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Short communication

Identification of nickel hyperaccumulator plants in the Valozoro (Madagascar) nickel deposit using BCF and TF indices

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Abstract
Heavy metal pollution, particularly nickel (Ni), poses significant threats to ecosystems and public health in industrial areas. This study investigated the Valozoro mining site in Madagascar to identify native Ni-hyperaccumulator plants suitable for phytoextraction. Sixteen herbaceous species were analyzed for Ni concentrations in roots and aerial parts, and their Bioconcentration Factor (BCF) and Translocation Factor (TF) were calculated. Three promising species were identified. Two were confirmed as hyperaccumulators: Senecio cochlearifolius (1252 mg/kg Ni, BCF=4.93, TF=2.03) and Helichrysum phylicaelofolium (1154 mg/kg Ni, BCF=4.54, TF=3.23). While Helichrysum aff. gymnocephalum remained below the 1,000 mg/kg hyperaccumulation threshold, its BCF and TF values (>1) indicate significant phytoremediation potential. H. phylicaelofolium is especially notable for its high translocation capacity. However, since the Helichrysum genus is widely used in Madagascar for traditional medicine, its high Ni accumulation raises urgent public health concerns. Use of these plants in herbal remedies could lead to heavy metal toxicity in local populations. Therefore, while H. phylicaelofolium, S. cochlearifolius, and H. aff. gymnocephalum are excellent candidates for soil decontamination, their integration into phytoremediation programs must include strict management and community awareness to prevent accidental medicinal use of contaminated biomass.

Keywords*:* *phytoextraction, nickel, Helichrysum, BCF, TF*

INTRODUCTION

Environmental pollution has been a growing global threat for several decades. Various industrial processes are responsible for significant contamination, generating substantial quantities of by-products. Hence the need to explore waste disposal methods that are simultaneously economical, ecological, and aesthetically acceptable is of great importance [1]. Extraction industries release a variety of waste, ranging from relatively simple heavy metals to complex organic compounds, thus affecting plant, animal, and human life in multiple ways [2]. Environmental monitoring studies and epidemiological surveys have recently revealed the presence of metals in ecosystems and biological tissues, raising concerns about metal exposure worldwide [3]. Although certain metals, such as manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), and nickel (Ni), are essential or beneficial micronutrients for microbes, plants, and animals, all metals exhibit significant toxic effects at high levels, representing a major public health issue [4].

Phytoremediation is considered to be an efficient method for heavy metal decontamination. Phytoremediation is an operational method for eliminating various soil contaminants, using plants to clean up polluted sites. This approach is divided into two main strategies. The first, called phytostabilization, involves the use of resistant plants to stabilize heavy metals contaminating the soil. This method reduces the mobility, toxicity, and bioavailability of these contaminants, without directly removing them. By preventing the dispersion of pollutants into the water cycle and the food chain, phytostabilization plays a crucial role in protecting ecosystems [5]. The second strategy, which is phytoextraction, relies on the ability of plant roots to absorb heavy metals from the soil. This process allows the harvesting of contaminated plants to remove the toxic elements [6].

