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## 4 **Implication of Plant-soil Relationships for Conservation and Restoration of** 5 **Copper-cobalt Ecosystems**

6

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21

### 22 **Abstract**

23 *Background:* Chemical soil factors play an important role in generating and maintaining plant diversity.  
24 Naturally metal-enriched habitats support highly distinctive plant communities consisting of many rare and  
25 endemic species. Species of these plant communities possess remarkable physiological adaptations and are now  
26 being considered key elements in the implementation of green technologies aimed at phytoremediation of  
27 contaminated soils and post-mined soils. Several studies have emphasised that industrial mineral extraction  
28 results in serious damage to ecosystems and serious threats to human health and leads to the extinction of  
29 metallophyte species. In the southeastern Democratic Republic of the Congo (DRC), mining activities represent

30 a threat to the long-term persistence of communities located on metalliferous copper and cobalt outcrops and  
31 their associated endemic metallophytes, which are currently considered some of the most critically endangered  
32 plants in the world.

33 *Scope:* Plant diversity conservation of metal-rich soils must assess soil-plant relationships at different scales  
34 (ecosystems, communities, and populations) to define *in-situ* and *ex-situ* conservation and restoration projects.  
35 This paper proposes a review of soil-plant relationships involved in plant diversity and endemism and their  
36 implications for biodiversity conservation and restoration.

37

38 **Keywords:** biodiversity conservation, chemical soil factors, endemism, heavy metals, metallophyte, restoration  
39 ecology, soil-plant interactions

40

41

## 42 **Introduction**

43 For decades, ecologists have attempted to understand the relationships between soil properties and plant  
44 diversity, as some soils are associated with high richness and endemism in plant species (Whittaker et al. 2001;  
45 Escudero et al. 2015). A number of plant diversity hotspots are associated with nutrient-poor environments,  
46 notably in the Fynbos in South Africa, the Kwongan in southwestern Australia, and the Campos Rupestres in  
47 Brazil (Cowling and Lombard 2002; Hopper and Gioia 2004; Lambers et al. 2010; Laliberté et al. 2013; Lambers  
48 2014; Silveira et al. 2015). Regions with naturally metal-rich soils may also exhibit landscape and environmental  
49 heterogeneity, promoting high richness and endemism in plant species, such as New Caledonia (Pillon et al.  
50 2010), Sabah (Malaysia) (van der Ent et al. 2015a), California (USA) (Brady et al. 2005), Cuba (Borhidi 1996),  
51 and southeastern Democratic Republic of Congo (DRC) (Küper et al. 2004; Faucon et al. 2010). The most  
52 widespread natural metalliferous outcrops across the world are ultramafic soils rich in nickel (Ni), chromium  
53 (Cr), iron (Fe), and magnesium (Mg) (Harrison and Rajakaruna 2011), but natural outcrops rich in Fe,  
54 manganese (Mn), copper (Cu), cobalt (Co), and other rare minerals also occur. These metalliferous outcrops  
55 support unique vegetation adapted to high metal concentrations (i.e., Ni, Cr, Fe, or Mg) and, in general, nutrient  
56 deficiency and cation imbalances. Vegetation occurring in metal-rich habitats may present an important  
57 proportion of endemic species (Rajakaruna 2004; Faucon et al. 2010; Arnacker 2011). These endemics  
58 significantly contribute to the biodiversity of some regions of the world (Kruckeberg et al. 1985; Jacobi et al.  
59 2007; Anacker et al. 2014; van der Ent et al. 2015b). For example, in California, endemic species from  
60 ultramafic rock outcrops represent close to 12% of the state endemic flora (Kruckeberg et al. 1990; Safford et al.  
61 2005); in New Caledonia, they correspond to almost 60% of the island's indigenous flora (Jaffré 1992).

62 In addition, metallicolous flora represents a remarkable biological resource for eco-technological applications,  
63 especially phytoremediation of contaminated soils (Boisson et al. *In press*; Shutcha et al. 2010; Shutcha et al.  
64 2015; van der Ent et al. 2015c). Whiting et al. (2004) considered these species an “El Dorado” of genetic  
65 material, which could be used for decontamination or ecological restoration of metal-contaminated sites.  
66 Implications in eco-technologies are particularly enhanced with the valorisation of metals in biomass from  
67 hyper-accumulators in green chemistry (Zn, Ni, Platinum - Pt, Cu, Co, and Manganese - Mn) as catalysts in  
68 organic synthesis of molecules of interest (Losfeld et al. 2012; Escande et al. 2014; Grison 2014).

69 As metallicolous vegetation often occurs on economically valuable mineral deposits, many are threatened by  
70 quarrying and mining activities (Erskine et al. 2012; Faucon et al. 2011; Whiting et al. 2004). Moreover,

71 extraction processes may lead to the contamination of adjacent nonmetalliferous habitats by industrial wastes  
72 (smelts, waste water, etc.), which might impact local plant diversity. Establishment of conservation and/or  
73 restoration programmes for plant biodiversity of metal-rich habitats (*in-situ* and *ex-situ* conservation) requires  
74 the understanding of relationships between soil and plant diversity. Knowledge of ecological and  
75 biogeochemical processes governing ecosystems, communities, and populations is fundamental to defining and  
76 applying ecological restoration of disturbed habitats (Palmer et al. 1997).

