Convergent patterns of tissue-level distribution of elements in different tropical woody nickel hyperaccumulator species from Borneo Island

Farida Abubakari¹, Jolanta Mesjasz-Przybyłowicz², Wojciech J. Przybyłowicz²,³ and Antony van der Ent¹,⁴*,

¹Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, Sir James Foots Building, Brisbane, QLD 4072, Australia, ²Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa, ³AGH University of Science and Technology, Faculty of Physics & Applied Computer Science, al. Mickiewicza 30, 30-059 Kraków, Poland, ⁴Universite de Lorraine – INRA, Laboratoire Sols et Environnement, UMR 1120, F-54505 Vandoeuvre-les-Nancy, France

*Corresponding author's e-mail address: a.vanderent@uq.edu.au

Abstract

The Malaysian state of Sabah on the Island of Borneo has recently emerged as a global hotspot of nickel hyperaccumulator plants. This study focuses on the tissue-level distribution of nickel and other physiologically relevant elements in hyperaccumulator plants with distinct phylogenetical affinities. The roots, old stems, young stems and leaves of Flacourtia kinabaluensis (Salicaceae), Actephila alanbakeri (Phyllanthaceae), Psychotria sarmentosa (Rubiaceae) and young stems and leaves of Glochidion brunneum (Phyllanthaceae) were studied using nuclear microprobe (micro-PIXE and micro-BS) analysis. The tissue-level distribution of nickel found in these species has the same overall pattern as in most other hyperaccumulator plants studied previously, with substantial enrichment in the epidermal cells and in the phloem. This study also revealed enrichment of potassium in the spongy and palisade mesophyll of the studied species. Calcium, chlorine, manganese and cobalt were found to be enriched in the phloem and also concentrated in the epidermis and cortex of the studied species. Although hyperaccumulation ostensibly evolved numerous times independently, the basic mechanisms inferred from tissue elemental localization are convergent in these tropical woody species from Borneo Island.

Keywords: Elemental distribution; elemental maps; hyperaccumulator; micro-PIXE; nuclear microprobe; X-ray microanalysis.

Introduction

Plants require some trace elements in minor quantities (e.g. Mn, Fe, Ni, Zn) for healthy growth, whereas excess of these can lead to toxicity symptoms (DalCorso et al. 2014). Other elements, such as Na, Al, Si and Co, although not essential, are known to be beneficial to some plant species (Pilon-Smits et al. 2009). Macronutrients (Mg, P, S, K and Ca) are needed for basic plant metabolism and to protect plants from various abiotic and biotic stresses (Shanker and Venkateswarlu 2011; Rowley et al. 2012; Morgan and Connolly 2013). Hyperaccumulators are plants that accumulate trace elements to extreme concentrations (e.g. Ni > 1000 μg g⁻¹) in their living shoots (Reeves 2003; van der Ent et al. 2013a). There are currently >500 nickel hyperaccumulator plant species.
species known globally, with the greatest number of species recorded in Cuba, New Caledonia and the Mediterranean Region (Baker and Brooks 1988; Reeves 2003; Reeves et al. 2017). At a global scale, the most common families of Ni hyperaccumulators in tropical regions are the Phyllanthaceae, Rubiaceae and Salicaceae (Reeves 2006). Nickel hyperaccumulator plants have the potential to be used in phytomining, an environmentally sustainable ‘green’ technology to produce Ni (Chaney 1983; Chaney et al. 1998, 2007; van der Ent et al. 2013b). In a phytomining operation, hyperaccumulator plants are grown on ultramafic soils, followed by harvesting, drying and incineration of the above-ground biomass to generate a commercial high-grade Ni bio-ore (Brooks and Robinson 1998; Chaney et al. 2007, 2018; van der Ent et al. 2013b, 2017c; Bani et al. 2015).

The ultramafic soils of the Malaysian state of Sabah on the Island of Borneo are renowned for high species richness (van der Ent et al. 2014), with over 5000 plant species known from the <1200 km² Kinabalu Park area (Beaman 2005) and 2854 plant species in 742 genera and 188 families recorded from the ultramafic soils in Kinabalu Park (van der Ent et al. 2014, 2016). In Sabah, 28 Ni hyperaccumulator species in 10 families and 17 genera are now known (van der Ent et al. 2019a, b), and most Ni hyperaccumulator species are from the order Malpighiales, predominantly in the families Phyllanthaceae, Salicaceae and Violaceae (van der Ent et al. 2019a, b).

Actephila alanbakeri and Glochidion brunneum (Fig. 1) are both members of the Phyllanthaceae family, which globally has the greatest numbers of Ni hyperaccumulating taxa (Reeves 2003) together with the closely related families Buxaceae (genus: Buxus) and Euphorbiaceae (genus: Leucocroton). Glochidion brunneum is widespread in Indonesia, Malaysia and the Philippines. In contrast, A. alanbakeri is a local endemic known from just two populations in Sabah near Kinabalu Park and Malawali Island. Glochidion brunneum is a medium-sized (up to 10 m tall) understory tree of lowland rainforest. It can accumulate up to 6200 µg g⁻¹ foliar Ni (van der Ent et al. 2015a). Actephila alanbakeri is a small (up to 3 m tall) woody shrub of disturbed habitats on eroded soils (Hypermagnesian Cambisols). This species may accumulate up to 14 700 µg g⁻¹ foliar Ni (van der Ent et al. 2015b). Psychotria sarmentosa is a member of Rubiaceae family (Fig. 1) and is a climber that occurs in lowland forest, mainly in disturbed areas. The species is widespread in Indonesia, Malaysia and the Philippines. It is a strong Ni hyperaccumulator which can attain up to 24 200 µg g⁻¹ foliar Ni (van der Ent et al. 2015a) (Fig. 1). Flacourtia kinabaluensis is a member of the Salicaceae family. It is a small tree (up to 8–12 m tall) that primarily occurs in riparian habitats. This species is a local endemic of the Kinabalu Park region of Sabah, Malaysia. It accumulates up to 7300 foliar µg g⁻¹ Ni (van der Ent et al. 2015a) (Fig. 1).

Previous studies regarding the distribution and chemical speciation of Ni in hyperaccumulators from Borneo (Sabah) have focussed on Rinorea cf. bengalensis, R. cf. javanica (Violaceae), Phyllanthus balgooyi, P. rufuschaneyi (previously designated as P. cf. securingoides) and Glochidion cf. sericeum (Phyllanthaceae) using nuclear microprobe (micro-proton-induced X-ray emission (micro-PIXE)) analysis with backscattering spectrometry (BS). Additionally, Ni distribution in these species has been studied with the use of synchrotron X-ray Fluorescence Microscopy (XFM) and X-ray Absorption Spectroscopy (XAS) techniques (Mesjasz-Przybyłowicz et al. 2016a; van der Ent et al. 2017a, 2018, 2020). The results showed that Ni was primarily concentrated in the epidermal areas of the leaves, and Ni in roots and stems of all three species was exceptionally enriched in the phloem. Nickel distribution in leaves, however, varies by species. In P. balgooyi the highest foliar Ni concentration was in the phloem, but in P. rufuschaneyi and R. bengalensis the highest foliar Ni concentration was in the epidermis and spongy mesophyll (R. cf. bengalensis). Phyllanthus balgooyi was unusual with extreme

Figure 1. Foliage of the Ni hyperaccumulator plants species studied. (A) Actephila alanbakeri, (B) Glochidion brunneum, (C) Psychotria sarmentosa and (D) Flacourtia kinabaluensis.
accumulation of Ni in the phloem with up to 169 g kg\(^{-1}\) Ni in the phloem sap (van der Ent and Mulligan 2015). This phloem sap concentration is second only to the New Caledonian tree *Pycandra acuminata*, which may contain up to 257 g kg\(^{-1}\) Ni in the latex (Jaffré et al. 1976). The chemical form of Ni was consistently associated with citrate and did not differ between the species in all of the tissues (roots, phloem and leaves) nor in the transport liquids (xylem and phloem) (van der Ent et al. 2017a). In *Phyllanthus serpentinus* and *Psychotria gabriellae* from New Caledonia, Ni-malate was reported as the dominant chemical form of Ni within the plant cells (Kersten et al. 1980), whereas citrate was found as the major ligand in several other hyperaccumulator plant species, e.g. *P. acuminata*, *Hybanthus caledonicus* (Lee et al. 1977, 1978; Kersten et al. 1980).

