Identification of Cu and Ni indicator plants from mineralised locations in Botswana

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Abstract

Plant species that accumulate high levels of metals in proportion to the metal content in the soil are of considerable interest in biogeochemical and biogeotaxonomic prospecting. This study was aimed at investigating copper and nickel accumulation in the plants Helichrysum candolleaum and Biophora diversifolia, to assess their potential use as mineral indicators in biogeochemical prospecting. Soils and plants were collected from copper-nickel mineralised areas in Botswana. Analyses of the soils and the respective plant parts (roots, stem, leaves and flowers) were carried out using ultrasonic slurry sampling electrothermal atomic absorption spectrometry (ETAAS), which allowed rapid determination of copper and nickel in small amounts of the samples.

The metal concentration in the soil was in the range of 40 μg/g-4% (w/w) for Cu and 60 μg/g-0.3% (w/w) for Ni. The concentration ranges of the elements in the plant parts were 6 μg/g-0.2% Cu and 3-210 μg/g Ni. At high soil metal content (greater than 2.5% (w/w) Cu and 0.1% (w/w) Ni), high levels of both nickel and copper were found in the shoots (leaves and flowers) of H. candolleaum. Concentrations as high as 0.2% (w/w) Cu were found in the leaves and flowers of H. candolleaum, indicating hyperaccumulation for this plant. For B. diversifolia, the metal concentrations did not exceed 100 μg/g for any plant part, for both metals. Both plant species tolerate high concentrations of metals and should therefore be categorized as metallophytes. In order to evaluate metal translocation from the soil to the shoots, metal leaf transfer coefficients (ratio of metal concentration in the leaf to metal concentration in the soil) were calculated. Our data suggest that the two plant species have different metal uptake and transport mechanisms, which needs to be investigated further. The present work also suggests that H. candolleaum may be used as a copper-nickel indicator plant in biogeochemical or biogeotaxonomic prospecting.

Keywords: Helichrysum candolleaum; Biophora diversifolia; Copper; Nickel; Indicator plants; Metallophytes
1. Introduction

Metal-tolerant plants have been extensively studied for various reasons, including their potential use in prospecting for minerals (Dunn et al., 1996; Brooks, 1998a) and in cleaning up or remediating heavy metal contaminated soils (i.e., phytoextraction, phytoremediation processes) (Ruaskin et al., 1997; Van der Lelie et al., 2001; Vassilev et al., 2001).

Biogeochemical and biogeobotanical prospecting are some of the methods in which metal-tolerant plants are used to indicate the presence of minerals (Brooks, 1998b; Reeves and Baker, 2000; Adnan, 2001). Biogeochemical prospecting involves chemical analysis of plants, as an alternative to soil analysis, in order to detect the presence of mineralization beneath the earth's surface (Brooks, 1972). For example, twigs of the black spruce (Picea mariana) were used to delineate uranium in Wollaston, Saskatchewan, Canada (Brooks, 1998b). In some cases where the plant grows only in mineralized areas, once the plant has been identified as a good mineral indicator, just its presence can be used to identify the minerals in the soil, i.e., geobotanical prospecting. For example, the ‘Zambian copper flower’ Becium centraliastrum (B. leonidas), has been used extensively in locating copper in the Shaba Province of Zaire (now the Democratic Republic of Congo) and the Zambian copper belt (Brooks, 1998b; Brummer and Woodward, 1999; Malaisse et al., 1999; Morrison et al., 1981).

