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### ► To cite this version:

Bastien Lange, Antony van Der Ent, Alan John Martin Baker, Guillaume Echevarria, Grégory Mahy, et al.. Copper and cobalt accumulation in plants: a critical assessment of the current status of knowledge: A critical assessment of the current state of knowledge. *New Phytologist*, 2017, 213 (2), pp.537-551. 10.1111/nph.14175 . hal-02265598

**HAL Id: hal-02265598**

**<https://hal.science/hal-02265598>**

Submitted on 10 Aug 2019

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# **Copper and cobalt accumulation in plants: a critical assessment of the current status of knowledge**

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- Total word count: 3 967

- Number of words per section:

I. Introduction : 517

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- Number of Figures: 4 (Fig. 1 and Fig. 4 in color)

33 - Number of Tables: 2

34

35 **Summary**

36

37 This review synthesizes contemporary understanding of Cu-Co tolerance and accumulation in  
38 plants. Accumulation of foliar Cu and Co to  $>300 \mu\text{g g}^{-1}$  is exceptionally rare globally, and  
39 known principally from the Copperbelt of Central Africa. Cobalt accumulation is also  
40 observed in a limited number of Ni hyperaccumulator plants occurring on ultramafic soils  
41 around the world. None of the putative Cu or Co hyperaccumulator plants appears to comply  
42 with the fundamental principle of hyperaccumulation as foliar Cu-Co accumulation is strongly  
43 dose-dependent. Abnormally high plant-tissue Cu concentration occurs only when plants are  
44 exposed to high soil Cu concentrations with a low shoot translocation factor. Most Cu tolerant  
45 plants are Excluders *sensu* Baker and therefore setting threshold values for Cu  
46 hyperaccumulation is not informative. Abnormal accumulation of Co occurs under similar  
47 circumstances in the Copperbelt of the DR Congo, however, Co tolerant plants behave  
48 physiologically as Indicators *sensu* Baker and sporadically coincides with Ni  
49 hyperaccumulation on ultramafic soils. Practical application of Cu-Co accumulator plants in  
50 phytomining is limited due to their dose-dependent accumulation characteristics, although for  
51 Co trials may be warranted on highly Co-contaminated minerals wastes because it its high  
52 metal value.

53

54 **Keywords** Accumulation, Cobalt, Copper, Hyperaccumulation, Metallophyte, Metal-  
55 tolerance, Phytomining, Toxicity.

## 56 **Introduction**

57

58 Plant species that can grow and reproduce on soil strongly enriched in trace elements (TE),  
59 *i.e.* metalliferous soils of natural or anthropogenic origin, have proved to be powerful model  
60 systems for studying the ecophysiology and evolution of adaptation to toxicity stresses  
61 (Baker, 1987). Metallophytes are species able to tolerate concentrations of such TE (*e.g.* Ni,  
62 Cu or Co) in soil that are toxic for most other species (Baker, 1981). These metallophytes can  
63 be restricted to metalliferous soils (*i.e.* obligate metallophytes) or occur also on ‘normal’ soils  
64 (*i.e.* facultative metallophytes) (Pollard *et al.*, 2014). Among these, some have the ability to  
65 actively accumulate certain TE in leaves, from either low or high available concentrations in  
66 soil without toxicity symptoms or growth inhibition, and are known as hyperaccumulator  
67 plants (Jaffré *et al.*, 1976; van der Ent *et al.*, 2013).

68

69 By far the greatest number of hyperaccumulators are known for Ni (>1000  $\mu\text{g g}^{-1}$  foliar Ni)  
70 and found on ultramafic soils (Reeves, 2003). (Hyper)accumulation of Cu and Co was first  
71 defined as >1000  $\mu\text{g g}^{-1}$  foliar Cu/Co (Malaisse *et al.*, 1978), but later revised downwards to  
72 >300  $\mu\text{g g}^{-1}$  (van der Ent *et al.*, 2013). More than 95% of the putative Cu-Co  
73 hyperaccumulators are found in the southeastern Democratic Republic of Congo (D. R.  
74 Congo) (see Brooks *et al.*, 1980), where there are a large number of natural Cu-Co outcrops;  
75 the so-called ‘Copper Hills’ of the ‘Katangan Copperbelt’. However, putative Cu-Co  
76 hyperaccumulators have also been reported from other countries on ultramafic soils enriched  
77 with Cu and Co. An overview of putative Cu and/or Co hyperaccumulator plants is provided  
78 in Fig. 1. Apart from the Katangan Copperbelt, high Co concentrations are usually found in  
79 ultramafic soils worldwide. Copper can also be present at high concentrations in some  
80 ultramafic soils. Putative Co hyperaccumulation on ultramafic soils has been reported for  
81 some individuals of two Ni-hyperaccumulating *Rinorea* species (Violaceae) (Brooks *et al.*,  
82 1977). A few cases of putative Cu hyperaccumulation have also been reported on Cu-enriched  
83 ultramafic soils in Sri Lanka, Brazil and Malaysia (Rajakaruna & Bohm, 2002; van der Ent &  
84 Reeves, 2015). Concluding, putative Cu and/or Co hyperaccumulation may occur in: Cu-Co  
85 outcrops (principally the Katangan Copperbelt), Cu-Co-enriched ultramafic soils (*e.g.* Goiás  
86 state, Brazil) and Co-enriched ultramafic soils (*e.g.* Barberton, South Africa).

87

88 High inter- and intra-specific variations in foliar/shoot Cu and/or Co have been reported  
89 among Cu-Co accumulators (Faucon *et al.*, 2009; Lange *et al.*, 2014). Some of the early

90 reports of Cu-Co (hyper)accumulation have been based upon analysis of herbarium materials  
91 (see *e.g.* Baker & Brooks, 1989; Reeves & Baker, 2000) and there is now substantial  
92 experimental data to suggest that many of these samples were contaminated with soil dust, so  
93 giving spurious high values to foliar dry matter analyses (Faucon *et al.*, 2007).

94

95 This review synthesizes recent advances and contemporary research on the understanding of  
96 Cu-Co tolerance and (hyper)accumulation, by (i) reviewing current state of knowledge about  
97 Cu-Co tolerance and (hyper)accumulation by physiological, ecological and biogeochemical  
98 approaches; (ii) presenting an updated global record of unusually high Cu-Co accumulation in  
99 plant species; (iii) evaluating possible applications for Cu-Co accumulating plants.