The current research aims to expand the knowledge base on tropical Ni hyperaccumulator plant species by investigating a number of species originating from different families: Rubiaceae (*P. sarmentosa*), Salicaceae (*F. kinabaluensis*) and from different genera from the *Phyllanthaceae family* (*G. bruneum, A. alanbakeri*) using micro-PIXE analysis. Specifically, the tissue-level distribution of Ni and other physiologically relevant elements in these species will be compared with information available for the *Rinorea* spp. and *Phyllanthus* spp. studied previously. Through this analysis we aimed to establish whether patterns of tissue-level elemental distribution are different in phylogenetically distant hyperaccumulator species, and hence whether basic underlying mechanisms of Ni hyperaccumulation may be distinct or similar.

**Materials and Methods**

**Collection and bulk analysis of plant tissue samples**

Young plants of *F. kinabaluensis*, *A. alanbakeri*, *G. bruneum* and *P. sarmentosa* were collected in their natural habitats in and near Kinabalu Park in Sabah (Malaysia) on the island of Borneo. These wild-collected plant specimens were subsequently potted in the nursery of the ‘Hyperaccumulator Botanical Garden’ at Monggis substation of Kinabalu Park and cultivated there for ~1 year. Individuals of *G. bruneum* were growing naturally near the nursery.

Tissue samples including roots, old stems, young stems and leaves of *F. kinabaluensis*, *A. alanbakeri*, *P. sarmentosa* and young stems and leaves of *G. bruneum* grown in cultivation at the Hyperaccumulator Botanical Garden were harvested. Branches, fruits and berries of *P. sarmentosa* and phloem of *A. alanbakeri* were also harvested. Root tissues were thoroughly washed with water to remove potentially particulate (soil) contamination. The plant tissue samples were cut out with a surgical stainless-steel knife directly from the living plants. The samples collected for micro-PIXE analysis were immediately flash-frozen in the field using a cold mirror technique in which the samples were pressed between a large block of copper metal cooled by liquid nitrogen (−196 °C) and a second block of copper attached to a Teflon holder. This ensured extremely fast freezing of the plant tissue samples to prevent cellular damage by ice crystal formation. The samples were then wrapped in aluminium foil and transported in a cryogenic container directly to iThemba LABS in South Africa for analysis. Phloem samples were collected by stripping sections of this tissue from beneath the bark.

Plant tissue subsamples were dried at 70 °C for 5 days in a dehydrating oven for bulk elemental analysis. The dried plant tissue samples were subsequently ground, and a 300-mg fraction was digested using 5 mL concentrated nitric acid (70 %) in a digestion microwave oven (Milestone Start D) for a 45-min programme, and after cooling diluted to 30 mL with ultrapure water. The samples were then analysed by ICP-AES (Varian Vista Pro II) for Na, Mg, Al, P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, and Zn.

**Nuclear microprobe elemental analysis of plant tissues**

Specimens were removed from the LN\(_2\) storage container and freeze-dried in a Leica EM CFD Cryosorption Freeze Dryer (Leica Microsystems AG, Austria). The freeze-drying process followed a long, 208-h programmed cycle to prevent shrinkage of the tissues. Freeze-dried plant tissues were then hand-cut with a steel razor blade and mounted on specimen holders covered with 0.5 % Formvar film and lightly coated with carbon to prevent charging. Elemental microanalyses were performed using the nuclear microprobe at the Materials Research Department, iThemba LABS, South Africa. The facility and methodology of measurements of biological materials have been reported elsewhere in detail (Prozesky et al. 1995; Przybyłowicz et al. 1999, 2005).

Nuclear microprobe elemental analysis uses a proton beam of 3 MeV energy, provided by a 6-MV single-ended Van de Graaff accelerator. The proton beam was focussed to a 3 × 3 μm\(^2\) spot and raster-scanned over the areas of interest, using square or rectangular scan patterns with a variable number of pixels (up to 128 × 128). Proton current was restricted to 100–150 pA to minimize specimen beam damage. Proton-induced X-ray emission and proton BS were used simultaneously. Proton-induced X-ray emission spectra were recorded with a Si(Li) detector manufactured by PGT (30 mm\(^2\) active area and 8.5 μm Be window) with an additional 125 μm Be layer as an external absorber. The effective energy resolution of the PIXE system (for the Mn K\(_{\alpha}\) line) was 150 eV, measured for individual spectra. The detector was positioned at a take-off angle of 135° and a working distance of 24 mm. The X-ray energy range was set between 1 and 40 keV. Backscattering spectrometry spectra were recorded with an annular Si surface barrier detector (100 μm thick) positioned at an average angle of 176°. Data were acquired in the event-by-event mode. The normalization of results was performed using the integrated beam charge, collected simultaneously from a Faraday cup located behind the specimen and from the insulated specimen holder. The total accumulated charge per scan varied from 0.51 to 3.82 μC.

The concentration and distribution of Si, P, S, Cl, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Br, Rb and Sr were quantified in the freeze-dried plant tissues of *F. kinabaluensis*, *A. alanbakeri*, *G. bruneum* and *P. sarmentosa*. These quantitative results were obtained by a standardless method using GeoPIXE II software package (Ryan et al. 1990a, b; Ryan 2000). The error estimates are extracted from the error matrix generated in the fit, and the minimum detection limits are calculated using the Currie equation (Currie 1968). The detailed calibration of detector efficiency, the thicknesses of selectable X-ray-attenuating filters and studies on the accuracy and precision have been reported elsewhere (van Achterbergh et al. 1995). The procedure reported there was used for the PGT Si(Li) detector used in the present study. The calibration of the analytical system was tested by measurements of standards (pure elements and synthetic glasses with known quantities of selected minor elements), the X-ray peaks of which cover practically the whole measurable energy range. Quantitative elemental mapping was performed using Dynamic Analysis method (Ryan and Jamieson 1993; Ryan et al. 1995; Ryan 2000). This method generates elemental images, which are (i) overlap-resolved, (ii) with subtracted background and (iii) quantitative, i.e. accumulated in μg g\(^{-1}\) dry weight units. Maps were complemented by data extracted from arbitrarily selected micro-areas within scanned plant tissue. Particle-induced X-ray emission and BS spectra were employed to obtain average concentrations from these micro-areas using a
full non-linear deconvolution procedure to fit PIXE spectra (Ryan et al. 1990a, b), with matrix corrections based on thickness and matrix composition obtained from the corresponding BS spectra, fitted with a RUMP simulation package (Dooolittle 1986) with non-Rutherford cross-sections for C, O, N.

**Electron microscopy of freeze-dried plant tissues**

Freeze-dried leaf samples (24 h at ~80 °C) were sputter-coated with carbon (25 nm) and mounted on stubs. The samples were then imaged with scanning electron microscopy (SEM) on a JEOL JSM-6610.

