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SPECIAL FEATURE

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Nickel hyperaccumulation in *Antidesma montis-silam*: from herbarium discovery to collection in the native habitat

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Abstract The majority of nickel hyperaccumulator plant species have been discovered by screening using a field spot test based on dimethylglyoxime. Recently, the use of a portable X-ray fluorescence spectroscopy instruments has enabled non-destructive analyses of existing herbarium collections. Given that the family Phyllanthaceae globally has the greatest numbers of hyperaccumulators, all available specimens from this family, including the speciose genus *Antidesma*, at the Forest Research Centre Herbarium in Sabah, Malaysia, were analysed. The results reveal 9 new manganese hyperaccumulators in the genus *Antidesma*, including *Antidesma puncticulatum*, with manganese concentrations reaching up to 46,400 μg g^{-1}. Prior to this study, only one manganese hyperaccumulator had been recorded in Sabah. The present study is the first to discover nickel hyperaccumulator plant species (four species) in the genus *Antidesma*, including *Antidesma montis-silam* with concentrations reaching up to 32,700 μg g^{-1}. Further collection and analyses of plant material of *Antidesma montis-silam* in the native habitat confirmed the high foliar nickel concentrations. The high nickel hyperaccumulating characteristic and ostensibly fast growth rate of *Antidesma montis-silam* infer potential for use in agromining technology. Some species in the genus *Antidesma* exhibit co-accumulation of manganese and nickel. This case-study shows how herbarium XRF screening can lead to discovery of taxa with unique properties.

Keywords Co-accumulation · Dimethylglyoxime · Hyperaccumulator · Nickel · XRF scanning

Introduction

Hyperaccumulators are plants that accumulate extraordinary concentrations of trace elements in their shoot while growing in their natural habitats (Reeves 1992; van der Ent et al. 2013). The worldwide 'standard reference plant' has elemental concentrations of Ni (1.5 μg g^{-1}), and Mn (200 μg g^{-1}) (Markert 1994; Dunn 2007), but in hyperaccumulators these concentrations can be thousands of times higher (see van der Ent et al. 2013). Understanding the ways in which hyperaccumulator plants take up and store metals is critical to optimizing their use in agromining (also called phytomining), a technology that uses hyperaccumulator plants to sequester valuable metals from sub-economic ore bodies such as ultramafic soils (Van der Ent et al. 2015a; Nkrumah et al. 2016). Although there are over 400 Ni hyperaccumulators species (> 0.1 Wt% shoot dry weight), there are just ~ 50 hypernickelophores (species with > 1 Wt% shoot dry weight) known globally that have the greatest utilization for agromining (Angle et al. 2001; Li et al. 2003). Many of these hypernickelophores occur in tropical regions such as Cuba, New Caledonia and Southeast Asia (Reeves 2003). Among the most promising of these species are a large number of taxa in the Phyllanthaceae. On a global scale, Ni hyperaccumulation occurs most frequently in the order Malpighiales, particularly in the families Dichapetalaceae, Phyllanthaceae, Salicaceae and Violaceae (Reeves 1992, 2003). The Malpighiales is one of the largest orders of flowering plants, containing approximately 16,000 species in 42 families globally, accounting for approximately 7.8% of Eudicots (Wurdack and Davis 2009). The Phyllanthaceae have the

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greatest numbers of hyperaccumulators with representatives in the genera *Actephila, Breynia, Cleistanthus, Glochidion* and *Phyllanthus* (Reeves 2003; Van der Ent et al. 2015b, c; Galey et al. 2017).

Some of the world's largest ultramafic exposures occur in Southeast Asia and in Sabah (Malaysia, on the island of Borneo) total 3500 km^2 (Proctor et al. 1988; Repin 1998). Sabah has an estimated 8000 plant species, of which, over half are known to occur on ultramafic soils (Van der Ent et al. 2015c). This region is a global hotspot for Ni hyperaccumulator plants with at least 25 different species discovered to date (Van der Ent et al. 2015b, c). The family Violaceae has two species of *Rinorea* (*R.* aff. *bengalensis* (Wall.) Gagnep. in Humbert and *R.* aff. *Javanica* Kuntze) that are Ni hyperaccumulators. In the genus *Phyllanthus* there are two known hyperaccumulators (*P.* cf. *securinegioides* Merr. and *P. balgooyi* Petra Hoffm. & A. J. M. Baker), at least 9 species in the genus *Glochidion*, one in the genus *Actephila* (*A. alanbakeri* Welzen and Ent), *Flacourtia kinabaluensis* Sleumer and *Xylosma luzonensis* Merr. in the Salicaceae and one species of *Dichapetalum* (*D. gelonioides* subsp. *tuberculatum* Leenh.) of the Dichapetalaceae. There are several Ni hyperaccumulators scattered in other families of other orders, including *Shorea tenuiramulosa* P.S.Ashton (Dipterocarpaceae), *Walsura pinnata* Hassk. (Meliaceae), *Kibara coriacea* (Blume) Hook. f. & A. Thomps (Monimiaceae), *Psychotria sarmentosa* Blume (Rubiaceae), *Mischocarpus sundaicus* Blume (Sapindaceae) (van der Ent et al. 2015b). In Sabah, nickel hyperaccumulators are restricted to circumneutral ultramafic soils with relatively high phytoavailable nickel concentrations (van der Ent et al. 2016a).

A feature of woody tropical Ni hyperaccumulator plants is the extreme enrichment of Ni in the phloem tissue, literally colouring the phloem green from Ni ions (Mesjasz-Przybylowicz et al. 2016; Van der Ent et al. 2017a). This phenomenon has so far been described from *Rinorea* aff. *bengalensis, R.* aff. *javanica (Violaceae), Dichapetalum gelonioides* subsp. *tuberculatum (Dichapetalaceae), Actephila alanbakeri, Phyllanthus* cf. *securinegoides,* and *P. balgooyi* (Phyllanthaceae) with the latter exuding a dark green phloem sap containing up to 16.9% Ni (van der Ent and Mulligan 2015; Mesjasz-Przybylowicz et al. 2016).