100

## 101 **Ecophysiology for tolerance and accumulation of Cu and Co**

102

### 103 *Strategies of tolerance*

104 Plants that can grow and reproduce on metalliferous soils can be classified based upon their  
105 TE uptake response when exposed to increasing soil concentrations of the element under  
106 consideration (Antonovics *et al.*, 1971). The three main strategies of tolerance are: ‘Excluder’,  
107 ‘Bioindicator’ and ‘Hyperaccumulator’ (Baker, 1981). Tolerance to certain TE and their  
108 accumulation patterns have been widely studied over the past decades, especially for Zn, Ni  
109 and Cd (Verbruggen *et al.*, 2009). Copper- and Co-tolerant populations have been reported in  
110 the literature for a large number of species collected from nature, but the capacity to grow  
111 under elevated Cu and/or Co concentrations without toxicity symptoms or growth inhibition  
112 has rarely been demonstrated experimentally (Table 1).

113

114 Most Cu tolerant plants respond ecophysiologicaly as Excluders *sensu* Baker with a very  
115 limited Cu translocation to the leaves (Macnair, 1981; Schat *et al.*, 1993; Song *et al.*, 2004;  
116 Chipeng *et al.*, 2010; Lange *et al.*, 2016). *Crassula helmsii* could accumulate more 9000 µg g<sup>-1</sup>  
117 <sup>1</sup> in its shoot at low Cu concentration in the nutrient solution would be the exception of Cu  
118 (hyper)accumulation (Küpper *et al.*, 2009). Cobalt accumulation exists in *Anisopappus*  
119 *chinensis* as foliar Co concentration increases in relation to Co soil concentration without a  
120 decrease of biomass or toxicity symptoms (Lange, 2016). None of the putative Cu or Co  
121 hyperaccumulator plants, however, appear to comply with the fundamental principle of  
122 hyperaccumulation that high shoot concentrations are maintained over a wide range, from low  
123 to high, of Cu or Co in the soil or growth medium (Van der Ent *et al.*, 2013).

124

125 *Physiology of Cu tolerance and accumulation*

126 Copper is an essential transition metal with two oxidation states under physiologically  
127 relevant conditions. Due to its ability to cycle between the oxidized Cu(II) and reduced Cu(I)  
128 states, it is involved in biological processes such as photosynthesis, respiration, oxygen  
129 superoxide scavenging, ethylene sensing, cell wall metabolism and lignification (Burkhead *et*  
130 *al.*, 2009). For the very reason that it is essential, Cu can also be highly toxic (Fernandes &  
131 Henriques, 1991). Free Cu catalyzes Fenton reactions that generate hydroxyl radicals causing  
132 damage to lipids, proteins and DNA (Cohu & Pilon, 2010). Copper has also been reported to  
133 interfere with iron homeostasis (Bernal *et al.*, 2012). An overall reduction of plant biomass,  
134 inhibition of root growth, chlorosis, bronzing and necrosis are the usual reported symptoms of  
135 a Cu excess due to increased production of Reactive Oxygen Species and harmful interactions  
136 at the cellular level.

137

138 Copper tolerance in metallophytes is thought to arise through adaptation of components of  
139 basic metallic homeostasis. Cellular Cu levels are controlled by interplay between the ATPase  
140 exporters and the Ctr family of Cu permeases (Leary & Winge, 2007). Most Cu-ions in cells  
141 are either compartmentalized or bound to proteins or metabolites. Copper is delivered to  
142 enzymes by specific chaperones while excess can be chelated by thiol-rich compounds such  
143 as metallothioneins which are low molecular weight cysteine-rich proteins, or phytochelatins,  
144 which are synthesized from glutathione. Long distance transport of Cu apparently involves  
145 nicotianamine, a ubiquitous metal chelator in higher plants, synthesized from three molecules  
146 of S-adenosyl-methionine (Zheng *et al.*, 2012).

147

148 As previously stated, most of Cu-tolerant plants behave as Excluders. The genetic control of  
149 Cu tolerance in *S. vulgaris* and *M. guttatus* seems to be governed by a single major gene while  
150 tolerance level seems to be controlled by a number of modifiers (Macnair, 1983; Schat & Ten  
151 Bookum, 1992). Major determinants of Cu tolerance in Cu-tolerant plants are not known or  
152 genetically confirmed. In *S. vulgaris*, Cu tolerance is associated with efficient ATP-dependent  
153 Cu efflux through the root plasma membrane (van Hoof *et al.*, 2001a). In *Arabidopsis*  
154 *thaliana*, which is sensitive to Cu, *HMA5* (HEAVY METAL ATPase 5) was shown to co-  
155 localize with a major QTL explaining 52% of the difference in Cu tolerance between two  
156 ecotypes (Kobayashi *et al.*, 2008). *HMA5* is a Cu ATPase with a role in Cu compartmentation  
157 and detoxification in roots by moving Cu from the symplasm to apoplasm (Andrés-Colás *et*

158 *al.*, 2006; del Pozo *et al.*, 2010). *HMA5* activity could correspond to the ATP-dependent Cu  
159 efflux highlighted in the study of van Hoof *et al.* (2001a) with *S. vulgaris*. Some authors have  
160 suggested a role for *HMA5* in xylem loading (Kobayashi *et al.*, 2008), which has been  
161 debatable since this transport was mostly maintained in the *HMA5* loss of function  
162 *Arabidopsis* mutant (Andrés-Colás *et al.*, 2006). Enhanced tolerance in *S. vulgaris* has also  
163 been associated with increased transcript levels of metallothionein 2B (van Hoof *et al.*,  
164 2001b).

165

166 Furthermore, Cu tolerance was found to be inversely correlated with long-term K<sup>+</sup> efflux in  
167 several species, sensitive or tolerant, including *M. guttatus* (Strange & Macnair, 1991;  
168 Murphy *et al.*, 1999). K<sup>+</sup> serves as a counter-ion for citrate that is released upon exposure to  
169 Cu excess, thus reducing Cu availability in the medium (Murphy *et al.*, 1999).

170

171 No molecular mechanism of (hyper)accumulation has been described yet in any of putative  
172 Cu hyperaccumulator plants. Similar to the hyperaccumulation of other TE, root sequestration  
173 is limited to allow mobility of Cu to the shoot. Due to its toxicity, Cu excess is released in  
174 shoot and immobilized by efficient chelation and sequestration mechanisms. In the aquatic  
175 plant *C. helmsii*, Cu was found to be almost exclusively bound by oxygen ligands, likely  
176 organic acids, and not sulphur ligands (Küpper *et al.*, 2009). The concentration of organic  
177 acids in CAM plants may be so high that it is not limiting for Cu complexation. These authors  
178 suggested that main storage sites of Cu were vacuoles and cell walls. Similar investigations  
179 have not yet been undertaken on land C3 Cu accumulator plants.