77 In southeastern DRC, natural copper and cobalt outcrops (Cu-Co outcrops) host remarkable herbaceous  
78 communities that comprise the so-called “copper flora”. These outcrops form isolated and scattered hills in a  
79 landscape matrix of Miombo woodland (Duvigneaud and Denaeyer-De Smet 1963; Duvigneaud 1959; Fig. 1)  
80 (<http://copperflora.org/eflora/>). The Cu-Co outcrops present a variety of habitats according to a variation of  
81 edaphic conditions, including the natural Cu-Co contamination level of the soil (Fig. 1). Mineralisation of the  
82 parent rocks and geological succession promotes the Cu and Co enrichment of the soils along the slope of the  
83 hills with concentrations of bioavailable Cu from 20 to 10,000 mg.kg<sup>-1</sup> and Co from 2 to 1,000 mg.kg<sup>-1</sup> toward  
84 the top of the Cu hills. These plant communities host more than 600 species tolerant to high Co and Cu  
85 concentrations (Leteinturier 2002). Among those tolerant species, 57 have been identified as endemic from the  
86 Cu-Co outcrops (i.e., Cu-Co endemics). The region, due to its extremely metal-rich subsoil, is currently at the  
87 forefront of mining activity. In DRC, 70% of metallophytes taxa (species or genera) are considered critically  
88 endangered (i.e., CR) and about 10% would already have disappeared (Faucon et al. 2010; 2012a). In addition,  
89 southeastern DRC is one of the principal regions across the world presenting both environmental and public  
90 health issues associated with soil, air, and water contaminations resulting from an important Cu, Co, and  
91 uranium (U) extraction (Banza et al. 2009; Manda et al. 2010; Cheyns et al. 2014). There is an urgent need to  
92 develop conservation measures as well as restoration projects for the biodiversity of Cu and Co outcrops.

93 This paper reviews recent advances in our understanding of relationships between soil and plant diversity in  
94 metalliferous outcrops located in southeastern DRC and the implication of these recent advances in defining  
95 conservation and restoration strategies for the Cu-Co ecosystems and their associated plant biodiversity.

96

97 **Patterns of Species Richness and Endemism on Copper-cobalt Outcrops**

98 High Cu and Co concentrations in soil are phytotoxic and represent a strong selection pressure for plant species,  
99 which may induce ecological isolation and promote the speciation process (Duvigneaud and Denaeyer-De Smet  
100 1963; Brooks and Malaisse 1990; Macnair and Gardner, 1998). Among the approximate 600 plant species of  
101 flora of Cu-Co outcrops, 32 are strictly Cu-Co endemic (i.e., absolute metallophyte occurring exclusively on Cu-  
102 Co rich soils), and 23 are broad Cu-Co endemics (i.e., facultative metallophyte with more than 75% of known  
103 populations occurring on Cu-Co rich soils) (Faucon et al. 2010). At outcrop scale, Cu and Co concentrations are  
104 a primary determinant of the richness of plant species. Outcrops with the highest Cu and Co concentrations in  
105 soil support the lowest total plant species richness (Duvigneaud and Denaeyer-De Smet 1963; Saad et al. 2012;  
106 Séleck et al. 2013). This pattern is in contrast to the richness of endemic metallophyte, which rises with the  
107 increase of Cu and Co concentration in the soil (Saad et al. 2012; Séleck et al. 2013). At the landscape scale, the  
108 spatial configuration of Cu-Co outcrops influences the richness of Cu-Co endemics of species. In a recent study  
109 of 34 Cu-Co outcrops varying in size (0.2 ha to 27.3 ha) and geographical isolation in a 30\*20 km landscape,  
110 Ilunga wa Ilunga et al. (unpublished results) demonstrated that Cu-Co endemic richness is positively correlated  
111 to the site surface, which might be associated with higher habitat diversity. Using species accumulation curves, it  
112 was demonstrated that the three largest outcrops encapsulate the total Cu-Co endemic richness (25 taxa) of the  
113 landscape, whereas at least 15 small Cu-Co outcrops are necessary to reach the same endemic richness.

114 How does endemic metallophyte richness in southeastern DRC compare with other metallicolous floras? The  
115 proportion of strict metallophyte endemic in the copper flora, estimated at 5%, is obviously low when compared  
116 with ultramafic floras of California, Cuba, and New Zealand but is rather similar to ultramafic floras of Italy or  
117 Great Dyke (Fig. 2). Endemic richness and the endemism percentage depend on a combination of different  
118 variables (Harrison et al. 2006). The history of geographical isolation is important to explain endemism patterns.  
119 Cuba and New Caledonia are oceanic islands with a long history of geographical isolation resulting in a high  
120 global level of endemism (Fig. 2). The age of exposure of metalliferous outcrops may also be an important factor  
121 to explain the proportion of endemics in a metallicolous flora (Harrison et al. 2004). Copper mineralisation in  
122 southeastern DRC dates from the late Cambrian period (about 620 myr, François 1973), but Cu-Co rich rocks  
123 have been exposed to plant colonisation for a much shorter period. Recent data indicate 2–3 myr as a likely age  
124 for Cu-Co outcrops (De Putter et al. 2010). This is similar to the age of exposure of California ultramafic  
125 outcrops (Harrison et al. 2004). Vegetation modification due to palaeoclimate variation might also be relevant to

