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1 **Copper tolerance and accumulation in two cuprophytes of South Central Africa:**
2 ***Crepidorhopalon perennis* and *C. tenuis* (Linderniaceae)**

3

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22

23 **ABSTRACT**

24 *Crepidorrhopalon perennis* is an endemic metallophyte restricted to only one site on Cu-rich
25 soils in Katanga. *Crepidorrhopalon tenuis* has a broader niche, from normal to Cu-rich soils,
26 but has high affinity for cupriferous habitats. Both plants have been considered as Cu-Co
27 accumulators. Cu tolerance and accumulation of *C. tenuis* were studied in axenic conditions
28 *in vitro* in four metallicolous populations and one non-metallicolous population whereas for
29 *C. perennis* only one population was investigated. Results showed a Cu tolerance of both *C.*
30 *tenuis* and *C. perennis*. Variation of tolerance among metallicolous and non-metallicolous
31 populations was also observed. The addition of Cu enhanced the growth of some
32 metallicolous populations under sterile conditions, hence confirming the high needs in copper
33 of metallicolous populations. This could represent a cost of tolerance which would explain the
34 high affinity of species for cupriferous habitats. On the other hand, *Crepidorrhopalon perennis*
35 did not show the same features. Its restricted distribution may be explained by a cost of Cu
36 tolerance. *Crepidorrhopalon perennis* and *C. tenuis* are not Cu hyperaccumulators and seem to
37 behave rather like excluder species.

38

39 **Key words:** copper tolerance, trace metals, copper accumulation, cost of tolerance

40

41 **Introduction**

42 Soil contamination by trace metals is a major environmental issue throughout the world
43 (Alloway, 1995; Smith and Huyck, 1999; Baize and Tercé, 2002) in the last century as a
44 consequence of the intensification of mining and mineral processing, which dispersed toxic
45 trace metals leading to large contaminated areas. In this context, new types of metalliferous
46 habitats, which did not exist or were rare before the industrial revolution, appeared on the
47 Earth (Allen and Sheppard, 1971; Ginocchio et al., 2002). The soils of such habitats have
48 concentrations of trace metals up to 1000 times higher than the “normal” soils, which hence
49 gives them a high phytotoxicity (Ernst, 1974; Baker et al., 2000; Reeves and Baker, 2000).
50 Only species that have adapted to these extreme soil conditions can grow in such

51 contaminated habitats. The trace metals tolerance of plants is nowadays a research topic
52 dealing mainly with the processes of adaptation to very restrictive environmental factors
53 (Antonovics et al., 1971). Copper and zinc tolerance has been largely studied in some
54 pseudometallophytes, hence contributing to a better understanding in terms of genetics and
55 evolution of trace metals tolerance of such plants. Several studies on copper tolerance have
56 highlighted the intensity of selection due to toxicity of trace metals (McNeilly and Bradshaw,
57 1968; Wu et al., 1975), heritability of tolerance (Macnair, 1981, 1983; Schat and Ten
58 Bookum, 1992; Schat et al., 1993), cost of copper tolerance (Macnair et al., 2000),
59 reproductive isolation (Macnair and Christie, 1983; Macnair and Gardner, 1998), and
60 speciation (Macnair and Gardner, 1998).