180

### 181 *Physiology of Co tolerance and accumulation*

182 Cobalt is a transition metal not essential for plants with seven possible oxidation states. In  
183 physiological conditions, the oxidation states of Co are mainly II and III, which makes Co a  
184 possible catalyzer of Fenton reactions. Beneficial effects of Co supply have been associated  
185 with symbiotic rhizobia that inhabit in the nodules of leguminous plants, and need vitamin  
186 B<sub>12</sub> (cobalamin) for the activity of several enzymes involved in nitrogen fixation. Other  
187 reported beneficial effects include retardation of leaf senescence through inhibition of  
188 ethylene biosynthesis, and increased drought resistance in seeds (reviewed by Pilon-Smits *et*  
189 *al.*, 2009). Toxicity of Co excess is linked to oxidative stress, inhibition of photosynthesis and  
190 iron deficiency (Palit *et al.*, 1994; Morrissey *et al.*, 2009). Cobalt has been reported to disrupt  
191 iron homeostasis and compete with iron for access to transporters in many organisms

192 including plants (Barras & Fontecave, 2011). Cobalt can be transported into root epidermal  
193 cells of *A. thaliana* by IRT1 (Korshunova *et al.*, 1999). Once inside cells, ferroportins IRON  
194 REGULATED1 (IREG1/FPN1) and IREG2/FPN2 play a role in Co detoxification.  
195 IREG2/FPN2 can transport Co<sup>2+</sup> inside vacuoles of root epidermal and cortical cells thereby  
196 sequestering Co in the outer most layers of roots. Mobile Co can be loaded into the xylem by  
197 FPN1 and translocated to the shoot (Morrissey *et al.*, 2009). Additionally, it is believed that  
198 *HMA3*, which is mainly expressed in roots, also transports Co<sup>2+</sup> inside vacuoles in addition to  
199 other metallic ions (Cd<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>) (Morel *et al.*, 2009).

200

201 Mechanisms of Co tolerance and accumulation are still poorly understood. Since some of Co  
202 accumulators seem also to accumulate Cu (Faucon *et al.*, 2007), some tolerance and/or  
203 accumulation mechanisms are believed to be shared. Considering the targets of Co toxicity,  
204 plants that (hyper)accumulate Co have most probably evolved adjustments of Fe homeostatic  
205 mechanisms. Such adaptation has already been observed in Zn and Cd hyperaccumulators  
206 showing modified regulation of genes involved in iron homeostasis (Krämer, 2010;  
207 Hanikenne & Nouet, 2011; Shanmugam *et al.*, 2011). Another critical aspect of Co  
208 (hyper)accumulation is expected to be an efficient Co detoxification system to avoid the  
209 accumulation of free ions that can induce oxidative stress. Cellular Co tolerance of *C.*  
210 *cobalticola* was associated with an increase in citric acid as well as in cysteine, without any  
211 further increase in phytochelatin levels (Oven *et al.*, 2002).

212

## 213 **Functional and evolutionary ecology of Cu and Co accumulation**

214

### 215 *Trace element (hyper)accumulation as a functional trait*

216 Functional traits are defined as “morpho-physio-phenological traits which impact fitness  
217 indirectly via their effects on growth, reproduction and survival” (Violle *et al.*, 2007). Under  
218 that definition, TE concentrations in leaf tissues qualify as functional traits. Hypotheses  
219 related to the ecological significance of TE accumulation should be reformulated in the  
220 broader framework of functional plant ecology (Delhaye *et al.*, 2016). Due to trade-offs  
221 between different traits, some trait combinations are more likely to occur than others. The  
222 range of possible traits has become known as the ‘Leaf Economics Spectrum’ (Wright *et al.*,  
223 2004). How (hyper)accumulation relates to the Leaf Economics Spectrum is still an open  
224 question, but the concept is being extended to elements other than carbon and nitrogen (Reich,  
225 2014). Is (hyper)accumulation more likely to evolve in association with traits favouring fast

226 resource capture or with traits favouring nutrient conservation? Trade-offs between the  
227 capacity to accumulate TE and the uptake of major nutrients might strongly constrain the  
228 evolution of TE accumulation. Recent findings confirm that TE accumulation is correlated to  
229 the capture/use of major nutrients (Lambers *et al.*, 2015), thus supporting the so-called  
230 hypothesis of ‘inadvertent uptake’ (Boyd & Martens, 1992). However, this hypothesis should  
231 be confirmed contrary to the hypothesis that TE (hyper)accumulation may serve as a defense  
232 against herbivores and pathogens (demonstrated for Ni, Zn and Cd hyperaccumulation)  
233 (Boyd, 2013; Cappa & Pilon-Smits, 2014). Functional leaf and root traits involved in nutrient  
234 acquisition and conservation should be studied to understand Cu-Co accumulation.

235

### 236 *Evolution of trace element accumulation*

237 Does evolution of increased TE accumulation capacity involve changes in the niche  
238 parameters? Circumstantial evidence suggests that TE exclusion is more frequently observed  
239 in broad-niched metallophytes compared to narrow-niched (Delhaye *et al.*, 2016). This  
240 observation is thought to be the consequence of the cost of tolerance and/or  
241 (hyper)accumulation (Maestri *et al.*, 2010). Future work should test if evolutionary transitions  
242 to (hyper)accumulation are linked to changes in competitive ability and niche metrics.  
243 Another hypothesis posits that lower occurrence of pathogenic fungi and herbivores in Cu-  
244 enriched soils may conceivably relax selection pressure of defences in Cu-tolerant populations  
245 (Chipeng *et al.*, 2010). Copper-tolerant populations would then be more susceptible to  
246 pathogenic fungi compared to non-tolerant populations.

247

### 248 **Inter- and intra-specific variations of shoot/foliar Cu and Co concentrations**

249

#### 250 *Phylogeny of variations in Cu and Co accumulation*

251 The absence of a suitable field test for Cu-Co in plant materials as exists for Ni has so far  
252 limited the widespread screening for significant accumulation of these elements. Furthermore,  
253 foliar Cu-Co concentrations are not routinely measured in many ecological studies around the  
254 world. Many of the records for abnormal Cu-Co concentrations in plants, therefore, result  
255 from targeted testing of plants from the Katangan Copperbelt, and for most part by atomic  
256 absorption spectrometry (and hence not obtaining multi-element data – but see Brooks *et al.*,  
257 1986). As a result, systematic analysis of the Cu-Co concentrations in plant species is limited.  
258 Only recently has systematic multi-element mass-screening of herbarium collections using  
259 non-destructive techniques (X-ray Fluorescence Spectroscopy) commenced (van der Ent,

260 2016). Therefore, at present it is difficult to objectively observe phylogenetic patterns of Cu  
261 and Co accumulation. Nevertheless, in Central Africa, substantial Cu-Co accumulation is  
262 mostly recorded in the Lamiaceae (Table 1). On ultramafic soils, accumulation of Co is a  
263 feature in a number of Ni hyperaccumulators, especially in the Brassicaceae, Violaceae and  
264 Phyllanthaceae (Table 1).

