improved by considering the above steps and optimizing the recovery of Ni.

4.4 Future perspectives in Ni phytomining from serpentine soils in Sri Lanka

With the growing demand for Ni in the metal and mineral industry and the exploration of alternative Ni resources, there is significant importance for the serpentine soils even if they contain low-grade Ni (Rosenkranz et al., 2019; Dang & Li, 2022). In this context, serpentinite deposits in Sri Lanka hold an important place due to the high enrichment of Ni in the soil compared to other serpentinite (Rajakaruna & Baker, 2004) regions (Nkrumah et al., 2016; van der Ent et al., 2016)(Bani, Echevarria, Zhang, et al., 2015; Cerdeira-Pérez et al., 2019)(Bani, Echevarria, Zhang, et al., 2015; Cerdeira-Pérez et al., 2019). The phytomining approach used in the present study has been experimented over the years with different serpentinite regions and has proven its effectiveness as an eco-friendly mining technique. With the current trends and added land values, phytomining has shifted towards agromining, where agricultural crops are grown in these soils along with Ni hyperaccumulators to produce both food and economically valuable metals (van der Ent et al., 2016; Chaney et al., 2021). As the first-ever Ni phytomining study in the Sri Lankan serpentinite deposits, the findings of the present study also emphasized the potential of implementing such practices in Sri Lanka. In addition, the present study is the first study to recognize *Apluda mutica* as a native hyperaccumulator species in Sri Lanka, even though geobotanical studies reported its presence in local serpentinite bodies (Rajakaruna & Baker, 2004). Also, the study observed that hyperaccumulator plants used in the study have the potential to remediate the toxicity effects of soil. Therefore, it is necessary to continue this research work by conducting more comprehensive studies in pilot scale to optimize the recovery percentage of Ni from the plant biomass. Development of such small-scale projects will ultimately benefit the economy of the country and encourage the community to sustainable resource consumption.

CHAPTER 5: CONCLUSIONS

Ni phytomining potential in serpentine deposits has been studied over many years due to the increasing demand for Ni in the global metal market and the depletion of high-grade Ni deposits. In this context, many studies have identified the potential hyperaccumulator plants, effective cultivation techniques, and extraction methods to gain the commercial benefits of this method.

The present study identified potential areas to implement *in-situ* Ni phytomining. Some areas showed elevated Ni concentrations which are close to the global cut-off grade in Ni-bearing laterite deposits (> 15, 000 mg/kg of Ni). As in previous literature, the average total Ni concentration in the soil and its bioavailable fraction have significantly affected the hyperaccumulation levels of the plants. The EDTA analysis in the soil determined that Ni is the most bioavailable metal among the trace metals found in the Ginigalpelessa serpentine soil. Analysis of BCF and TF values along with the bioavailable fraction of Ni and Co in soil showed that *A. mutica* has the highest potential for phytomining among native species. As the pioneering research for Ni phytomining in Sri Lanka, these findings were first reported in the present study and named *A. mutica* as a Ni hyperaccumulator in the serpentinite environments. Tree species, such as *M. tinctoria*, *P. suberifolium*, *V. pinnata,* and *A. indica*, found in the deposits may be considered as excluders that can tolerate and survive in the serpentine soil without accumulating high concentrations of Ni and other metals in their biomass.

Phytomining pot trials carried out in the present study observed that the mixing percentage of the compost significantly affected the Ni accumulation in plant biomass. It was confirmed by the high negative correlation between compost treatment and the Ni accumulation levels by the selected plants. Moreover, *C. verrucosa* and *A. mutica* bio-ores were identified as the best candidates for Ni phytomining since both bio-ores were concentrated with nearly >5,000 mg/kg of Ni. Compared to the other two plants, *B. juncea* and *I. cylindrica* showed low potential for Ni phytomining. Among the high-potential species *A. mutica* was selected for the leaching experiments since the plant was highly abundant in the deposit and was easy to access. Moreover, it was observed that the hyperaccumulation ability of these plants can be used to remediate the toxicity effects caused by Ni and Co from any contaminated soils.

