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1 **Herbarium X-ray Fluorescence screening for nickel, cobalt** 2 **and manganese hyperaccumulator plants in the flora of Sabah** 3 **(Malaysia, Borneo Island)**

4
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14 15 **ABSTRACT**

16 Sabah (Malaysia) on the Island of Borneo has high plant diversity (>8000 species) occurring on a
17 wide range of soils, including ultramafic soils which are known to host hyperaccumulator plants. In
18 this study a new approach (“Herbarium X-Ray Fluorescence Ionomics”) was used to obtain elemental
19 data from herbarium specimens using non-destructive X-Ray Fluorescence spectroscopy analysis. In
20 total ~7300 specimens were thus analysed for nickel, cobalt, and manganese concentrations at the
21 Herbarium of the Forest Research Centre (FRC) in Sepilok, Sabah. The measurements led to
22 recording a total of 759 specimens (originating from 17 families in 30 genera and 74 species) as trace
23 element hyperaccumulators, including 28 nickel hyperaccumulator species (in 10 families, 17
24 genera), 12 cobalt hyperaccumulator species (in 3 families, 7 genera), and 51 manganese
25 hyperaccumulator species (in 12 families, 24 genera). The outcomes of this research demonstrate that
26 handheld XRF is highly useful approach for hyperaccumulator plant discovery in herbarium
27 collections that has the potential to add vast numbers of hyperaccumulating taxa to the global
28 inventory.

29
30 **Keywords:** *hyperaccumulators, phylogenetic, ultramafic, Phyllanthaceae, Malpighiales.*

33 1. INTRODUCTION

34 Some rare plants restricted to ultramafic soils have evolved ecophysiological mechanisms to
35 accumulate trace elements in extreme concentrations and are known as hyperaccumulator plants
36 (Jaffré *et al.* 1976; Reeves, 2003; van der Ent *et al.* 2013a). Nominal threshold values for
37 ‘hyperaccumulation status’ have been established for various elements, including 1000 $\mu\text{g g}^{-1}$ for Ni,
38 300 $\mu\text{g g}^{-1}$ for Co, and 10 000 $\mu\text{g g}^{-1}$ for Mn (Reeves 2003; van der Ent *et al.* 2013a).
39 Hyperaccumulators can be either ‘facultative’ or ‘obligate’ hyperaccumulator plants (Pollard *et al.*
40 2014). Obligate hyperaccumulating taxa are exclusively found on ultramafic soils where they
41 consistently hyperaccumulate, whereas facultative hyperaccumulator taxa can also grow on non-
42 ultramafic soils where they do not hyperaccumulate (Pollard *et al.* 2014; van der Ent *et al.* 2013a).
43 To date more than 700 hyperaccumulator species have been reported globally of which ~ 70% are Ni
44 hyperaccumulators (Reeves *et al.* 2018). Hyperaccumulators with >1 w% (*i.e.* 10 000 $\mu\text{g g}^{-1}$ Ni) are
45 also called ‘hypernickelophores’ and approximately 50 such taxa are presently known (Jaffré and
46 Schmid 1974; Reeves *et al.* 2018). Global hotspots for hyperaccumulator plants include Brazil, Cuba,
47 and New Caledonia (Reeves *et al.*, 1999; 2007; Isnard *et al.*, 2016).

48
49 North Borneo (principally the Malaysian state of Sabah) is one of the top five biodiversity centres in
50 the world (Barthlott *et al.* 2007) and the influence of ultramafic soils on biodiversity in this region
51 have been studied extensively in recent years (van der Ent *et al.*, 2015b, 2018). Plant adaptation to
52 ultramafic soils represents a valuable experiment system for evolutionary ecologists and geneticists
53 (Brady *et al.*, 2005). Ultramafic soils contain toxic concentrations of some elements (*e.g.* Mg and Ni)
54 and a lack of mineral nutrients (*e.g.* P and K), resulting in edaphic stresses for plants (Proctor, 2003;
55 Galey *et al.*, 2017). Ultramafic outcrops are relatively widespread and cover 4.6 % (3500 km^2) of the
56 total land area of Sabah (Repin, 1998; van der Ent *et al.*, 2013b, 2015b). In Sabah, 33 plant species
57 are currently known as Ni hyperaccumulators, seven species as Co hyperaccumulators, and 14 species
58 as Mn hyperaccumulators (van der Ent *et al.*, 2015; 2016; 2018d; Nkrumah *et al.*, 2018).

59
60 Globally the discovery of hyperaccumulator plants has been hindered by the lack of systematic
61 screening of plant species and is highly biased towards particular regions (Reeves 2003; van der Ent
62 *et al.* 2013a). The greatest numbers of Ni hyperaccumulator plants are from Malpighiales order (Jaffré
63 *et al.*, 2013; Reeves *et al.*, 2018). The Malpighiales is one the most diverse groups of flowering plants,
64 comprising about 8% of all eudicots and 6% of all angiosperms and has approximately 40 families
65 containing over 16 000 species (Davis *et al.*, 2005; Korotkova *et al.*, 2009; Wurdack and Davis,
66 2009). The Malpighiales has its greatest diversity in the Neo- and Old-World Tropics (Berry *et al.*,

67 2016), and contains the highest proportion of known of Ni hyperaccumulator species in Cuba and
68 New Caledonia (Reeves 2003; Jaffré *et al.* 2013).

69

70 The value of herbaria as references for taxonomic and biogeographical information on floras is widely
71 established, but herbaria may also supply information on the elemental profile in stored specimens
72 (van der Ent and al., 2016; Nkrumah and al., 2018a). This is not a new concept, and the use of
73 herbarium specimens for discovering hyperaccumulator plants goes back to the 1970s, and tropical
74 hyperaccumulators plants were studied first by Brooks and co-workers from the mid-1970s (Brooks
75 *et al.*, 1977; Jaffré *et al.*, 1979; Kersten *et al.*, 1979). Until the 1980's, testing for hyperaccumulation
76 used mainly atomic absorption spectrophotometry (AAS), which can only analyse one element at a
77 time, which limited discovery of the range of possible hyperaccumulated elements (*i.e.* frequently only
78 Ni was analysed). Nowadays, trace elements in plant samples can be analysed using techniques that
79 capture quantitative data from a suite of different elements simultaneously, such as inductively
80 coupled plasma atomic emission spectrometry (ICP-AES) and inductively-coupled plasma mass
81 spectrometry (ICP-MS). For example, fragments of herbarium specimens have been analysed with
82 ICP-AES for suites of elements in the flora of the DR Congo (Yang *et al.*, 1985), selected families of
83 plants in New Caledonia, and more recently in search of Mn hyperaccumulators in selected lineages
84 in Australia (Fernando *et al.*, 2009). However, as small fragments (typically 20–30 mg) of herbarium
85 material needs to be excised for the analysis, this is a destructive method of analysis, which is
86 ultimately not compatible with preserving herbarium specimens for prosperity. Moreover, plant
87 material acid digestion (or ashing) followed by ICP-AES analysis is relatively expensive and time-
88 consuming. In contrast, X-ray fluorescence spectrometry (XRF) is a non-destructive technique to
89 quantitatively determine multi-elemental concentrations within a sample. Handheld XRF devices
90 have in-built X-ray source (miniature tube) that produces an incident beam that causes a sample to
91 emit a spectrum of fluorescent X-rays which can be interrogated to determine the concentrations of
92 elements present. In order to obtain reliable quantitative data, the XRF instrument has to be
93 appropriately calibrated (Markowicz 2008), which is particularly important for non-standard matrix
94 samples such as herbarium material (Gei *et al.* 2018). The non-destructive nature and short duration
95 of the measurements (*e.g.* 30 seconds), makes it possible to perform elemental analysis on a vast
96 number of stored herbarium specimens (Gei *et al.*, 2018). This approach has been termed “Herbarium
97 X-ray Fluorescence Ionomics” and enables to obtain elemental information from entire phylogenetic
98 lineages, and as such is expected to be transformative for the discovery of hyperaccumulator plants
99 (van der Ent *et al.*, 2019).

