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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  $\text{km}^2$ ) 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  $\mu$ 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 mm<sup>2</sup> 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  $\mu$ g g<sup>-1</sup> (but typically >300  $\mu$ g g<sup>-1</sup> 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% HNO<sub>3</sub>. 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<sup>-1</sup>) and Fe (>2500 µg g<sup>-1</sup>) 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 ( $\pm 3$  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 (Phylomatic 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 Mn<sup>2+</sup> 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<sup>-1</sup>. 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<sup>-1</sup> 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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464

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## 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: <sup>1</sup>number of specimens from unidentified species from this study between brackets. <sup>2</sup>number of hyperaccumulator specimens from this study between parentheses. <sup>3</sup>number 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: <sup>1</sup>number of hyperaccumulator specimens from this study between parentheses. <sup>2</sup>number of hyperaccumulator specimens from unidentified species from this study between brackets.

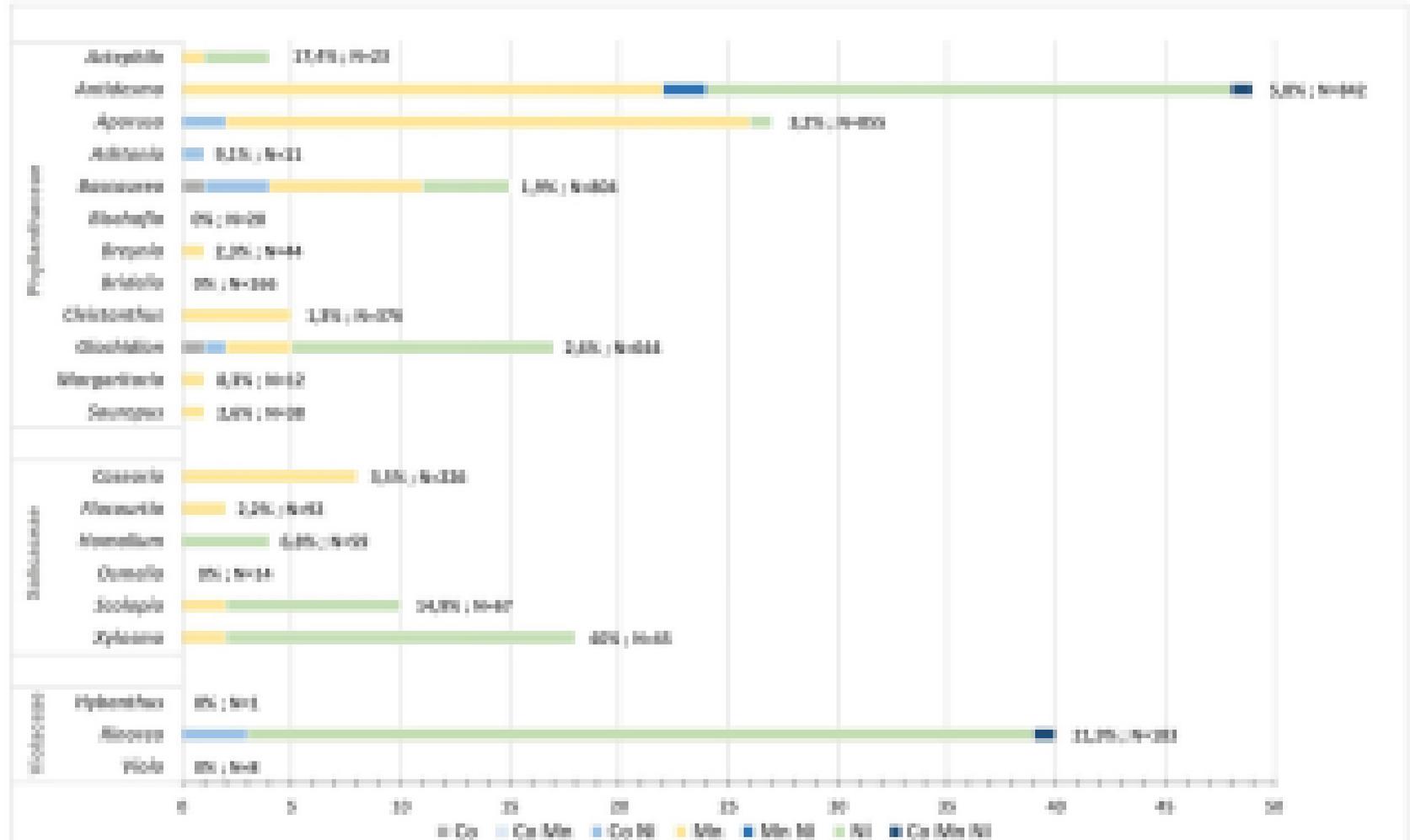
**Table 3.** Families and selected genera found on ultramafic and non-ultramafic substrates showing the corrected XRF values of their ranges, mean values, the total number of specimens and t-test (*p*-value at 0.05). "-" is below the element-specific LOD ( $<190 \mu\text{g g}^{-1}$  Ni,  $<140 \mu\text{g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