61 Copper tolerance is little known particularly in species that grow only or show a
62 frequency and/or high abundance on copper soils (Brooks and Malaisse, 1990; Harper et al.
63 1997; 1998), i.e. the cuprophytes (Duvigneaud and Denaeyer-De Smet, 1963; Ernst, 1974,
64 1990). Tolerance to extreme Cu concentrations in soils (1-50 g kg⁻¹) was demonstrated
65 experimentally only for a few cuprophytes such as *Haumaniastrum katangense*, *H. robertii*,
66 *Aeolanthus biformifolius* (Lamiaceae) (Morrison et al., 1979; Chipeng et al., 2010; Peng et al.,
67 in press); *Mimulus guttatus* (Scrophulariaceae) (Allen and Sheppard, 1971; Macnair, 1983);
68 *Silene cobalticola* (Caryophyllaceae) (Baker et al., 1983) and *Elsholtzia haichowensis* (Lou et
69 al., 2004). The limited number of experimental studies in these cuprophytes, under the
70 intrinsic value of biological material, may be partly explained by difficulty of cultivation in
71 soils with low Cu concentrations (Malaisse and Brooks, 1982; Paton and Brooks, 1996). In
72 fact, cuprophytes are known almost exclusively on copper soils of southern central Africa
73 (Duvigneaud and Denaeyer-De Smet, 1963; Brooks and Malaisse, 1985). Among these,
74 *Crepidorrhopalon tenuis* (S. Moore) Eb. Fisch. (syn.: *Lindernia damblonii* P.A. Duvign. and *L.*
75 *tenuis* S. Moore) (Linderniaceae) grows on soils with a wide range of Cu concentrations,
76 including occasionally non-metalliferous soils (Faucon et al., 2009, 2011a). In contrast,
77 *Crepidorrhopalon perennis* (P.A. Duvign.) Eb. Fisch. (syn.: *Lindernia perennis* (P.A. Duvign.)
78 is endemic to a single metalliferous site (Faucon et al., 2010) and occupies a narrow niche,
79 growing on soils with extremely high concentrations of Cu and Co (Faucon et al., 2011a;
80 Faucon et al., in press). In this context, the *C. perennis*-*C. tenuis* species pair appears to be a
81 very promising model to investigate the relationship between the degree of tolerance of plant

82 populations, copper bioavailable content of sites and the ecological isolation of an endemic
83 species.

84 In *Crepidorhodon perennis* and *C. tenuis*, genuine cuprophily was assessed by
85 examining the relationship between the concentration of copper in soil and plant biomass
86 (Faucon et al., 2009, 2011a). It appeared that copper-tolerant populations showed lower
87 fitness on non-metalliferous soil than non-tolerant populations and *vice versa* on copper soil.
88 It seems that needs in essential metals are higher for tolerant populations than for non-tolerant
89 plants (Macnair et al., 2000). Strict endemism on copper-rich soils could be explained by a
90 “cost of copper tolerance” (Harper et al., 1997; Macnair et al., 2000; Tadros, 1957; Kazakou
91 et al., 2008). However, the existence of a cost of copper tolerance was demonstrated only in
92 *Mimulus guttatus* and remains highly controversial. Another hypothesis is that cuprophytes
93 are very sensitive to pathogens of soil (Malaisse and Brooks, 1982; Paton and Brooks, 1996;
94 Chipeng et al., 2010).

95 Thirty-two cuprophytes apparently have the capacity to accumulate copper in their top growth
96 at concentrations above the threshold of Cu hyperaccumulation ($>1000 \text{ mg kg}^{-1}$, Baker and
97 Brooks, 1989) (Reeves and Baker, 2000; Reeves, 2006). Nevertheless, There has been
98 extensive discussion of the reliability of earlier data on Cu and Co accumulation, the wide
99 variation of these element concentrations in a number of the African species, and the effects
100 of contamination by dusts of secondary minerals of elements such as Cu, e.g. in Reeves and
101 Baker (2000). The validity of these concerns was demonstrated in the work of Faucon et al.
102 (2007). Macnair (2000) states that Cu hyperaccumulation has not yet been observed in
103 culture, but Küpper et al. (2009) found that *Crassula helmsii* could accumulate more 9000 mg
104 kg^{-1} in its shoot at $10 \mu\text{M}$ ($=0.6 \text{ ppm}$) Cu^{2+} in the nutrient solution. A large inter and intra-
105 specific variation of foliar concentrations of Cu was observed in the supposed
106 hyperaccumulators (Malaisse and Grégoire, 1978; Brooks et al., 1987; Malaisse et al., 1979,
107 1994, 1999; Paton and Brooks, 1996; Faucon et al., 2007; Faucon et al., 2009). In
108 *Crepidorhodon perennis* and *C. tenuis*, the range of Cu concentrations in leaf was
109 respectively, 500 to 1500 mg kg^{-1} and 100 to 980 mg kg^{-1} (Morrison, 1980; Faucon et al.,
110 2007; Faucon et al., 2009).