100

101 The flora of Sabah was already known to be rich in hyperaccumulator plant species (van der Ent et
102 al., 2015; 2016; 2018d), but these discoveries originated from field-collected material during
103 ecological investigations. It was envisaged that the flora of Sabah would harbour numerous more
104 hyperaccumulator species hitherto unknown, that could be discovered through analysis of herbarium
105 specimens. Therefore, in this study ~7300 herbarium specimens were measured with handheld XRF
106 for Ni, Co and Mn concentrations at the Forest Research Centre (FRC) Herbarium at Sepilok in
107 Sabah, Malaysia. The aim was to prospect for novel hyperaccumulator plants to contribute to a
108 broader understanding of the phylogenetic incidence of hyperaccumulation, and to allow for the
109 identification of species potentially suitable for utilization in phyto-technologies, such as agromining
110 (*e.g.* the use of hyperaccumulator plants to obtain Ni from ultramafic soils).

111

112 **2. MATERIALS AND METHODS**

113

114 **2.1 Selection of herbarium specimens for the XRF scanning**

115 Given that the FRC Herbarium contains ~350 000 specimens (~8000 species or 10 000 taxa) from
116 the state of Sabah, a selection had to be made. All specimens in the Malpighiales families
117 Phyllanthaceae, Salicaceae and Violaceae (totalling 5592 specimens) were selected. The selection
118 further contained the genus *Walsura* (Meliaceae – 98 specimens), the genus *Mischocarpus*
119 (Sapindaceae – 81 specimens), and the family Sapotaceae (all genera – 383 specimens). This selection
120 of specimens was based on previous studies on hyperaccumulator plants in Sabah (van der Ent et al.,
121 2015; 2016; 2018d). Furthermore, single specimens from all species (1183 specimens) known to
122 occur on ultramafic soils in Sabah were selected to provide a wide phylogenetic assay of the incidence
123 of hyperaccumulation. In total, this resulted in 7337 specimens that were measured.

124

125 **2.2 XRF calibration for measurement of herbarium specimens**

126 The Thermo Fisher Scientific Niton XL3t 950 GOLDD+ analyser uses a miniaturised X-ray tube (Ag
127 anode, 6–50 kV, 0–200 μ A max) as its excitation source. The X-ray tube irradiates the sample with
128 high-energy X-rays which excite (characteristic) fluorescent X-rays in the sample. These fluorescent
129 X-rays are detected and quantified with a large 20 mm² Silicon Drift Detector (SDD, 185 eV, up to
130 60 000 counts per second). It can detect elements from Mg to U within 15–60 seconds with absolute
131 detection limits of 50–100 μ g g⁻¹ (but typically >300 μ g g⁻¹ depending on the element in real-life
132 samples). The instrument incorporates Compton Normalisation; appropriate for the relatively low
133 elemental concentrations found in plant material in a low-density (*i.e.* cellulose) matrix.

134

135 A total of 590 dried plant samples were used from earlier ecological studies in Sabah (van der Ent *et*
136 *al.*, 2015b; van der Ent *et al.*, 2016b). These plant samples covered concentration ranges ranging from
137 'normal' to abnormal (*i.e.* hyperaccumulation) and includes known Ni, Co and Mn hyperaccumulator
138 samples. From each sample, 6 mm diameter leaf discs were extracted using a paper punch. The XRF
139 analysis was undertaken on a sheet of 'herbarium cardboard' on a pure titanium plate (~99.995%, 2
140 mm thick × 10 × 10 cm) to provide a uniform background and block transmitted X-rays. The XRF
141 analysis used the 'Soils Mode' in the 'Main filter' configuration for 60s duration. After scanning, the
142 leaf samples were weighed and pre-digested in 1 mL 70% HNO₃. The samples were subsequently
143 digested at 125°C for 2 hours and diluted to 30 mL with ultra-pure water before analysis with ICP-
144 AES (Varian Visa Pro II), which included measurements of the following elements: Ni, Co, Cr, Cu,
145 Zn, Mn, Fe, Mg, Ca. The quality control included the NIST 1515 ('Apple Leaves') standard and
146 blanks.

147

148 **2.3 Herbarium specimen and sample contamination**

149 Each herbarium specimen was measured for 30 seconds in 'Soil Mode' with the 'Main filter' and the
150 raw XRF data was corrected using the regression formulas obtained from the empirical, calibration.
151 The pure titanium plate was located under the herbarium specimens to provide a uniform background
152 and to block penetrating X-rays. Consistently, old/mature leaves (*i.e.* the most basal leaves on a
153 specimen) were measured, and care was taken to fully cover the 6 mm measurement area of the XRF.

154

155 The potential for contamination of leaves with soil particulates presents a problem for the accurate
156 determination of elemental concentrations in plant material (Gei *et al.*, 2017). This risk is highest for
157 ground-herbs, and lesser for tall trees, but cannot be entirely avoided. Concomitant apparent high
158 foliar concentrations of Cr (>60 µg g⁻¹) and Fe (>2500 µg g⁻¹) are useful indicators for soil
159 contamination, as these elements are major constituents of ultramafic soils. Any suspect samples with
160 anomalously high Cr and Fe were excluded from the dataset. Another possible problem is the practise
161 of some tropical collectors to use methylated spirit for temporarily preserving sample collections to
162 limit decomposition of the specimens during transport, which can potentially 'wash out' soluble
163 elements from the plant material. These specimens are typically readily identified by their
164 characteristic de-colouration ('yellowing'), and were treated with caution in the dataset. Note that
165 affected specimens would have lower apparent Ni, Co, Mn concentrations, and hence would not lead
166 to 'false-positives' for detecting hyperaccumulator plants.

167

168 Even though the handheld XRF instrument can accurately measure Zn concentrations, and Zn has
169 been measured previously in herbarium specimens (Gei *et al.*, 2017; Nkrumah *et al.*, 2018a.b), Zn

170 data has been excluded from this dataset because some specimens at the FRC Herbarium have been
171 glued to the cardboard sheets with a glue that has high concentrations of Zn (>1 Wt%). The type of
172 glue that is Zn-rich was used mainly on older specimens (pre-1980s), but the identity of specimens
173 that were affected could not reliably be established.

174

175 **2.4 Statistical analyses**

176 The apparent limits of detection (LOD) for Ni, Co and were estimated by visual inspection of the log-
177 transformed linear regression models of the XRF data against corresponding ICP-AES measurements
178 and set at: <190 $\mu\text{g g}^{-1}$ for Ni, <140 $\mu\text{g g}^{-1}$ for Co and <336 $\mu\text{g g}^{-1}$ for Mn. The residuals *vs.* fitted
179 values were inspected for each linear regression analysis, and outliers (± 3 SD of the residual) were
180 identified and removed. Secondary linear regression models were then derived after the samples with
181 XRF values <LOD were removed. Herbarium specimens with XRF values < LOD were excluded
182 from the dataset, and of the total 7337 specimens that were measured, 6775 specimens remained in
183 the final dataset. The XRF-ICP-AES correction factors were then applied to all of the raw XRF
184 measurements obtained from the herbarium specimens.

185

186 The data were analysed using Xlstat (Microsoft professional 2016) and comprised of computing range
187 and means, and graphing plots. The phylogenetic data was analysed using the packages “ape” and
188 “phylo4d” in R and the software PHYLOGENETIC (Phyloomatic tree R20120829 Plants).

189

190 **3. RESULTS**

191

192 **3.1 General statistics and phylogenetic patterns**

193 Overall, 6775 specimens (representing 119 different families) originating from ultramafic and non-
194 ultramafic soils were analysed by XRF for Ni, Co and Mn and this yielded 759 specimens (in in 17
195 families, 30 genera and 74 species) as trace element hyperaccumulators. This includes 28 Ni
196 hyperaccumulator species (in 10 families, 17 genera), 12 Co hyperaccumulator species (in 3 families,
197 7 genera), and 51 Mn hyperaccumulator species (in 12 families, 24 genera). Some specimens
198 hyperaccumulated a combination of Ni, and/or Co and/or Mn simultaneously. All specimens
199 hyperaccumulating Ni and Co originated from ultramafic areas, whereas Mn hyperaccumulators are
200 widespread and very few specimens originated from known ultramafic areas. Hyperaccumulators of
201 Ni, Co, and Mn in the Malpighiales (Table 1) and for other clades (Table 2), and hyperaccumulation
202 records are broken down into families and genera. The Phyllanthaceae had the greatest number of
203 hyperaccumulators among the families tested, with nine genera containing hyperaccumulators and
204 four genera containing hyperaccumulators of all three elements. The second highest number of

205 hyperaccumulating genera was the Salicaceae, with four genera, but only Ni and Mn were
206 hyperaccumulated in this family. This study examined the accumulation patterns of Ni, Co and Mn
207 in 24 families, 78 genera and 351 species from the Malpighiales, and a phylogenetic tree was
208 constructed based on these results (Figure 1).