In Botswana, biogeochemical studies have been done in the Ghanzi and Ngwako Pan areas in the late 1960s to late 1970s, in order to investigate unknown but inferred copper mineralization hidden underneath the calcrete and Kalahari sand covers (Cole and Le Roex, 1978). In the Ghanzi area, Cole and co-workers were able to use Helichrysum tepliechi DC (Composite family) to indicate copper mineralization in areas of shallow overburden; while in the Ngwako Pan area, Ngamiland, the deeper rooting indicator shrubs such as the blue flowering Echolium lugareae (Acanthaceae family) were used to locate copper obscured by a thick blanket of wind-blowen sand (Cole and Le Roex, 1978). At about the same time, E. lugareae was found at the Palaborwa and Messiina copper mines (Cole and Le Roex, 1978). H. lepolepis has also been found in areas where copper and nickel concentration of surface soil are high, such as Solibe, Phikwe and Matsitama areas in eastern Botswana and other places in South Africa and Namibia where copper was found (Cole, 1971). Although these species were found in areas where there was copper mineralization (0.1% Cu), the copper accumulated was only slightly higher than in normal plants. Another record of biogeochemical work was in the early 1980s by Kausel in the Ngwako Pan area, who found out that the occurrence of the blue flowering species, Monocha milneanum, related well with the occurrence of the copper bearing orebody near the surface, although the copper concentration in the plant was not higher than normal (Kausel, 1991).

In the early 1990s, work aimed at identifying plants that tolerate high copper and nickel content started at the University of Botswana (Takava et al., 1997), with plants from the north-east of Botswana (see Fig. 1) Survey studies conducted in our laboratories (Takava, 1995, Takava et al., 1997; Ramoehla, 2002) have revealed elevated levels of copper and nickel in Helichrysum capulitum (H. lepolepis family) and Blitum diversissimum (Nees) C. B. Clarke (Acanthaceae family) amongst other plants.

Baker (1981) proposed two different strategies that metal-tolerant plants can use to cope with high levels of metals in the soil they grow in (exclusion from the shoot) and accumulation (in the shoot). Based on these strategies, Baker (1981) suggested three types of plant-soil relationships: excluders, accumulators and indicators.

Excluders are defined as plants that restrict transport of metals to the shoot, and maintain relatively low metal concentration in the shoot over a wide range of metal concentrations in the soil. High metal accumulation may be found in the roots of excluder plants and their leaf to root metal concentration ratio is less than 1.0 (Baker, 1981; Baker and Brooks, 1989; Terry and Raffoel, 2000). There can be another type of exclusion, whereby metals are restricted from entering the plant (Tilstone and Menair, 1997).

The accumulators show a tendency or ability to translocate and accumulate high levels of metal in the above-ground plant parts, from both low and high soil metal concentration levels, without any associated toxicity symptoms, i.e., their leaf to root metal concentration ratio is greater than 1.0 (Baker, 1981; Baker and Brooks, 1989; Tilstone and Menair, 1997; Terry and Raffoel, 2000). For indicator
plants, an intermediate response to high metal concentration in the soil is shown, and the metal in plants reflects the concentration in the soil, i.e., the plant to soil metal concentration ratio is relatively constant (Baker, 1981).

Hyperaccumulators are defined as plants that accumulate 100-fold more metal in their shoots than normal plants (Brooks et al., 1977, 1980; Brooks, 1998a; Reeves and Baker, 2000; Terry and Barcelos, 2000). For Cu, Co, Cr, Ni or Pb...
this concentration is >1000 mg kg\(^{-1}\) (Baker and Brooks, 1989). Hyperaccumulators can simply be viewed as accumulator plants that show an extreme behaviour of affinity towards metal uptake and translocation to the shoots. This unique characteristic of hyperaccumulators has sparked great research interest, and the continuous search for this type of plants, especially for possible use in the remediation of heavy metal contaminated soil. Although most hyperaccumulators are endemic to metal-rich soils, there are some that grow in soils with a low metal content, such as the Arabidopsis Hailleri, which is a Zn- and Cd-accumulator (Bort et al., 2009). According to Brooks (1998a), good biogeochemical indicator plants have an "approximately linear relationship between elemental content in the plants and the concentration of the same element in the soil". This suggests that not every hyperaccumulator plant would be a good mineral indicator.