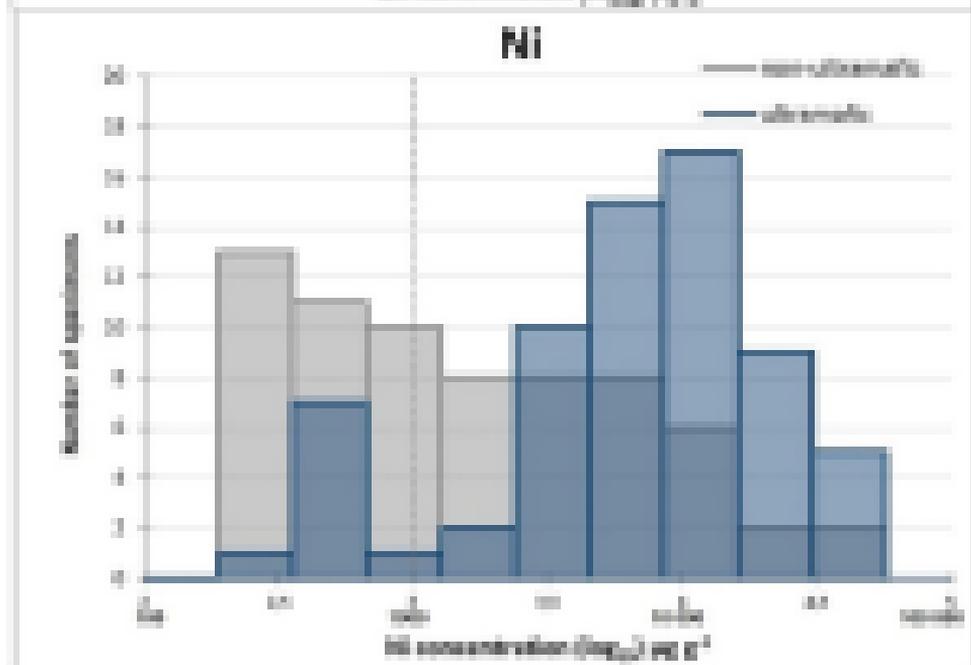
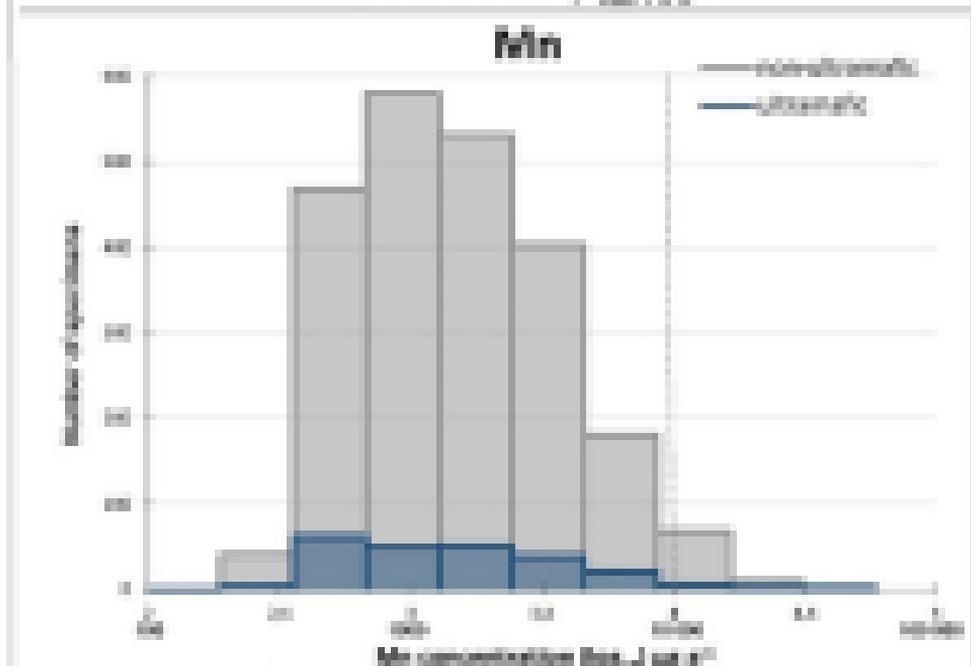
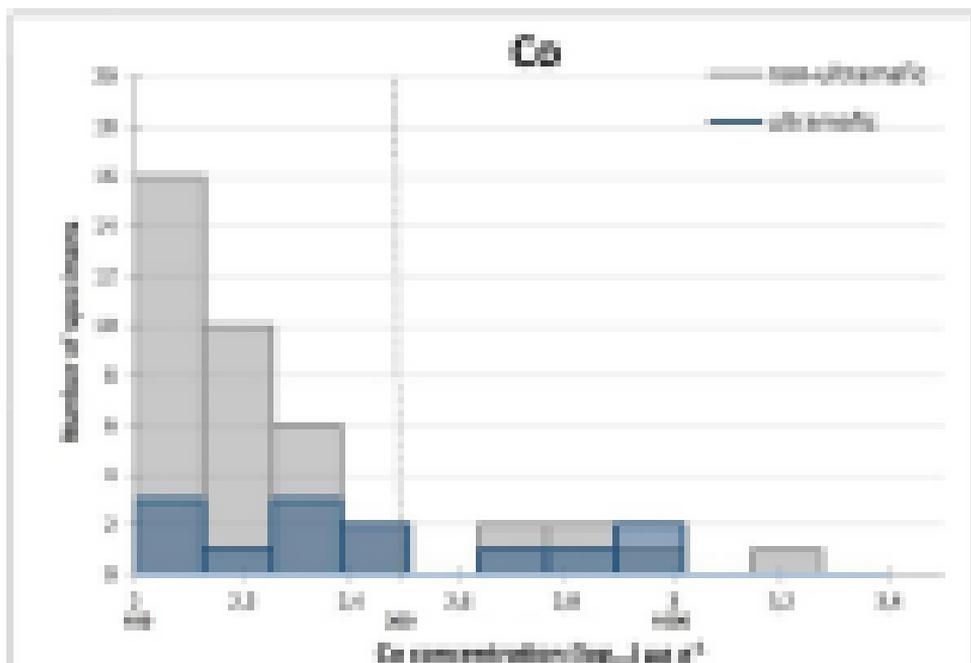
## FIGURE CAPTIONS