**Results**

**Bulk chemistry of the studied hyperaccumulator plants**

The results of the ICP-AES in plant tissues of A. alanbakeri, G. brunneum, P. sarmentosa and F. kinabaluensis are shown in Tables 1 and 2. The mean foliar Ni concentration in A. alanbakeri was 4500 μg g⁻¹ (range from 280 to 14 700 μg g⁻¹), 20 500 μg g⁻¹ in P. sarmentosa (range from 9500 to 29 600 μg g⁻¹), 770 μg g⁻¹ in F. kinabaluensis and 3900 μg g⁻¹ in G. brunneum (range from 2180 to 5540 μg g⁻¹) (Table 1). In the roots Ni concentration was 970 μg g⁻¹ in A. alanbakeri and 1090 μg g⁻¹ in P. sarmentosa. In the old stems its concentration was between 3100 and 8430 μg g⁻¹ in P. sarmentosa (mean value 6050 μg g⁻¹), while in the young stems the ranges of concentrations were from 580 to 1700 μg g⁻¹ in A. alanbakeri (mean value 1030 μg g⁻¹) and from 6200 to 8800 μg g⁻¹ in P. sarmentosa (mean value 7400 μg g⁻¹). The mean foliar concentration of K was 17 100 μg g⁻¹ in A. alanbakeri (range from 8800 to 22 400 μg g⁻¹), 2880 μg g⁻¹ in P. sarmentosa (range from 1260 to 4620 μg g⁻¹), 14 000 μg g⁻¹ in F. kinabaluensis and 5060 μg g⁻¹ in G. brunneum (range from 4150 to 6280 μg g⁻¹) (Table 2). Actephylia alanbakeri, P. sarmentosa and F. kinabaluensis had foliar Ca concentrations ranging between 1520 and 11 300 μg g⁻¹ (mean value 5720 μg g⁻¹), between 2600 and 13 500 μg g⁻¹ (mean value 6900 μg g⁻¹) and 10 500 μg g⁻¹, respectively, and between 4170 and 5300 μg g⁻¹ in G. brunneum (mean value 4720 μg g⁻¹). Foliar Mn concentration in A. alanbakeri ranged between 50 and 530 (mean value 180 μg g⁻¹), whereas in P. sarmentosa it was between 680 and 1550 μg g⁻¹ (mean value 1100 μg g⁻¹), between 80 and 140 μg g⁻¹ in G. brunneum (mean value 110 μg g⁻¹) and 90 μg g⁻¹ in F. kinabaluensis. Values for Cr, Fe, Co, Cu and Zn were low in the leaves of all of the studied species (Table 1).

**Nuclear microprobe microanalyses of the studied hyperaccumulator plants**

The results of the nuclear microprobe analysis in anatomical regions of the roots, old stems, young stems and leaves are shown in Tables 3–6, Figs 2–5 and Supporting Information—Figs S1–S5.

**Roots**

The concentrations of Ni in roots of A. alanbakeri ranged between 100 and 370 μg g⁻¹; in P. sarmentosa they were between 50 and 100 μg g⁻¹, and between 70 and 190 μg g⁻¹ in F. kinabaluensis (Table 3), whereas Ni was predominately concentrated in the phloem of F. kinabaluensis and A. alanbakeri. It also showed high enrichment in the epidermis of A. alanbakeri and F. kinabaluensis and in some parts of P. sarmentosa (Fig. 2; see Supporting Information—Figs S1 and S2).

The concentrations of Cl in the roots of A. alanbakeri ranged between 3060 and 6300 μg g⁻¹, between 750 and 4070 μg g⁻¹ in P. sarmentosa and between 670 and 1780 μg g⁻¹ in F. kinabaluensis (Table 3) with enrichment in the cortex of A. alanbakeri and F. kinabaluensis, whereas in P. sarmentosa it was concentrated in the cortex and phloem (Fig. 2; see Supporting Information—Figs S1 and S2). The concentrations of K in A. alanbakeri were between 9460 and 14 800 μg g⁻¹, whereas in P. sarmentosa it ranged from 3620 to 4600 μg g⁻¹ and between 5600 and 9110 μg g⁻¹ in F. kinabaluensis (Table 3) with strong enrichment in the cortex and phloem of all three species (Fig. 2; see Supporting Information—Figs S1 and S2).

It was much more concentrated in the phloem of A. alanbakeri in comparison with the two other species [see Supporting Information—Fig. S2]. The concentrations of Ca in A. alanbakeri ranged from 345 to 890 μg g⁻¹, in P. sarmentosa from 900 to 2030 μg g⁻¹, and from 740 to 1300 μg g⁻¹ in F. kinabaluensis (Table 3) with high enrichment in the cortex and phloem of A. alanbakeri and F. kinabaluensis and in some parts of the phloem of P. sarmentosa (Fig. 2; see Supporting Information—Figs S1 and S2).

Manganese concentrations in the roots of A. alanbakeri were between 30 and 55 μg g⁻¹, between 10 and 30 μg g⁻¹ in P. sarmentosa and between 6 and 65 μg g⁻¹ in F. kinabaluensis (Table 3), with strong enrichment in the epidermis and cortex of A. alanbakeri and F. kinabaluensis, but low and evenly spread in P. sarmentosa (Fig. 2; see Supporting Information—Figs S1 and S2). The concentrations of Co in roots of all of the three species were low with comparable values; in A. alanbakeri they ranged between 6 and 20 μg g⁻¹, did not exceed 15 μg g⁻¹ in P. sarmentosa and were between 6 and 15 μg g⁻¹ in F. kinabaluensis (Table 3). Cobalt was enriched in the epidermis, cortex and phloem of A. alanbakeri and in the epidermis and cortex of F. kinabaluensis, but more evenly spread throughout the sections of P. sarmentosa (Fig. 2; see Supporting Information—Figs S1 and S2).

**Old stems**

The concentration of Ni was 700 μg g⁻¹ in the old stem of A. alanbakeri, 250 μg g⁻¹ in P. sarmentosa and between 100 and 500 μg g⁻¹ in F. kinabaluensis (Table 4), with enrichment in the cortex and phloem of F. kinabaluensis and A. alanbakeri and in the pith and xylem of P. sarmentosa (Fig. 3; see Supporting Information—Fig. S3). The concentration of Cl in the old stem of A. alanbakeri was 1670 μg g⁻¹, whereas in P. sarmentosa it was 30 000 μg g⁻¹ and between 520 and 540 μg g⁻¹ in F. kinabaluensis (Table 4) with enrichment in the cortex of A. alanbakeri and F. kinabaluensis and in the cortex, phloem and xylem of P. sarmentosa (Fig. 3; see Supporting Information—Fig. S3). The K concentration in P. sarmentosa was 27 400 μg g⁻¹, whereas in A. alanbakeri it was 11 100 μg g⁻¹ and between 7720 and 8230 μg g⁻¹ in F. kinabaluensis (Table 4) with the highest enrichment in the cortex and phloem of F. kinabaluensis and A. alanbakeri (Fig. 3; see Supporting Information—Fig. S3). In P. sarmentosa, this element was more evenly spread and showed enrichment in the part of xylem (Fig. 3). The concentration of Ca in old stem of A. alanbakeri was 1800 μg g⁻¹ and 2440 μg g⁻¹ in P. sarmentosa. In F. kinabaluensis its concentration ranged from 2340 to 2960 μg g⁻¹ (Table 4), with enrichment in the cortex and phloem of A. alanbakeri and some ‘dots’ of enrichment in the cortex, phloem and xylem of P. sarmentosa, whereas in F. kinabaluensis it was more concentrated in the phloem (Fig. 3; see Supporting Information—Fig. S3).

Manganese concentrations in the old stems of F. kinabaluensis were between 15 and 100 μg g⁻¹, 60 μg g⁻¹ in A. alanbakeri and 20 μg g⁻¹ in P. sarmentosa (Table 4) with Mn enriched in the cortex of A. alanbakeri, and F. kinabaluensis while it was much more evenly spread in the cortex, xylem and phloem of P. sarmentosa (Fig. 3; see Supporting Information—Fig. S3). The concentration of Co in F. kinabaluensis was between 4 and 14 μg g⁻¹, whereas in A. alanbakeri it was 3 μg g⁻¹ and below the limit of detection (<5 μg g⁻¹) in P. sarmentosa (Table 4).