126 explain the endemism level in metalicolous floras. During the Holocene, tropical Africa experienced dramatic  
127 climate fluctuations, including a dry-cool period about 18,000 cal yr BP (Van Zinderen et al. 1988, Vincens et al.  
128 2005), with a deglacial period between 16,500 and 12,300 cal yr BP, including a retreat of montane elements at  
129 higher altitudes on the plateau under warmer conditions. This led to evolutionary divergences between  
130 populations isolated on the Cu-Co outcrops. These relatively recent divergences might explain the low  
131 percentage of endemic species observed in the copper flora. The close morphological resemblance between Cu-  
132 Co endemic plant species and more widespread counterparts, such as *Vigna dolomitica* and *V. reticulata* (Maxted  
133 et al. 2004), *Crotalaria peschiana* and *C. subcaespitosa* (Polhill 1982), *Acalypha cupricola*, *A. fuscescens*, and  
134 *A. dikuluwensis* (Levin et al. 2007), and *Silene burchellii* and *S. cobalticola* (Malaisse 1983), suggests that the  
135 speciation processes may be ongoing in the southeastern DRC region. This also supports the hypothesis that  
136 most of Cu-Co endemic species are neo-endemics (Malaisse 1983; Brooks et al 1985; 1990). This implies a  
137 recent divergence under intense ecological isolation (Macnair and Gardner 1998; Rajakaruna 2004). Another  
138 factor that may contribute to the low metallophyte endemism in southeastern DRC is the relatively low total  
139 surface of mineralised soils. Based on a typical site surface of a few tenths of a hectare, the total area of the Cu-  
140 Co outcrops may not exceed 100 km<sup>2</sup>, which is a small surface compared to the thousands of km<sup>2</sup> of ultramafic  
141 soil existing in California, Cuba, or New Caledonia (Harrison and Rajakaruna 2011). Eventually, ecological  
142 isolation and selective forces acting on populations occurring on Cu-Co rich soils may be overestimated. Unlike  
143 ultramafic soils, Cu-Co rich soils of southeastern DRC are relatively rich in nutrients (P, Ca, and Mg) (Faucon et  
144 al. 2011; Séleck et al. 2013), and Cu soil toxicity might be mitigated by organic matter and other metals in  
145 oxidated forms (Lange et al. 2014; Pourret et al. 2015).

146 Even if metal-rich habitats are favourable environments for evolutionary divergence, the exceptionally high  
147 endemism occurring in such habitats remains intriguing (Kay et al. 2011). Intrinsic characteristics of metal-  
148 tolerant taxa may be responsible for the limitation of their ecological niche. One hypothesis for high endemism  
149 in metal-rich habitats might be a low ability of metallophyte to colonise non-metalliferous habitats due to  
150 constitutive needs in metals (Tadros 1957; Kay et al. 2011). For some Cu-Co endemic taxa, it has been  
151 demonstrated that biomass and fitness increase with Cu concentration in soil (Chipeng et al. 2009). Metal  
152 tolerance may also represent an adaptive cost responsible for a disadvantage of metallophyte in non-  
153 metalliferous soils (Wu 1990; Macnair et al. 2000) with a reduced competitive ability of metallophyte in non-  
154 metalliferous habitats. Some studies support the hypothesis that the restricted distribution of metallophyte on

155 metal-rich soils could be due to their low resistance to pathogens. Metal toxicity constitutes a strong selection  
156 pressure against pathogens and herbivores, limiting pathogen and herbivory pressures on metallophytes (Noret et  
157 al. 2005). As a result, metal tolerant taxa may also be characterised by a decrease in defence against herbivory  
158 and pathogens, which could limit their colonisation of non-metalliferous habitats (Faucon et al. 2012b; Kazakou  
159 et al. 2008). Very few data exist for Cu flora, but it has been shown that the Cu-Co endemic *Crepidorhopalon*  
160 *perennis* is able to grow on substrate without Cu only in axenic conditions (i.e., without soil biota) (Faucon et al.  
161 2012b). However, this hypothesis is still controversial because some species of bacteria and fungi are perfectly  
162 adapted to metal-rich soils (Wakelin et al. 2014; Stefanowicz et al. 2008).

163 As a result of the complex interactions among physiological, ecological, and evolutionary factors, the ecological  
164 niches of Cu-Co endemic from southeastern DRC vary widely (Faucon et al. 2011; 2012a). Congeneric species  
165 may present highly distinct edaphic niches. *Crepidorhopalon perennis* (P.A. Duvigneaud) Eb. Fisch.  
166 (Linderniaceae), a Cu-Co endemic, occurs on soils richer in Cu compared to its pseudo-metallophyte congener  
167 *C. tenuis* (S. Moore) Eb. Fisch. (Faucon et al. 2011; 2012b). Boisson et al. (unpublished results) recently  
168 demonstrated that among eight Cu-Co endemic species present on the same Cu-Co outcrops, five have their  
169 optimum niches in the lowest concentrations of Cu ( $< 300 \text{ mg Cu.kg}^{-1}$ ) and Co ( $55 \text{ mg Co.kg}^{-1}$ ) and only two  
170 have their optimum niches in the highest concentrations of Cu ( $> 5,000 \text{ mg Cu.kg}^{-1}$ ). Species with their  
171 optimums in the higher Cu/Co concentrations also present the largest Cu/Co niche width. Ilunga wa Ilunga et al.  
172 (2013) found a similar pattern for non-endemic tolerant species on a Cu-Co outcrop in southeastern DRC. In  
173 addition, Ilunga wa Ilunga et al. (2013) demonstrated niche differentiation in relation to physical soil  
174 characteristics, namely rock cover and percentage of stones in the soil.