In this work, we investigate accumulation of copper and nickel in plants in order to assess their potential use as mineral indicators. This is because Botswana's economy is largely dependent on mining (diamonds, soda ash and Cu–Ni); Botswana is amongst Africa's top three mineral producers by value (Etosse et al., 2003). Furthermore, most of the land (two-thirds) consists of the Kalahari desert, which is covered with wind-blown sand, but may have mineral deposits that would be difficult to delineate using ordinary geological methods. Therefore, better and effective ways of mineral exploration, which are more environmentally friendly than earth drilling, should be found. A study was undertaken to investigate metal accumulation in two plant species, H. candolleanum and B. diversispina, collected from four Cu–Ni mineralized areas in Botswana. Attention was paid to these two plant species because earlier (unpublished) survey studies done in our laboratory suggested that these plants accumulate high amounts of copper and nickel. Analyses of the plant parts enabled us to determine the 'sink' of the metal taken up by the plants and consequently determine the type of metal tolerance, based on whether the plant was able to translocate the metal to the upper parts.

Ultrasonic slurry sampling electrothermal atomic absorption spectroscopy (ETAAS) methodology, developed in our earlier work (Takuwa et al., 1997) was applied for the determination of Cu and Ni. This method has the advantage that it offers straightforward analysis of slurried samples without prior sample decomposition and minimal sample loss through volatilization (Franz and Anderson, 1996). Biogeochemical prospecting relies on "cheap and rapid methods" of chemical analysis (Brooks, 1972, 1983; Brummer and Woodward, 1992) and given the advantages stated above, slurry sampling ETAAS method became the method of choice. The results obtained in this study indicate higher accumulation levels of copper and nickel than usually found in normal plants. Besides, work done in our laboratories (Takuwa, 1995; Takuwa et al., 1997; Ramocha, 2002), to the best of the author's knowledge, H. candolleanum and B. diversispina are not among the plants that have previously been reported to accumulate copper and nickel. Takuwa et al. (1997) did some analysis of H. candolleanum and B. diversispina plant species collected from Selkirk. However, the focus of their study was on method development, not soil–plant relationship, and therefore they analysed only one plant for each plant species. The metal content in the soil for the plants they analysed was not indicated.

2. Materials and methods

2.1. Equipment and apparatus

Milli-Q water purification system, A10 (Millipore, South Africa) was used for production of ultrapure water. Grinding of the soil and plant samples was done with MM 2000 mixer mill (Retsch, Haan, Germany) equipped with two 10 ml grinding cups and two 10 mm beads in each cup, all made of zirconium oxide. For homogenizing of the slurried samples, an ultrasonic processor, VT 50T Vibra Cell (Series Materials Inc., Danbury CT, USA) with a 3 mm (0.1) titanium probe was used. A Varian Spectra
2.2. Reagents and materials

Freshly prepared ultrapure water (18 MΩ cm⁻¹) was always used. The reagents used were: Triton X-100 (BDH, Poole, England), metal ion stock solutions of 1000 mg/L (Saarchem, Johannesburg, South Africa) and supra pure concentrated nitric acid (65%) (Merck, Germany).

Certified reference materials (CRMs), Chinese soils GBW 07407 (yellow-red soil), GBW 07406 (laterite) and Fluka Olive leaves BCR 62 (Olea Europea), were obtained from the Laboratory of Government Chemists (Teddington, England) and were used as received.

2.3. Sampling and samples

2.3.1. Sampling locations

All plants and soils were collected from four Cu-Ni mineralized areas in the North-Eastern part of Botswana as indicated in the map of Botswana (Fig. 1). Also indicated in the map are the Global Positioning System (GPS) locations obtained from the eTrex personal navigator (Garmin, 2000), a differential GPS accurate to within 5 m. Selkirk is an active copper mine; Nakalakwana is an abandoned copper excavation site, while Thakadu and Malaka are abandoned copper mines in the Matsitama area. Fig. 2 shows photographs of some of the sampling areas taken during sampling.

2.3.2. Sampling criteria

The criteria used for sampling was to collect samples that were young and growing as close to the ore body as possible. However, in some cases there were no plants growing on or near the ore body and therefore plant samples were collected.

Fig. 2. Photographs of some sampling areas showing plants growing: a) plants growing directly on the ore hill and b) the ore hill from a distance.
where they were available. For example, *H. candollei-num* plant species was found growing and hence collected from reddish soils, i.e., either the ore pit in Thakadu or at the foot of the ore hill in Selkirk, while *B. diversispina* was found in three places: Selkirk (away from the ore hill), Malaka and Nakalakwana in soils with relatively low metal content. Sampling was done twice: on 13th to 14th November 2002 and on 12th to 13th March 2003 on the same sampling locations.