In F. kinabaluensis there was a clear enrichment of this element in the cortex and phloem, while in A. alanbakeri there was some....
Table 1. Concentrations of beneficial and trace elements in plant tissues in *Actephila alanbakeri*, *Glochidion brumneum*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis* (values as ranges and [means] in µg g\(^{-1}\) dry weight) analysed by ICP-AES.

| Species                     | n  | Na  | Al   | Cr   | Mn   | Fe   | Co   | Ni   | Cu   | Zn   |
|-----------------------------|----|-----|------|------|------|------|------|------|------|------|
| **Leaves**                  |    |     |      |      |      |      |      |      |      |      |
| *Actephila alanbakeri*      | 6  | 30–910 [305] | 15–60 [45] | 3.0–30 [20] | 50–530 [180] | 40–100 [80] | 10–55 [30] | 280–14 700 [4500] | 3.0–25 [15] | 20–100 [50] |
| *Psychotria sarmentosa*     | 5  | 90–2400 [1480] | 165–3770 [1200] | 30–140 [95] | 680–1550 [1100] | 30–125 [60] | 4.0–15 [10] | 9500–29 600 [20 500] | 4.0–15 [5] | 50–100 [75] |
| *Flacourtia kinabaluensis*  | 1  | 220 | 50   | 60   | 90   | 70   | 85   | 770  | 25   | 50   |
| *Glochidion brumneum*       | 3  | 180–570 [395] | 30–60 [40] | 5–10 [8] | 80–140 [110] | 10–65 [30] | 20–40 [30] | 2180–5540 [3900] | 2 [2] | 10–30 [20] |
| **Branches**                |    |     |      |      |      |      |      |      |      |      |
| *Psychotria sarmentosa*     | 1  | 1050 | 65   | 15   | 40   | 10   | 10   | 4820 | 5    | 30   |
| **Fruit**                   |    |     |      |      |      |      |      |      |      |      |
| *Psychotria sarmentosa*     | 1  | 850 | 430  | 10   | 290  | 25   | 10   | 3920 | 10   | 20   |
| **Berries**                 |    |     |      |      |      |      |      |      |      |      |
| *Psychotria sarmentosa*     | 1  | 95  | 70   | 10   | 50   | 10   | 10   | 6820 | 5    | 40   |
| **Wood**                    |    |     |      |      |      |      |      |      |      |      |
| *Actephila alanbakeri*      | 1  | 200 | 5    | 5    | 40   | 15   | 10   | 270  | 2    | 10   |
| **Young stems**             |    |     |      |      |      |      |      |      |      |      |
| *Actephila alanbakeri*      | 3  | 240–390 [300] | 5–110 [10] | 1.0–2.0 [2.0] | 70–290 [210] | 10–30 [20] | 5.0–10 [10] | 580–1700 [1030] | 2.0–3.0 [2.5] | 20–70 [40] |
| *Psychotria sarmentosa*     | 3  | 1060–3150 [1960] | 110–150 [140] | 30–50 [40] | 100–270 [150] | 15–30 [20] | 6.0–9.0 [7.5] | 6200–8800 [7400] | 3.0–6.00 [5.00] | 20–90 [50] |
| **Old stems**               |    |     |      |      |      |      |      |      |      |      |
| *Psychotria sarmentosa*     | 3  | 220–1510 [1050] | 30–320 [135] | 20–170 [80] | 30–220 [150] | 15–20 [20] | 10–15 [10] | 3100–8430 [6050] | 3.0–10 [4.50] | 20–30 [20] |
| **Phloem**                  |    |     |      |      |      |      |      |      |      |      |
| *Actephila alanbakeri*      | 1  | 250 | 30   | 2    | 350  | 50   | 15   | 2500 | 4    | 120  |
| **Roots**                   |    |     |      |      |      |      |      |      |      |      |
| *Actephila alanbakeri*      | 1  | 40  | 65   | 15   | 30   | 300  | 8    | 970  | 2    | 40   |
| *Psychotria sarmentosa*     | 1  | 1500 | 405  | 20   | 70   | 270  | 8    | 1090 | 5    | 15   |
enrichment in the epidermis, in comparison with the other tissue parts (Fig. 3; see Supporting Information—Fig. S3).

**Young stems.** The Ni concentration in the young stem of *A. alanbakeri* was 1160 µg g⁻¹, whereas in *G. brunneum* it was 175 µg g⁻¹, 190 µg g⁻¹ in *P. sarmentosa* and 100 µg g⁻¹ in *F. kinabaluensis* (Table 5). In *A. alanbakeri*, there was a strong Ni enrichment in the phloem and relatively low enrichment in the pith and primary xylem (Fig. 4). In *G. brunneum*, Ni was more evenly spread throughout the whole stem section, with slight enrichment in the pith and xylem (Fig. 4). The enrichment in the pith and xylem was much more pronounced in *P. sarmentosa* (Fig. 4), this pattern was also visible in *F. kinabaluensis* (Fig. 4), but less clear because of overall lower concentration of Ni in the measured section.

The concentration of Cl in young stem of *A. alanbakeri* was 1600 µg g⁻¹, whereas in *G. brunneum* it was 13 300 µg g⁻¹, 6900 µg g⁻¹ in *P. sarmentosa* and 700 µg g⁻¹ in *F. kinabaluensis* (Table 5) with Cl concentrated in the cortex of *A. alanbakeri*, *P. sarmentosa*, *F. kinabaluensis* and in the cortex, phloem and xylem of *G. brunneum* (Fig. 4). The highest concentration of K in the young stems was in *G. brunneum*, where it reached 21 400 µg g⁻¹, whereas in *A. alanbakeri* it was 11 700 µg g⁻¹, 13 500 µg g⁻¹ in *P. sarmentosa* and 13 600 µg g⁻¹ in *F. kinabaluensis* (Table 5) with strong enrichment in the cortex, phloem, xylem and pith of *A. alanbakeri*, in the cortex and phloem of *G. brunneum*, *P. sarmentosa* and *F. kinabaluensis*, and some enrichment in the xylem and phloem of *P. sarmentosa* (Fig. 4). There was significant depletion of this element in the pith of *G. brunneum* in comparison with the other species. The concentration of Ca in young stem of *A. alanbakeri* was 2950 µg g⁻¹, 2570 µg g⁻¹ in *F. kinabaluensis*, 1540 and 1370 µg g⁻¹ in *P. sarmentosa* (Table 5). Many small ‘dots’ (Ca-oxalate crystals) were visible in the pith and phloem of *P. sarmentosa* and *F. kinabaluensis*. In *P. sarmentosa*, there was an overall enrichment in the epidermis, cortex and phloem, whereas in *A. alanbakeri* it was more concentrated in the epidermis, phloem and pith (Fig. 4).

The concentrations of Mn were very low in all studied species, at the 15–30 µg g⁻¹ level with the exception of *A. alanbakeri*, where the average value for the whole section was 155 µg g⁻¹ (Table 5), with enrichment in the epidermis, cortex and phloem (Fig. 4). The concentrations of Co were even lower, below the limits of detection with the exception of *A. alanbakeri* where Co was found at the 3 µg g⁻¹ level (Table 5).