265

#### 266 *Genetic variability of Cu and Co accumulation*

267 The substantial phenotypic variation of Cu-Co accumulation recorded in Cu-Co metallophytes  
268 is expressed between species, populations, and individuals within a population (Faucon *et al.*,  
269 2007). For Cu, these variations can be of genetic origin. Peng *et al.* (2012) demonstrated the  
270 foliar Cu variability for six distinct metalicolous populations of *Haumaniastrum katangense*.  
271 Six times as much Cu was obtained in non-metallicolous compared to metalicolous plants of  
272 *Crepidorhopalon tenuis* (approx. 115  $\mu\text{g g}^{-1}$ ) (Faucon *et al.*, 2012a). For a given concentration  
273 in the growth medium, Cu accumulation variation could be related to variations in the degree  
274 of tolerance, with the less Cu-accumulating populations the most tolerant (Ouzounidou *et al.*,  
275 1994; Gonnelli *et al.*, 2001; Weng *et al.*, 2005).

276

277 In the case of Co, accumulation variation have been demonstrated between three closely  
278 related *Silene* taxa (Baker *et al.*, 1983). The most tolerant taxon was the less accumulating for  
279 a given soil Co concentration. The intra-specific variation of Co accumulation by  
280 metallophytes from natural Co-enriched soils has not yet been tested. For the facultative  
281 metallophyte *Anisopappus chinensis*, the strong variation in the degree of Co tolerance  
282 between populations suggests intra-specific variation of accumulation (Lange, 2016). It may  
283 be hypothesized that a lower accumulation variation within metalicolous populations than  
284 within non-metallicolous populations exists, as a result of a long-term directional selection in  
285 metalicolous populations (Dechamps *et al.*, 2007).

286

#### 287 *Copper and Co chemical speciation in metalliferous soils and accumulation by plants*

288 The Cu-Co outcrops have high total Cu-Co soil concentrations (up to 27793  $\mu\text{g g}^{-1}$  and 3451  
289  $\mu\text{g g}^{-1}$ , respectively; Pourret *et al.* 2016) from which a fraction is mobile (1145–5225  $\mu\text{g g}^{-1}$   
290 and 39–2146  $\mu\text{g g}^{-1}$ , respectively; Fig. 2a, 3a). This permits high Cu-Co accumulation by *A.*  
291 *chinensis* (Fig. 2b, 3b; up to 736  $\mu\text{g g}^{-1}$  foliar Cu and 2822  $\mu\text{g g}^{-1}$  foliar Co). Moreover, Cu  
292 accumulation variations (by *C. perennis* and *A. chinensis*) were mostly controlled by Cu  
293 adsorbed onto Mn and Fe oxides (Lange *et al.*, 2014). Mobility is higher in soils impacted by

294 mining. Overall, the strong affinity of Mn oxides for Co may explain the lower Co mobility in  
295 Mn-rich soils (Collins & Kinsela, 2011). Cobalt accumulation (in *C. perennis* and *A.*  
296 *chinensis*) was strongly influenced by free Co and by Co adsorbed onto the OM and Fe oxide  
297 fractions (Lange *et al.*, 2014).

298  
299 Copper and Co concentrations in soils from Goiás (513–2015  $\mu\text{g g}^{-1}$  and 430–1230  $\mu\text{g g}^{-1}$ ,  
300 respectively) only have a small mobile fraction (0.05–4.25  $\mu\text{g g}^{-1}$  and 7–134  $\mu\text{g g}^{-1}$ ,  
301 respectively; Fig. 2a, 3a). The Ni-hyperaccumulator *Pfaffia sarcophylla* (Amaranthaceae)  
302 displays high foliar Cu concentrations (105–298  $\mu\text{g g}^{-1}$ ) on Niquelândia 7 soil but far lower  
303 concentrations (8–26  $\mu\text{g g}^{-1}$ ) on Niquelândia 5 soil (van der Ent & Reeves, 2015; Fig. 2b).  
304 *Piriqueta sp.* (Turneraceae) can accumulate up to 149  $\mu\text{g g}^{-1}$  foliar Co (van der Ent & Reeves,  
305 2015; Fig. 3b).

306  
307 Soils from Barberton have lower Cu-Co concentrations (50–150  $\mu\text{g g}^{-1}$  and 95–272  $\mu\text{g g}^{-1}$ ,  
308 respectively) with a very low mobile fraction (0.01–1.86  $\mu\text{g g}^{-1}$  and 2–13  $\mu\text{g g}^{-1}$ , respectively;  
309 Fig. 2a, 3a). No Cu accumulation has been found. When soils are waterlogged (*i.e.* as at  
310 Groenvally, Fig. 3), Co is mainly associated with amorphous Fe oxides. Soluble Co/Ni ratio  
311 becomes then higher (*i.e.* 1/5) than in typical ultramafic soils (1/20–1/100) and *Berkheya*  
312 *coddii* may thus accumulate >600  $\mu\text{g g}^{-1}$ . Cobalt is preferred over Ni by *B. coddii* when both  
313 are supplied at isomolar ratios as for *Alyssum* species (Homer *et al.*, 1991). Cobalt  
314 (hyper)accumulation in ultramafic soils is triggered by the available Co/Ni ratio.

315  
316 *Influence of the rhizosphere biota on trace element uptake by plants*

317 Soil-plant-microbe interactions are widely investigated for the study of soil-plant processes  
318 (Huang *et al.*, 2014; Dessaux *et al.*, 2016). Soil microbial community has the capacity to  
319 mobilize/immobilize trace element, and thus, to affect the soil TE speciation (reviewed by  
320 Gadd, 2004) and availability (Cuypers *et al.*, 2013). However, the rhizosphere biota influence  
321 on Cu-Co accumulation remains poorly understood. It should be emphasised that natural soil  
322 properties are difficult to replicate synthetically and directly influence accumulation patterns  
323 in plants. The most appropriate method to study strategies of tolerance, therefore, is using  
324 natural soils from the habitat of the species under investigation (van der Ent *et al.*, 2015a).

325  
326 A diversity of metal-tolerant bacteria and fungi are naturally present in metalliferous soils  
327 (including plant growth-promoting bacteria, endophytic bacteria and Arbuscular Mycorrhizal

328 Fungi (AMF)), having in some cases a beneficial effect on plant establishment, growth and  
329 TE tolerance and accumulation (Sessitsch *et al.*, 2013; Lenoir *et al.*, 2016; Ma *et al.*, 2016).  
330 However, the majority of studies have focused on Ni- and Zn-accumulating plants. For Cu,  
331 Chen *et al.* (2005) demonstrated that Cu-tolerant bacteria strains from the rhizosphere of *E.*  
332 *splendens* had a positive effect on both the Cu mobility in soil and uptake by the plant. In the  
333 same species, the presence and diversity of AMF had a positive effect on the uptake of Cu  
334 from a Cu-contaminated soil ( $\pm 15\%$ ) (Wang *et al.*, 2005; 2007).