Hyperaccumulator species are commonly used to maximize the efficiency of contaminant adsorption [7]. The capacity of roots to capture these pollutants is a key element for the success of phytoextraction [8].
This last technique involves the periodic harvest and the extraction of plants that have accumulated metallic trace elements or heavy metals in their aerial parts [9]. Although many plants do not tolerate these conditions, hyperaccumulating ones have developed strategies to survive and accumulate these metals in hostile environments [10]. Thus, phytoextraction is widely employed to fight the increasing contamination of soil, air, and water by heavy metals, which is fuelled by anthropogenic activities, notably industrial, agricultural, and mining activities, as well as by soil erosion due to geological and ecological phenomena [11].
If not conducted with the right measures, the application of phytoremediation techniques may raise ecological concerns. Some ecologists warn against the risk that certain plants used for that purpose may invade surrounding ecosystems, disrupting ecological functions, threatening indigenous biodiversity, and leading to negative repercussions on the local economy and human health [12]. To address this problem, scientists are striving to explore the use of wild plants already present on the targeted site, which constitutes a more sustainable approach [13].
Plants of the genus *Helichrysum*, notably *Helichrysum italicum*, are famous for their various medicinal properties. They possess anti-inflammatory effects, used to treat joint and muscle pain, as well as antioxidant properties, helping to combat cellular aging. Their essential oil is also antiseptic and healing, facilitating the recovery of wounds, burns, and cuts. Certain species, like *Helichrysum petiolare*, are used to relieve respiratory ailments [14]. In aromatherapy, they are employed to reduce stress and improve sleep [15].
Helichrysum is also valued for its anti-inflammatory and healing effects, as well as its antioxidant properties, which help prevent skin aging [14,15]. It is also used to treat respiratory disorders and digestive and urinary infections, thanks to its decongestant and antimicrobial effects.
Due to the capacity of *Helichrysum* species to accumulate nickel, it is essential to take precautions when using them in infusions, decoctions, or in the form of balms. Excessive consumption of these preparations can lead to adverse health effects, including digestive issues, allergic reactions, and respiratory problems. It is important to verify the origin of the plants to avoid heavy metal contamination. Before using balms, it is recommended to perform a skin patch test to prevent any allergic reaction. People sensitive to nickel must consult a health professional before using these products.
The identification of hyperaccumulator plants in Madagascar opens up new perspectives for multidisciplinary research (agronomy, chemistry, geology, etc.). The main objective of our study was to identify hyperaccumulator plants in the nickel deposit of Valozoro, located in the south centre region of the island, called Haute Matsiatra. This site is currently undergoing mining extraction projects. This site has been selected as our study area to prevent ecological risks and to restore deteriorating environmental conditions.

MATERIALS AND METHODS

Study Area

The study area is located on the island of Madagascar, in the southern plateau, defined by the geographical coordinates 20°50'16" S and 47°14'25"E (Fig. 1). The relief is characterized by rounded hills, framed by narrow valleys and bounded to the South by the Vatofotsy massif, to the Southeast by the Akoholahy massif, to the Northwest by the Vorombato massif, to the North by partially oriented hills, to the West by the Faliandro hill, and to the East by a high hill [14÷16].
Historically, the site was covered by Tapia forests (*Uapaca bojeri*), an endemic Malagasy plant associated with magnesium-rich soils. Currently, the vegetation is mainly constituted by savannas. The deposit is bordered to the North by a pine reforestation area and to the South by a eucalyptus forest, which is disturbed by the exploitation of tourmaline by small-scale miners [15]. This site is part of the central highlands, characterized by a tropical climate with two distinct seasons: a cold and dry season from April to September, and a hot and humid season from November to March. Annual

rainfall varies between 1100 mm and 1500 mm, spread over 120 to 140 days. According to meteorological data from 2000, the temperature fluctuates between 2°C and 30°C, with an annual average of 19.8°C [16]. The high rainfall and elevated temperatures favour the rapid chemical decomposition of the rocks, which is essential for the formation of nickeliferous laterites in a moderate relief where erosion is limited [17].