#### 175 **Soil and Plant Communities' Co-variation.**

176 The physiognomy of vegetation varies on Cu-Co outcrops from the top to the bottom along a topographical  
177 gradient corresponding roughly to the Cu/Co gradient (Fig. 1). At the top, chasmophytic vegetation generally  
178 develops on poorly mineralised rocks (i.e., plant communities colonising the cracks and fissures of low  
179 mineralised rock with Cu concentrations of  $250\text{-}900 \text{ mg kg}^{-1}$ ). Steppe vegetation colonises the upper part of the  
180 outcrops with the highest Cu soil concentrations (ranging from  $3,500$  to  $10,000 \text{ mg kg}^{-1}$ ). Finally, steppic  
181 savannah vegetation develops on the intermediate and foothill slopes and flat periodically flooded savannahs  
182 (dembo) at the bottom of the outcrops with Cu concentrations varying from  $100$  to  $3,500 \text{ mg kg}^{-1}$  (Duvigneaud



183 and Denaeyer-De Smet 1963; Brooks et al. 1985; Saad et al. 2012; Séleck et al. 2013) (Fig. 1). However,  
184 vegetation proved to be more complex than a simple succession of physiognomic plant formations along  
185 correlated Cu and Co gradients. More detailed studies revealed a mosaic of plant communities differing in  
186 species assemblages within and among Cu-Co outcrops (Saad et al. 2012; Séleck et al. 2013; Ilunga wa Ilunga et  
187 al. 2013).

188 Although (extractable) Cu and Co soil concentrations are the main factors correlated to variation in species  
189 composition among plant communities on Cu-Co outcrops, the mosaic of plant communities is also related to  
190 complex variations of other inter-correlated chemical factors. For example, in addition to differences in Cu-Co  
191 concentrations, steppic communities present higher values for pH, C, N, and extractable Ca and P (acetate-  
192 EDTA) and lower values for the C:N ratio and extractable Fe and K concentrations compared to steppic  
193 savannah communities. Besides the potential direct effect on species assemblages, soil factors, such as pH, C, Ca  
194 and Mn, can also contribute indirectly to floristic variation and heterogeneity of plant communities in Cu  
195 outcrops (Saad et al. 2012; Ilunga wa Ilunga et al. 2013; Séleck et al. 2013) because they can influence Cu and  
196 Co availability and toxicity (Lange et al. 2014; Pourret et al. 2015). Cobalt has a high affinity for manganese  
197 oxides (MnOx). A higher MnOx concentration in soil can decrease Co availability and toxicity (Collins et al.  
198 2011). In the same way, high concentrations of iron oxides and organic matter in soils can reduce Cu availability  
199 (Kabala et al. 2001). Characterisation of metal speciation in soils also suggests a strong relationship between Cu  
200 and Co speciation in soils and structures of plant communities on a single Cu-Co outcrop (Fig. 3). A steppe  
201 community (Community 3 in Fig. 3) is associated with high concentrations of Cu and Co fractions that are  
202 considered available (i.e., Cu-Free, Cu-FeOx (Cu-iron oxides), Cu-MnOx, Co-Free, and Co-FeOx) (Lange et al.  
203 2014). A second steppe community (Community 2 in Fig. 3) presents the highest concentrations of unavailable  
204 Cu and Co fractions (i.e., Cu-OM and Co-MnOx) and less Cu-Co-tolerant species. Variation of Cu and Co  
205 chemical forms in soils may create spatial heterogeneity of soil properties that promotes a diversity of plant  
206 assemblages (Fig. 4). It may be hypothesised that the diversity of Cu and Co chemical forms in soil decreases  
207 metal availability and toxicity and promotes plant species diversity.

208 Nutrient content does not seem restrictive for the vegetation of Cu-Co outcrops; the amount of the essential  
209 macronutrients is higher on Cu-Co outcrops than on non-metalliferous soils in southeastern DRC (Saad et al.  
210 2012; Séleck et al. 2013). This differs from ultramafic soils where deficiencies in N, P, K, and Ca have been  
211 suggested as a potential reason for limited plant productivity (O'Dell et al. 2006; Whittaker 1954). It can be thus

212 inferred that selection pressure may be more strongly influenced by metal toxicity than by a high variation of  
213 nutrient content. However, the covariation of plant communities with nutrients, partly independent from metal  
214 availability (Seleck et al. 2013), suggests the need for a deeper exploration of the effect of N and P through the  
215 examination of the variation of the foliar N:P ratio and experimentation on P limitations along a Cu/Co gradient,  
216 where both the diversity and productivity of plant species should be measured (Aerts and Chapin 1999).