2.3.3. Plant description

Fig. 3 shows photographs of the plants. *H. candollei-num* was collected from Thakadu and Selkirk and the species was deposited at the University of Botswana Herbarium with voucher number 03SELHC1 and herbarium number HBC1 318. It is a small short (about 20 cm high, but can grow to a height of about 45 cm) herb, with a woody base, glossy silver-white flowers that are pinkish in the inside and with hairy leaves. *H. candollei-num* belongs to the Asteraceae family and grows in the Pretoria/Magaliesburg region, South Africa, all year round (von Wyk and Malan, 1988). Eastern Zimbabwe and in other parts of Southern Africa (Huxley, 1992).

*B. diversispina* was collected from Malaka and Nakalakwana and was identified by Dr. Raj Verle-

sen, Kew Herbarium Royal Botanical Gardens, UK with voucher number, 03 MAK BD1. *B. diversispina* is a shrub, with purple-blush flowers. This plant was also found at Selkirk, about 150-200 m away from the ore body (metal-rich soil). *B. diver-

![A. Helichrysum candollei-num from Thakadu](image1)

![B. Blytharis diversispina from Nakalakwana](image2)

Fig. 3. The photographs of the plants under study growing in their natural environment.

2.3.4. Sample collection and preparation

Relatively young plants were dug from the ground using gloves and a hand spade. After removing soil lumps, the plants were washed with deionised water.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Copper</th>
<th>Nickel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>222.6</td>
<td>232.0</td>
</tr>
<tr>
<td>Slit width (mm)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Sample volume (mL)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Calibration range (mg/L)</td>
<td>0.4-2.0</td>
<td>0.2-1.0</td>
</tr>
</tbody>
</table>

### Table 1

(a) Instrumental parameters

(b) Furnace programmes for determination of Cu and Ni by slurry sampling ICP-AES

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature (°C)</th>
<th>Bake (s)</th>
<th>Hold time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying 1</td>
<td>80</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Drying 2</td>
<td>110</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Assembling</td>
<td>Cu: 800 Ni: 500</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Activation</td>
<td>Cu: 2300 Ni: 2600</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cleaning</td>
<td>Cu: 2600 Ni: 2700</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*B. diversispina* belongs to the family Acanthaceae, which consists of 250 genera and 2500 species (Arnold and de Wet, 1975). The genus *Blytharis* is known to have 166 species, of which 80 are distributed from Africa to East Indies. About 50 species are found in Southern Africa and 11 are present in Botswana (Barnes et al., 1994). *B. diversispina* is quite widespread over much of Eastern and Northeastern Botswana.
and rinsed with a water jet from a water bottle onto stubborn areas. Excess water was shaken off the plant samples and the samples were placed into labelled polyethylene bags. For each plant sample, soil from a depth of about 15 cm from where each plant was sampled, was collected into labeled polyethylene bags.

The plant parts (roots, stem, leaves, flowers) were pruned using tweezers. Thereafter, both the plant and soil samples were treated the same way. The samples were left to air-dry in petri-dishes for a few days. The dried samples were ground manually with anise pestle and mortar and further ground in the mixer mill for 60 min to a fine powder (less than 63 μm).

2.4. Analytical procedures

All laboratory equipment was cleaned with (1+1) nitric acid and rinsed with deionized water. The slurry sampling ETAAS procedure, which is discussed in detail in our previous work (Takahawa et al., 1997), is briefly outlined below. Reference and sample materials (soil or plant of particle size less than 63 μm) were accurately weighed (1.9–4.0 mg) into the 2 ml autosampler vials and 1 ml of the diluent (5% (v/v) HNO₃ and 0.05% Triton X-100) was added. Before injection into the graphite furnace, the samples were manually homogenized using a hand held ultrasonic probe (30 W, 25 s). Five individual slurry preparations (n=5) were analysed for each sample material. The parameters and furnace programme used in the ETAAS determination of Cu and Ni in the slurries (soil and plants) are given in Table 1.