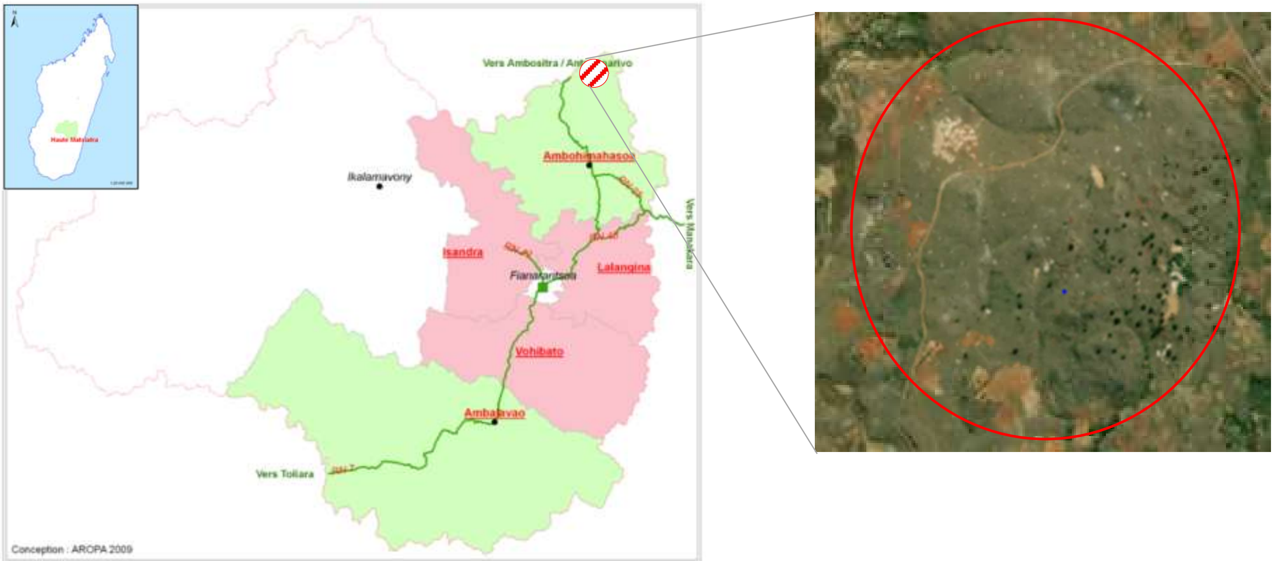

Fig. 1. Location of the studied area on the Madagascar Map

Plant sampling method

A previous study on potential hyperaccumulating plants in the Malagasy highlands identified the existence of 1,000 potential species [18]. Based on this list, a survey of the species present at the study site was conducted using literature data, leading to the selection of 30 potential hyperaccumulator plants likely to grow in this region. The on-site verification, allowed selecting among these 30 species some herbaceous plants, which are spontaneously present in the region. Herbaceous species were preferred to shrub species due to their faster growth, which allows for periodic harvesting. The collected samples were divided into two parts, with one part for metal analysis and another part for the creation of herbarium specimens to confirm their scientific names.

Identification of the sampled plants

The identification of the scientific names of the sixteen plant samples collected at the Valozoro site was carried out through morphological observation and comparison with reference specimens available at the Herbarium of the Tsimbazaza Botanical and Zoological Park. Each sample was examined based on its vegetative characteristics (leaves, stems, inflorescences, fruits, and seeds) to determine its taxonomic classification. Botanical identification keys of Madagascar were used to confirm the identifications. This method allows assigning exact scientific name to each plant, thus ensuring the reliability of the floristic data collected at the Valozoro site.

Sample preparation for analysis

Samples for analysis were carefully washed with distilled water, cut into small pieces to facilitate packaging, and then placed in plastic bags. The samples were dried in the sun for three weeks until they reached a constant weight, then stored in a moisture-free environment for adequate preservation. Before analysis, the samples were coded, re-humidified by soaking in distilled water for one hour, dried in an oven at 80°C for 24 hours (ISOTEMP Muffle Furnace, Thermo Scientific 550 series), then crushed, sieved through a 106 µm mesh, and stored in clean, labelled vials [19].

Sample analysis method

The samples were analyzed by Atomic Absorption Spectrometry (AAS). A mass of 0.1 g of solid was introduced into a Teflon tube with 8.5 mL of 63% nitric acid and 1.5 mL of 50% hydrogen peroxide

(VWR) [20, 21]. Once sealed, the tubes were placed in a microwave digestion system (Milestone Start D Microwave Digestion System) and the mineralization was performed as follows: temperature was increased to 120°C in 10 min (10°C min^{-1}), followed by a further temperature increase to 180°C in 10 min (6°C min^{-1}), and then held at 180°C for 15 min. The volume of the resulting solutions was then brought up to 50 mL by dilution with ultra-pure water [20].