209

210 **3.2 Nickel hyperaccumulation**

211 In total, 271 specimens had Ni concentrations greater than the LOD ($<190 \mu\text{g g}^{-1}$), of those 163
212 specimens exceeded hyperaccumulation threshold ($>1000 \mu\text{g g}^{-1}$), with the highest value recorded in
213 *Dichapetalum gelonioides* with $45\,600 \mu\text{g g}^{-1}$ Ni. As such, only 4% of the specimens, had detectable
214 Ni. The incidence of Ni hyperaccumulation is shown in Figure 3. A total of 10 families with 17 genera
215 and 28 species were identified as Ni hyperaccumulators. Nickel hyperaccumulation was recorded in
216 unrelated families, such as the Achariaceae (*Hydnocarpus* – 2 spp.), Annonaceae (*Marsypopetalum*
217 – 1 sp.), Dipterocarpaceae (*Shorea* – 1 sp.), Fabaceae (*Dalbergia* – 1 sp.), Loganiaceae (*Strychnos* –
218 1 sp.) and Meliaceae (*Walsura* – 2 spp.), but is most frequent in the Phyllanthaceae (genera: *Actephila*,
219 *Antidesma*, *Aporusa*, *Ashtonia*, *Baccaurea*, *Cleistanthus*, *Glochidion*, *Phyllanthus*). Particularly
220 interesting new records include an unidentified species of *Hydnocarpus* (Achariaceae) with up to
221 $3200 \mu\text{g g}^{-1}$ Ni, *Marsypopetalum pallidum* (Annonaceae) with $6000 \mu\text{g g}^{-1}$ Ni. The rare dipterocarp
222 *Shorea tenuiramulosa* first reported by Proctor et al. (1989) from Mt Silam on Sabah's east coast as
223 a Ni hyperaccumulator was not detected in this survey, but it is a weak Ni hyperaccumulator with up
224 to $1790 \mu\text{g g}^{-1}$ Ni (van der Ent and Mulligan 2015). Values for *Dichapetalum gelonioides*
225 (Dichapetalaceae) are extremely wide-ranging ($340\text{--}45\,600 \mu\text{g g}^{-1}$ Ni), which may be explained by
226 the omission of infraspecific ranks in this study. The various *Dichapetalum* subspecies differ greatly
227 in their hyperaccumulation characteristics, for example subsp. *sumatranum* and *pilosum* do not
228 accumulate Ni, but Zn (Nkrumah et al. 2018b).

229

230 Some known Ni hyperaccumulator plants species, such as *Kibara coriacea* (Monimiaceae) with up
231 to $5840 \mu\text{g g}^{-1}$ Ni (van der Ent and Mulligan 2015) and *Actephila alanbakeri* (Phyllanthaceae) with
232 up to $14\,700 \mu\text{g g}^{-1}$ Ni (van der Ent et al. 2016) were not detected because the FRC Herbarium does
233 not hold any specimens of these taxa. This survey added another species of *Actephila*, *A. excelsa*, as
234 a strong Ni hyperaccumulator with $5200\text{--}14\,600 \mu\text{g g}^{-1}$ Ni. Another such instance is the known Ni
235 hyperaccumulator *Walsura pinnata* (Meliaceae) which can accumulate up to $8000 \mu\text{g g}^{-1}$ Ni, with the
236 new recording *W. pachycaulon* with $690\text{--}8600 \mu\text{g g}^{-1}$ Ni.

237

238 *Antidesma montis-silam* with $4600\text{--}32\,700 \mu\text{g g}^{-1}$ Ni is a rare taxon principally known from Mt Silam
239 on Sabah's east coast, which has been the subject of a recent field-based study (Nkrumah et al. 2017).

240 Other Ni hyperaccumulating species in this genus include *A. neurocarpum* (up to 21 500 $\mu\text{g g}^{-1}$ Ni),
241 *A. riparium* (up to 2900 $\mu\text{g g}^{-1}$ Ni) and *A. puncticulatum* (up to 3500 $\mu\text{g g}^{-1}$ Ni). The genus *Aporosa*
242 also yielded several Ni hyperaccumulators, including *A. lucida* with up to 10 400 $\mu\text{g g}^{-1}$ Ni (and up
243 to 980 $\mu\text{g g}^{-1}$ Co), all except *A. chalarocarpa* were unknown previously. Numerous Ni
244 hyperaccumulating species of *Glochidion* all of which were previously recorded, were also detected
245 (see Suppl Info for details). Note that several *Glochidion* taxa from Mount Kinabalu Park (*G. cf.*
246 *mindorensis*, *G. cf. sericeum*, *G. "panatanran"*, *G. "bambangan"*, *G. "Nalumad"*). There are at the
247 Sabah Parks Herbarium (SNP) and could not be matched with specimen sheets the FRC Herbarium,
248 likely because there are yet undescribed taxa endemic to Mount Kinabalu. The genus *Glochidion*,
249 which consists of more than 300 species mainly distributed in the Indo-Pacific region, is in desperate
250 need of a modern revision for Borneo, with regional herbaria typically housing thousands of
251 unidentified *Glochidion* specimens. The FRC Herbarium only has specimens of six *Phyllanthus* taxa:
252 *P. lamprophyllus*, *P. balgooyi* (synonym *P. hellwigii*), *P. kinabaluicus*, *P. rufuschaneyi* (formerly *P.*
253 *cf. securinegioides* and identified as *P. gracilipes*), *P. urinaria* and *P. amarus*. The latter two a pan-
254 tropical weeds, whereas *P. lamprophyllus* is a common species along rivers in Sabah. The other two
255 taxa are hypernickelophores, whereas *P. kinabaluicus* is a hyper-endemic to Mount Kinabalu (and a
256 Ni accumulator with 700 $\mu\text{g g}^{-1}$ Ni, unpublished data).

257

258 In the large genus *Psychotria* (Rubiaceae) three taxa (*P. densifolia*, *P. gracilis*, *P. sarmentosa*)
259 hyperaccumulate Ni, up to 24 500 $\mu\text{g g}^{-1}$, but it is uncertain whether these taxa are actually distinct
260 given the taxonomical uncertainties in this genus. The large family Sapindaceae, with the well-known
261 fruits *Nephelium lappaceum* (Rambutan) and *Dimocarpus longan* (Longan), only yielded one Ni
262 hyperaccumulator in *Mischocarpus sundaicus*, which was previously known and can accumulate up
263 to 4420 $\mu\text{g g}^{-1}$ Ni (van der Ent et al. 2015f), although lower values up to 2400 $\mu\text{g g}^{-1}$ Ni were recorded
264 with XRF analysis. The Violaceae (genus: *Rinorea*) has two Ni hyperaccumulating taxa (*R.*
265 *bengalensis*, *R. javanica*) which were previously known and again detected in this study. They can
266 accumulate up to 22 200 $\mu\text{g g}^{-1}$ and 9680 $\mu\text{g g}^{-1}$ Ni respectively (van der Ent et al. 2017a). The
267 Salicaceae has four genera (*Flacourtia*, *Homalium*, *Scolopia*, *Xylosma*) with Ni hyperaccumulators.
268 Of these two unidentified *Scolopia* spp. (likely *S. luzonensis*) accumulates up to 9200 $\mu\text{g g}^{-1}$ Ni,
269 whereas *S. spinosa* accumulates 1240 $\mu\text{g g}^{-1}$ Ni.