Until recently, Ni hyperaccumulator plant species have been identified by screening using a field spot test based on dimethylglyoxime (DMG). Recently, the use of portable X-Ray Fluorescence Spectroscopy (XRF) instruments have enabled non-destructive analyses of existing herbarium collections (Gei et al. 2018). This method is fast (< 30 s) and hence makes it possible to perform systematic screening of entire phylogenetic lineages, which potentially results in the discovery of more hyperaccumulator plant species. Using XRF scanning, 6480 herbarium specimens were analysed at the Forest Research Centre (FRC) in Sabah (Herbarium Code: SAN) (Van der Ent et al. unpublished). We did not attempt taxonomical revisions because most specimens have been identified by botanical experts at the SAN herbarium, and we followed the taxonomical nomenclature in The Plant List (http://www.theplantlist.org/). Apart from representatives from many different families, all available specimens from the Phyllanthaceae were scanned. Given that this family globally hosts the greatest numbers of hyperaccumulators, the explicit aim was to uncover further hyperaccumulators that may exist in the Sabah flora. Up to now, no Ni hyperaccumulators are known from the genus *Antidesma*.

Antidesma (Phyllanthaceae) is a highly variable genus (~ 150 species) of dioecious shrubs and trees common in the understorey of the tropical rain forest (Baker et al. 1998). The genus is distributed in the palaeotropics from West Africa to the Pacific, with the greatest numbers of species (~ 90) occurring in Malesia (the biogeographical region encompassing the Malay Peninsula and the Malay Archipelago) especially Borneo (Baker et al. 1998). *Antidesma* are hardwoods and can have a bole up to 30 m high with a diameter up to 1 m, whereas the leaves are usually evergreen and 1.5–60 by 0.4–30 cm in size (Hoffmann 2006). The inflorescences are raceme-like 0.5–35 cm long with the flowers usually light coloured, and the immature fruits green, maturing to bright colours (Hoffmann 2006). Many species of *Antidesma* are ubiquitous in lowland mixed dipterocarp forests on a variety of different soils derived from sedimentary, volcanic and ultramafic bedrock. *Antidesma montis-silam* Airy Shaw is a tree up to 20 m with a clear bole up to 10 m and diameter up to 22 cm (Airy Shaw 1973). The bark is whitish, ~ 6 mm thick, and the leaves are oblong 15–33 by 6–11 cm (Airy Shaw 1973). The infructescences are 14–28 cm long, and the fruits are ellipsoid 10–14 by 6–10 mm (Hoffmann 2006).

The present study focussed on herbarium screening of all specimens in the genus *Antidesma* held at the FRC herbarium in Sabah, which led to the discovery of Ni hyperaccumulation in *Antidesma montis-silam*, which is known only from a few collections at the type locality. We then undertook field survey to investigate the rhizosphere soil chemistry as well as the elemental concentrations in the various plant parts of *Antidesma montis-silam* occurring in the native habitat.

Materials and methods

X-ray fluorescence spectroscopy measurements of herbarium *Antidesma* specimens

A Thermo Fisher Scientific Niton XL3t instrument (Thermo Scientific, Boston, USA) was used to measure the foliar elemental concentrations of herbarium specimens. The instrument contains a miniature X-ray tube [Ag anode (6–50 kV, 0–200 μA max)], with a geometrically optimized large area silicon drift detector (SDD). It can detect elements from Mg to U within 15–60 s with detection limits 50–100 μg g^{-1} for transition elements such as Ni. Dried herbarium leaf specimens were subjected to an incident beam of X-rays for 30 s in Soils Mode (which uses Compton Normalization). For calibration purposes, a total of 590 leaf samples were selected from known Co, Mn, Ni, and Zn hyperaccumulator species that had been collected from ultramafic and mineralized soils in Sabah, Malaysia (van der Ent et al. 2017b). Leaf rounds of 6 mm diameter (to match the diameter of the incident X-ray beam) were analysed for 30 s in the Soils Mode with Main Filter for three replicated readings per samples. After XRF analyses, these samples were weighed and digested in 4 mL of 70% HNO_3 in a microwave for 1 h at 125°C and diluted to 30 mL, and analysed with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Varian Vista Pro II) for Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, S, and Zn. The limit of detection (LOD) for Co, Mn, Ni, and Zn was estimated by visual inspection of the initial linear regression models. Secondary linear regression models were derived after samples that had XRF concentrations below the LOD were removed. The residuals vs fitted values were inspected for each linear regression analysis, and outliers (± 3 SD of the residual) were identified and removed. The resulting secondary linear regression models were used to calibrate the XRF concentrations of Co, Mn, Ni, and Zn.

Collection and bulk analysis of plant tissues

Plant tissue samples of *A. montis-silam* (leaves, twigs, branches, stems, wood, bark/phloem and roots) for bulk chemical analysis were collected in the natural habitat in Sabah, Malaysia. These samples were dried at 70 °C for 5 days in a drying oven and subsequently packed for transport to Australia and gamma irradiated at Steritech Pty. Ltd. in Brisbane following Australian Quarantine Regulations. The dried plant tissue samples were subsequently ground and digested using 4 mL HNO_3 (70%) and 1 mL H_2O_2 (30%) in a microwave oven (Milestone Start D) for a 45-min programme and diluted to 30 mL with ultrapure water (Millipore 18.2 MΩ cm at 25 °C) before analysis with ICP-AES (Varian Vista Pro II) for Ni, Co, Cr, Cu, Zn, Mn, Fe, Mg, Ca, Na, K, S and P.