<table>
<thead>
<tr>
<th>Place</th>
<th>Status</th>
<th>Soil concentration range</th>
<th>Where collected</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selkirk</td>
<td>Active Cu-Ni mine</td>
<td>0.06-0.3 Ni (% w/w), 0.3-4.2 Cu (% w/w)</td>
<td>Collected at the foot of the ore hill</td>
<td>Severe mineralization</td>
</tr>
<tr>
<td>Thaladu</td>
<td>Abandoned Cu-Ni mining site</td>
<td>0.009-0.015 Ni, 1.5-4.2 Cu</td>
<td>Collected along the ore pit (20–30 m)</td>
<td>Severe mineralization</td>
</tr>
<tr>
<td>Maleka</td>
<td>Abandoned Cu-Ni mining site</td>
<td>0.007-0.014 Ni, 0.004-0.05 Cu</td>
<td>Collected 15–20 m from the ore body</td>
<td>Slightly mineralised</td>
</tr>
<tr>
<td>Nakalakwana</td>
<td>Abandoned Cu-Ni excavation site</td>
<td>0.006-0.009 Ni, 0.004-0.008 Cu</td>
<td>Collected 15–20 m from the ore body</td>
<td>Within normal conc. for Cu, Higher than normal conc. for Ni</td>
</tr>
</tbody>
</table>

### 3. Results and discussions

#### 3.1. Quality control

For every set of real soil and plant samples, a suitable CRM was also analysed. The recoveries obtained for soil CRMs were in the ranges: 96–102% for Cu and 83–101% for Ni. For plant CRM, the recoveries were: 98–105% for Cu and 89–111% for Ni. The RSDs were below 5% for Cu and below 8% for Ni.

#### 3.2. Metal concentration in soils

The results, as shown in Tables 2 and 3, indicate that most of the soil samples, where the plants were collected, had copper and nickel concentrations higher than normal soils, i.e., higher than 5–100 μg/g Cu and 20 μg/g Ni (Adriano, 2001). The soils from Selkirk and Thaladu showed severe copper mineralization. Maleka and Nakalakwana areas had normal to slightly elevated levels of copper in the soils. The soils collected from Selkirk and Thaladu contained more copper than nickel. Selkirk soils had higher nickel concentrations than Thaladu soils, which had nickel concentration of the same order of magnitude as soils from Maleka and Nakalakwana (Table 2). Table 3 also shows results for the metal transfer coefficients (TCs) that will be discussed in the later sections.

#### 3.3. Metal accumulation in plants

The sampling and sample preparation of plants, can introduce high errors in the final analytical results.
Table 3
Total soil copper and nickel concentration (n=5) and the metal transfer coefficient (ratio of the leaf metal concentration to the soil metal concentration) (TC), for B. diversispina and H. castillejanum collected from Bulbank (B), Thabak (T), Matasha (M) and Nabululwe swe (N) sampling locations.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Copper</th>
<th></th>
<th>Nickel</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Soil conc. (g/kg)</td>
<td>TC</td>
<td>Sample</td>
</tr>
<tr>
<td>B. diversispina</td>
<td>N1</td>
<td>0.064</td>
<td>0.4</td>
<td>N1</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>0.004</td>
<td>0.4</td>
<td>N2</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>0.008</td>
<td>0.2</td>
<td>M3</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>0.02</td>
<td>0.1</td>
<td>N1</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>0.08</td>
<td>0.08</td>
<td>M2</td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>0.06</td>
<td>0.06</td>
<td>M1</td>
</tr>
<tr>
<td></td>
<td>M5</td>
<td>0.06</td>
<td>0.06</td>
<td>M4</td>
</tr>
<tr>
<td>H. castillejanum</td>
<td>S1</td>
<td>0.3</td>
<td>0.0</td>
<td>T4</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0.6</td>
<td>0.05</td>
<td>T3</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>1.5</td>
<td>0.04</td>
<td>T2</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>1.5</td>
<td>0.04</td>
<td>T1</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>2.6</td>
<td>0.03</td>
<td>T5</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>2.9</td>
<td>0.08</td>
<td>S3</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>3.4</td>
<td>0.06</td>
<td>S1</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>4.3</td>
<td>0.05</td>
<td>S2</td>
</tr>
</tbody>
</table>

* For each plant sample, the first letter (N, M, S and T) represents the sampling location (the number that follows is arbitrary).