The Ni leaching experiments carried out in the present study observed a trend in the H_2SO_4 concentrations and the pulp density of *A. mutica* plant ash. The Ni-rich bio-ore produced from open burning showed higher leaching efficiency than the incinerated biomass. The highest leaching efficiency was exhibited as 59% under 100 g/L of pulp density and 5 mol/L H_2SO_4 concentration where the agitation speed (300 rpm), leaching reagent (H_2SO_4 acid), and temperature (room temperature) was maintained constant throughout the leaching experiments. The 200 g/L of pulp density showed poor leaching efficiency since the acid concentration was insufficient to leach out the total Ni content from the Ni-rich bio-ore. Even though the Ni concentration in the incinerated bio-ore was high, leachates of both ash samples showed relatively similar Ni concentrations. Therefore, a high leaching efficiency was depicted from the open burnt ash samples after calculations. When considering the cost-effectiveness, energy efficiency, and simplicity of processing, open burnet ash samples can be employed in Ni recovery. With the present findings, the leaching optimization experiments can be carried out to determine the best lixiviant concentration and pulp density in future studies. Moreover, the low leaching efficiency observed in the leaching results may have caused due to the formation of the alluminum silicate precipitate, which masks the leaching of Ni to the solution. Therefore, further investigations are required in this regard to optimize the leaching capacity of the Ni-rich bio-ore considering the effect of other parameters.

CHAPTER 6: RECOMMENDATIONS

Even though the concept of Ni phytomining is new to Sri Lanka, many global studies have showed the significance of the method and the applicability in recovery of Ni from low-grade soil at commercial scale. The current findings of the study would be the groundwork to implement such eco-friendly extraction techniques to gain the advantages at commercial scale. Since this method removes the metal toxicity from the serpentine soil and enhances soil nutrient conditions, it facilitates the cultivation of agricultural crops along with the hyperaccumulators.

Therefore, more research efforts particularly in optimizing the hyperaccumulation and recovery percentage of Ni are required in the future under both *in-situ* and *ex-situ* conditions in the Ginigalpelessa serpentinite deposit, in order to implement a sustainable phytomining approach. Leaching experiments from Ni-rich bio-ores also need to be improved to optimize the leaching efficiencies. It is recommended to carry out the leaching experiments widely, considering all the parameters to investigate their effect on another. Since some areas in the deposit contain Ni that is close to the global cut-off grade for Ni laterites, those areas need to be further studied to discover the Ni enrichment in the vertical profile. In addition, Ni recovery from the bio-ore should be optimized in a sustainable manner, considering the leaching parameters, such as acid concentration and pulp density. Moreover, a cost benefit analysis should be conducted along with the field experiments to determine the economic potential of this method to establish a small-scale Ni phytomining industry in Sri Lanka.

Industrial-scale phytomining projects can directly support to achieve the United Nation's sustainable development goals (SDGs) such as good health and well-being (Goal 3) and sustainable consumption and production patterns (Goal 12). Since phytomining leads to create a new supply chain for Ni, particularly to use in the renewable and sustainable energy sector to mitigate climate change, it also supports achieving affordable and clean energy (Goal 7) and climate action (Goal 13) (United Nations, 2023).

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Appendix -1

XRD results of serpentinite rocks and soil samples in Ginigalpelessa serpentinite deposit