Collection of rhizosphere soil samples for bulk analysis

Rhizosphere soil samples were collected from near the roots of *A. montis-silam* plants. Soil sub-samples (~ 300 mg) were digested using 9 mL 70% HNO_3 and 3 mL 37% HCl per sample in a digestion microwave (Milestone Start D) for a program of 1.5 h, and diluted to 45 mL with ultrapure water before analysis to obtain pseudo-total elemental concentrations. Soil pH was obtained in a 1–2.5 soil to water mixture after 2 h shaking. Exchangeable trace elements were extracted in 0.1 M $Sr(NO_3)_2$ at a soil-solution ratio of 1:4 (10-g soil with 40 mL solution) and 2 h shaking time (adapted from Kukier and Chaney (2001)). As a means of estimating potentially phytoavailable trace elements, the DTPA-extractant was used according to Becquer et al. (1995) which was adapted from the original method by Lindsay and Norvell (1978), with the following modifications: excluding TEA, adjusted at pH 5.3, 5 g soil with 25 mL extractant, and extraction time of 1 h. The soil digests/extracts were analysed with ICP-AES (Varian Vista Pro II) for Ni, Co, Cr, Cu, Zn, Mn, Fe, Mg, Ca, Na, K, S and P.

Statistical analyses

Statistical analyses were performed using Statistica version 13.2 (StatSoft Inc., www.statsoft.com) and Microsoft Excel 2013. Concentration values of Ni, Mn and Zn given in μg g^{-1} presented as boxplots and in tables (-values are given in ranges and means in μg g^{-1}). Key to symbols of boxplots: open squares are the ± mean, whiskers are ± standard deviation, circles are outliers and asterisks are extreme outliers. The mean ± standard deviation was determined using descriptive analysis tool, and significant difference was tested using one-way analysis of variance (one-way ANOVA) with confidence level of 95% in the Analysis Toolpak in Microsoft Excel 2013.

Results

Elemental accumulation in the genus *Antidesma*

The elemental accumulation in the genus *Antidesma* revealed by systematic scanning of all the *Antidesma* specimens held at the Forest Research Centre Herbarium in Sepilok, Sabah, Malaysia using X-ray fluorescence spectroscopy (XRF) is shown in Fig. 1 and Table S1. The results show unusually high concentrations of trace elements in various species within the genus *Antidesma*. The concentrations of Mn in *Antidesma puncticulatum* Miq. are extremely high, reaching up to 46,400 μg g^{-1} (mean concentration is 8300 μg g^{-1} Mn) which exceeds the threshold (10,000 μg g^{-1}) (van der Ent et al. 2013) set for Mn hyperaccumulation. The present study also revealed several other *Antidesma* species with maximum Mn concentrations exceeding 10,000 μg g^{-1}, these include *A. coriaceum* Tul. (100–16,700 μg g^{-1} with a mean of 1700 μg g^{-1} Mn), *A. ghesaembilla* Gaertn. (140–14,600 μg g^{-1} with a mean of 5100 μg g^{-1} Mn), *A. leucopodum* Airy Shaw (70–13,400 with a mean of 1900 μg g^{-1} Mn), *A. montanum* Blume, *A. neurocarpum* Miq. (70–11,400 with a mean of 2600 μg g^{-1} Mn), *A. neurocarpum var. linearifolium* (Pax & K.Hoffm.) Petra Hoffm. (230–13,800 μg g^{-1} with a mean of 5300 μg g^{-1} Mn), *A. stipulare* Blume