270

271 The majority of Ni hyperaccumulators are 'obligate' (e.g. hyperaccumulate Ni consistently for all
272 specimens), for example *Phyllanthus rufuschaneyi* with 25 samples ranging from 2196–23 300 $\mu\text{g g}^{-1}$
273 Ni (van der Ent et al. 2015f) or high but variable (but with some specimens below the Ni
274 hyperaccumulation threshold i.e. $>1000 \mu\text{g g}^{-1}$ Ni) such as *Rinorea bengalensis* with 45 specimens

275 ranging from 250–32 200 (with a mean of 7400 $\mu\text{g g}^{-1}$ Ni), or *Walsura pinnata* with 22 specimens
276 290–8000 ranging from 290–8000 (with a mean of 2300 $\mu\text{g g}^{-1}$ Ni). Only a few Ni hyperaccumulators
277 are probably truly 'facultative', such as *Antidesma neurocarpum* with 196 specimens ranging from
278 370–21 500 $\mu\text{g g}^{-1}$ Ni (with a mean of 2900 $\mu\text{g g}^{-1}$ Ni). However, for most other Ni hyperaccumulating
279 species not enough specimens (most species were represented by less than 5 specimens) were
280 available to draw any definitive conclusions about obligate or facultative hyperaccumulation status.
281 The frequency distribution of Ni concentrations in the herbarium specimens (Figure 2) is distinct
282 from Co and Mn (see further below). There are two separate modes on ultramafic and non-ultramafic
283 soils (confirmed by statistical tests shown in Table 3). On non-ultramafic soils the distribution of Ni
284 follows a pseudo 'log-normal' distribution with a geometric mean of $\sim 320 \mu\text{g g}^{-1}$. On ultramafic soils,
285 two groups exist, the first follows a log-normal distribution with a geometric mean of $\sim 500 \mu\text{g g}^{-1}$
286 (e.g. below the Ni hyperaccumulation threshold), whereas the second group also follows a log-normal
287 distribution and has a geometric mean of $\sim 10\,000 \mu\text{g g}^{-1}$ (e.g. 'hypernickelophores'). The two groups
288 are clearly separated at $\sim 1000 \mu\text{g g}^{-1}$ which is the recognised threshold to define Ni
289 hyperaccumulation. Statistical tests reveal that Ni concentrations in specimens from ultramafic and
290 non-ultramafic soils are significantly different (Table 3). Only the genus *Baccaurea* (Phyllanthaceae)
291 and the family Salicaceae show no significant difference between non-ultramafic and ultramafic soils.

292

293 **3.3 Cobalt hyperaccumulation**

294 In total, 107 specimens had Co concentrations greater than the LOD ($<140 \mu\text{g g}^{-1}$), of those 23
295 specimens exceeded the hyperaccumulation threshold ($>300 \mu\text{g g}^{-1}$), with the highest value recorded
296 in *Ashtonia excelsa* with $1500 \mu\text{g g}^{-1}$ Co. The frequency distribution shows that only a very limited
297 number of specimens are clearly separated based on their concentrations (Figure 2). There were only
298 three families, nine genera and nine species which had Co concentrations $>300 \mu\text{g g}^{-1}$ (Table 1 and
299 2). The Phyllanthaceae family emerged as the main Co (hyper)accumulator group. The known Co
300 hyperaccumulator *Glochidion* cf. *sericeum* was not detected in this study, but that can be explained
301 because the FRC Herbarium does not hold any specimens of this taxon, since they are deposited at
302 the Sabah Parks Herbarium (van der Ent et al., 2018c). In the family Phyllanthaceae, samples
303 originating from ultramafic soils tended to have higher foliar Co, notably in the genus *Baccaurea*
304 (Table 3).

305

306 **3.4 Manganese hyperaccumulation**

307 In total 4218 specimens had Mn concentrations greater than the LOD ($<336 \mu\text{g g}^{-1}$), of those 123
308 specimens exceeded the hyperaccumulation threshold ($>10\,000 \mu\text{g g}^{-1}$), with the highest value
309 recorded in *Antidesma puncticulatum* with $46\,480 \mu\text{g g}^{-1}$ Mn. As such, about one third of the

310 specimens measured, originating from 46 families, had detectable Mn. Although hyperaccumulation
311 of Mn is relatively rare, concentrations exceeding $550 \mu\text{g g}^{-1}$ are frequent in the flora of Sabah. A
312 total of 12 families, 24 genera and 51 species were Mn hyperaccumulators (Tables 1 & 2). The
313 Phyllanthaceae had the greatest numbers of Mn hyperaccumulators with six genera and 27 species.
314 Manganese hyperaccumulation is clearly phylogenetically scattered, and in most instances just a
315 single or a few specimens of a species were found with inordinately high Mn values. For example,
316 *Diospyros singaporensis* (Ebenaceae) with $19\,700 \mu\text{g g}^{-1}$ Mn, *Chrozophora oblongifolia*
317 (Euphorbiaceae) with $11\,600 \mu\text{g g}^{-1}$ Mn, *Gymnacranthera* sp. (Myristicaceae) with $10\,200 \mu\text{g g}^{-1}$ Mn,
318 and *Syzygium fastigiatum* (Myrtaceae) with up to $28\,800 \mu\text{g g}^{-1}$ Mn. Such instances may be explained
319 by local variations in soil Mn availability in which the particular specimen grew. In other cases, Mn
320 hyperaccumulation is a more consistent trait with numerous specimens of the same species having
321 extremely high values. Some of these are especially strong Mn hyperaccumulators, such as *Casearia*
322 *tuberculata* with up to $38\,200 \mu\text{g g}^{-1}$ Mn, *Baccaurea sumatrana* with up to $21\,700 \mu\text{g g}^{-1}$ Mn,
323 *Neoscortechinia philippinensis* (Euphorbiaceae) with three specimens with $3600\text{--}11\,800 \mu\text{g g}^{-1}$ Mn,
324 and *Aporosa illustris* with up to $26\,600 \mu\text{g g}^{-1}$ Mn. The genus *Antidesma* is noteworthy for the
325 numbers of Mn hyperaccumulating specimens, including *A. neurocarpum* with 192 specimens
326 ranging from $370\text{--}13\,900 \mu\text{g g}^{-1}$ Mn (mean of $2830 \mu\text{g g}^{-1}$ Mn), and *A. tomentosum* with 112
327 specimens ranging from $390\text{--}17\,400 \mu\text{g g}^{-1}$ Mn (mean of $3190 \mu\text{g g}^{-1}$ Mn). The genus *Walsura* in the
328 Meliaceae family is of similar interest because three taxa (*W. decipiens*, *W. pachycaulon*, *W. pinnata*)
329 are strong Mn hyperaccumulators with up to $21\,000 \mu\text{g g}^{-1}$, $45\,200 \mu\text{g g}^{-1}$ Mn and $30\,700 \mu\text{g g}^{-1}$
330 Mn respectively.

331

332 In all species, however, the mean Mn value does not exceed the Mn hyperaccumulation threshold
333 (*i.e.* $>10\,000 \mu\text{g g}^{-1}$ Mn). Nickel and Mn hyperaccumulation in *Antidesma*, focussing on *A. montis-*
334 *silam*, has been subject of a dedicated study (Nkrumah et al. 2017). Yet other taxa have very wide-
335 ranging Mn values, for example *Mischocarpus pentapetalus* (Sapindaceae) with 51 specimens
336 ranging from $650\text{--}19\,400 \mu\text{g g}^{-1}$ Mn. Interesting, the related *M. pinnata* is a strong Ni
337 hyperaccumulator. Another example is *Cleistanthus sumatranus* (Phyllanthaceae) with 54 specimens
338 ranging from $350\text{--}11\,300 \mu\text{g g}^{-1}$ Mn.

339

340 The frequency distribution of Mn concentrations in the herbarium specimens (Figure 2) shows that
341 almost all the specimens fall in a log-normal distribution with a geometric mean of $1000 \mu\text{g g}^{-1}$. There
342 does not seem to be an effect of ultramafic soils in the pattern of foliar Mn concentrations. However,
343 statistical differences between specimens from the Violaceae family from ultramafic and non-
344 ultramafic soils were found ($p < 0.05$) (Table 3). The Violaceae originating from non-ultramafic soils

345 had Mn concentrations higher than the Violaceae originating from ultramafic soils ($p < 0.01$). Also,
346 the Phyllanthaceae had more Mn hyperaccumulator species growing on non-ultramafic soils than on
347 ultramafic soils.