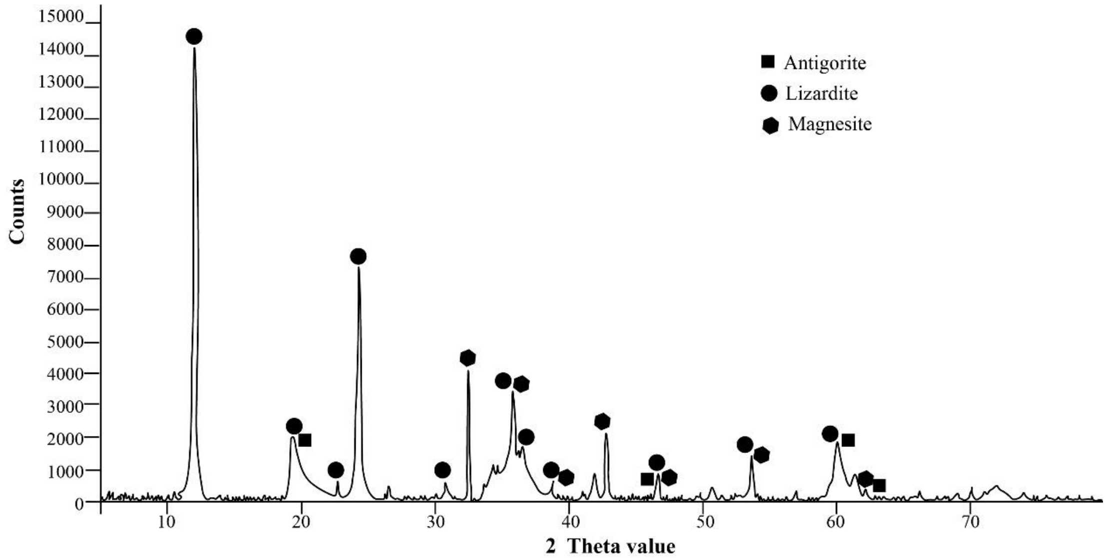

Figure 1. The XRD results of serpentinite rock samples at GP 19 location in Ginigalpelessa serpentinite deposit

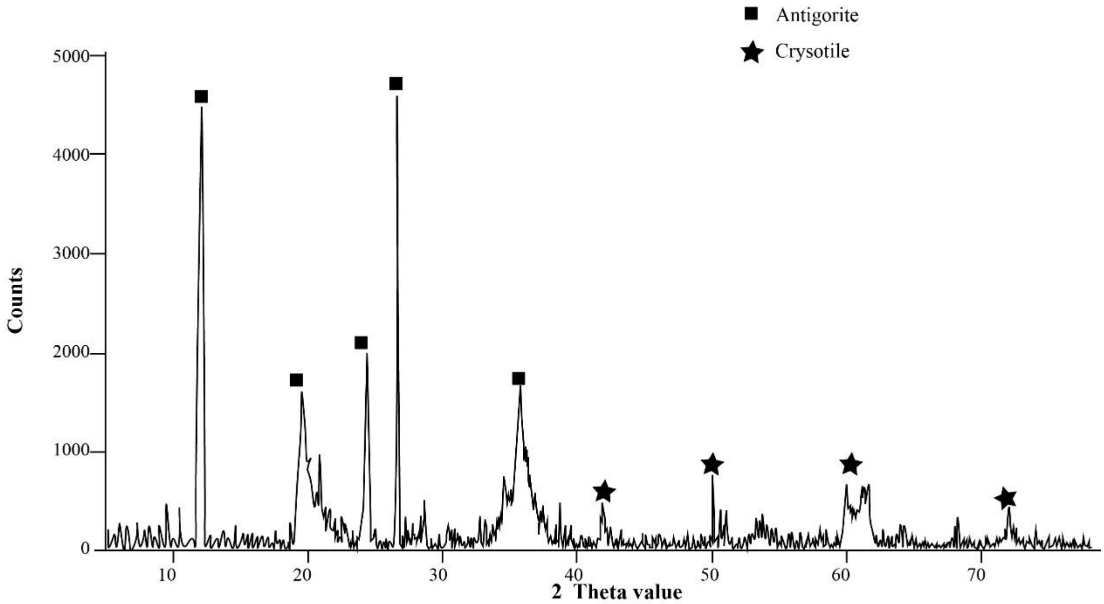

Figure 2. The XRD results of serpentine soil samples at GP 19 location in Ginigalpelessa serpentinite deposit