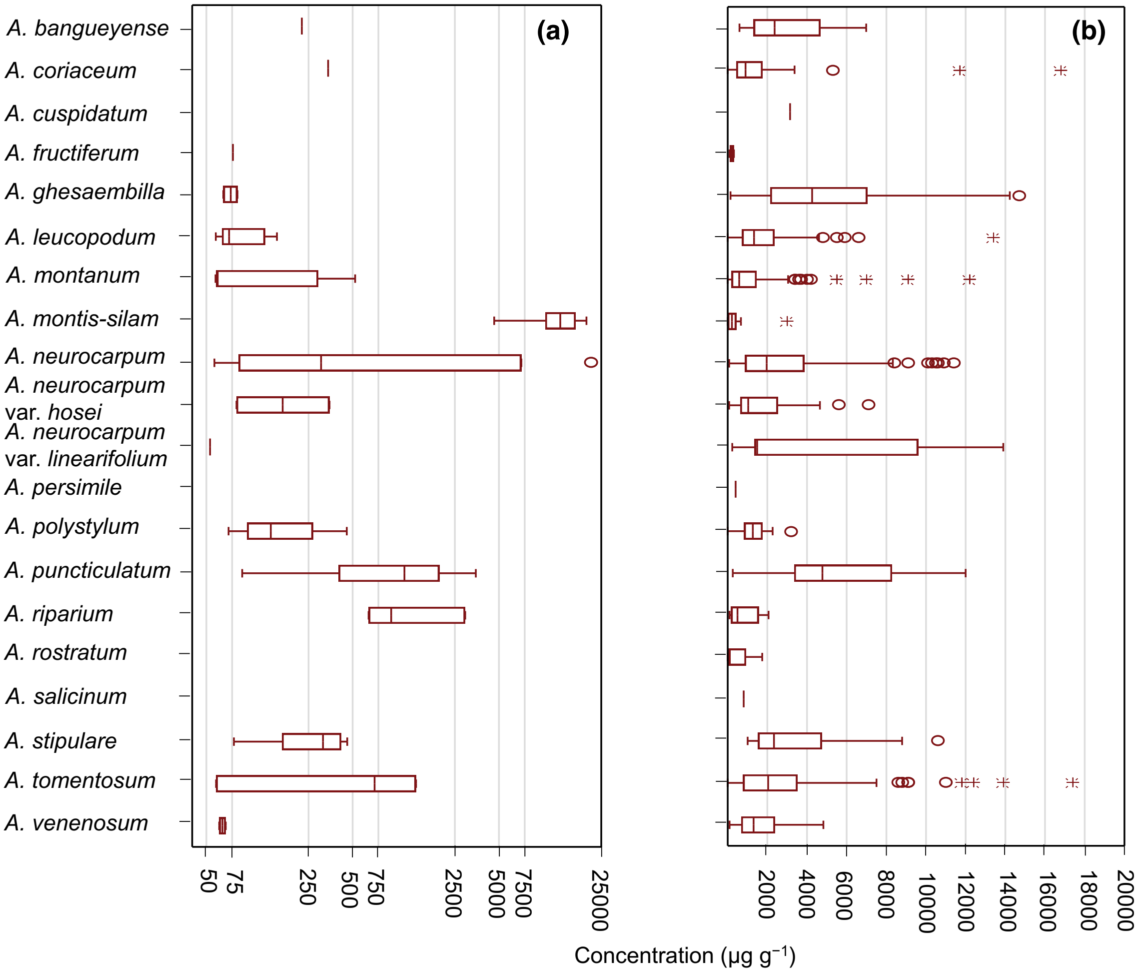

Fig. 1 **a** Nickel, and **b** manganese accumulation in the genus *Antidesma* revealed by systematic scanning of all the *Antidesma* specimens held at the Forest Research Centre Herbarium in Sepilok, Sabah, Malaysia using X-ray fluorescence spectroscopy (XRF) technique. Concentration values of Ni and Mn given in μg g^{-1} (using correction factor based on empirical calibration of the XRF values—see below under Table S1) presented as boxplots. Key to symbols: open squares are the ± mean, whiskers are ± standard deviation, circles are outliers and asterisks are extreme outliers. Species with elemental concentrations below the limit of detection have no boxplot(s) for the concerned element(s)

(1000–10,600 μg g^{-1} with a mean of 4000 μg g^{-1} Mn), and *A. tomentosum* Blume (60–17,400 μg g^{-1} with a mean of 2800 μg g^{-1} Mn). Therefore, the genus *Antidesma* in Sabah contains 9 Mn hyperaccumulators.

For Ni accumulation, most of the species within the genus *Antidesma* had unremarkable foliar Ni concentrations. Prior to the present herbarium XRF scanning, no *Antidesma* species was known to accumulate Ni at concentrations exceeding the threshold (i.e. > 1000 μg g^{-1}) set for Ni hyperaccumulation. The present study is the first to discover Ni hyperaccumulator plant species in the genus *Antidesma*. *Antidesma montis-silam* has extremely high foliar Ni concentrations reaching up to 32,700 μg g^{-1}. Apart from *A. montis-silam*, the present study reveals three other species in the genus *Antidesma* that have elevated foliar Ni concentrations, with mean values exceeding 1000 μg g^{-1} (set as threshold for Ni hyperaccumulation): *Antidesma neurocarpum* (50–21,600 μg g^{-1} with a mean of 4900 μg g^{-1} Ni), *A. puncticulatum* (80–3400 μg g^{-1} with a mean of 1100 μg g^{-1} Ni), and *A. riparium* Airy Shaw (600–3000 μg g^{-1} with a mean of 1500 μg g^{-1} Ni). Interestingly, the present study also reveals co-accumulation of more than one transition element: *Antidesma puncticulatum* and *A. coriaceum* have hyperaccumulator concentrations of both Mn and Ni.

The habitat and rhizosphere soils of *Antidesma montis-silam*

Antidesma montis-silam is a hyper-endemic species distributed only in eastern Sabah (Sandakan and Tawau divisions) in primary mixed dipterocarp forest at 70–700 m altitude (Fig. 2). It occurs on ultramafic soils at the base of Mount Silam, and the mean soil pH in the root zone is near neutral (mean pH value of 6.18) which is a characteristic of well-buffered ultramafic hypermagnesian Cambisols (Table 1). The rhizosphere soil

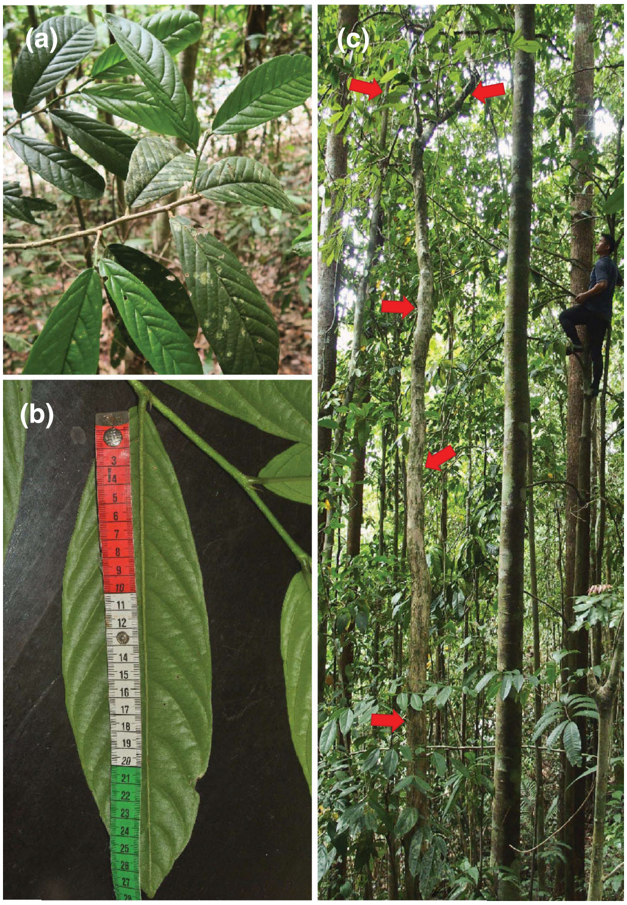

Fig. 2 *Antidesma montis-silam* in the native habitat in Sabah. **a** detail of a terminal branch of *Antidesma montis-silam* showing older and young leaves; **b** mature leaf of *Antidesma montis-silam* approximately 27 cm long and 8 cm wide; and **c** large individual of *Antidesma montis-silam* in the habitat in the Mount Silam Forest Reserve, note the light corky bark and small crown, as indicated with arrows. Jemson Miun from the Forest Research Centre climbs a nearby tree to obtain samples from the *Antidesma montis-silam* tree