335

336 Endophytic bacteria associated with *H. katangense* and *C. tenuis* (Katangan Copperbelt) have  
337 been characterized (Kabagale *et al.*, 2010). However, no studies have yet been investigating  
338 the relationships between Cu-Co accumulation by these plants and their rhizosphere biota  
339 diversity/activity. Hypothetical influence of rhizosphere biotic processes on Cu-Co speciation,  
340 availability and uptake is displayed in Fig. 4. Further studies should be performed, especially  
341 *in situ* characterization of the rhizosphere microbial community from a Cu-Co metallophyte in  
342 relationship to Cu-Co uptake and accumulation.

343

#### 344 **Phytomining technology using Cu-Co (hyper)accumulator plants**

345

346 Phytomining involves extracting target elements from the soil by accumulation in the plant  
347 biomass, which are then harvested and processed for recovering the target elements from the  
348 “bio-ore” (Chaney *et al.*, 1998; van der Ent *et al.*, 2015b). The feasibility of phytomining has  
349 been demonstrated at field scale (Brooks & Robinson, 1998; Angle *et al.*, 2001; Chaney *et al.*,  
350 2007; van der Ent *et al.*, 2015b, Bani *et al.*, 2015), but is limited to Ni.

351

352 Copper accumulation was tested using *A. chinensis* from southeastern D. R. Congo, and some  
353 individuals could accumulate up to 5 g Cu plant<sup>-1</sup> DW and 1 335  $\mu\text{g Cu g}^{-1}$  DW, *i.e.* a yield of  
354 437 g Cu ha<sup>-1</sup> year<sup>-1</sup> (Grison *et al.*, in press). The amount of Cu phytoextracted would be of  
355 the same magnitude as other species such as *E. splendens* (Yang *et al.*, 2005). Given the low  
356 economic value of Cu, combined with very limited achievable yields, it is unlikely that Cu  
357 phytomining will be commercially viable.

358

359 Cobalt phytomining was first proposed in the early 1990s as a possibility using Ni-  
360 hyperaccumulator species on ultramafic soils (Homer *et al.*, 1991), but it was noted that the  
361 presence of Ni limits the uptake of Co in most Ni-hyperaccumulator plants (Malik *et al.*,

2000). *Alyssum* species can grow with shoot concentrations  $>1000 \mu\text{g g}^{-1}$  in Co-contaminated soils (Malik *et al.*, 2000) and *B. coddii* can have foliar Co concentrations of  $>2000 \mu\text{g g}^{-1}$  (Keeling *et al.*, 2003). In the Copper Flora, *Haumaniastrum robertii*, with leaf Co concentration can  $>4000 \mu\text{g g}^{-1}$  (Morrison *et al.*, 1981), might be a good candidate for Co phytomining. *B. coddii* could also be a candidate in Co-enriched tailings due to its high biomass and foliar Co (up to  $5000 \mu\text{g g}^{-1}$ , unpublished results). Cobalt phytomining may, therefore, be considered for Co-contaminated lands because of the possible attainable yields and the high metal product value (LME is USD \$24,000/tonne in March 2016).

370

### 371 **Conclusions and Outlook**

372

373 Accumulation of Cu and/or Co in leaves is exceptionally rare globally and principally known  
374 from the Cu-Co outcrops of the Copperbelt of Central Africa. None of the putative Cu or Co  
375 hyperaccumulator plants appears to comply with the fundamental principle of  
376 hyperaccumulation that uptake and accumulation characteristics are not dose-dependent. In  
377 the case of plants growing on ultramafic soils, we postulate that Co accumulation coincides  
378 with Ni accumulation operating on similar physiological pathways. True Cu  
379 hyperaccumulation does not appear to exist as Cu tolerant plants are essentially "Excluders"  
380 *sensu* Baker and there is no physiological mechanism of Cu hyperaccumulation known in  
381 these species. The occasional observations of high foliar Cu concentrations can be explained  
382 by stress and the breakdown of tolerance mechanisms reducing Cu storage capacity in the  
383 roots, leading to a release of Cu in the plant shoot, which highly Cu-tolerant genotypes can  
384 survive up to a certain level.

385

386 The ecophysiology of Co tolerance and accumulation is still poorly understood compared to  
387 the state of knowledge on Cu regulation in plants, and investigations of the interactions with  
388 simultaneous tolerance/accumulation of Co and Cu makes a strong science case. Application  
389 of next-generation sequencing technologies to research in non-model organisms as Cu- and  
390 Co-tolerant and Co accumulating plants is expected to uncover novel adaptation mechanisms  
391 (Verbruggen *et al.*, 2013). The ecological significance of Cu-Co accumulation should be  
392 investigated in relation to the "leaf economics spectrum", and what the adaptive significance  
393 of Cu-Co accumulation might be. Adaptation to local soil, by characterizing the soil microbial  
394 communities and compare the response of Cu-Co-tolerant *versus* non-tolerant populations to  
395 the pathogen pressure by reciprocal transplantation should be investigated.

396

397 The application of Co accumulator plants in phytomining is limited because their  
398 accumulation characteristics mean that only ‘ore-grade soils’ may yield sufficient  
399 accumulation in the shoots to develop this technology. Cobalt phytomining may be considered  
400 for strongly Co-contaminated soils and tailings, because of the high metal product value  
401 compared to Cu.

402

### 403 **Acknowledgments**

404

405 The Belgian Fund for Scientific Research (FRS-FNRS, Belgium) is acknowledged for  
406 financial support to B.L. as research fellow of the “Fonds pour la Recherche dans l’Industrie  
407 et l’Agriculture”. A.V.D.E. was the recipient of a post-doctoral scholarship from the French  
408 National Research Agency through the national “Investissements d’avenir” program (ANR-  
409 10-LABX-21 - LABEX RESSOURCES21), and currently an Australian Research Council  
410 Post Doctoral Fellowship (DE160100429).

411

### 412 **Author contributions**

413

414 B.L., A.V.D.E. and M.P.F. conceived the manuscript. B.L., A.V.D.E., A.J.M.B., G.E., G.M.,  
415 F.M., P.M., O.P., N.V. and M.P.F. wrote the manuscript with B.L. coordinating the writing  
416 process.

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728 **Table captions**

729

730 **Table 1.** Species for which the capacity of population(s) to grow under elevated  
731 concentrations of Cu and/or Co has been experimentally demonstrated.

732 The reported concentrations correspond to the higher experimental treatment, or correspond to  
733 the concentration above which plants' growth was inhibited. CB = Katangan Copperbelt.

734

735 **Table 2.** Updated list of species recorded with at least one healthy specimen with a foliar  
736 concentration  $>300 \text{ mg kg}^{-1}$  DW.