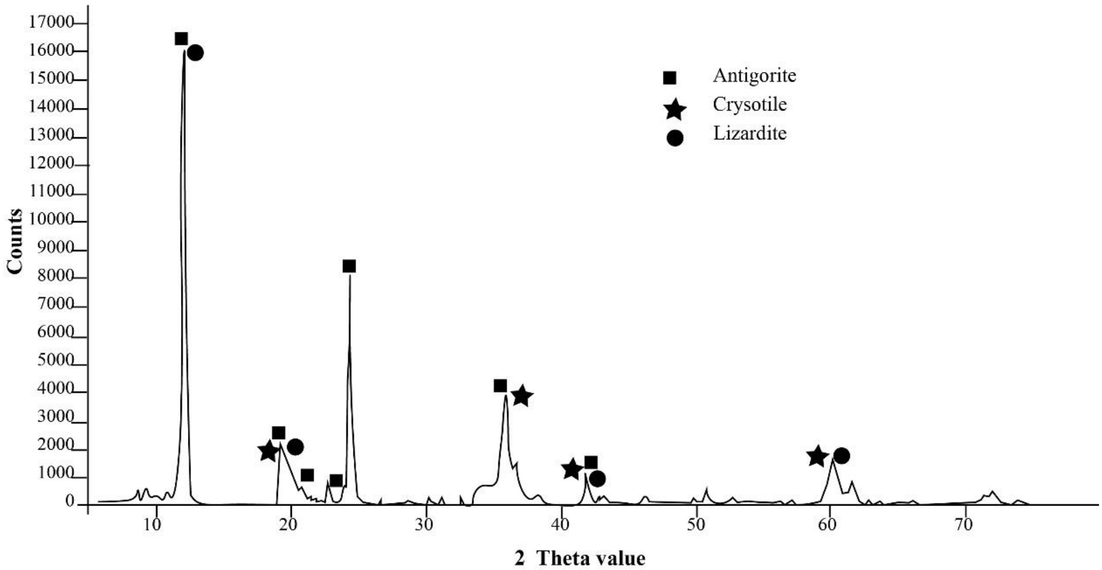

Figure 3. The XRD results of serpentinite rock samples at GP 7 location in Ginigalpelessa serpentinite deposit

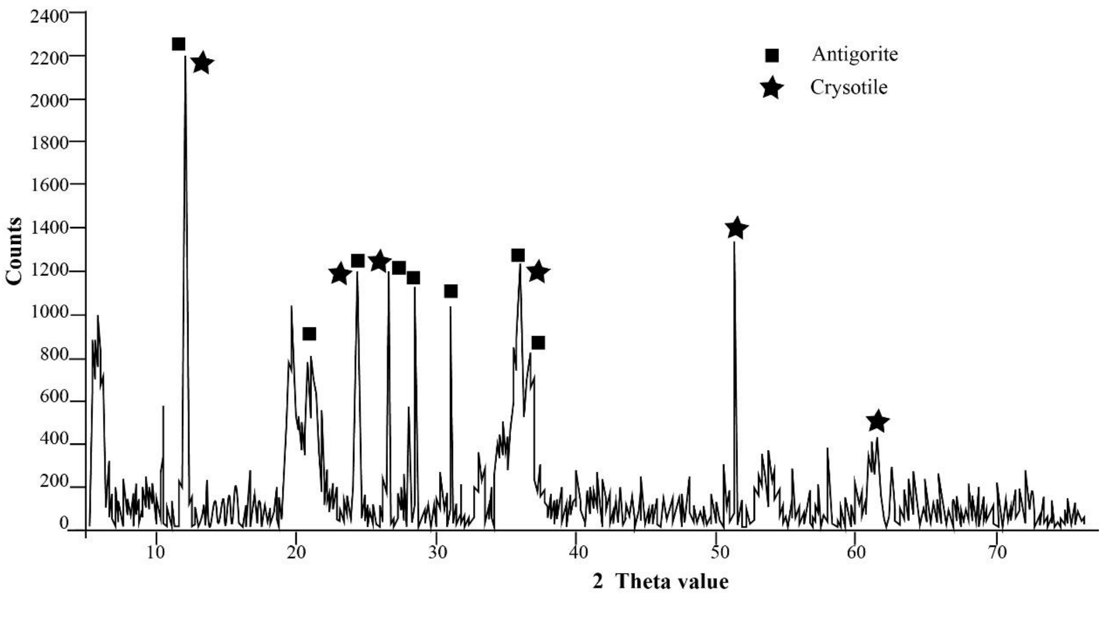

Figure 4. The XRD results of serpentine soil samples at GP 7 location in Ginigalpelessa serpentinite deposit