chemistry reveals high total concentrations of the trace elements Cr, Fe and Mn (mean > 1000 µg g^{-1}) as expected for ultramafic soils, but comparatively low concentrations of Co and Zn (mean < 50 µg g^{-1}). The total concentrations of the major nutrient elements Ca, K and P are low (mean < 300 µg g^{-1}), relatively high Mg concentrations (mean > 30,000 µg g^{-1}), with extremely high mean Mg:Ca ratio (144:1). The total soil Ni concentrations are relatively low (mean 1070 µg g^{-1}), whereas the potentially plant available Ni concentrations, as given by DTPA (Table 1), are moderately high (mean 140–200 µg g^{-1}) in comparison to other ultramafic soils in Sabah (Van der Ent et al. 2016a) (Fig. 2).

Elemental concentrations in plant parts of *Antidesma montis-silam*

The results of the bulk elemental concentrations are given in Table 2. The foliar tissue concentrations of most trace elements are unremarkable, with the exception of Ni. The mean concentrations of Co, Mn and Zn in the different plant parts are all below 150 µg g^{-1}; Co is particularly low (mean < 10 µg g^{-1}). However, the major element concentrations of the foliar tissues are high, particularly Ca, K and P (Table 2), despite the very low soil concentrations (Table 1). Considering the foliar Ni concentrations (Fig. 3), the leaf tissues have the highest, with concentrations up to 18,100 µg g^{-1}. Apart from the leaf tissues, high Ni concentrations are also recorded in other plant parts. The Ni concentrations in the young twigs (4000–12,600 µg g^{-1}) are much higher than in the old twigs (1700–5400 µg g^{-1}) with high significant difference ($P < 0.01$). Moreover, the Ni concentrations in the branches, bark/phloem and stems (mean > 2000 µg g^{-1}) are enriched, compared to that in the wood (mean 950 µg g^{-1}), which may be explained due to the absence of phloem tissue in the wood.

Table 1 Rhizosphere soil chemistry in the natural habitat of *Antidesma montis-silam* (the number of samples is two with ranges and means provided)

Elements	Elemental concentrations (µg g^{-1})		
	Total	DTPA-extractable	$Sr(NO_3)_2$-extractable
Mg	30,400–32,600 (31,500)	890–960 (930)	950–970 (960)
P	60–150 (100)	1.0–2.0 (1.0)	0.5–2.0 (1.5)
K	20–30 (25)	10–30 (20)	15–30 (25)
Ca	120–310 (220)	–	60–130 (100)
Cr	1070–1120 (1100)	0.05–0.15 (0.10)	0.05–0.1 (0.05)
Mn	1040–1660 (1350)	200–390 (300)	2.5–4.0 (3.1)
Fe	27,000–27,800 (27,400)	70–130 (104)	0.5–1.0 (0.5)
Co	10–60 (30)	7.0–15 (10)	0.5
Ni	920–1200 (1070)	140–210 (170)	9.0–11 (9.0)
Zn	30–40 (30)	2.0–3.0 (3.0)	0.1

The soil pH is 6.17–6.19 (mean is 6.18), and the elemental concentrations of the solutions extracted by total, diethylenetriaminepentaacetic acid (DTPA) and strontium nitratrate ($Sr(NO_3)_2$) methods are given in µg g^{-1}

Table 2 Bulk elemental concentrations in different plant tissues in *Antidesma montis-silam* (values are given in ranges and means in μg g^{-1}, and *n* is the number of samples)

Plant tissue	*n*	Elemental concentrations (μg g^{-1})					
		Mg	Al	P	S	K	Ca
Old leaves	9	3030–7100 (4660)	14–75 (40)	330–1105 (575)	960–3450 (1925)	570–2580 (1360)	2270–7070 (3890)
Young leaves	8	2175–4690 (3830)	6.0–15 (10)	630–1465 (1035)	865–1865 (1405)	3410–8605 (5490)	1915–6960 (3260)
Twigs	4	1470–7100 (3200)	10–20 (15)	730–1885 (1335)	285–735 (460)	1960–6750 (3760)	1690–4960 (3055)
Green twigs	5	2500–5470 (3390)	5.5–20 (10)	1040–2590 (1665)	650–1215 (965)	2425–7230 (3990)	600–16,500 (8870)
Branches	6	520–2250 (1390)	6.0–20 (15)	495–4005 (1420)	195–815 (435)	470–4440 (2050)	520–13,200 (4400)
Bark/Phloem	6	620–4500 (1690)	10–45 (20)	175–475 (265)	360–1040 (560)	800–1890 (1270)	1950–23,530 (11,160)
Stems	4	440–2260 (1470)	2.5–15 (10)	250–465 (360)	160–850 (570)	380–1200 (870)	125–8200 (4975)
Wood	3	185–835 (415)	2.0–5.5 (4.0)	90–1160 (485)	110–385 (230)	170–770 (430)	80–1290 (725)
Roots	6	1695–8980 (4045)	35–1100 (370)	160–1030 (450)	280–725 (495)	310–1130 (650)	445–14,125 (5550)