### Leaves

The concentrations of Ni in the leaves of *G. brunneum* were 1060 µg g⁻¹, the only one species where the hyperaccumulation threshold was exceeded in the leaves’ sections; between 270 and 740 µg g⁻¹ in *A. alanbakeri*, from 60 to 250 µg g⁻¹ and from 85 to 360 µg g⁻¹ in *P. sarmentosa* and *F. kinabaluensis*, respectively (Table 6). Nickel was mainly distributed in the lower and upper epidermal cells of the leaves of all the studied species. However, in *A. alanbakeri*, Ni was also

### Table 2. Concentrations of macroelements in plant tissues in *Actephila alanbakeri*, *Glochidion brunneum*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis* (values as ranges and [means] in µg g⁻¹ dry weight) analysed by ICP-AES.

| Species                        | n  | Mg                      | P                        | S                        | K                        | Ca                        |
|-------------------------------|----|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| **Leaves**                     |    |                         |                          |                          |                          |                          |
| *Actephila alanbakeri*         | 6  | 1280–6500 [4100]        | 300–1440 [852]           | 610–2770 [1770]          | 8800–22 400 [17 100]     | 1520–11 300 [5720]       |
| *Psychotria sarmentosa*        | 5  | 4380–8100 [6000]        | 130–600 [330]            | 1360–1900 [1700]         | 1260–4620 [2880]         | 2600–13 500 [6900]       |
| *Flacourtia kinabaluensis*     | 1  | 5400                    | 810                      | 2100                     | 14 000                   | 10 500                   |
| *Glochidion brunneum*          | 3  | 1320–2710 [2160]        | 160–210 [190]            | 530–700 [640]            | 4130–6280 [5060]         | 4170–5300 [4720]         |
| *Psychotria sarmentosa*        | 3  | 1000–2090 [1500]        | 85–410 [210]             | 840–1260 [1010]          | 3710–6710 [5000]         | 575–6530 [2730]          |
| *Actephila alanbakeri*         | 6  | 3370                    | 530                      | 1040                     | 5420                     | 3960                     |
| *Psychotria sarmentosa*        | 1  | 490                     | 65                       | 370                      | 2430                     | 850                      |
| *Flacourtia kinabaluensis*     | 5  | 4380–8100 [6000]        | 130–600 [330]            | 1360–1900 [1700]         | 1260–4620 [2880]         | 2600–13 500 [6900]       |
| *Glochidion brunneum*          | 3  | 1320–2710 [2160]        | 160–210 [190]            | 530–700 [640]            | 4130–6280 [5060]         | 4170–5300 [4720]         |
| *Psychotria sarmentosa*        | 3  | 1000–2090 [1500]        | 85–410 [210]             | 840–1260 [1010]          | 3710–6710 [5000]         | 575–6530 [2730]          |
| **Branches**                   |    |                         |                          |                          |                          |                          |
| *Actephila alanbakeri*         | 1  | 520                     | 270                      | 290                      | 1670                     | 200                      |
| *Psychotria sarmentosa*        | 1  | 3720                    | 325                      | 515                      | 7800                     | 2660                     |
| **Fruit**                      |    |                         |                          |                          |                          |                          |
| *Psychotria sarmentosa*        | 1  | 3720                    | 325                      | 515                      | 7800                     | 2660                     |
| **Berries**                    |    |                         |                          |                          |                          |                          |
| *Actephila alanbakeri*         | 1  | 4260                    | 290                      | 840                      | 12 800                   | 2090                     |
| **Old stems**                  |    |                         |                          |                          |                          |                          |
| *Actephila alanbakeri*         | 1  | 1260                    | 350                      | 215                      | 3900                     | 310                      |
| *Psychotria sarmentosa*        | 1  | 650                     | 110                      | 380                      | 910                      | 1370                     |

Supporting Information—Fig. S3.
Table 3. Average elemental concentrations (micro-PIXE, µg g\(^{-1}\) dry weight) in the sections of the roots of *Actephila alanbakeri*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis* (values as ranges and [mean values]).

| n | Si (µg g\(^{-1}\)) | P (µg g\(^{-1}\)) | S (µg g\(^{-1}\)) | Cl (µg g\(^{-1}\)) | K (µg g\(^{-1}\)) | Ca (µg g\(^{-1}\)) | Cr (µg g\(^{-1}\)) | Mn (µg g\(^{-1}\)) | Fe (µg g\(^{-1}\)) | Co (µg g\(^{-1}\)) | Ni (µg g\(^{-1}\)) | Cu (µg g\(^{-1}\)) | Zn (µg g\(^{-1}\)) |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Actephila alanbakeri** | | | | | | | | | | | | | |
| 3 | 4810–9830 [6060] | 330–625 [515] | 1890–3060 [2317] | 3060–6300 [4863] | 9460–14 800 [12 553] | 335–890 [585] | 30–40 [33] | 30–55 [45] | 770–1370 [1077] | 6–20 [12] | 100–370 [253] | 3.5–4.5 [4] | 40–70 [53] |
| **Psychotria sarmentosa** | | | | | | | | | | | | | |
| 2 | 1710–9200 [5455] | 200–220 [210] | 2670–9800 [6235] | 750–4070 [2410] | 3620–4600 [4110] | 900–2030 [1465] | 1–40 [20] | 10–30 [20] | 120–1340 [730] | 1–15 [7.5] | 50–100 [75] | <2.1 | 6.5–20 [13.3] |
| **Flacourtia kinabaluensis** | | | | | | | | | | | | | |
| 3 | 1640–9500 [4900] | 160–250 [200] | 480–11 700 [4300] | 670–17 800 [1120] | 5600–91 100 [7170] | 740–13 000 [1030] | 7–85 [39] | 6–65 [37] | 250–1580 [820] | 6–15 [9] | 70–190 [120] | <3.5 | 10–30 [20] |

Table 4. Average elemental concentrations (micro-PIXE, µg g\(^{-1}\) dry weight) in the sections of the old stems of *Actephila alanbakeri*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis*. For single measurements errors of analysis (± SD) are shown in brackets. For n > 1 the results are shown as ranges and [mean values].

| n | Si (µg g\(^{-1}\)) | P (µg g\(^{-1}\)) | S (µg g\(^{-1}\)) | Cl (µg g\(^{-1}\)) | K (µg g\(^{-1}\)) | Ca (µg g\(^{-1}\)) | Cr (µg g\(^{-1}\)) | Mn (µg g\(^{-1}\)) | Fe (µg g\(^{-1}\)) | Co (µg g\(^{-1}\)) | Ni (µg g\(^{-1}\)) | Cu (µg g\(^{-1}\)) | Zn (µg g\(^{-1}\)) |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Actephila alanbakeri** | | | | | | | | | | | | | |
| 1 | 4070 (425) | 380 (30) | 620 (30) | 1670 (20) | 11 100 (50) | 1800 (30) | 15 (1.0) | 60 (2) | 500 (10) | 3 (3) | 700 (20) | 3.5 (1.0) | 90 (8) |
| **Psychotria sarmentosa** | | | | | | | | | | | | | |
| 1 | 870 (250) | 1050 (80) | 1310 (54) | 30 000 (270) | 27 400 (170) | 2440 (70) | 30 (1) | 20 (5) | 80 (7) | <5 | 250 (20) | 5 (2) | 10 (1) |
| **Flacourtia kinabaluensis** | | | | | | | | | | | | | |
| 2 | 5100–13 100 [9100] | 310–417 [364] | 440–1970 [1205] | 520–540 [530] | 7720–8230 [7975] | 2340–2960 [2650] | 5.5–40 [23] | 15–100 [58] | 410–1520 [965] | 4–14 [9] | 100–500 [300] | <3 | 20–35 [28] |
Table 5. Average elemental concentrations (micro-PIXE, µg g\(^{-1}\) dry weight) in the sections of the young stems of *Actephila alanbakeri*, *Glochidion brunneum*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis*. Errors of analysis (+ SD) are shown in brackets.