(Hall, 1995; Kovacheva et al., 2000). Contamination from soil may contribute to this error, especially in the roots which are difficult to clean adequately. Therefore, soil particles must be removed before chemical analysis. However, there is some debate regarding the need to wash plant samples (Hall, 1995). Some work-
ers find it unnecessary to wash leaves from places of uncovered mineralization (Dunn, 1992), whereas for plants collected from arid areas or close to active mining areas washing is clearly necessary (Brooks, 1998c). Metal complexing agents such as EDTA (Ottaviani and Magnati, 1986), organic solvents such as dichloroform (Hall, 1995) and water (Takowa et al., 1997), have been used as washing solutions in the analysis of plants. The decision whether to wash, and how, lies with the individual researchers after assessing their specific situation (the type of plant, and the extent to which sampling environment has contaminated the plant) (Dunn, 1992; Hall, 1995). In this work, washing with water, as described in Section 2.3.4, was considered adequate.

Fig. 4 shows copper and nickel concentrations in the respective plant parts (roots, stem, leaves and flowers) for both H. candidissimum and B. diversispina arranged for each graph, from left to right, according to increasing metal concentration in the soil. Both plants had metal concentrations higher than concentrations normally found in plants and therefore should be categorized as metalophytes. The amount of copper required for normal plant growth is 5–20 μg/g; concentrations below 5 μg/g are considered deficient and above 20 μg/g toxic (Adriano, 2001; Kabata-Pendias and Pendias, 1984; Loneragan and Robson, 1981). Plants that tolerate high levels of copper, i.e., indicator plants, have a copper concentration range of 20–500 μg/g (Adriano, 2001). Nickel concentration in plants growing in normal soils ranges from 0.1 to 1.0 μg/g (Baker and Brooks, 1989) and seldom exceeds 5 μg/g (Adriano, 2001), but for plants growing in serpentine areas, nickel concentration is usually around 50 μg/g, seldom exceeding 100 μg/g (Brooks, 1972; Adriano, 2001).

3.3.1 H. candidissimum plant species

At relatively low metal concentration in the soil, H. candidissimum accumulated most nickel in the root or stem (i.e., from T4 to S3, Fig. 4A), while copper accumulated mostly in the roots (i.e., from S1 to T1, Fig. 4D). For both metals, the leaf to root metal concentration ratio was less than 1.0, for soil metal concentrations of about 0.07% or less Ni and about 1.5% Cu or less, as illustrated in Fig. 5A and B, respectively. Above 0.1% Ni and 2.5% Cu, H. can-

![Diagram A](image1)

![Diagram B](image2)

![Diagram C](image3)

![Diagram D](image4)

**Fig. 5.** A plot of leaf to root metal concentration ratio (w/w) for *Helichrysum candidissimum* (A and B) and *Baccharis diversispina* (C and D) plant against metal concentration in the soil collected from Seilkid, Thaladu, Malata and Natakamuri sampling locations.
**B. diversispina** plant species

This plant species was collected from areas with relatively low soil copper and nickel concentrations (40–500 μg/g Cu and 60–140 μg/g Ni) at Malaka and Nakalakwana. Unlike *H. candollei*, it was never found growing in Cu-rich places such as the ore pit or the ore hill.

The Ni accumulation pattern in *B. diversispina* is less conclusive (Figs. 4C and 5C). The results show that Cu was evenly distributed in all plant parts except in the stem where it accumulated the least in all cases except one (Fig. 4D). This is further demonstrated by a constant leaf to root metal concentration ratio of 1.0 (i.e., 0.7 to 1.2, see Fig. 5D), except for one case. These results suggest that, at the soil Cu concentrations investigated, Cu accumulation for *B. diversispina* did not depend on the Cu concentration in the soil.