Plant tissue	*n*	Elemental concentrations (μg g^{-1})				
		Mn	Fe	Co	Ni	Zn
Old leaves	9	40– 120 (80)	20–150 (80)	7.0–15 (10)	6530–16,500 (10,740)	35–70 (50)
Young leaves	8	45–75 (65)	15–40 (25)	7.0–15 (10)	3840–18,120 (9715)	50–70 (55)
Twigs	4	20–50 (35)	10–25 (15)	6.0–15 (9.5)	1756–5440 (3760)	30–60 (40)
Green twigs	5	40–90 (70)	15–120 (40)	5.0–15 (9.0)	4010–12,600 (7900)	45–90 (75)
Branches	6	7.5–30 (20)	7.0–30 (20)	5.0–10 (8.0)	1060–5400 (2480)	15–40 (30)
Bark/Phloem	6	25–200 (70)	20–170 (55)	5.5–10 (7.0)	3330–6600 (4890)	40–100 (70)
Stems	4	4.5–105 (55)	4.0–25 (15)	7.0–9.5 (8.5)	890–9155 (5200)	10–130 (75)
Wood	3	3.5–10 (7.0)	3.0–10 (8.5)	4.5–10 (7.5)	490–1335 (945)	9.3–15 (13)
Roots	6	15–215 (125)	105–2705 (1215)	6.5–15 (10)	1350–3735 (2790)	15–85 (40)

The digest and extracts were analysed with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Elemental concentrations are given in μg g^{-1}

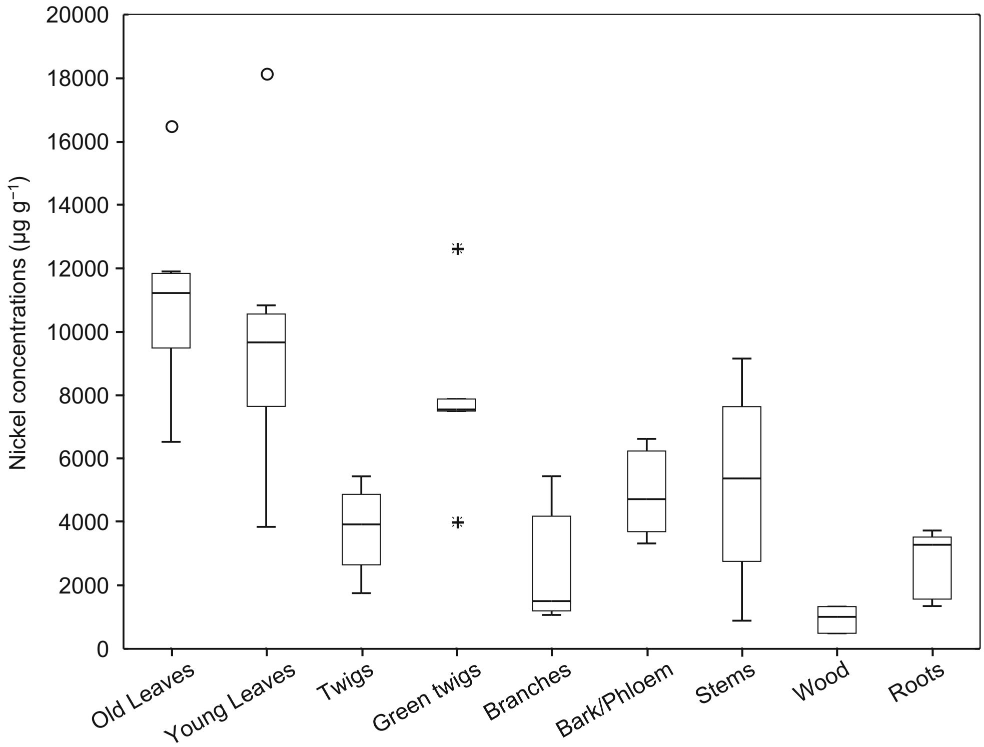

Fig. 3 Nickel concentrations in different plant tissues in *Antidesma montis-silam* analysed with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) presented as boxplots. Key to symbols: open squares are the ± mean, whiskers are ± standard deviation, circles are outliers and asterisks are extreme outliers

Discussion

Hyperaccumulator plant species are of interest for their unusual physiology and biochemistry (Baker and Brooks 1989; Broadhurst et al. 2004, 2009; Tappero et al. 2007; van der Ent et al. 2017a), ecological significance and function (Boyd 2007, 2012), as well as for their potential use in phytoremediation (Chaney et al. 1997; McGrath et al. 2001) and agromining (van der Ent et al. 2015a; Nkrumah et al. 2016, 2018). The increased scientific interest as well as the potential applications of plant species with abnormal concentrations of trace element(s) in the shoots, underscores the need for extensive surveys to discover more taxa with unique properties. The identification of more hyperaccumulator plants is important for advancing the evolutionary understanding of the hyperaccumulation phenomenon, for adding potentially suitable 'metal crops' to the inventory of 'agrominers', and for conservation of these rare plant species.

In the tropics, nickel hyperaccumulation is a distinctive feature in the order Malpighiales, particularly in the families Dichapetalaceae, Phyllanthaceae, Salicaceae and Violaceae (Reeves 1992, 2003). The greatest number of hyperaccumulators occur in the family Phyllanthaceae with representatives in the genera, *Actephila, Breynia, Cleistanthus, Glochidion* and *Phyllanthus* (Reeves 2003; Van der Ent et al. 2015b, c; Galey et al. 2017). Plant species with extraordinary shoot Ni concentrations occur on ultramafic outcrops, with some species being 'obligate' whereas others are 'facultative'. 'Obligate' hyperaccumulator plant species are restricted to ultramafic substrates, and always hyperaccumulate Ni, whereas 'facultative' hyperaccumulators grow on a range of soils, with only ultramafic populations hyperaccumulating (Brooks and Wither 1977; Pollard et al. 2014). Sabah has emerged as a global hotspot for Ni hyperaccumulator plant species after the discovery of 25 hyperaccumulator plant species (van der Ent et al. 2015b, c) reported to date. In addition to *Antidesma montis-silam*, the present study revealed three Ni hyperaccumulator plant species in the genus *Antidesma*: *A. neurocarpum*, *A. puncticulatum* and *A. riparium*. Prior to the new discoveries, there was no *Antidesma* species known to hyperaccumulate Ni (> 1000 µg g^{-1}). The discovery of Ni hyperaccumulation in *A. montis-silam*, which is known only from a few collections at the type locality, provides a case-study of how herbarium XRF screening can lead to discoveries of taxa with unique hyperaccumulation characteristics.