217 Variations of Cu and Co concentrations in soil also involve variations in trait responses of plant species. Delhaye  
218 et al. (unpublished results) showed that the gradient of soil metal concentrations is associated with a pattern of  
219 trait substitution rather than high intraspecific trait variation. At the community-level, trait variation results in a  
220 shift in the abundances of various life forms. Xylopod species (i.e., with underground storage organs) are  
221 dominant in the lower part of the Cu-Co outcrops in communities occurring on deeper soils that are less rich in  
222 metal, whereas annual species are dominant in the upper part of the Cu-Co outcrop in communities located on  
223 more shallow soils with high metal concentrations (Séleck et al. 2013).

224

## 225 **Implication of Plant-soil Relationships for Conservation and Restoration of Plant Biodiversity in Cu-Co** 226 **Outcrops in Southeastern DRC**

227 The first strategy from a biodiversity conservation point of view would include the preservation of a proportion  
228 of Co-Cu outcrops in protected areas. As demonstrated by endemism patterns at the landscape scale, the  
229 preservation of a limited number of Cu-Co outcrops occurring in southeastern DRC should allow the  
230 conservation of a set of populations of most Cu-Co endemic species. In contrast, the preservation of the diversity  
231 of plant communities in untouched protected Cu-Co outcrops will be far more challenging. The high diversity of  
232 plant communities as well as the variability of plant assemblages among outcrops would need to preserve a large  
233 proportion of Cu-Co outcrops if ecosystem diversity must be addressed. This may turn out to be a limited and  
234 economically unrealistic option, considering the increasing demand for Cu and Co in the world and the high  
235 contribution of the Cu-Co market to the economy of the DRC.

236 A valuable alternative to preserving the extraordinary biological heritage of Cu-Co outcrops is to set up, prior to  
237 ecological restoration and rehabilitation of sites at mine closure, an *ex-situ* conservation strategy at the level of  
238 plant communities with topsoil and community translocation and at the level of individual species with  
239 translocation of individuals in restored habitats, conservation and multiplication in botanical gardens, and long

240 term *ex-situ* seed banks for most characteristic species (<http://www.copperflora.org>; Conservation; Godefroid et  
241 al. 2013) (Fig. 5).

242 In this review, we have highlighted that extractable Cu-Co soil concentrations and other edaphic soil factors (i.e.,  
243 pH, C, N, Ca, Mn, and Fe) are the main drivers structuring plant communities, and their variation is partly  
244 responsible for the heterogeneity of communities observed at the scale of the Cu-Co outcrops. Alteration and/or  
245 modifications of edaphic conditions of Cu-Co outcrops therefore have important consequences on vegetation  
246 composition and structure. Plant communities of Cu-Co outcrops are poorly or not resilient to strong  
247 anthropogenic disturbances, such as mineral extraction, made either by artisanal miners or mining companies  
248 (Ilunga wa Ilunga et al. 2015). The comparison, in terms of species composition and/or abundance of functional  
249 traits, between primary plant communities occurring on Cu-Co outcrops and secondary plant communities  
250 resulting from the re-colonisation of disturbed areas (i.e., altered areas after mineral extraction with potentially  
251 deposition of mining waste) did not show any functional resilience of the primary communities, even after 30  
252 years of degradation by mining (Faucon et al. 2011; Ilunga wa Ilunga et al. 2015). Conservation or restoration of  
253 the soil factors is therefore necessary and crucial to conserve plant community on Cu-Co outcrops.

254 Plant community translocation projects through the transfer of topsoil and vegetation mats have been set up in  
255 southeastern DRC by Tenke Fungurume Mining, a mining company (Fig. 5). The initial results of these  
256 translocations demonstrate that vegetation mat translocation is the most efficient method to preserve the  
257 biodiversity of Cu-Co outcrops compared to topsoil transfer (Le Stradic et al. *In press*). In contrast to topsoil  
258 transfer, vegetation mat translocation allows transference of numerous species with fewer non-target species,  
259 probably due to a higher competition with the already established vegetation. For steppic savannahs, the seed  
260 bank is poor in species and seeds, and little emergence of target species (i.e., copper flora species) occurs in the  
261 topsoil, while ruderal species quickly colonise bare ground areas. Results are more encouraging for the steppe,  
262 given that high metal concentrations (i.e., Cu and Co) appear to limit the development of ruderal species, and a  
263 greater number of annual species provides a more rapid vegetation cover from the first year with some target  
264 species of copper flora (i.e., species present in the pristine Cu-Co communities), such as *Bulbostylis cupricola*  
265 Goetgh. or *Haumaniastrum robertii* (Robyns) P.A. Duvign. and Plancke. Steppic communities present shallow  
266 soils, favouring the transfer of the plant community without root damage. In contrast, vegetation mat  
267 translocation failed to transfer structuring xylopod species (i.e., dominant species) with important underground  
268 systems, such as *Cryptosepalum maraviense* (Fabaceae). The absence of xylopods in translocated ecosystems

269 may modify underground competition relationships within the community and promote the dominance of  
270 Poaceae species. While edaphic conditions are essential in order to restore Cu-Co communities properly, biotic  
271 filters and species interactions are also necessary to structure plant communities occurring on Cu-Co outcrops,  
272 and more research on this topic is necessary.