Biological factors

The potential for contaminated soil cleans up can be evaluated using two key factors: the bioconcentration factor (BCF) and the translocation factor (TF) [22, 23].

BCF, also known as the bioaccumulation factor or biological absorption coefficient, is defined as the ratio of the total concentration of a specific element, such as a heavy metal, present in the harvested plant tissue to the concentration of that element in the soil where the plant grows (1). In other words, the BCF measures the efficiency with which a plant can accumulate heavy metals from the soil. This parameter directly gives an indication on its potential as a phytoremediator [24]. This relationship is expressed by the following formula:

$$\text{BCF} = (\text{nickel concentration in plant tissue}) / (\text{nickel concentration in soil}) \quad (1)$$

TF is defined as the ratio of the total concentration of an element in the aerial parts of the plant, such as stems and leaves, to its concentration in the roots (2). This factor is essential for understanding how plants transport absorbed heavy metals from the soil to their upper parts [25, 26]. It is calculated as follows:

$$\text{TF} = (\text{nickel concentration in the aerial parts}) / (\text{nickel concentration in roots}) \quad (2)$$

All concentrations are measured in mg/kg dry weight.

TF values less than 1 indicate that the metals are primarily accumulated in the roots, while values greater than 1 suggest significant accumulation in the aerial parts of the plant [26]. Specifically, effective phytoextraction is indicated when both TF and BCF values are greater than 1. Conversely, if the TF is less than 1 while the BCF is greater than 1, it suggests that phytostabilization is occurring, and metals are immobilized within the roots, without being transferred to the aerial parts. Furthermore, any species exhibiting a BCF greater than 1 possesses the fundamental characteristics of a heavy metal hyperaccumulator, making it an especially valuable candidate for environmental remediation applications [26÷28].

RESULTS AND DISCUSSION

Analysis of plant species collected from the Valozoro site reveals significant differences in their capacity for Ni accumulation and translocation, reflected by concentrations measured in the aerial parts and roots, as well as by the calculated BCF and TF. The resulting analysis data are summarized in Table 1.

Metal analysis indicated varied plant responses to the presence of trace metals in the collected soils, which underscores the importance of studying the interaction between vegetation and the metallic elements present in the soil.

Two species particularly stand out as potential nickel hyperaccumulators: *Helichrysum phylicaelofolium*, with a concentration of 1154 mg/kg in the aerial parts, 357 mg/kg in the roots, a high BCF of 4.54, and a TF of 3.23; and *Senecio cochlearifolius*, with 1252 mg/kg in the aerial parts, 617 mg/kg in the roots, a BCF of 4.93, and a TF of 2.03. These values exceed the commonly accepted thresholds for hyperaccumulating plants Ni > 1000 mg/kg, BCF > 1, TF > 1, which confirms their ability to efficiently extract nickel from the soil and translocate it to the aerial tissues.

The two species identified at Valozoro, *Helichrysum phylicaelofolium* (1154 mg/kg) and *Senecio cochlearifolius* (1252 mg/kg), display nickel concentrations that indisputably classify them as hyperaccumulators according to the criteria established by Baker and Brooks [29], namely >1,000 mg/kg in the aerial parts. Plants belonging to the *Asteraceae* family show a high potential for bioaccumulation. Specifically, the genera *Helichrysum* and *Alyssum* are cited as possessing good accumulator activity. *Alyssum bertolonii*, a well-known serpentine species, reaches 10900 mg/kg, a value lower than that observed in the two Malagasy species.