| Element | *Actephila alanbakeri* | *Glochidion brunneum* | *Psychotria sarmentosa* | *Flacourtia kinabaluensis* |
|---------|------------------------|-----------------------|------------------------|--------------------------|
| Si      | 4420 (390)             | 140 (140)             | 1100 (115)             | 1010 (100)               |
| P       | 1620 (130)             | 350 (55)              | 510 (40)               | 640 (35)                 |
| S       | 2000 (110)             | 1740 (40)             | 1850 (25)              | 2020 (80)                |
| Cl      | 1600 (40)              | 13 300 (80)           | 6900 (30)              | 700 (40)                 |
| K       | 11 700 (80)            | 21 400 (90)           | 13 500 (80)            | 13 600 (80)              |
| Ca      | 2950 (30)              | 1540 (60)             | 1370 (30)              | 2570 (50)                |
| Cr      | 14.5 (1)               | <1.5                  | 95 (3)                 | <2.2                     |
| Mn      | 155 (6)                | 30 (4)                | 15 (5)                 | 15 (1.0)                 |
| Fe      | 770 (15)               | 4 (3)                 | 65 (5)                 | 90 (5)                   |
| Co      | 3 (3)                  | <7                    | <3                     | <5                       |
| Ni      | 1160 (30)              | 175 (10)              | 190 (10)               | 100 (10)                 |
| Cu      | 5 (1)                  | 4.5 (0.5)             | 2.0 (0.5)              | <3.5                     |
| Zn      | 230 (6)                | 9.0 (0.5)             | 10 (1)                 | 30 (2)                   |

Table 6. Average elemental concentrations (micro-PIXE, µg g\(^{-1}\) dry weight) in the sections of the leaves of *Actephila alanbakeri*, *Glochidion brunneum*, *Psychotria sarmentosa* and *Flacourtia kinabaluensis*. For single measurements errors of analysis (+ SD) are shown in brackets. For n > 1 the results are shown as ranges and [mean values].

| n | Si          | P       | S         | Cl       | K       | Ca      | Cr      | Mn      | Fe      | Co      | Ni      | Cu      | Zn      |
|---|-------------|---------|-----------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|   | n=2        | 190–480 | 220–1000  | 580–1090 | 4200–4620 | 12 800–15 800 | 700–6800 | 1.4–11 | 90–390 | 20–580 | 0.2–7 | 270–740 | <5 15–61 | 38 |
|   | n=1        | 2420 (300) | <85   | 1170 (40) | 17 300 (100) | 38 200 (170) | 3940 (100) | <3      | 110 (10) | 25 (10) | <20    | 1060 (60) | <5 20 (2) |
|   | n=3        | 740–1850 | 300–670  | 1250–2500 | 12 400–16 400 | 2300–7800 | 690–1970 | 1.1–40 | 25–120 | 80–200 | <2    | 60–250 | <3.5 5.5–45 | 22 |
|   | n=3        | 410–2780 | 250–280  | 400–1250 | 1100–4300 | 2650–10 300 | 900–3470 | 0.4–6 | 4–15 | 180–250 | 2–15 | 85–360 | <5 8–45 | 21 |
Figure 2. Micro-PIXE quantitative elemental maps of root cross-sections of Actepha alanbakeri, Psychotria sarmentosa and Flacourtia kinabaluensis. Concentration scale in wt% dry weight or in μg g⁻¹ dry weight. Abbreviations of anatomical features: C, cortex; E, epidermis; P, phloem; and X, xylem.
strongly enriched in the spongy and palisade mesophyll (Fig. 5; see Supporting Information—Fig. S5). Nickel concentration was lower in the phloem and xylem of all of the species (Fig. 5; see Supporting Information—Figs S4 and S5).

The concentration of Cl in A. alanbakeri ranged between 4200 and 4620 µg g⁻¹, 17 300 µg g⁻¹ in G. brunneum, between 12 400 and 16 400 µg g⁻¹ in P. sarmentosa and from 1100 to 4300 µg g⁻¹ in F. kinabaluensis (Table 6) with enrichment in the xylem of...
A. alanbakeri, spongy mesophyll of G. brunneum, upper and lower epidermis and palisade mesophyll of P. sarmentosa and xylem and phloem of F. kinabaluensis (Fig. 5; see Supporting Information—Figs S4 and S5). Potassium concentrations in the leaves of G. brunneum were 38 200 µg g⁻¹, between 12 800 and 15 800 µg g⁻¹ in A. alanbakeri, between 2650 and 10 300 µg g⁻¹ in F. kinabaluensis and from 2300 to 7800 µg g⁻¹ in P. sarmentosa. In all of the studied species, K was strongly enriched in the spongy
Figure 5. Micro-PIXE quantitative elemental maps of leaf cross-sections of *Actephila alanbakeri*, *Glochidion brunneum*, *Psychotria sarmentosa* and *Flacourtia kinabalensis*. Concentration scale in wt% dry weight or µg g⁻¹ dry weight. Abbreviations of anatomical features: UE, upper epidermis; LE, lower epidermis; PM, palisade mesophyll; SM, spongy mesophyll; P, phloem; and X, xylem.
and palisade mesophyll. There was also enrichment of K in the phloem of *F. kinabaluensis* and *G. brunneum* and in the xylem of *P. sarmentosa* (Fig. 5; see Supporting Information—Figs S4 and S5). Calcium in *A. alanbakeri* was between 700 and 6800 µg g⁻¹, 3340 µg g⁻¹ in the leaves of *G. brunneum*, between 900 and 3470 µg g⁻¹ in *F. kinabaluensis* and between 690 and 1970 µg g⁻¹ in the leaves of *P. sarmentosa* (Table 6) with enrichment in the spongy and palisade mesophyll of *A. alanbakeri* and *G. brunneum* and also in the upper and lower epidermis of *G. brunneum*, and in palisade mesophyll and phloem of *F. kinabaluensis* (Fig. 5; see Supporting Information—Figs S4 and S5). Small ‘dots’ highly enriched in Ca were also visible in the spongy and palisade mesophyll of *A. alanbakeri* and in the upper epidermis of *F. kinabaluensis* (Fig. 5).

The plant tissue elemental concentrations reported in this study originate from samples collected from wild populations of *P. sarmentosa*, *G. brunneum*, *A. alanbakeri*, and *F. kinabaluensis* growing on ultramafic soil at Kinabalu Park. In comparison, the plant tissue samples used for the nuclear microprobe investigations originated from plants grown on ultramafic soil in a horticultural setting (Hyperaccumulator Botanical Garden) at Kinabalu Park. The ultramafic potting soil (Mollic Leptosol Hypermagnesic) contains far lower concentrations of plant-available Ni than the ultramafic soil (Hypermagnesic Cambisol) from the native populations (van der Ent et al. 2017b).

In combination with the small pot size (2- to 3-L), this resulted in relatively low (<3000 µg g⁻¹) foliar Ni concentrations compared to wild material (>10 000 µg g⁻¹). This finding was unexpected, but has since been observed in a dedicated experiment on the effect of pot size on Ni hyperaccumulation in the temperate herb *Alyssum corsicum* (Chaney et al. 2017). Elements other than Ni may have also been affected, but conceivably only P and K, which are plant-essential macronutrients that are typically present in limited amounts in the ultramafic soils of Sabah (van der Ent et al. 2016).