273 *Ex-situ* conservation of individual species also introduces great challenges. In this review, we have highlighted  
274 interspecific variations in realised ecological niches of Cu-Co endemics in relation to Cu and Co concentrations  
275 in soils but also other chemical and physical soil factors. This suggests that conservation strategies need to be  
276 species-specific and cannot be generalisable for all endemic metallophytes, particularly if the aim of the *ex-situ*  
277 conservation strategy is reintroducing and self-sustaining populations in restored habitats. In the short term,  
278 detailed studies are needed to characterise both the fundamental and realised niches of endemic metallophytes  
279 from Cu-Co outcrops (Schenk 2008). Characterisation of the fundamental niches of Cu-Co endemics (i.e., the  
280 physiological tolerance of a species in the absence of biotic interactions) will be crucial to developing *ex-situ*  
281 conservation and multiplication programmes in botanical gardens before reintroduction in restored habitats.

282 Although species conservation priorities are generally based on rarity and species extinction threats (IUCN  
283 criteria), conservation strategies for Cu-Co metallophytes should also integrate the conservation of genetic  
284 diversity within the region. Some genetic variation of adaptive traits exists between populations of the same  
285 species. Genetic variation between populations of Cu tolerance and accumulation has been demonstrated in two  
286 pseudo-metallophytes, *Crepidorhapon tenuis* and *Haumaniastrum katangense* (Faucon et al. 2012b; Peng et al.  
287 2012). This genetic variability of Cu tolerance and accumulation is an opportunity to select the most tolerant  
288 populations and define phytoremediation processes (phytoextraction or phytostabilisation).

## 289 **Conclusion**

290 This review highlights advances in knowledge of plant diversity of natural Cu-Co outcrops at different scales or  
291 ecological levels (flora, ecosystems, plant species communities, and plant populations) in order to provide  
292 guidelines and identify knowledge gaps to define biodiversity conservation programmes. In mining regions  
293 where there is an obvious conflict of interest between economically important mining activities and conservation  
294 of plant biodiversity of natural metalliferous outcrops, there is an urgent need to define science-based strategies  
295 for biodiversity conservation, including *in-situ* and *ex-situ* approaches, ecosystem reconstruction, and post-  
296 mining restoration (Fig 5). *Ex-situ* conservation prior to mining activities could be undertaken on available areas

297 adjacent to mineralised outcrops with Cu-Co rich soils or Cu-Co enriched soils. Plant diversity, especially  
298 metallophytes, even in *ex-situ* conservation areas, is a genuine resource for phytoremediation of degraded post-  
299 mining areas. Future challenges are to conciliate biodiversity conservation and ecological engineering for  
300 phytoremediation of Cu-Co contaminated soils generated by mining activities. This goal will be reached only if a  
301 strong cooperation between scientists, field conservationists, and mining companies is set up. Considering the  
302 urgency of the situation, scientific studies should be a full part of conservation strategies. In turn, learning from  
303 true conservation experience needs to be designed now (research by design) and will be a key method for  
304 increasing our scientific knowledge of this exceptional biological resource.

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313

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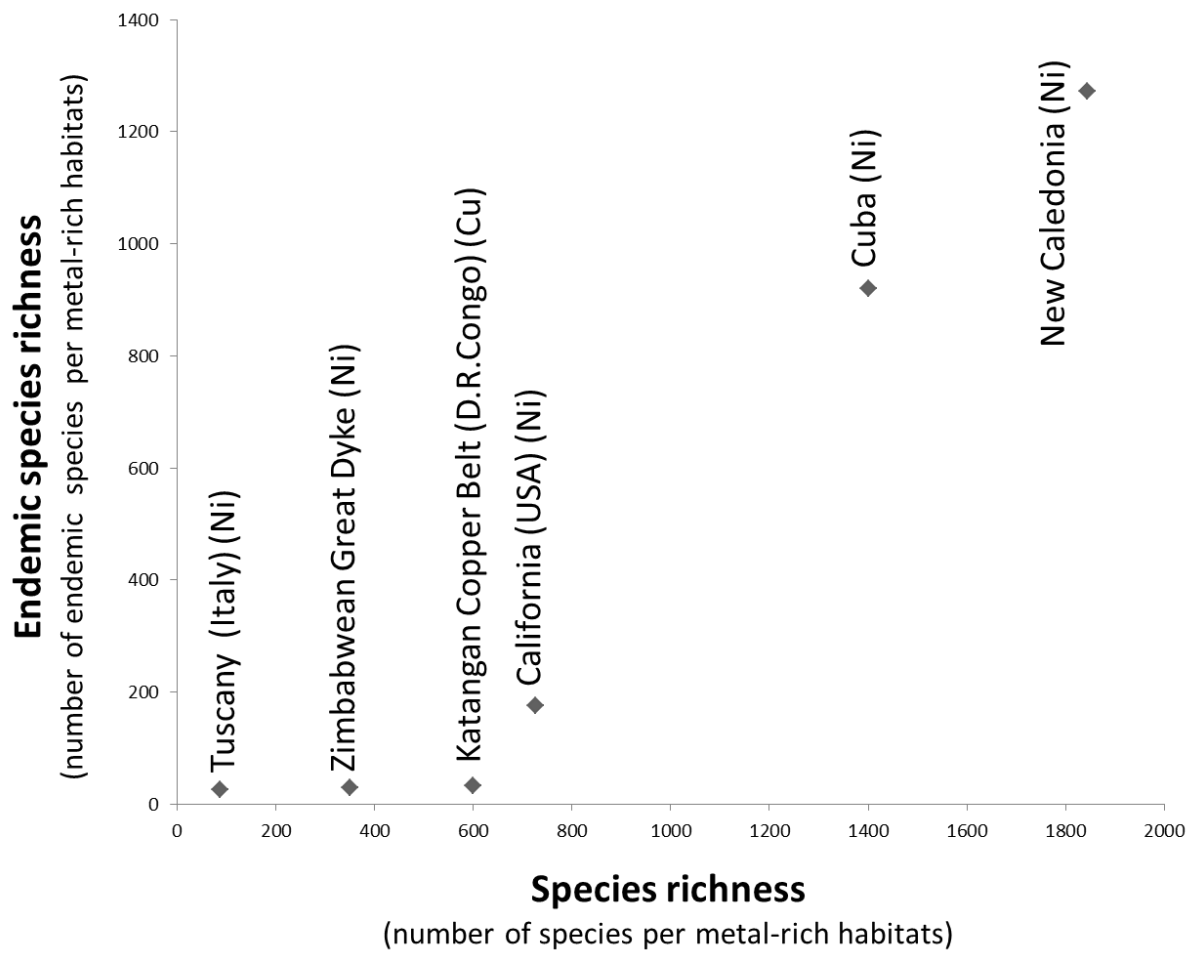