Table 1. Results of sample analyses, BCF and TF

Scientific name	Ni (mg/kg)		BCF	TF	Geographical position
	Aerial parts	Roots			
Helichrysum retrosum	130	157	0.51	0.83	-20° 50' 18"S, 47° 14' 19"E
Helichrysum gymnocephallum	330	423	1.30	0.78	-20° 50' 17"S, 47°14' 28"E
Erica floribunda	95	138	0.37	0.69	-20° 50' 17"S, 47° 14' 14"E
Helichrysum bracteiferum	197	313	1.12	0.63	-20° 50' 15"S, 47°14' 15"E
Stenocline ericoides	102	146	0.40	0.70	-20° 50' 17"S, 47° 14' 15"E
Helichrysum phylicaelofolium	1154	357	4.54	3.23	-20° 50' 20"S, 47° 14' 26."E
Erica sp	102	144	0.40	0.71	-20° 50' 19"S, 47° 14' 16"E
Psiadia salviaefolia	95	127	0.37	0.75	-20° 50' 14"S, 47° 14' 14"E
Solanium nigrum	98	146	0.38	0.67	-20° 50' 23"S, 47° 14' 25"E
Helichrysum madagascariensis	129	274	0.51	0.47	-20° 50' 18"S, 47° 14' 25"E
Pteridium aquilinum	192	249	0.76	0.77	-20° 50' 15"S, 47° 14' 14"E
Tetradenia fruticosa	204	249	0.80	0.82	-20° 50' 14"S, 47° 14' 14"E
Helichrysum aff. gymnocephalum	348	279	1.37	1.25	-20° 50' 14"S, 47° 14' 13"E
Senecio cochlearifolius	1252	617	4.93	2.03	-20° 50' 24"S, 47° 14' 30"E
Helichrysum cordifolium	348	378	1.37	0.92	-20° 50' 16"S, 47° 14' 14"E
Gaertnera brevipedicilleta	212	379	0.84	0.56	-20° 50' 24"S, 47° 14' 30"E

Similarly, *Alyssum caricum*, with 12500 mg/kg [29], has a content equivalent to that of *Senecio cochlearifolius*, and slightly higher than that of *Helychrysum phylicaelofolium*.
In contrast, *Alyssum corsicum* (18100 mg/kg) and *Alyssum markgrafii* (19100 mg/kg) show higher levels, although the values recorded in Valozoro remain in a comparable order of magnitude. The two Malagasy species also fall within the upper range of concentrations reported for *Alyssum murale*, which varies between 4730 and 20100 mg/kg [29, 30].
A species from the genus *Berkheya* was also cited by Mesjasz-Przybyłowicz et *al*. in 2004. Berkheya coddii reaches 18000 mg/kg, which remains higher, but in comparable proportions [31].
Regarding other botanical families, *Isatis pinnatiloba* (Brassicaceae), native to Turkey, accumulates only 1441 mg/kg [32], which is approximately nine times less than the two Valozoro species.
Tropical hyperaccumulators such as *Rinorea bengalensis* (1.8% or 18000 mg/kg) or *Dichapetalum gelonioides* (27% or 270000 mg/kg) exhibit significantly higher concentrations [33, 34], but the Malagasy species remain in the high range of plant accumulations reported globally.
It is worth noting that *Phyllanthus cf. securinegoides*, discovered in Malaysia, shows 23000 mg/kg in its leaves [35, 36], nearly double the Malagasy values.
However, these results remain significant in the regional context, particularly when compared to other species identified in Madagascar.
For example, *Asteropeia mcphersonii* only reaches 1250 mg/kg, *Clerodendrum arenarium* (5490 mg/kg), and *Keraudrena macrantha* as well as *Croton trichotomus* approximately 1000 mg/kg [18], levels which are significantly lower than the values measured in *Helychrisum phylicaelofolium* and *Senecio cochlearifolius*.
In the literature, two species have demonstrated the best potential: *Alyssum markgrafii* and *Pycnandra acuminata* (up to 25% Ni in the latex [37]).
Although the two species *Helychrisum phylicaelofolium* and *Senecio cochlearifolius* do not reach the extreme concentrations observed in the latter, they show promising accumulation activities within the Madagascan context. They rival the efficiency of several reference species, positioning them as potential candidates for phytoextraction strategies on moderately contaminated soils.
Other species from the Valozoro site also exhibit BCF values greater than 1, indicating a good capacity for nickel absorption from the soil. However, their nickel concentrations in the aerial parts remain significantly below the hyperaccumulation threshold (1000 mg/kg), which excludes them from this category. This is notably the case for *Helichrysum aff. gymnocephalum* (Aerial Ni: 348