**3.4. Metal transfer coefficients**

In order to evaluate metal translocation from the soil to the shoots, i.e., find out how much of the total metal concentration in the soil the plant actually took up and transferred to the shoots, an operationally defined transfer coefficient (TC), also known as transfer function (Adriano, 2001; Henning et al., 2001), was calculated. It is defined as the ratio of the metal concentration in the plant [M]_{plant} to the total metal concentration in the soil [M]_{soil} (Adriano, 2001; Henning et al., 2001).
In this study we used the metal concentration in the leaf, and therefore \([M]_{\text{leaf}}\) is replaced by \([M]_{\text{exp}}\) i.e., \(\text{TC} = [M]_{\text{exp}}/[M]_{\text{at}}\). The results are shown in Table 3.

As can be observed from Table 3, Cu TCs for \(B.\) diversifolia decreased as the soil Cu concentration increased, suggesting that the uptake and translocation of Cu to the leaves is not proportional to the soil Cu concentration. The Ni TCs for \(B.\) diversifolia did not show any conclusive pattern, an observation similar to the one made above in Figs. 4C and 5C. Transfer coefficients for the \(H.\) candollei plant varied little over a wide metal concentration range in the soil, for both metals (0.04–0.06 for Cu, and 0.04–0.1 for Ni), except for Cu in the case of Cu and T3 in the case of Ni. These results suggest metal uptake that is dependent on the soil metal concentration for both metals in \(H.\) candollei.

Further investigations on the metal uptake and its dependency on the soil concentrations (especially \(B.\) diversifolia at elevated concentrations) as well as metal uptake and transport mechanisms for the two plant species are warranted. Mechanisms for metal uptake are known to be both metal and species specific (Kabata-Pendias and Pendas, 1984; Andreano, 2001). The different mechanisms for metal uptake by plant roots may involve processes such as cation exchange by the roots, transport inside cells by chelating agents, and other carriers and rhizosphere effects (Kabata-Pendias and Pendas, 1984).

4. Conclusions

Of the four sampling locations, Selkirk and Thakadu exhibited severe copper mineralization with soil metal concentrations in the range 0.5–4% (w/w), while Malaka and Nkalukwana had normal to slightly copper mineralized soils (10–500 μg/g). All soils had nickel concentration above the normal average concentration of 20 μg/g Ni, with Selkirk soils having the highest nickel content –0.3% (w/w).

Both plant species showed tolerance to high copper–nickel content and therefore should be categorized as metallophytes. The concentration ranges of the elements in the plant parts were: 20 μg/g–0.2% (w/w) Cu and 6–230 μg/g Ni for \(H.\) candollei, and 6 μg/g–40 μg/g Cu and 3–90 μg/g Ni for \(B.\) diversifolia. \(H.\) candollei plant species has the tendency to accumulate and translocate Cu and Ni to the shoots (an accumulator) at high metal concentrations in the soil, above 2.5% (w/w) Cu and 0.1% (w/w) Ni. Concentrations larger than 0.1% (w/w) Cu were found in the leaves and flowers of \(H.\) candollei plant species, and therefore it is a possible hyperaccumulator, but only at high soil Cu concentrations. \(B.\) diversifolia showed an even distribution of Cu in all plant parts except the stem. The present work suggests that of the two plant species, \(H.\) candollei is the better candidate as a copper/nickel indicator that may be used in biogeochemical and biogeobotanical prospecting.

Our data also shows that the two species have different mechanisms of dealing with metal tolerance. This was illustrated at high soil metal content, by the leaf to root metal concentration ratio of greater than 1.0 for both metals in \(H.\) candollei, indicating metal accumulation. Furthermore, a constant leaf to root Cu concentration ratio, and decreasing Cu TCs with increasing Cu soil concentration, were observed for \(B.\) diversifolia. There was no significant difference in the leaf metal transfer coefficients for both metals over a wide range of metal content in the soil for \(H.\) candollei, suggesting metal uptake and translocation mechanism that is dependent on the soil content. The significantly different trends suggest different Cu uptake and transport mechanisms for the two plant species. Further work to investigate metal uptake and translocation mechanisms of the two plant species under varying soil (substrate) conditions, preferably in a laboratory setup, needs to be done.

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References