737 Were considered in this list plants that have received a washing protocol only (clearly  
738 mentioned in the publication). When available, a range of variation is given. Otherwise,  
739 values are single measurements, or mean values with standard deviation. *NA* = Not Available  
740 data. *Ex situ* = measurement on plants growing under experimental conditions. CB =  
741 Katangan Copperbelt.

742

743 **Figure captions**

744

745 **Figure 1.** Visual appearance of selected Cu- and/or Co-accumulator plants.

746 (a) *Haumaniastrum robertii* (Lamiaceae) (D. R. Congo). Photo credit: copperflora.org; (b)

747 *Hibiscus rhodanthus* (Malvaceae) (D. R. Congo). Photo credit: copperflora.org; (c)

748 *Anisopappus chinensis* (Asteraceae) (D. R. Congo). Photo credit: B. Lange; (d) *Rinorea*

749 *bengalensis* (Violaceae) (S-E Asia). Photo credit: A. van der Ent; (e) *Crepidorhopalon tenuis*

750 (Linderniaceae) (D. R. Congo). Photo credit: B. Lange; (f) *Berkheya codii* (Asteraceae)

751 (South-Africa). Photo credit: A. van der Ent; (g) *Alyssum murale* (Brassicaceae) (USA). Photo

752 credit: A. van der Ent.

753

754 **Figure 2.** Copper speciation in soils (modeled after Pourret *et al.*, 2015) and accumulation in  
755 metallophytes from various origins.

756 (a) Proportion of Cu species in considered soils; (b) Inorganic Cu concentrations (mobile

757 concentrations considered as available) in soils. Accumulated concentrations in plants are

758 added when available. F5: Fungurume 5; G2: Goma 2; Et: Etoile; Niq: Niquelândia; Gr:

759 Groenvaly; So: Songimvelo; AM: Agnes Mine.

760

761 **Figure 3.** Cobalt speciation in soils (modeled after Pourret *et al.*, 2015) and accumulation in  
762 metallophytes from various origins.

763 (a) Proportion of Co species in considered soils; (b) Inorganic Co concentrations in soils

764 (mobile concentrations considered as available). Accumulated concentrations in plants are

765 added when available. F5: Fungurume 5; G2: Goma 2; Et: Etoile; Niq: Niquelândia; Gr:

766 Groenvaly; So: Songimvelo; AM: Agnes Mine.

767

768 **Figure 4.** Hypothetical rhizosphere processes influencing Cu and Co availability and uptake  
769 by metallophytes originating from naturally Cu- and Co-enriched soils.

770 LOA = Low-molecular-weight Organic Acids. The proportions of the different Cu and Co

771 fractions in the soil are related to the font size (Lange *et al.*, 2014; Pourret *et al.*, 2015).

Table 1

	Origin	Experimental demonstration of Cu tolerance, or of the capacity to grow under elevated concentration of Cu	Experimental demonstration of Co tolerance or of the capacity to grow under elevated concentration of Co	Growth medium	References
<b>Asteraceae</b>					
<i>Anisopappus chinensis</i> Hook.f. & Arn.	CB (D.R. Congo)	up to 500 $\mu\text{g g}^{-1}$ (total Cu)	up to 50 $\mu\text{g g}^{-1}$ (total Cu)	Soil	Lange <i>et al.</i> , 2014
<b>Caryophyllaceae</b>					
<i>Minuartia hirsuta</i> (M.Bieb.) Hand.-Mazz.	Kilkis (Greece)	up to 80 $\mu\text{M CuSO}_4$	-	Nutrient solution	Ouzounidou <i>et al.</i> , 1994
<i>Silene burchelli</i> Otth ex DC. (Ecotype)	Luitia (D. R. Congo)	>1 000 $\mu\text{g g}^{-1}$ (total Cu)	up to 800 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> , 1983
<i>Silene burchelli</i> var. <i>angustifolia</i> Sond.	Kundelungu Plateau (D. R. Congo)	up to 500 $\mu\text{g g}^{-1}$ (total Cu)	up to 100 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> , 1983
<i>Silene cobalticola</i> P.A. Duvign. & Plancke	CB (D.R. Congo)	>1 000 $\mu\text{g g}^{-1}$ (total Cu)	>1 000 $\mu\text{g g}^{-1}$ (total Co)	Soil	Baker <i>et al.</i> , 1983
<i>Silene vulgaris</i> (Moench) Garcke	Ontario (Canada)	-	up to 5 $\text{mg l}^{-1}$	Nutrient solution	Paliouris & Hutchinson, 1991
	Marsberg – Imsbach - Harlingerode (Germany)	up to 8 $\mu\text{M}$	-	Nutrient solution	Schat & Ten Bookum, 1992
	Imsbach (Germany)	19 to 8 645 $\mu\text{g g}^{-1}$ (total Cu)	-	Soil	Song <i>et al.</i> , 2004

**Crassulaceae**

<i>Crassula helmsii</i> (Kirk) Cockayne	Oer-Erkenschwick (Germany)	up to 20 $\mu\text{M}$	-	Nutrient solution	Küpper <i>et al.</i> , 1996
<b>Lamiaceae</b>					
<i>Aeolanthus biformifolius</i> De Wild.	CB (D.R. Congo)	up to 9 000 $\mu\text{g g}^{-1}$ (total Cu)	up to 670 $\mu\text{g g}^{-1}$ (total Co)	Soil	Morrison <i>et al.</i> , 1979
<i>Elsholtzia haichowensis</i> Sun.	Yangtze River (China)	0.32 to 500 $\mu\text{M}$	-	Nutrient solution	Lou <i>et al.</i> , 2004
<i>Elsholtzia splendens</i> Nakai ex F.Maek.	Zhejiang (China)	up to 1 000 $\mu\text{g g}^{-1}$ (total Cu)	-	Soil	Jiang <i>et al.</i> , 2004
	Zhejiang (China)	up to 5 146 $\mu\text{g g}^{-1}$ (total Cu)	-	Soil	Song <i>et al.</i> , 2004
<i>Haumaniastrum katangense</i> (S. Moore) Duvign. & Plancke	CB (D.R. Congo)	up to 100 $\mu\text{M}$	-	Nutrient solution	Chipeng <i>et al.</i> , 2010; Peng <i>et al.</i> , 2012
		up to 1 400 $\mu\text{g g}^{-1}$	up to 600 $\mu\text{g g}^{-1}$	Soil	Morrison <i>et al.</i> , 1979
<i>Haumaniastrum robertii</i> (Robyns) P.A. Duvign. & Plancke	CB (D.R. Congo)	up to 8 500 $\mu\text{g g}^{-1}$ (total Cu)	up to 4 000 $\mu\text{g g}^{-1}$ (total Co)	Soil	Morrison <i>et al.</i> , 1979
<b>Linderniaceae</b>					
<i>Crepidorhopalon tenuis</i> (S. Moore) Eb. Fisch.	CB (D.R. Congo)	up to 60 $\mu\text{M}$	-	MS medium	Faucon <i>et al.</i> , 2012a
<i>Crepidorhopalon perennis</i> (P.A. Duvign.) Eb. Fisch.	CB (D.R. Congo)	up to 60 $\mu\text{M}$	-	MS medium	Faucon <i>et al.</i> , 2012a