These new discoveries were followed by sampling of fresh plant material and the collection of habitat data and samples. Concentrations of trace elements can greatly differ in plants parts. The present study has shown that *A. montis-silam* has highly distinctive tissue Ni concentrations with the highest accumulation in the leaf tissues, reaching up to 18,100 µg g^{-1} with a mean concentration of 10,200 µg g^{-1} Ni (Fig. 3). These extreme foliar Ni concentrations put *A. montis-silam* amongst the highest foliar Ni concentrations recorded globally. Moreover, *A. montis-silam* falls into a category of hyperaccumulator plant species termed 'hypernickelophores' (species with > 1 Wt% shoot dry weight; only ~ 150 species are recorded globally). The foliar Ni concentrations in *A. montis-silam* exceed that in several Ni hyperaccumulator plant species in Sabah, including *R. aff. bengalensis* (mean 12,800 µg g^{-1} Ni) (van der Ent et al. 2015b), and the recently discovered *A. alanbakeri* (mean 5800 µg g^{-1} Ni) (van der Ent et al. 2016b). However, there are some few species with foliar Ni concentrations exceeding that in *A. montis-silam*: *Phyllanthus* cf. *securinegoides* (mean 23,300 µg g^{-1} Ni), *Glochidion* sp. 'Bambangan' (mean 16,700 µg g^{-1} Ni) (Phyllanthaceae) and *Psychotria sarmentosa* (mean 24,200 µg g^{-1} Ni) (Rubiaceae) (van der Ent et al. 2015b). The Ni concentrations in the twigs are also remarkable. The discovery of *A. montis-silam* as a new Ni 'hypernickelophore' has potential implications for economical Ni agromining operations in Southeast Asia; it is clear that the leaves and twigs, particularly the young twigs (mean 7900 µg g^{-1} Ni), will play major role in such operations. Apart from the high foliar Ni concentrations in *A. montis-silam*, this species also accumulates remarkable concentrations of Ca, K and P (Table 2), despite the very low soil concentrations of these elements (Table 1). However, the high foliar concentrations of these major elements may not be favourable in economic agromining operations as they are 'contaminants' in the eventual 'bio-ore' (van der Ent et al. 2015a). Furthermore, the accumulation of these major elements suggests the need to replenish the soil through fertilizer application during agromining operations to avoid depletion in the soil and subsequent loss of fertility (Bani et al. 2015; Chaney et al. 2007; Nkrumah et al. 2016, 2018).

In the branches, bark/phloem and stems of *A. montis-silam* there is enrichment in the Ni concentrations compared to the wood, due to the presence of phloem tissue in the former plant parts. This observation is in agreement with previous studies that have reported striking differences in Ni concentrations between bark and wood: *Pycnandra acuminata* (Pierre ex Baill.) Swenson & Munzinger contained 24,500 µg g^{-1} Ni (bark) and 1700 µg g^{-1} Ni (wood) (Jaffré et al. 1976); *Psychotria gabriellae* (Baill.) Guillaumin contained 52,400 µg g^{-1} Ni (bark) and 2 300 µg g^{-1} Ni (wood) (Jaffré and Schmid 1974); and *Phyllanthus* cf. *securinegoides* contained 6000 µg g^{-1} Ni (bark) and 800 µg g^{-1} Ni (wood) (van der Ent and Mulligan 2015). The phloem tissue in these tropical Ni hyperaccumulator trees is well developed, and acts as a 'Ni-sink'; the latex can reach up to 14.7% Ni in *P. acuminata* (Schaumlöffel et al. 2003), and 16.9% Ni in the phloem sap of *P. balgooyi* (van der Ent and Mulligan 2015; Mesjasz-Przybylowicz et al. 2016).

Apart from new discoveries of Ni hyperaccumulation in the genus *Antidesma*, the present study also reveals accumulation of other trace elements (i.e. Mn) in this genus. The criterion for Mn hyperaccumulation is 10,000 μg g^{-1} Mn (Reeves 2003; van der Ent et al. 2013), and the present study has made 9 new discoveries in the genus *Antidesma* that are Mn hyperaccumulator plant species. These species include *A. coriaceum* (with up to 16,700 μg g^{-1} Mn), *A. ghesaembilla* (with up to 14,600 μg g^{-1} Mn), *A. leucopodum* (with up to 13,400 μg g^{-1} Mn), *A. montanum* (with up to 12,100 μg g^{-1} Mn), *A. neurocarpum* (with up to 11,400 μg g^{-1} Mn), *A. neurocarpum* var. *linearifolium* (with up to 13,800 μg g^{-1} Mn), *A. puncticulatum* (with up to 46,400 μg g^{-1} Mn), *A. stipulare* (with up to 10,600 μg g^{-1} Mn), and *A. tomentosum* (with up to 17,400 μg g^{-1} Mn). These discoveries place Sabah as a global hotspot for Mn hyperaccumulator plant species; as only one Mn hyperaccumulator had been recorded in Sabah prior to this study, *Eugenia* sp. (with up to 13,700 μg g^{-1} Mn) (Myrtaceae) (Proctor et al. 1989). Globally, only 11 Mn hyperaccumulator plant species are reported in the literature, with the majority found in New Caledonia (nine species) (Reeves 2006). These include *Maytenus bureaviana* (Loes.) Loes. (with up to 33,750 μg g^{-1} Mn) (Celastraceae), and *Macadamia neurophylla* (Guillaumin) Virot (with up to 55,200 μg g^{-1} Mn) (Proteaceae) (Jaffré 1977, 1979). Furthermore, Fernando et al. (2009) analysed six eastern Australian genera from the Queensland Herbarium collection, and found four new Mn hyperaccumulators in the genus *Gossia* (Myrtaceae) in addition to the previously known *G. bidwilli* (Benth.) N.Snow & Guymer (with up to 21,500 μg g^{-1} Mn) (Bidwell et al. 2002).