348

349 **3.5 Simultaneous hyperaccumulation of two or more elements**

350 The genus *Baccaurea*, and especially *B. odoratissima* with up to 8000 $\mu\text{g g}^{-1}$ Ni, is of interest because
351 of its ability to also accumulate Co up to 960 $\mu\text{g g}^{-1}$. *Ashtonia excelsa*, with only one taxon in Borneo,
352 also stands out because it accumulates up to 8100 $\mu\text{g g}^{-1}$ Ni and 1500 $\mu\text{g g}^{-1}$ Co. *Walsura pinnata*
353 (Meliaceae) is another taxon that can hyperaccumulate Ni (up to 8000 $\mu\text{g g}^{-1}$), Co (up to 4580 $\mu\text{g g}^{-1}$
354 in van der Ent et al. 2015b) and Mn (up to 30 700 $\mu\text{g g}^{-1}$). *Rinorea bengalensis* and *R. javanica*
355 incidentally also have high Co values, with up to 630 $\mu\text{g g}^{-1}$ and 670 $\mu\text{g g}^{-1}$ respectively (Brooks et al.
356 1977).

357

358 Hyperaccumulation of Co is relatively rare, mostly because soils with high available Co are
359 infrequent (Lange et al., 2017; van der Ent et al., 2018c, d). In this study, only a few specimens from
360 the same genera in the Phyllanthaceae hyperaccumulated Co alone. Other species identified in this
361 study as Co hyperaccumulators also hyperaccumulated Ni and Mn simultaneously. Furthermore, in
362 most ultramafic soils worldwide, Co correlates with Ni (van der Ent & Mulligan, 2015; Tashakor et
363 al., 2018; van der Ent et al., 2018c,d).

364

365 **3.6 New hyperaccumulator plant discoveries**

366 The herbarium XRF scanning in this study led to the recording of a total of 28 Ni hyperaccumulator
367 species from 10 families and 17 genera (Suppl. Table 2). Among the Ni hyperaccumulators were 10
368 species that can be classified as ‘hypernickelophores’ of which eight were already known prior to this
369 study (Baker et al., 1992; van der Ent et al., 2015b, 2016c; Bouman et al., 2018; Nkrumah et al.,
370 2018a). The ‘hypernickelophores’ were from the Dichapetalaceae, Phyllanthaceae, Violaceae and
371 Rubiaceae, and the two newly reported hypernickelophores were from the genus *Xylosma*
372 (Salicaceae); *Xylosma luzoniensis* and *X. sumatranum*. Moreover, the recently reported
373 hypernickelophore *Antidesma montis-silam* was discovered through herbarium XRF scanning and
374 plant material was subsequently collected in the field and analysed, confirming the findings from the
375 XRF survey (Nkrumah et al., 2018a). The present study found that the highest Ni concentrations in
376 herbarium specimens in *Dichapetalum gelonioides* subsp. *tuberculatum* with 4.56 Wt% Ni. This
377 taxon was already the species with the highest Ni concentration previously reported with 2.7 Wt%
378 (Nkrumah et al. 2018b). *Dichapetalum gelonioides* subsp. *sumatranum* and *D. gelonioides* subsp.
379 *pilosum* are strong Zn hyperaccumulators on normal soils, reaching up to 15 700 $\mu\text{g g}^{-1}$ and 26 400

380 $\mu\text{g g}^{-1}$ foliar Zn (Baker et al., 1992; Nkrumah et al., 2018b), and although extremely high Zn were
381 detected in this study in *Dichapetalum* spp. (and Zn-rich glue could be eliminated in these cases after
382 careful inspection of the specimens), we omitted Zn values from the overall dataset for consistency
383 (as the presence or absence of the problematic glue could not be established in all cases).

384

385 This study also recorded a total of 51 Mn hyperaccumulator species from 13 families and 24 genera
386 (Suppl. Table 3), of which 33 were previously known to be hyperaccumulators (van der Ent et al.,
387 2018d). The present record for Mn hyperaccumulation in Sabah is *Antidesma puncticulatum* with 4.6
388 Wt% Mn (Nkrumah et al. 2017), followed by *Walsura pachycaulon* with 4.5 Wt% Mn, and *Casearia*
389 *tuberculata* with 3.8 Wt% Mn. Additionally, 14 Co hyperaccumulator species from three families
390 and eight genera (Suppl. Table 4) were found, of which seven were previously known to be
391 hyperaccumulators (van der Ent et al., 2018d). The highest Co concentration in herbarium specimens
392 was recorded in *Ashtonia excelsa* with 1500 $\mu\text{g g}^{-1}$ foliar Co.

393

394 **4. DISCUSSION**

395

396 Hyperaccumulator plants have been studied from many different angles for their unusual
397 biochemistry and ecophysiology (Baker and Brooks 1989; van der Ent et al., 2017), as well as for
398 their potential utility in agromining applications (van der Ent et al., 2013b, 2015a). The identification
399 of new hyperaccumulators can provide information about the evolution of hyperaccumulation in the
400 Plant Kingdom. At the global scale Ni hyperaccumulator plants are known primarily to occur in the
401 Brassicales (in the northern hemisphere temperate and Mediterranean climates) and Malpighiales (in
402 tropical climates of the Old and New World) (Reeves, 1992, 2003; Jaffré et al., 2013). Although Ni
403 hyperaccumulation has been most intensively studied at global scale (Reeves et al., 2018), as well as
404 in the Sabah (van der Ent et al., 2018), this study led to the discovery of numerous hyperaccumulators
405 for Mn and several for Co. This places Sabah among the top regions in the world for the diversity of
406 Ni hyperaccumulators after Cuba with 120 species (Reeves et al., 2007) and New Caledonia with 65
407 species (Jaffré et al., 2013). This study also confirmed that Ni hyperaccumulation is edaphically
408 restricted to ultramafic soils and a phylogenetic proponency for the Malpighiales. In these respects,
409 Ni hyperaccumulation is therefore a distinct phenomenon from Mn hyperaccumulation.

410

411 This study led to the recording of 51 hyperaccumulator species and hence added significantly to the
412 global inventory of Mn hyperaccumulator plants (only 14 Mn hyperaccumulator species were
413 previously known from Sabah). These Mn hyperaccumulator species are phylogenetically scattered
414 and occur, amongst others, in the Celastraceae, Clusiaceae, Euphorbiaceae, Meliaceae,

415 Phyllanthaceae, Salicaceae and Violaceae. Manganese is not specifically accumulated in plants
416 occurring on ultramafic soils, in stark contrast to Ni (and Co), although ultramafic soils in Sabah are
417 significantly higher in Mn than other ‘normal’ soils (van der Ent et al., 2017a). Given that Mn
418 hyperaccumulation is phylogenetically widely distributed, and not associated with ultramafic soils, it
419 is likely it is far more common than previously thought (Reeves 2006). There are at least two
420 mechanisms response for Mn hyperaccumulation: (i) rhizosphere acidification from the release of
421 carboxylic acids (*i.e.* citrate) by roots for P uptake which can induce Mn accumulation (Lambers et
422 al., 2015), as shown for *Lupinus albus* and *Phytolacca americana* (Jiang et al., 2015; DeGroote et al.,
423 2018), and (ii) resulting from the mineralogical changes of Mn oxides in soils during
424 oxidation/reduction cycles. Soil reduction results in dissolution of Mn oxides and increased
425 availability of Mn under the Mn²⁺ ion. This mechanism has been identified as a cause for Co
426 hyperaccumulation in Stagnosols by facultative Co hyperaccumulators (Co is extremely
427 ‘manganesophilic’ in soils) (Lange *et al.*, 2017).