mg/kg, BCF: 1.37, TF: 1.25), *Helichrysum gymnocephalum* (330 mg/kg, BCF: 1.30, TF: 0.78), *Helichrysum faradifani* (197 mg/kg, BCF: 1.12, TF: 0.63), and *Helichrysum cordifolium* (348 mg/kg, BCF: 1.37, TF: 0.92).

These species show good potential for phytoextraction in contexts of moderate pollution. Their BCF higher than 1 confirms good root absorption, while TF values ranging between 0.6 and 1.25 indicate partial but significant translocation to the aerial organs.

However, the use of plants in phytoremediation, although effective for decontaminating soils, raises ecological concerns, particularly regarding the transfer of heavy metals, such as nickel, into food chains. Plants that accumulate a large quantity of heavy metals in their aerial parts, such as *Philipia floribunda*, could pose risks to herbivorous fauna. By consuming these plants, these animals risk ingesting high concentrations of nickel, which could lead to toxic effects on their health [38]. Furthermore, these metals can move up the food chain, thereby affecting predators and disrupting the local ecosystem. It is therefore essential to monitor contamination levels in both plants and the animals that consume them, in order to prevent bioaccumulation in trophic chains.

Plants that primarily accumulate in their roots, such as those of the genus *Helichrysum*, are better suited for phytostabilization applications, where the metal is confined to the soil, thus reducing the risk of transfer to fauna. This limits the risk of food chain contamination, as the nickel is not found in the aerial parts of the plants consumed by herbivores [39]. Moreover, this approach prevents the heavy metal from entering other compartments of the ecosystem, which is particularly crucial in sensitive environments, such as agricultural land or grazing areas.

Phytotherapy, the use of medicinal plants to treat various ailments, can also be influenced by the presence of heavy metals in the plants. Certain plants used in phytotherapy, particularly those that accumulate heavy metals like nickel, may have their therapeutic properties altered by these contaminants [40]. For example, if a medicinal plant used for its anti-inflammatory or antioxidant properties accumulates high levels of nickel, it could make its use dangerous for human health [41]. It is therefore essential to ensure that the plants used in phytotherapy are not contaminated with heavy metals, especially when they come from polluted soil areas.

Hyperaccumulating plants, such as *Alyssum murale* and *Thlaspi caerulescens*, which are capable of absorbing large quantities of nickel, can potentially become a problem if they are used for medicinal applications without adequate heavy metal control. However, some research suggests that approaches such as prior decontamination of the plants or their cultivation in less polluted soils could allow them to be used safely in phytotherapeutic products [42, 43].

CONCLUSIONS

The work conducted on the Valozoro site allows the identification of two major Malagasy species, *Helichrysum phylicaelofolium* and *Senecio cochlearifolius*, as powerful nickel (Ni) hyperaccumulators. Their Ni concentrations in the aerial parts (reaching 1154 mg/kg and 1252 mg/kg respectively), with high Bioconcentration Factors and Translocation Factors, classify them in the high range of global plant accumulation and make them highly promising candidates for nickel phytoextraction from contaminated soils. However, the detection of this hyperaccumulation capacity, particularly in the *Helichrysum* genus which is widely used in phytotherapy, underscores the critical need to evaluate and prevent the risks of nickel contamination of the food chains and health products. This requests increased vigilance in any future phytoremediation strategy and traditional use.

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