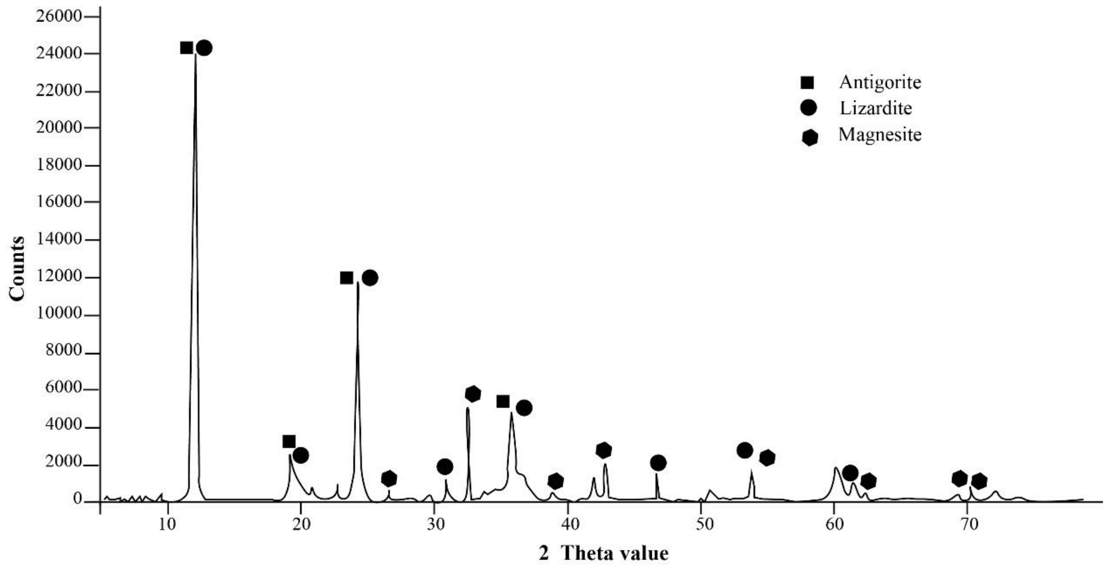

Figure 5. The XRD results of serpentinite rock samples at GP 26 location in Ginigalpelessa serpentinite deposit

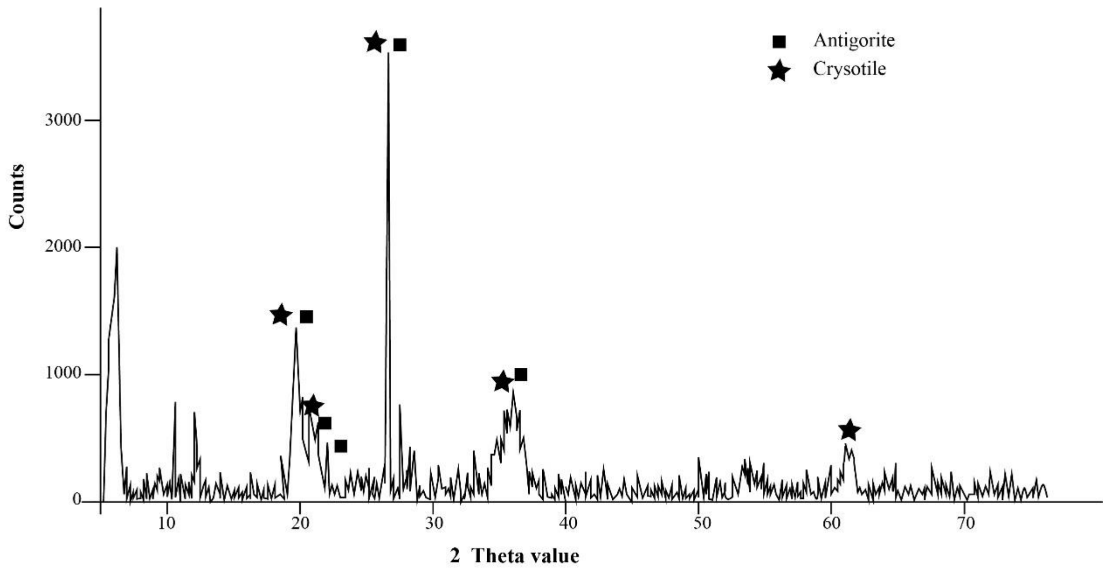

Figure 6. The XRD results of serpentine soil samples at GP 26 location in Ginigalpelessa serpentinite deposit