520 **Fig. 1** a) b) and c) General overview of copper and cobalt outcrops in Southeastern DRC (Democratic Republic  
521 of Congo), geographically isolated in the landscape matrix dominated by Miombo woodland on non-  
522 metalliferous soils; d) chasmophytic vegetation at the top of Cu-Co outcrops; e) et f) steppes on soils with high  
523 Cu-Co content; g) steppic savannah on the slope and h) steppic savannah on downslope with lower Cu-Co  
524 content.

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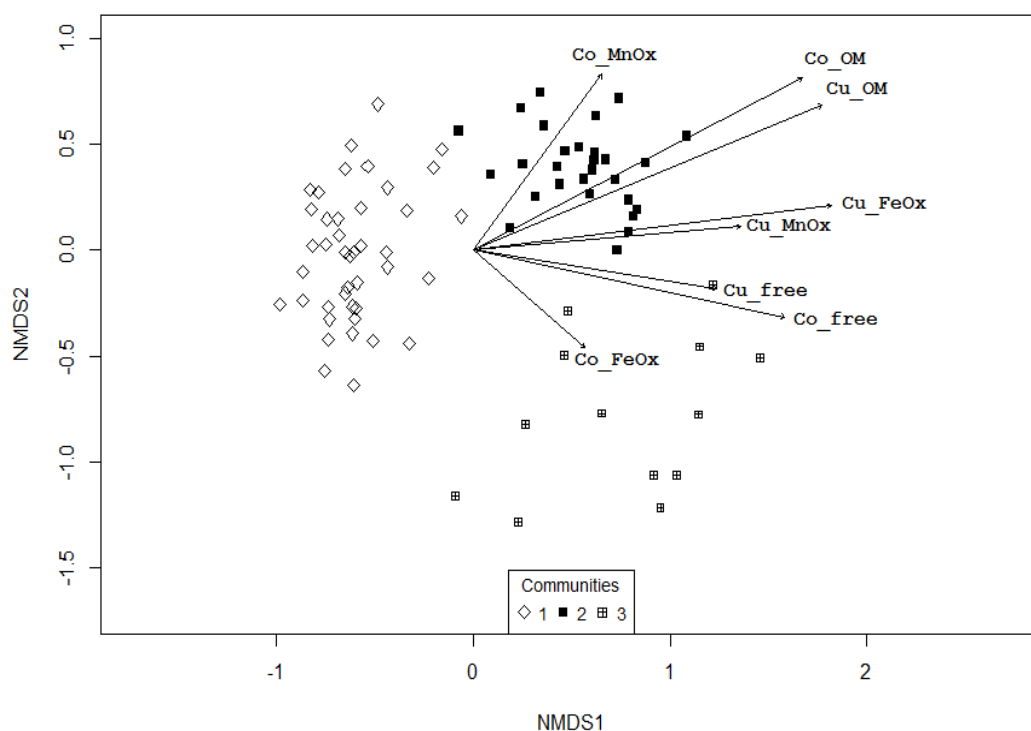




526

527 **Fig. 2** Relationship between total plant species richness and metallophyte endemic richness on natural metal rich  
 528 soils in six metal world regions. Data are from Faucon et al. 2010; Wild 1965; Jaffré 1992; Borhidi 1996;  
 529 Kruckeberg 1984; Selvi 2007.