**Phrymaceae**

<i>Mimulus guttatus</i> Fischer ex DC.	California (USA)	1.0 parts/10 <sup>6</sup>	-	Nutrient solution	Allen & Sheppard, 1971; Macnair, 1983
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**Poaceae**

<i>Agrostis capillaris</i> L.	Parys Mountain, Anglesey (UK) Black Forest (Germany)	up to 15 µM	-	Nutrient solution	Jowett, 1958
<i>Agrostis gigantea</i> Roth. (one clone)	Ontario (Canada)	up to 20 mM	up to 170 mM	Nutrient solution	Hogan & Rauser, 1979
<i>Agrostis stolonifera</i> L.	Piesky (Slovakia)	up to 15 µM	-	Nutrient solution	Jowett, 1958
<i>Calamagrostis epigejos</i> (L.) Roth	Legnica (Poland)	up to 2 mM	-	Nutrient solution	Lehmann & Rebele, 2004
<i>Cynodon dactylon</i> (L.) Pers.	Guangdong (China)	up to 0.5 mg l <sup>-1</sup>	-	Nutrient solution	Shu <i>et al.</i> , 2002
<i>Deschampsia cespitosa</i> (L.) Beauv.	Coniston smelter population, Ontario (Canada)	0.3 µg cm <sup>-3</sup>	-	Nutrient solution	Cox & Hutchinson, 1980
<i>Paspalum distichum</i> L.	Guangdong (China)	up to 1 mg l <sup>-1</sup>	-	Nutrient solution	Shu <i>et al.</i> , 2002

Table 2

	Foliar Cu ( $\mu\text{g g}^{-1}$ DW)	Foliar Co ( $\mu\text{g g}^{-1}$ DW)	Origin of samples	Site	Restriction to metalliferous soils	Life-cycle	References
<b><u>MAGNOLIOPSIDA</u></b>							
<b>Amaranthaceae</b>							
<i>Celosia trigyna</i> L.	345 to 603	75 to 501	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<i>Pandiaka carsonii</i> Baker (C.B. Clarke)	273 to 445	6 to 7	<i>In situ</i>	CB (D.R. Congo)	Facultative	Perennial	Lefèbvre <i>et al.</i> , unpublished ; Duvigneaud & Denaeyer-De Smet, 1963 Delhayé <i>et al.</i> , 2016
	740	NA	<i>In situ</i>	CB (D.R. Congo)			
	1 870	NA	<i>In situ</i>	CB (D.R. Congo)			
<b>Apocynaceae</b>							
<i>Carissa spinarum</i> L.	702 (leaf+stem)	1.6 (leaf+stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<b>Asclepiadaceae</b>							
<i>Calotropis gigantea</i> L. R. Br.	582 (leaf+stem)	0.8 (leaf+stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<b>Asteraceae</b>							
<i>Anisopappus chinensis</i> Hook.f. & Arn.	4 to 2800	3 to 1300	<i>In situ</i>	CB (D.R. Congo)	Facultative	Perennial	Lange <i>et al.</i> , 2014
<i>Berkheya coddii</i> Roessler	NA	40 to 2116	<i>Ex situ</i>	South-Africa	Obligate	Perennial	Keeling <i>et al.</i> , 2003
<i>Vernoniastrum latifolium</i> (Steetz) H. Rob.	269 to 1942	82 to 549	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<b>Boraginaceae</b>							
<i>Onosma stenosphon</i> Boiss.	300 to 657	NA	<i>In situ</i>	Sarcheshmeh (Iran)	Facultative	Biennial to perennial	Ghaderian & Ravandi, 2012

<b>Brassicaceae</b>							
<i>Alyssum corsicum</i> Duby	NA	1 080 ± 260 (shoot)	<i>Ex situ</i>	Greece	Facultative	Perennial	Malik <i>et al.</i> , 2000
<i>Alyssum heldreichii</i> Hausskn.	NA	<5 to >1 000	<i>Ex situ</i>	NA	Obligate	Perennial	Homer <i>et al.</i> , 1991
<i>Alyssum murale</i> Waldst. & Kit.	NA	2 070 ± 252 (shoot)	<i>Ex situ</i>	NA	Facultative	Perennial	Tappero <i>et al.</i> , 2007
	NA	1 320 ± 300 (shoot)	<i>Ex situ</i>	Greece			Malik <i>et al.</i> , 2000
<i>Alyssum serpyllifolium</i> Desf.	NA	<5 to >1 000	<i>Ex situ</i>	NA	Facultative	Perennial	Homer <i>et al.</i> , 1991
<i>Alyssum tenium</i> Halácsy	NA	<5 to >1 000	<i>Ex situ</i>	NA	Obligate	Perennial	Homer <i>et al.</i> , 1991
<i>Alyssum troodii</i> Boiss.	71	10 to 2 325	<i>Ex situ</i>	Republic of Cyprus	Obligate	Perennial	Homer <i>et al.</i> , 1991
<b>Caryophyllaceae</b>							
<i>Silene cobalticola</i> P.A. Duvign. & Plancke	1660	NA	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Duvigneaud & Denaeyer-De Smet, 1963
<b>Cornaceae</b>							
<i>Nyssa sylvatica</i> var. <i>biflora</i> (Walt.) Sarg.	NA	0 to 438	<i>Ex situ</i>	Southeastern USA	Facultative	Perennial	McLeod & Ciravolo, 2007
	NA	300	<i>Ex situ</i>	Florida (USA)	Facultative	Perennial	Malik <i>et al.</i> , 2000
<i>Nyssa sylvatica</i> var. <i>sylvatica</i> Marsh.	NA	800	<i>Ex situ</i>	Florida (USA)	Facultative	Perennial	Malik <i>et al.</i> , 2000
<b>Crassulaceae</b>							
<i>Crassula helmsii</i> (Kirk) Cockayne	9200 ±1500	NA	<i>Ex situ</i>	Oer-Erkenschwick (Germany)	Facultative	Perennial	Küpper <i>et al.</i> , 2009
<b>Euphorbiaceae</b>							
<i>Acalypha cupricola</i> Robyns	390 to 2890	207 to 904	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Faucon <i>et al.</i> , 2007
<i>Croton bonplandianus</i> Baill.	2 163 (leaf+stem)	2 (leaf+stem)	<i>In situ</i>	Ginigalpelessa (Sri	Facultative	Perennial	Rajakaruna & Bohm, 2002

Lanka)