428

429 This study also led to the recording of 12 Co hyperaccumulator species from three families and seven
430 genera. Seven Co hyperaccumulator species were already known previously (Reeves, 2006; van der
431 Ent et al., 2015b, 2018c, d), and those recorded in this study occur in the Meliaceae (genus *Walsura*),
432 Phyllanthaceae, and Violaceae (genus *Rinorea*). The highest Co concentration was found in *Ashtonia*
433 *excelsa* with 1500 µg g⁻¹. The results of this study confirmed that Co hyperaccumulation is relatively
434 rare, and that some taxa in the Phyllanthaceae (*e.g.* *Baccaurea membranacea*) can accumulate both
435 Co and Ni at concomitantly high concentrations.

436

437 Finally, the outcomes of this research demonstrate that handheld XRF is highly useful approach for
438 hyperaccumulator plant discovery in herbarium collections that has the potential to add vast numbers
439 of hyperaccumulating taxa to the global inventory. This research represents the largest single
440 contribution to the global knowledgebase on hyperaccumulator plants, and includes a substantial
441 expansion in phylogenetic records, many of the recorded genera being previously unknown to contain
442 hyperaccumulator plants. This reiterates the importance of systematic screening across different
443 families, as undertaken in this study, to avoid a bias. The current main limitation is the lack of
444 sensitivity for transition elements present at concentrations < 300 µg g⁻¹ and for light elements
445 (elements with a Z <20 such as Al). However, technological advances, including monochromatically
446 excited XRF, will enable higher sensitivity for most elements of the Periodic Table. Newly discovered
447 hyperaccumulators may eventually be used for genetic and ecophysiological experiments under
448 controlled conditions to advance our fundamental understanding of trace element hyperaccumulation
449 (Gei *et al.*, 2018; Nkrumah *et al.*, 2018a). The discovery of hypernickelophores (>1 Wt% Ni) is

450 promising for future advances in the development of Ni agromining. The two species of *Xylosma*
451 discovered in this study are fast growing trees and might prove as useful as other species that are
452 currently being used in agromining in Sabah, such as *Phyllanthus rufuschaneyi* (Bouman *et al.*, 2018).

453

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455

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464

465 **REFERENCES**

466

467 Baker, A.J.M., and R.R. Brooks, 1989. Terrestrial Higher Plants Which Hyperaccumulate Metallic
468 Elements, *Biorecovery*, 81–126.

469

470 Baker, A.J.M., Proctor, J., van Balgooy, M.M.J. et al., 1992. Hyper accumulation of nickel by the
471 flora of the ultramafics of Palawan, Republic of the Philippines, The vegetation of ultramafic
472 serpentine. *First International Conference on serpentine ecology*, 291–304.

473

474 Barthlott, W., Hostert, A., Kier, G. et al., 2007. Geographic patterns of vascular plant diversity at
475 continental to global scales. *Erdkunde* 614, 305–315.

476

477 Berry, P.E., Van Ee, B., Kainulainen, K. et al., 2016b. *Croton cupreolepis* Euphorbiaceae, a new
478 coppery-lepidote tree species from eastern Madagascar. *Systematic Botany* 41, 977–982.

479

480 Bouman, R., van Welzen, P., Sumail, S. et al., 2018. *Phyllanthus rufuschaneyi*: a new nickel
481 hyperaccumulator from Sabah with potential for tropical phytomining. *Botanical Studies* 59, 9.

482

483 Brady, K.U., Kruckeberg, A.R. and Bradshaw, H.D. Jr. 2005. Evolutionary ecology of plant
484 adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics*, 243–266.

485 Brooks, R.R., Lee, J., Reeves, R.D. et al., 1977. Detection of nickeliferous rocks by analysis of
486 herbarium specimens of indicator plants. *Journal of Geochemical Exploration* 7, 49–57.
487

488 Brooks RR, Naidu SM, Malaisse F, Lee J. 1986. The elemental content of metallophytes from the
489 copper/cobalt deposits of Central Africa. *Bulletin de la Société Royale de Botanique de Belgique T.*
490 119, Fasc. 2, 179-191.
491

492 DeGroot, K.V., Pollard, A.J., McCartha, G.L., 2018. Interactions of the manganese
493 hyperaccumulator *Phytolacca americana* L. with soil pH and phosphate. *Ecological Research* 33 4,
494 1–7.
495

496 Fernando DR, Guymer G, Reeves RD, Woodrow IE, Baker AJ, Batianoff GN. 2009. Foliar Mn
497 accumulation in eastern Australian herbarium specimens: prospecting for ‘new’ Mn
498 hyperaccumulators and potential applications in taxonomy. *Annals of Botany* 103: 931–939.
499

500 Galey, M.C., van der Ent, A., Iqbal, M.C.M. et al., 2017. Ultramafic geoecology of South and
501 Southeast Asia. *Botanical Studies* 58:18.
502

503 Gei, V., Erskine, P.D., Echevarria, G. et al. 2017. A systematic assessment of the occurrence of
504 trace element hyperaccumulation in the flora of New Caledonia. *New Phytologist*. Submitted.
505

506 Gei, V., Erskine, P.D., Harris, H.H. et al., 2018. Tools for the Discovery of Hyperaccumulator Plant
507 Species and Understanding Their Ecophysiology in: Reeves, R.D., van der Ent, A., Baker, A.J.M.
508 *Agromining: Farming for Metals, Mineral Resource Reviews*, 1-312.
509

510 Isnard S, L’Huillier L, Rigault F, Jaffré T. 2016. How did the ultramafic soils shape the flora of the
511 New Caledonian hotspot? *Plant and Soil* 403: 53–76.
512

513 Jaffré T, Schmid M. 1974. Accumulation du nickel par une Rubiacée de Nouvelle Calédonie,
514 *Psychotria douarrei* (G. Beauvisage) Däniker. *C R Acad Sci Paris D* 278:1727– 1730.
515

516 Jaffré, T., Brooks, R.R., Lee, J., Reeves, R.D., 1976. *Sebertia acuminata*: A Hyperaccumulator of
517 Nickel from New Caledonia, *Science*: 193, 579–580.
518

519 Jaffré, T., Pillon, Y., Thomine, S. et al., 2013. The metal hyperaccumulators from New Caledonia
520 can broaden our understanding of nickel accumulation in plants. *Frontiers in Plant Science* 4: 279.
521

522 Jiang, C.A., Wu, Q.T., Goudon, R. al., 2015. Biomass and metal yield of co-cropped *Alyssum*
523 *murale* and *Lupinus albus*. *Australian Journal of Botany*: 63, 159–166.
524

525 Kersten, W., Brooks, R., Reeves, R., et al., 1979. Nickel uptake by New Caledonian species of
526 *Phyllanthus*. *Taxon* 28: 529–534.
527

528 Korotkova, N., Schneider, J. V., Quandt, D., et al., 2009. Phylogeny of the eudicot order
529 Malpighiales: Analysis of a recalcitrant clade with sequences of the petd group II intron. *Plant*
530 *Systematics and Evolution* 282, 201–228.
531

532 Lambers, H., Haye, P.E., Laliberté, E. et al., 2015. Leaf manganese accumulation and phosphorus–
533 acquisition efficiency. *Trends in Plant Science*: 20, 1360–1385.
534

535 Lange, B., van der Ent, A., Baker et al., 2017. Copper and cobalt accumulation in plants: a critical
536 assessment of the current status of knowledge. *New Phytologist*: 213 2., 537–551.
537

538 Markowicz, A.A., 2008. Quantification and correction procedures. In: Potts PJ, West M eds.
539 *Portable X-ray fluorescence spectrometry capabilities for in situ analysis*. Royal Society of
540 *Chemistry, Cambridge*.
541

542 Nkrumah, P., Echevarria, G., Erskine, P.D. et al., 2018a. The discovery of nickel hyper-
543 accumulation in *Antidesma montis-silam*: from herbarium identification to field re-discovery.
544 *Ecological Research* 33:3, 675–685.
545

546 Nkrumah, P., van der Ent, A., Echevarria, G. et al., 2018b. Contrasting nickel and zinc
547 hyperaccumulation in subspecies of *Dichapetalum gelonioides* from Southeast Asia. *Scientific*
548 *Reports*, 8, 9659
549

550 Nkrumah, P.N., Chaney, R.L., Morel, J.L., 2018. Agronomy of ‘Metal Crops’ Used in Agromining
551 in: Reeves, R.D., van der Ent, A., Baker, A.J.M. *Agromining: Farming for Metals, Mineral*
552 *Resource Reviews*.
553