The results from this study showed that the highest Ni concentrations are in the foliar epidermal cells of all four species. Similarly, in *Rinorea cf. bengalensis* and *P. rufuschaneyi*, Ni is also enriched in the foliar epidermal cells (van der Ent et al. 2017a). Boyd and Martens (1992) postulated that Ni enrichment

**Discussion**

The plant tissue elemental concentrations reported in this study originate from samples collected from wild populations of *P. sarmentosa*, *G. brunneum*, *A. alanbakeri*, and *F. kinabaluensis* growing on ultramafic soil at Kinabalu Park. In combination, the plant tissue samples used for the nuclear microprobe investigations originated from plants grown on ultramafic soil in a horticultural setting (Hyperaccumulator Botanical Garden) at Kinabalu Park. The ultramafic potting soil (Mollic Leptosol Hypermagnesic) contains far lower concentrations of plant-available Ni than the ultramafic soil (Hypermagnesic Cambisol) from the native populations (van der Ent et al. 2017b). In combination with the small pot size (2- to 3-L), this resulted in relatively low (<3000 µg g⁻¹) foliar Ni concentrations compared to wild material (>10 000 µg g⁻¹). This finding was unexpected, but has since been observed in a dedicated experiment on the effect of pot size on Ni hyperaccumulation in the temperate herb *Alyssum corsicum* (Chaney et al. 2017). Elements other than Ni may have also been affected, but conceivably only P and K, which are plant-essential macronutrients that are typically present in limited amounts in the ultramafic soils of Sabah (van der Ent et al. 2016).

The results from this study showed that the highest Ni concentrations are in the foliar epidermal cells of all four species. Similarly, in *Rinorea cf. bengalensis* and *P. rufuschaneyi*, Ni is also enriched in the foliar epidermal cells (van der Ent et al. 2017a). Boyd and Martens (1992) postulated that Ni enrichment
in epidermal cells deter herbivory. However, Mesjasz-Przybyłowicz et al. (2016a) argued that this hypothesis would only make sense if symmetrical accumulation of Ni took place in both the upper and lower epidermis, as insect herbivores feed on both sides. Localization of Ni in epidermal cells has further been hypothesized to aid in osmoregulation and drought tolerance by increasing the water potential in the leaves (Severne 1974; Baker and Walker 1990; Boyd and Martens 1992; Mesjasz-Przybyłowicz et al. 1996). Preferential localization of Ni in the upper epidermal cell has also been suggested by Robinson et al. (2003) to act as a protection for the underlying chlorophyll against ultraviolet radiation. Nickel enrichment in the epidermal parts of leaves is a typical distribution pattern encountered in the majority of studied Ni hyperaccumulator plants to date from diverse phylogenetic and geographical affinities, e.g. Senecio coronatus, S. anomalochrous and Berkhuya zeyheri subsp. rehmannii var. rogersiana (Mesjasz-Przybyłowicz et al. 1994, 1996, 2001) from South Africa; Hybanthus floribundus subsp. floribundus (Kachenko et al. 2008) and Stuckhousia tryonii (Bhatia et al. 2004) from Australia; Alyssum murale (Broadhurst et al. 2004a; McNear et al. 2005), A. bertoloni, A. lesiacium and Noccaea goeingense (Küpfer et al. 2001) from Europe. An exception to this rule is Berkhuya coddii (Asteraceae) where Ni is strongly enriched in the leaf veins and mesophyll, whilst the concentrations in the epidermis are relatively lower (Budka et al. 2005; Mesjasz-Przybyłowicz and Przybyłowicz 2011, 2020).

Different species of the Ni hyperaccumulator, Alyssum, have been reported to have Ni concentrated along with other elements in certain plant tissues. Accumulation of Ni, Mn and Ca was reported by Broadhurst et al. (2004b) at the base of Alyssum leaf trichomes. Subsequent studies by Broadhurst et al. (2009) also revealed a concentration of Ni and Mn only in trichome bases and in cells adjacent to the trichomes of A. murale and A. corsicum. In this study, the epidermal distribution of Ni as observed in the studied species is similar to that of the distribution of Ca and Mn in P. sarmentosa and G. brunneum, respectively. Simultaneous accumulation of other potentially toxic trace elements in plant tissues other than Ni such as Mn, Zn and Co has further been suggested by Boyd (2012) to deter herbivory.

Moreover, this study also revealed similar distribution patterns of K in the spongy and palisade mesophyll of the studied species. Calcium was also found enriched in the spongy and palisade mesophyll of G. brunneum, whereas in A. alanbakeri, both Ca and Ni were strongly enriched in the spongy and palisade mesophyll. This pattern of distribution may be explained by the essential requirement for K, Ca and Ni as plant nutrients by the studied species. Some Mn enrichment in the spongy and palisade mesophyll of A. alanbakeri and in the upper epidermis of F. kinabaluisensis was also found in this study. On the other hand, Mn was found sequestered in the palisade mesophyll cells of the Mn hyperaccumulators Glossia biduillii, Viroto nephylla, Macadamia integrifolia and M. tetraphylla (Fernando et al. 2006a,b) where the authors indicated this to be due to the species high demand for Mn as part of the active centre of the oxygen-evolving complex. In G. fragransissima, Co and Zn were found primarily localized in foliar epidermal cells whilst Mn and Ni were concentrated in the palisade leaf (Fernando et al. 2013). Previous studies by Brooks et al. (1981) and Bidwell et al. (2002) have revealed that the Mn hyperaccumulators Alyxia sp. and G. biduillii accumulate Mn at the expense of K and Mg. Cobalt distribution in A. murale was concentrated in the apoplast, which forms a Co-rich mineral precipitates on the foliar surface (Tapper et al. 2007). In comparison for Glochidion cf. sericeum, Co exudate was reported on the leaf surface in the form of lesions (van der Ent et al. 2018), where it was argued by the later authors to be due to the exposure of aerial oxygen that consequently led to oxidation of Co²⁺ to Co³⁺ on their leaf surfaces. However, in this study, minor Co was observed in the epidermis and spongy mesophyll of A. alanbakeri and F. kinabaluisensis.

The phloem bundles are important tissues of Ni accumulation for the woody hyperaccumulators, such as P. balgooyi, P. rufuschaneyi and Rninea cf. bengalensis, with up to 169 g kg⁻¹ Ni in the phloem sap in P. balgooyi (Mesjasz-Przybyłowicz et al. 2016a; van der Ent et al. 2017a). High concentrations of Ni in the phloem have also been reported in herbaceous plants such as S. coronatus (Mesjasz-Przybyłowicz et al. 1997, 2007), A. murale (McNear et al. 2005; Tapper et al. 2007), B. coddii (Orlowska et al. 2013) and B. zeyheri subsp. rehmannii var. rogersiana (Mesjasz-Przybyłowicz et al. 2016b). This aligns with the results of this study on F. kinabaluisensis, F. sarmentosa and A. alanbakeri with Ni enrichment in the phloem. In comparison with the New Caledonian Ni hyperaccumulator plants including Homalium fraxii (Phyllanthaceae), Hybanthus austrocaledonicus (Rubiaceae) and P. gabriellea (Salicaceae), Ni is also strongly localized in the epidermal cells and phloem bundles (Gei et al. 2020; Paul et al. 2020), and likewise in Geissois pruinosus (Cunoniaceae). However, P. acuminate (Sapotaceae) has Ni-rich laticifers, which constitute an independent network of cells parallel to the vascular bundles (Gei et al. 2020). In addition to the elevated concentrations of K, Ca, Cl, Mn and Co in the phloem of the studied species, these elements have also been found in the present study to be enriched in the cortex and epidermis of young stems, old stems and roots. Even though Ni hyperaccumulation has ostensibly evolved numerous times independently in distant phylogenetic lineages in different areas around the world, the physiological mechanisms, as inferred from elemental localization, are convergent in these tropical woody species from Borneo Island.

Supporting Information

The following additional information is available in the online version of this article—

**Figure S1.** Micro-PIXE elemental maps of Flacourtia kinabaluisensis root section. Concentration scale in wt% dry weight or μg g⁻¹ dry weight.

**Figure S2.** Micro-PIXE elemental maps of Actephila alanbakeri root section. Concentration scale in wt% dry weight or μg g⁻¹ dry weight.