Evidence from the present study suggests co-accumulation of trace elements with some species hyperaccumulating both Mn and Ni (*A. puncticulatum*). The accumulation of either Mn or Ni suggests that the uptake mechanisms involve the expression of similar Fe transporters of the IREG/Ferroportin (FPN) family (see Merlot et al. 2014). These transporters are known to be involved in the hyperaccumulation of these elements, and small changes in the regulation of their expression among species of *Antidesma* could explain why this genus expresses such frequent hyperaccumulation of these two elements.

It follows that the conservation of these unique species is necessary and timely. The principal occurrence of *A. montis-silam* is in the Mount Silam Forest Reserve near Lahad Datu. The floristics, ecology, biogeochemistry and hydrology of this small coast mountain (summit at 884 m asl) has been extensively studied (Proctor et al. 1988, 1989; Bruijnzeel et al. 1993). Mount Silam is part of the Silam-Beeston complex, which is about 30 km long and up to 6 km wide (Leong 1974). The forests at lower altitude had a large stature and resembled lowland evergreen rain forest on other substrata with 104 species in a 0.4-ha plot at 480 m (Proctor et al. 1988). Mount Silam is renowned for the range of (hyper)endemics and species with an extremely narrow distribution on ultramafic soils including trees such as *Pittosporum silamense* Sugau, Pittosporaceae (Sugau 1994), *Ardisia silamensis* Utteridge, Julius & Suzana, Myrsinaceae (Utteridge et al. 2014), *Madhuca silamensis* Yii & P.Chai, Sapotaceae (Yii and Chai 2001), *Timonius ophioliticus* J.Chen, Rubiaceae (Chen et al. 2015), *Syzygium silamense* P.S.Ashton and *Syzygium ultramaficum* P.S.Ashton, Myrtaceae (Ashton 2006a), *Tristaniopsis kinabaluensis* subsp. *silamensis* P.S.Ashton, Myrtaceae (Ashton 2006b), *Callicarpa hispida* (Moldenke) Bramley, Lamiaceae (Bramley 2009), the orchid *Corybas serpentinus* J.Dransf., Orchidaceae (Dransfield et al. 1986), the palm *Benstonea serpentinica* Callm. & Buerki, Pandanaceae (Callmander and Buerki 2016), and the bamboo *Dinochloa darvelana* S.Dransf., Poaceae (Dransfield 1989). The rare Sabah-endemic tree *Borneodendron aenigmaticum* Airy Shaw (Euphorbiaceae) which is an ultramafic obligate species is especially common on Mount Silam. Some of these exceedingly rare species, such as *Shorea tenuiramulosa* P. S. Ashton (Dipterocarpaceae) known from Mount Silam have also been found to occur on very poor soils elsewhere in Borneo (Ashton 1982; Proctor et al. 1988). Further, *A. montis-silam* occurs principally in protected Forest Reserves (Silam FR and Sepagaya FR), and the restriction of this species to just two populations, the small area of occupancy (< 10 km^2) makes it a very rare species. There appears to be a small number of individuals, as no more than 50 individuals have been observed to date, although no information is available about the population-dynamics and hence trends in population size. As there is no currently available evidence of decline or fluctuations in the extent of its occurrence (EOO), the area of occurrence (AOO), or the number of mature individuals, the conservation status of the species is currently classified as ‘Near Threatened’ (IUCN 2001).

Future investigations employing state of the art techniques such as nuclear microprobe, metabolomics techniques and synchrotron X-ray fluorescence microscopy may provide insights into the biopathways of trace elements in hyperaccumulator plant species in the genus *Antidesma*. Regarding species that exhibit co-accumulation of more than one transition element, classical dosing experiments in which Ni and Mn are supplied in isomolar ratios are useful for unravelling ecophysiological responses. Considering the potential of *A. montis-silam* for agromining operations, future trials on propagation and agronomy, as demonstrated for *Phyllanthus* cf. *securinegoides* and *Rinorea* aff. *bengalensis* (Nkrumah et al. unpublished), could be worthwhile. It is clear that analyses of herbarium specimens either by ICP-AES measurements of leaf fragments or by employing the XRF technique are important methods to prospect for ‘new’ hyperaccumulators. This may then be followed by sampling of fresh plant material of the discovered hyperaccumulator plant species and the collection of habitat data and samples, as shown for *A. montis-silam* in the present study (Fig. 4). Notably, the field survey confirmed the Ni hyperaccumulation status

Fig. 4 **a** Type specimen of *Antidesma montis-silam* (collected in 1965) held at the Leiden Herbarium in The Netherlands, and **b** field sampling of plant material of *Antidesma montis-silam* (in 2016) in the Mount Silam Forest Reserve, Sabah, Malaysia. Antony van der Ent poses with the first live individual of *Antidesma montis-silam* found

of *A. montis-silam* revealed by the herbarium XRF screening of all specimens in the genus *Antidesma* held at the FRC herbarium in Sabah.

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