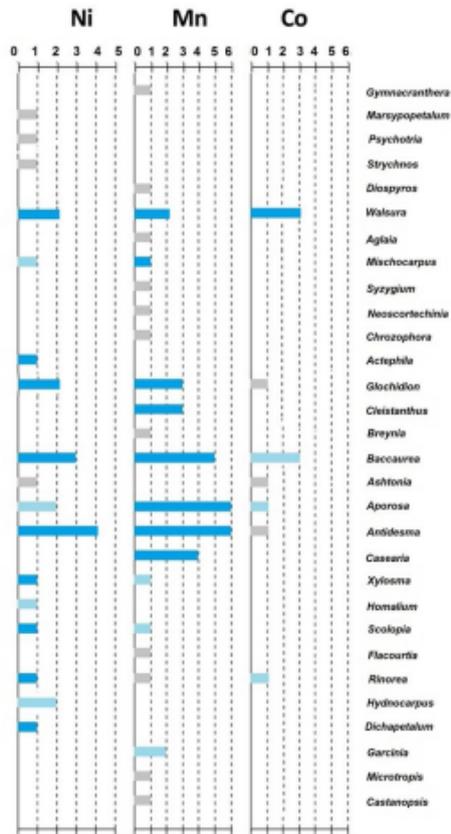
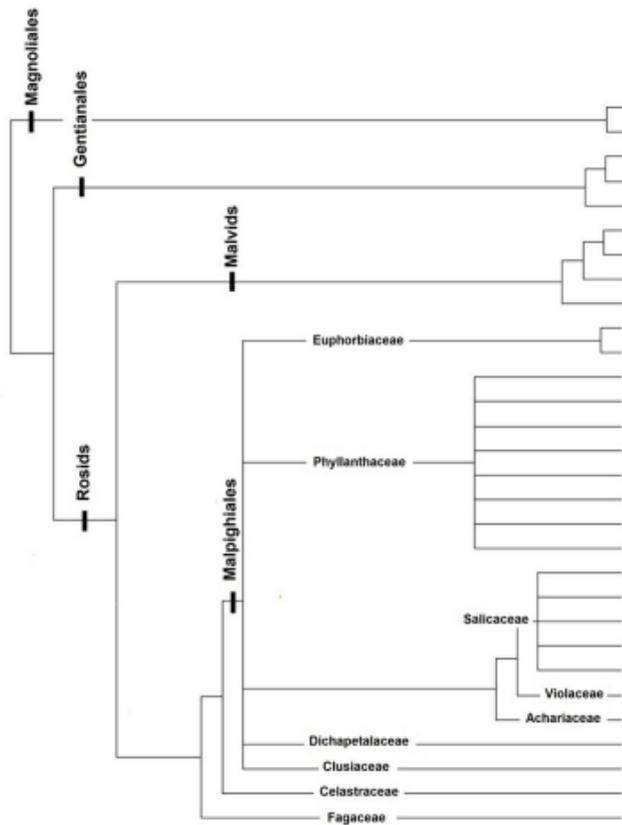
**Figure 1.** Bar graphs showing a breakdown of combinations of Ni, Co and Mn hyperaccumulation in the Phyllanthaceae, Salicaceae and Violaceae genera. Shown are the number of specimens and percentages and non-hyperaccumulator number of specimens measured by XRF.

**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 <sup>1</sup>	Range of foliar concentrations ( $\mu\text{g g}^{-1}$ )			Mean of foliar concentrations ( $\mu\text{g g}^{-1}$ ) <sup>2</sup>			Total number of hyperaccumulator species <sup>3</sup>		
				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 ( $\mu\text{g g}^{-1}$ ) <sup>1</sup>			Number of hyperaccumulator species <sup>2</sup>		
					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