554 Pollard, A.J., Reeves, R.D. and A.J.M. Baker, 2014. Facultative hyperaccumulation of heavy metals
555 and metalloids. *Plant Science* 217–218, 8–17.
556

557 Proctor, J., 2003. Vegetation and soil and plant chemistry on ultramafic rocks in the tropical Far
558 East. *Perspectives in Plant Ecology Evolution and Systematics* 61–2, 105–124.
559

560 Proctor, J., Phillipps, C., Duff, G.K., Heaney, A., Robertson, F.M. 1989. Ecological studies on
561 Gunung Silam, a small ultrabasic mountain in Sabah, Malaysia. II. Some forest processes. *Journal*
562 *of Ecology* 77, 317–331.
563

564 Reeves, R.D., 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction.
565 *Plant and Soil*, 57–65
566

567 Reeves, R.D., 2006. Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G,
568 Goncharova N Eds. *Phytoremediation of Metal–Contaminated Soils*, NATO Science Series IV.:
569 *Earth and Environmental Sciences*. *Springer*, Dordrecht, The Netherlands 68, 25–52.
570

571 Reeves RD, Baker AJM, Borhidi A, Berazaín R. 1999. Nickel hyperaccumulation in the serpentine
572 flora of Cuba. *Annals of Botany* 83: 1–10.
573

574 Reeves RD, Baker AJM, Becquer T, Echevarria G, Miranda ZJG. 2007. The flora and
575 biogeochemistry of the ultramafic soils of Goiás state, Brazil. *Plant and Soil* 293: 107–119.
576

577 Reeves, R.D., Baker, A.J.M, Jaffré, T. et al., 2018. A global database for hyperaccumulator plants
578 of metal and metalloid trace elements. *New Phytologist* 218, 407–411.
579

580 Repin, R. 1998. Serpentine ecology in Sabah, Malaysia. *Sabah Parks Journal* 1, 19–28.
581

582 van der Ent, A., Baker, A.J.M., Reeves, R.D. et al., 2013a. Hyperaccumulators of metal and
583 metalloid trace elements: facts and fiction. *Plant and Soil* 362, 319–334.
584

585 van der Ent, A., Baker, A. J. M., van Balgooy, M. M. J. et al., 2013b.. Ultramafic nickel laterites in
586 Indonesia Sulawesi, Halmahera.: Mining, nickel hyperaccumulators and opportunities for
587 phytomining. *Journal of Geochemical Exploration* 128, 72–79.
588

589 van der Ent, A., Baker, A. J. M., Reeves, R.D. et al., 2015a. Agromining: farming for metals in the
590 future? *Environmental Science and Technology* 49, 4773–4780.
591
592 van der Ent, A., Erskine, P.D., Sumail, S., 2015b. Ecology of nickel hyperaccumulator plants from
593 ultramafic soils in Sabah Malaysia. *Chemoecology* 255, 243–259.
594
595 van der Ent, A., Mulligan, D., 2015c. Multi-element concentrations in plant parts and fluids of
596 Malaysian nickel hyperaccumulator plants and some economic and ecological considerations.
597 *Journal of Chemical Ecology* 41, 396–408.
598
599 van der Ent, A., Echevarria, G., Tibbett, M., 2016a. Delimiting soil chemistry thresholds for nickel
600 hyperaccumulator plants in Sabah Malaysia. *Chemoecology* 26:2, 67–82.
601
602 van der Ent, A., Erskine, P.D., Mulligan, D.R. et al., 2016b. Vegetation on ultramafic edaphic
603 islands in Kinabalu Park Sabah, Malaysia. in relation to soil chemistry and altitude. *Plant and Soil*,
604 403, 77–101.
605
606 van der Ent, A., van Balgooy, M., van Welzen, P. 2016c. *Actephila alanbakeri* Phyllantaceae.: a
607 new nickel hyperaccumulating plant species from localised ultramafic outcrops in Sabah Malaysia.
608 *Botanical Studies*: 57, 6.
609
610 van der Ent, A., Callahan, D.L., Noller, B.N. et al, 2017a. Nickel biopathways in tropical nickel
611 hyperaccumulating trees from Sabah Malaysia. *Scientific Reports* 7:41861.
612
613 van der Ent, A., Przybyłowicz, W.J., de Jonge, M.D. et al., 2017b. X-ray elemental mapping
614 techniques for elucidating the ecophysiology of hyperaccumulator plants. *New Phytologist*, 1–24.
615
616 van der Ent, A., Cardace, D., Tibbett, M. et al., 2018a. Ecological implications of pedogenesis and
617 geochemistry of ultramafic soils in Kinabalu Park Malaysia. *Catena* 160, 154–169.
618
619 van der Ent, A., Echevarria, G., Pollard, A.J., Erskine, P.D., 2018b. X-Ray Fluorescence Ionomics
620 of Herbarium Collections. *Scientific Reports*. In Press.
621

622 van der Ent, A., Mark, R., de Jonge, M.D. et al., 2018c. Simultaneous hyperaccumulation of nickel
623 and cobalt in *Glochidion cf. sericeum* Phyllanthaceae: elemental distribution and speciation.
624 Scientific Reports 8, 9683.
625
626 van der Ent, A., Mulligan, D.R., Repin, R. et al., 2018d. Foliar elemental profiles in the ultramafic
627 flora of Kinabalu Park Sabah, Malaysia. Ecological Research 33:3, 659–674.
628
629 Wurdack, K.J., Davis, C.C., 2009. Malpighiales phylogenetics: Gaining ground on one of the most
630 recalcitrant clades in the angiosperm tree of life. American Journal of Botany 96, 1551–1570.
631
632 Yang X, Brooks R, Jaffré T, Lee J. 1985. Elemental levels and relationships in the Flacourtiaceae of
633 New Caledonia and their significance for the evaluation of the ‘serpentine problem’. Plant and Soil
634 87: 281–291.

TABLE CAPTIONS

Table 1. Co, Mn and Ni hyperaccumulator genera from the Malpighiales. The number of specimens, ranges and means above the hyperaccumulator threshold are shown. "-" is below the element-specific hyperaccumulation threshold ($>300 \mu\text{g g}^{-1}$ Co, $>1000 \mu\text{g g}^{-1}$ Ni, $>10\,000 \mu\text{g g}^{-1}$ Mn). Notes: ¹number of specimens from unidentified species from this study between brackets. ²number of hyperaccumulator specimens from this study between parentheses. ³number of hyperaccumulator specimens from unidentified species from this study between brackets.

Table 2. Co, Mn and Ni hyperaccumulator genera from Orders (excluding Malpighiales). With the number of specimens and foliar ranges above the hyperaccumulator threshold. "-" is below the element-specific hyperaccumulation threshold ($>300 \mu\text{g g}^{-1}$ Co, $>1000 \mu\text{g g}^{-1}$ Ni, $>10\,000 \mu\text{g g}^{-1}$ Mn). Notes: ¹number of hyperaccumulator specimens from this study between parentheses. ²number of hyperaccumulator specimens from unidentified species from this study between brackets.

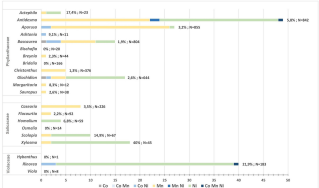
Table 3. Families and selected genera found on ultramafic and non-ultramafic substrates showing the corrected XRF values of their ranges, mean values, the total number of specimens and t-test (*p*-value at 0.05). "-" is below the element-specific LOD ($<190 \mu\text{g g}^{-1}$ Ni, $<140 \mu\text{g g}^{-1}$, $<336 \mu\text{g g}^{-1}$ Mn). *values from all the genera in the family.