**Fabaceae**

<i>Senna auriculata</i> L.	885 (leaf+stem)	1 (leaf+stem)	<i>In situ</i>	Ussangoda (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<i>Tephrosia villosa</i> Pers.	1 858 (shoot)	0.2	<i>In situ</i>	Indikolapelessa (Sri Lanka)	Facultative	Annual to perennial	Rajakaruna & Bohm, 2002
<i>Vigna dolomitica</i> R. Wilczek	222 to 1000	241 to 540	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Faucon <i>et al.</i> , 2007

**Lamiaceae**

<i>Aeolanthus biformifolius</i> De Wild.	0 to 78	0 to 1 900	<i>Ex situ</i>	CB (D.R. Congo)	Obligate	Perennial	Morrison <i>et al.</i> , 1978
<i>Elsholtzia haichowensis</i> Sun.	NA	17 to 391	<i>In situ</i>	Yangtze River (China)	Obligate	Annual	Tang <i>et al.</i> , 1999
<i>Elsholtzia splendens</i> Nakai ex F.Maek.	<10 to >500	NA	<i>Ex situ</i>	Zhuji (China)	Facultative	Annual	Yang <i>et al.</i> , 2002
<i>Ocimum tenuiflorum</i> L.	2 265 (leaf+stem)	11 (leaf+stem)	<i>In situ</i>	Yodhagannawa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<i>Haumaniastrum katangense</i> (S. Moore) Duvign. & Plancke	187 to 317	224 to 772	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<i>Haumaniastrum robertii</i> (Robyns) P.A. Duvign. & Plancke	62 to 6159	62 to 326	<i>In situ</i>	CB (D.R. Congo)	Obligate	Annual	Duvigneaud & Denaeyer-De Smet, 1963
	28	>7 200	<i>Ex situ</i>	CB (D.R. Congo)			Morrison <i>et al.</i> , 1979

**Linderniaceae**

<i>Crepidorhopalon tenuis</i> (S. Moore) Eb. Fisch.	0 to 2524	8 to 605	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<i>Crepidorhopalon perennis</i> (P.A. Duvign.) Eb. Fisch.	80 to 1384	61 to 1105	<i>In situ</i>	CB (D.R. Congo)	Obligate	Short-lived perennial	Faucon <i>et al.</i> , 2007

**Malvaceae**

<i>Abutilon indicum</i> Sweet	915 (leaf+stem)	1 (leaf+stem)	<i>In situ</i>	Indikolapelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<i>Hibiscus rhodanthus</i> Gürke	102 to 515	42 to 274	<i>In situ</i>	CB (D.R. Congo)	Facultative	Perennial	Faucon <i>et al.</i> , 2007
<i>Triumfetta welwitschii</i> Mast.	111 to 704	162 to 1971	<i>In situ</i>	CB (D.R. Congo)	Facultative	Perennial	Faucon <i>et al.</i> , 2007
<b>Onagraceae</b>							
<i>Epilobium hirsutum</i> L.	1300 to 1581	NA	<i>In situ</i>	Sarcheshmeh (Iran)	Facultative	Perennial	Ghaderian & Ravandi, 2012
<b>Orobanchaceae</b>							
<i>Alectra sessiliflora</i> (Vahl) O. Ktze.	45 to 769	21 to 866	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<i>Buchnera henriquesii</i> Engl.	142 to 242	404 to 930	<i>In situ</i>	CB (D.R. Congo)	Facultative	Annual	Faucon <i>et al.</i> , 2007
<b>Phrymaceae</b>							
<i>Mimulus guttatus</i> Fischer ex DC.	120 to 780	NA	<i>Ex situ</i>	USA	Facultative	Annual and perennial	Tilstone & Macnair, 1997
<b>Phyllanthaceae</b>							
<i>Aporosa chalarocarpa</i> Airy Shaw	NA	468	<i>In situ</i>	Sabah (Malaysia)	Facultative	Perennial	Van der Ent <i>et al.</i> , 2015c
<i>Glochidion cf. sericeum</i> (Blume) Zoll. & Moritzi	NA	442 to 1 310	<i>In situ</i>	Sabah (Malaysia)	Facultative	Perennial	Van der Ent <i>et al.</i> , 2015c
<b>Poaceae</b>							
<i>Agrostis stolonifera</i> L.	100 to 1000	NA	<i>In situ</i>	Prescot (UK)	Facultative	Perennial	Wu <i>et al.</i> , 1974
<i>Polypogon fugax</i> Nees ex steud.	1550 to 4012	NA	<i>In situ</i>	Sarcheshmeh (Iran)	Facultative	Annual	Ghaderian & Ravandi, 2012
<b>Polygonaceae</b>							
<i>Rumex acetosa</i> Linn.	340 to 1102	NA	<i>In situ</i>	Yangtze River (China)	Obligate	Perennial	Tang <i>et al.</i> , 1999

<b>Solanaceae</b> <i>Hyoscyamus senecionis</i> Willd.	87 to 331	NA	<i>In situ</i>	Sarcheshmeh (Iran)	Facultative	Perennial	Ghaderian & Ravandi, 2012
<b>Sterculiaceae</b> <i>Waltheria indica</i> L.	1 504 (leaf+stem)	1.3 (leaf+stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Short-lived perennial	Rajakaruna & Bohm, 2002
<b>Verbenaceae</b> <i>Clerodendrum infortunatum</i> L.	2 280 (leaf+stem)	0.6 (leaf+stem)	<i>In situ</i>	Ginigalpelessa (Sri Lanka)	Facultative	Perennial	Rajakaruna & Bohm, 2002
<b>Violaceae</b> <i>Rinorea bengalensis</i> (Wall.) O.Ktze.	NA	0.5 to 545	<i>In situ</i>	S.E. Asia	Facultative	Perennial	Brooks <i>et al.</i> , 1977
<i>Rinorea javanica</i> (Bl.) O.Ktze.	NA	3 to 670	<i>In situ</i>	S.E. Asia	Facultative	Perennial	Brooks <i>et al.</i> , 1977
<b><u>LILIOPSIDA</u></b>							
<b>Commelinaceae</b> <i>Commelina communis</i> Linn.	19 to 587	NA	<i>In situ</i>	Yangtze River (China)	Facultative	Annual	Tang <i>et al.</i> , 1999
<i>Commelina zigzag</i> P.A. Duvign. & Dewit	1 210	NA	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Duvigneaud & Denaeayer-De Smet, 1963
<i>Cyanotis longifolia</i> Benth. var. <i>longifolia</i>	7.5 to 286	2.3 to 1 697	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Delhaye <i>et al.</i> , unpublished
<b>Cyperaceae</b> <i>Ascolepis metallorum</i> P.A. Duvign. & G. Léonard	1200	NA	<i>In situ</i>	CB (D.R. Congo)	Obligate	Perennial	Duvigneaud & Denaeayer-De Smet, 1963