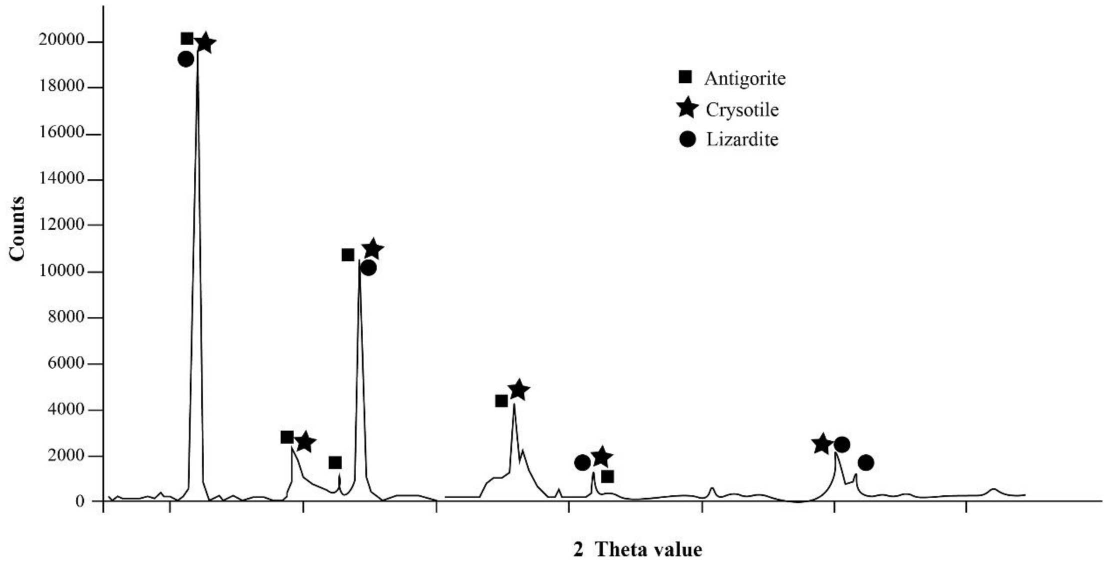

Figure 7. The XRD results of serpentinite rock samples at GP 32 location in Ginigalpelessa serpentinite deposit

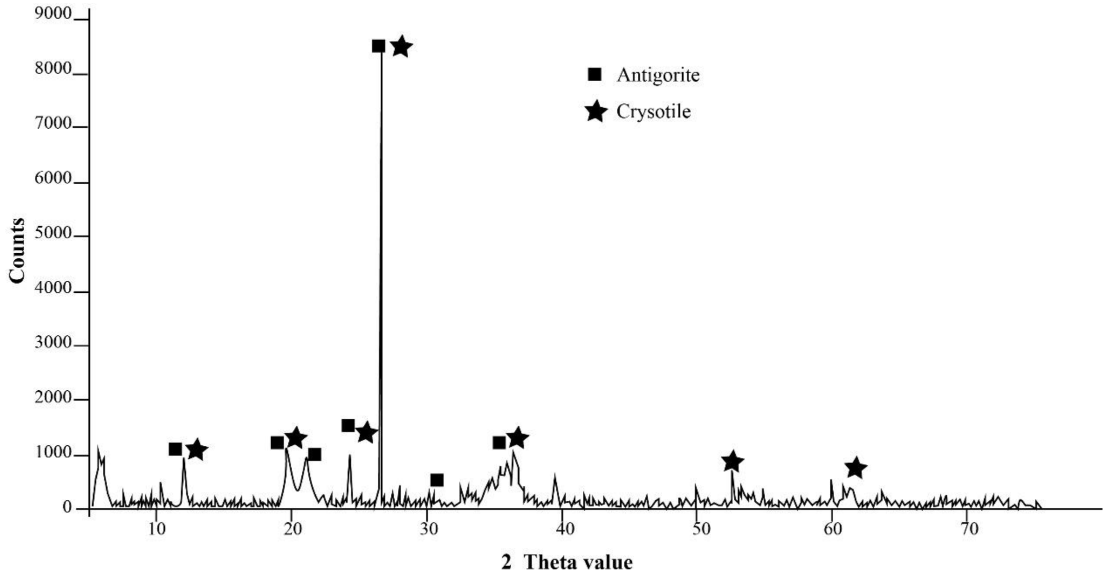

Figure 8. The XRD results of serpentine soil samples at GP 32 location in Ginigalpelessa serpentinite deposit