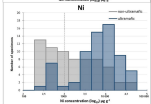
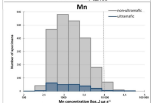
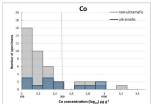
FIGURE CAPTIONS

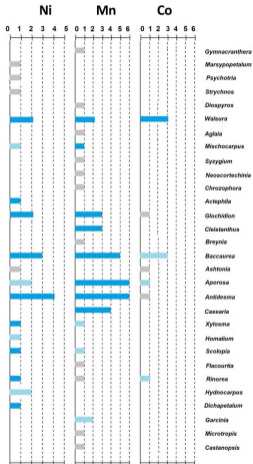
Figure 1. Bar graphs showing a breakdown of combinations of Ni, Co and Mn hyperaccumulation in the Phyllanthaceae, Salicaceae and Violaceae genera. Shown are the number of specimens and percentages and non-hyperaccumulator number of specimens measured by XRF.

Figure 2. Frequency distribution of Ni, Co and Mn elemental concentrations in herbarium specimens originating from ultramafic (shaded blue) and non-ultramafic soils (shaded grey). Concentration scales (X-axis) \log_{10} transformed and actual values in $\mu\text{g g}^{-1}$. Numbers of specimens on Y-axis. Dotted line denotes the element-specific hyperaccumulation threshold ($>300 \mu\text{g g}^{-1}$ Co, $>1000 \mu\text{g g}^{-1}$ Ni, $>10\,000 \mu\text{g g}^{-1}$ Mn).

Figure 3. Phylogenetic tree of Ni, Co and Mn hyperaccumulator taxa in the flora of Sabah. The tree was constructed using PHYLOGENETIC (Phylomatic tree R20120829 Plants) and shows orders, clades, families and genera with hyperaccumulators.







Number of hyperaccumulator taxa

N=1
 1 < N < 5
 N > 5

TABLE 1

Order	Family	Genera	Number of specimens/ number of taxa ¹	Range of foliar concentrations ($\mu\text{g g}^{-1}$)			Mean of foliar concentrations ($\mu\text{g g}^{-1}$) ²			Total number of hyperaccumulator species ³		
				Co	Mn	Ni	Co	Mn	Ni	Co	Mn	Ni
Malpighiales	Achariaceae	<i>Hydnocarpus</i>	18/10	–	–	3000–3200	–	–	3100 (3)	–	–	[3]
	Dichapetalaceae	<i>Dichapetalum</i>	98/5 [2]	–	–	1000–45 600	–	–	15 900 (20)	–	–	1
	Phyllanthaceae	<i>Actephila</i>	23/2 [5]	–	–	2600–14 600	–	12 800 (1)	7500 (3)	–	[1]	1
		<i>Antidesma</i>	842/23 [98]	–	10 100–46 500	1000–32 700	560 (1)	14 600 (25)	9500 (27)	1	8 [2]	5 [2]
		<i>Aporosa</i>	855/34 [74]	500–980	10 300–26 700	4000–10 400	740 (2)	14 300 (24)	6500 (3)	1 [1]	6 [1]	2 [1]
		<i>Ashtonia</i>	11/1	–	–	–	1500 (1)	–	8100 (1)	1	–	1
		<i>Baccaurea</i>	804/29 [44]	320–960	10 200–21 700	1000–8000	690 (4)	14 000 (7)	4400 (7)	3	5	3
		<i>Cleistanthus</i>	376/31 [1]	–	10 400–11 300	–	–	10 700 (5)	–	–	3	–
		<i>Glochidion</i>	644/30 [126]	590–780	10 200–12 600	1900–11 600	690 (2)	11 200 (3)	7100 (13)	1 [1]	3	1 [12]
		<i>Casearia</i>	226/12 [47]	–	10 100–38 200	–	–	14 200 (8)	–	–	5 [3]	–
	Salicaceae	<i>Homalium</i>	59/3 [17]	–	–	1400–5700	–	–	2700 (4)	–	–	[4]
		<i>Scolopia</i>	67/3 [13]	–	14 800–16 600	1200–9200	–	15 700 (2)	4700 (8)	–	[2]	[8]
		<i>Xylosma</i>	45/3 [13]	–	13 600–15 400	1300–23 000	–	14 500 (2)	7800 (16)	–	[2]	[16]
	Violaceae	<i>Rinorea</i>	188/12 [9]	300–620	–	1100–32 200	470 (4)	13 400 (1)	8500 (40)	1	1	1 [1]

TABLE 2

Clades	Order	Family	Genera	Number of specimens/ number of taxa	Range foliar concentrations ($\mu\text{g g}^{-1}$)			Mean foliar concentrations ($\mu\text{g g}^{-1}$) ¹			Number of hyperaccumulator species ²		
					Co	Mn	Ni	Co	Mn	Ni	Co	Mn	Ni
Asterids	Ericales	Ebenaceae	<i>Diospyros</i>	27/26	–	–	–	–	19 700 (1)	–	–	1	–
	Gentianales	Rubiaceae	<i>Psychotria</i>	7/7	–	–	–	–	–	20 700 (1)	–	–	1
Rosids	Fagales	Fagaceae	<i>Castanopsis</i>	3/3	–	–	–	–	18 200 (1)	–	–	1	–
	Magnoliales	Annonaceae	<i>Marsypopetalum</i>	1/1	–	–	–	–	–	6000 (1)	–	–	1
	Myrtales	Myrtaceae	<i>Syzygium</i>	33/33	–	–	–	–	28 800 (1)	–	–	1	–
	Sapindales	Meliaceae	<i>Walsura</i>	101/4	300–720	10 200–45 200	1300–8600	400 (9)	17 100 (23)	4500 (12)	1 [5]	1 [9]	1 [1]
		Sapindaceae	<i>Mischocarpus</i>	76/3	–	10 100–19 400	1200–2400	–	13 800 (6)	1900 (3)	–	1	1

TABLE 3

Order	Families	Genera	Co $\mu\text{g g}^{-1}$					Mn $\mu\text{g g}^{-1}$					Ni $\mu\text{g g}^{-1}$				
			Ultramafic		Non-ultramafic			Ultramafic		Non-ultramafic			Ultramafic		Non-ultramafic		
			Mean (n)	Range	Mean (n)	Range	<i>p</i> -value	Mean (n)	Range	Mean (n)	Range	<i>p</i> -value	Mean (n)	Range	Mean (n)	Range	<i>p</i> -value
Malpighiales	Dichapetalaceae	<i>Dichapetalum</i>	–	–	–	–	–	1100 (2)	400–1900	1300 (39)	300–4300	0.83	23 9 (8)	8000–45 600	10 600 (12)	1000–37 100	0.02
	Phyllanthaceae	*	450 (9)	100–980	240 (30)	100–1500	0.11	2500 (193)	400 – 46 500	2300 (2136)	300–21 700	0.97	7800 (36)	300–32 800	2500 (36)	300–32 600	0.01
		<i>Antidesma</i>	340(2)	100–560	150 (9)	100–300	0.73	3100 (67)	400–46 500	2700 (490)	300–17 400	0.97	9700 (21)	400–32 800	3600 (14)	300–32 600	0.01
		<i>Aporosa</i>	560 (2)	100–0.98	170 (5)	100–200	0.57	3400 (43)	400–26 600	2700 (647)	300–19 800	0.11	10 400 (1)	–	500 (3)	300–600	–
		<i>Baccaurea</i>	810 (2)	700–1000	240 (8)	100–800	0.22	1800 (20)	400–11 600	1800 (279)	300–21 700	0.89	4000 (6)	400–8000	1000 (11)	300–5900	0.08
	Salicaceae	*	–	–	–	–	–	2800 (51)	400–38 200	2400 (273)	300–16 600	0.02	4000 (29)	200–2300	3400 (6)	400–10 400	0.98
	Violaceae	<i>Rinorea</i>	200 (4)	130–300	260 (10)	100–600	0.95	1700 (33)	400–6200	2100 (100)	300–13 400	0.01	10 700 (23)	500–32 200	3900 (20)	200–14 300	0.01
Sapindales	Meliaceae	<i>Walsura</i>	210 (3)	110–330	230 (34)	100–700	0.81	5400 (14)	400 – 15 300	7600 (81)	400–45 200	0.25	3900 (10)	300–8000	1300 (17)	300–8600	0.01
	Sapindaceae	<i>Mischocarpus</i>	–	–	–	–	–	4700 (7)	1700–8500	4200 (67)	400–19 400	0.28	23 900 (8)	8000–45 600	10 600 (12)	1000–37 100	0.02