**Figure S3.** Micro-PIXE elemental maps of Flacourtia kinabaluisensis old stem section.

**Figure S4.** Micro-PIXE elemental maps of Psychotria sarmentosa leaf section.

**Figure S5.** Micro-PIXE elemental maps of Actephila alanbakeri leaf section.

Sources of Funding

This research was undertaken at the nuclear microprobe facility of iThemba Laboratory for Accelerator Based Sciences in South Africa. F.A. is the recipient of a UQ Graduate School Scholarship (UQGSS) from The University of Queensland. W.J.P. and J.M.-P. are recipients of the South African National Research Foundation incentive grants no. 114693 and 114694, respectively.
Conflict of Interest
None declared.

Contributions by the Authors
A.V.D.E. and J.M.P. planned and designed the research. J.M.P. collected the specimens and performed cryo-preparation in the field. J.M.P. and W.P. conducted the micro-PIXE experiments and analysed the PIXE data. All authors wrote the manuscript.

Acknowledgements
We acknowledge Sabah Parks for permission to conduct research in Kinabalu Park, and the Sabah Biodiversity Council for research permits. We thank Rimi Repin, Rosiitt Karim, Sukabin Sumail (Sabah Parks) and Postar Miun (Sabah Forestry Department) for their help and expertise in the field. The authors acknowledge the facilities, and the scientific and technical assistance, of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy and Microanalysis, The University of Queensland.

Literature Cited
Baker AJM, Brooks RR. 1988. Botanical exploration for minerals in the humid tropics. *Journal of Biogeography* 15:211–229.
Baker AJM, Walker PL. 1990. Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ, ed. *Heavy metal tolerance in plants: evolutionary aspects*. Boca Raton, FL: CRC Press Inc., 155–157.
Bani A, Echevarria G, Zhang X, Benizri E, Laubie B, Morel JL, Simonnot MO. 2015. The effect of plant density in nickel-phytomining field experiments with *Alyssum murale* in Albania. *Australian Journal of Botany* 63:72–77.
Beamhan JH. 2005. Mount Kinabalu: hotspot of plant diversity in Borneo. *Biologische Skrifter* 55:103–127.
Bhatia NP, Walsh KB, Orlic I, Siegeler A, Ashwath N, Baker AJM. 2004. *Alyssum murale* and *Alyssum corsicum* as hyperaccumulator of nickel from New Caledonia. *Taxon* 53:420–423.
Budka D, Mesjasz-Przybyłowicz T, Tylko G, Przybyłowicz WJ. 2005. Freeze-substitution methods for Ni localization and quantitative analysis in *Berkheya cordifolia* leaves by means of PXE. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* 231:338–344.
Chaney RL. 1983. Plant uptake of inorganic waste. Land treatment of hazardous wastes. In: Parr JE, Marsh PB, Kla JM, eds. *Land treatment of hazardous wastes*. Park Ridge, IL: Noyes Data Corp, 50–76.
Chaney RL, Angle JS, Baker AJM, Li JM. 1998. Method for phytomining of nickel, cobalt and other metals from soil. US Patent # 5711784.
Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL. 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *Journal of Environmental Quality* 36:1429–1443.
Chaney RL, Baker AJ, Morel JL. 2018. The long road to developing agromining/phytomining. In: A. van der Ent et al. (eds.), *Agromining: farming for metals*. Mineral Resources Reviews, Springer International Publishing AG, 1–17.
Chaney RL, Baklanov I, Paul A. 2017. Effect of soil volume on Ni hyperaccumulation from serpentine soil by *Alyssum curvispica*. Presented at the 9th International Conference on Serpentine Ecology, Pogradec, Albania (4–9 June).
Currie LA. 1968. Limits for qualitative detection and quantitative determination. Application to radiochemistry. *Analytical Chemistry* 40:586–593.
DalCorso G, Manara A, Piasentini S, Furioli A. 2014. Nutrient metal elements in plants. *Metallomics* 6:1770–1788.
Doolittle IR. 1986. A semiautomatic algorithm for Rutherford backscattering analysis. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* 15:227–231.
Fernando DR, Bakkaus EJ, Perrier N, Baker AJ, Woodrow IE, Batianoff GN, Collins RN. 2006a. Manganese accumulation in the leaf mesophyll of four tree species: a PIXE/EDAX localization study. *The New Phytologist* 171:751–757.
Fernando DR, Batianoff GN, Baker AJ, Woodrow IE. 2006b. In vivo localization of manganese in the hyperaccumulator *Gossia bidwillii* (Benth.) N. Snow & Guymer (Myrtaceae) by cryo-SEM/EDAX. *Plant Cell and Environment* 29:1012–1020.
Fernando DR, Marshall AT, Forster PJ, Hoebbe SE, Siegeler R. 2013. Multiple metal accumulation within a manganese-specific genus. *American Journal of Botany* 100:690–700.
Gei V, Echevarria G, Erskine PD, Montarges-Pelletier E, Isnard S, Fogliani B, Jaffre T, Spiers KM, Garrevoet J, van der EnT A. 2020. Soil chemistry, elemental profiles and elemental distribution in nickel hyperaccumulator species from New Caledonia. *Plant and Soil* 457 (1–2): 293–320.
Jaffre T, Brooks RR, Lee J, Reeves RD. 1976. Seberia acuminata: a hyperaccumulator of nickel from New Caledonia. *Science* 193:579–580.
Kuchenko AG, Singh B, Bhatia NP, Siegeler R. 2008. Quantitative elemental localization in leaves and stems of nickel hyperaccumulating shrub *Hybanthus floribundus* subsp. *floribundus* using micro-PIXE spectroscopy. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* 266:667–676.
Kersten WJ, Brooks RR, Reeves RD, Jaffre A. 1980. Nature of nickel complexes in *Psychotria douareti* and other nickel-accumulating plants. *Phytochemistry* 19:1963–1965.
Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP. 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum leucanthemum*, *Alyssum bertoloni* and *Thlaspi goessense*. *Journal of Experimental Botany* 52:2291–2300.
Lee J, Reeves RD, Brooks RR, Jaffre T. 1977. Isolation and identification of a citrato-complex of nickel from nickel-accumulating plants. *Phytochemistry* 16:1503–1505.
Lee J, Reeves RD, Brooks RR, Jaffre T. 1978. The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry* 17:1033–1035.
McNair DH Jr, Pelletier E, Everhart J, Chaney RL, Sutton S, Newville M, Rivers M, Sparks DL. 2020. Application of quantitative fluorometry and absorption-edge computed microtomography to image metal compartmentalisation in *Alyssum murale*. *Environmental Science & Technology* 39:2210–2218.
some economic and ecological considerations. *Journal of Chemical Ecology* 41:396–408.
van der Ent A, Mulligan DR, Repin R, Erskine PD. 2019a. Foliar elemental profiles in the ultramafic flora of Kinabalu Park (Sabah, Malaysia). *Ecological Research* 33:659–674.
van der Ent A, Ocenar A, Tisserand R, Sugau JR, Erskine PD, Echevarria G. 2019b. Herbarium X-ray fluorescence screening for nickel, cobalt and manganese hyperaccumulation in the flora of Sabah (Malaysia, Borneo Island). *Journal of Geochemical Exploration* 202:49–58.
van der Ent A, Repin R, Sugau J, Wong KM. 2014. The ultramafic flora of Sabah: an introduction to the plant diversity on ultramafic soils. Kota Kinabalu, Malaysia: Natural History Publications (Borneo); Sabah Parks.
van der Ent A, van Balgooy M, van Welzen P. 2015b. *Actephila alanbakeri* (Phyllanthaceae): a new nickel hyperaccumulating plant species from localised ultramafic outcrops in Sabah (Malaysia). *Botanical Studies* 57:6.