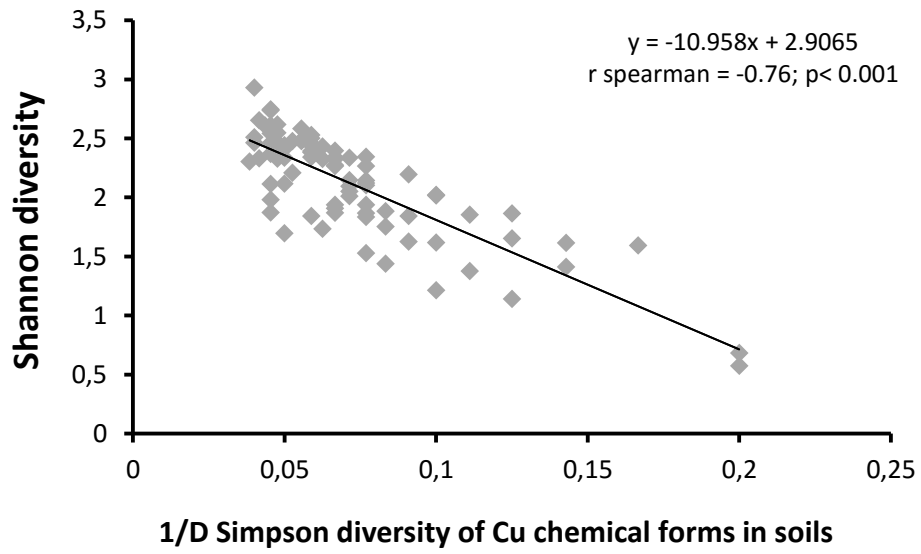
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532 **Fig. 3** NonMetrical Multidimensional scaling (NMDS) ordination diagram of plots (n=83) distributed in three  
 533 different communities on a Cu-Co outcrop called Fungurume V. Fitted copper and cobalt fractions are  
 534 overlain by using the envfit command of vegan package (R statistical software, Oksanen et al. 2011). MnOx:  
 535 manganese oxides. FeOx: iron oxides. Community 1: Steppic savannah with *Cryptosepalum maraviense*  
 536 (*Caesalpiniaceae*); *Loudetia simplex* (*Poaceae*) et *Scleria bulbifera* (*Cyperaceae*) (Figure 1 photo g and h).  
 537 Community 2: Steppe with *Hyparrhenia diplandra* (*Poaceae*); *Schizachyrium brevifolium* (*Poaceae*); *Justicia*  
 538 *elegantula* (*Acanthaceae*), *Michrochloa altera* (*Poaceae*); *Xerophyta equisetoides* (*Velloziaceae*) and *Bulbostylis*  
 539 *cupricola* (*Cyperaceae*) (Figure 1 photo f). Community 3: Steppe with *Pandiaka carsonii* (*Amaranthaceae*);  
 540 *Ascolepis metallorum* (*Cyperaceae*); *Anisopappus davyi* (*Asteraceae*) (Fig. 1).

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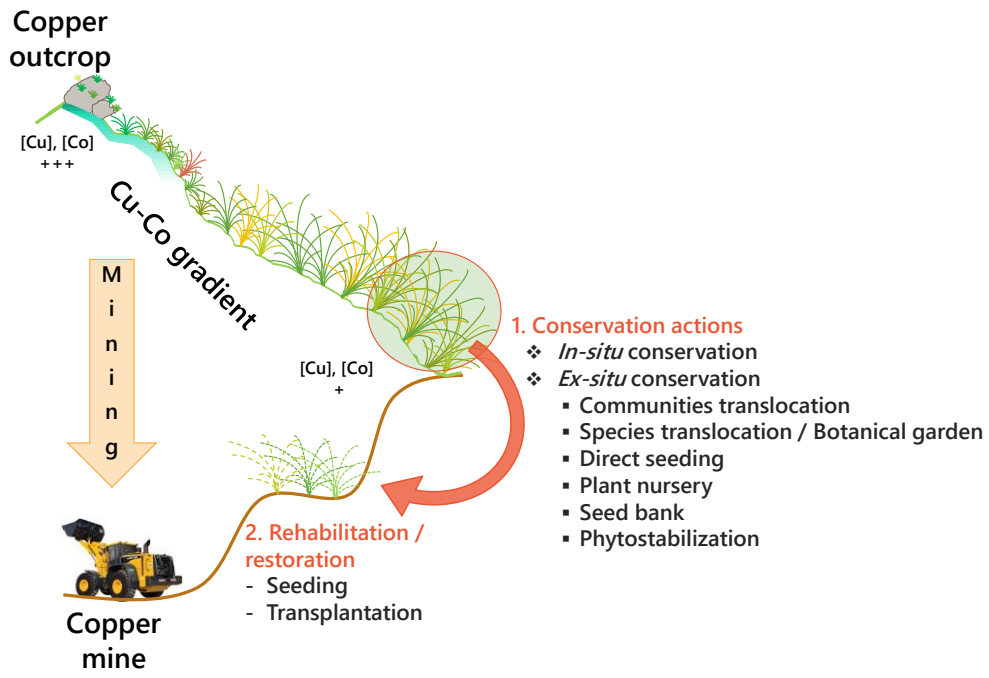
542

543 **Fig. 4** Relationship between 1/D Simpson diversity ( $D = \text{Simpson's dominance index}$ ) of copper chemical forms  
 544 in soils and Cu Shannon diversity of higher plant species ( $n=83$ ). Copper fractions in soils were modeled by  
 545 speciation modeling (WHAM 6) from method of Pourret et al. 2015.

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**Fig. 5** Global strategy of biodiversity of natural Cu and Co outcrops in a mining region