111 In this paper, we examine the tolerance and accumulation of copper in controlled
112 conditions *in vitro* of four metalicolous populations and one non-metallicollous of *C. tenuis*

113 and in the single population of *C. perennis*. To our knowledge, this is the first study
114 comparing the Cu tolerance among metallicolous and non-metallicolous in cuprophytes from
115 Katanga (Democratic Republic of Congo). We specifically address the following questions:(i)
116 is there inter-population variation in the ability to tolerate Cu in *C. tenuis*? (ii) do both
117 cuprophytes show an increase in their performance in response to high Cu concentrations?
118 (iii) can the restricted endemism of *C. perennis* be explained by a cost of tolerance? and (iv)
119 are *C. perennis* et *C. tenuis* genuine Cu hyperaccumulators and is there any inter-population
120 variation of the ability to accumulate Cu in *C. tenuis*?

121

122

123

124 **Material and methods**

125

126 *Site harvest and plant material*

127

128 Seeds were harvested in six populations in Upper-Katanga (Dem. Rep. of Congo) in April
129 2008 (Table 1) and a pool of representative seeds of the morphological variability of each
130 population was established. Seeds of *Crepidorhopalon perennis* (CP) were collected in its
131 single population located at the Mine de l'Etoile, 10 km northeast of Lubumbashi. This
132 endemic cuprophyte is a pioneer species of mining debris or reworked mining substrates and
133 grows on soils having concentrations of Cu higher than the habitats occupied by the
134 populations of *C. tenuis* (CTC) (range of Cu, for CP = 2161-55300 mg kg⁻¹, CTC=201-12557
135 mg kg⁻¹) (Faucon et al. 2009, 2011a). *Crepidorhopalon tenuis* has a much broader
136 geographical range, sometimes occurring on nonmetalliferous soils (Brooks and Malaisse,
137 1985; Fischer, 1999). *Crepidorhopalon tenuis* has its ecological optimum in Cu and Co rich
138 soils contaminated by metallic atmospheric fallout from ore-smelter or substrate (often mine
139 debris) disturbed and reworked by mining activities (Faucon et al., 2011a). The non-
140 metallicolous population considered in this study is one of the few known in the plant
141 communities of woodland clearings (miombo) on laterite soil in Katanga.

142

143 *Method of culture*

144

145 *In vitro* culture was performed in a growth chamber at 20°C on Murashige and Skoog (MS)
146 medium (Murashige and Skoog, 1962). MS medium contained EDTA that can reduce
147 bioavailability of copper (Christensen et al., 2008). Seeds were immersed in 70% ethanol for
148 5 min, followed by a 10 min soak in solution of 5% bleach and 0.1% SDS (sodium dodecyl
149 sulfate). After three successive washes with sterile ultrapure water, seeds (approximately 30
150 per dish) were sown on 10 mL of MS spiked with CuSO₄ at a concentration of 60 µmol L⁻¹
151 (this concentration was chosen based on the results of Lequeux et al. (2010) and Chipeng et
152 al. (2010). A control test (no added CuSO₄) at 0.1 µmol L⁻¹ CuSO₄ was also processed in
153 parallel. After one month of incubation, 12 individuals per population were transplanted into
154 pots of 50 mL of MS, six without added CuSO₄ and six containing 60 µmol L⁻¹ CuSO₄ (n total
155 individuals = 72). After eight weeks, the dry mass of shoot plants was measured. The biomass
156 was used as a rough estimate of fitness because it was correlated with the number of seeds (r
157 = 0.78, n = 123 ind. x three fruits, P < 0.05).

158

159 *Mineral analysis*

160 After eight weeks of pot culture, plants were harvested, washed with 1% Alconox
161 solution (Alconox Inc.) during 2 min, rinsed with demineralised water and then dried at 60°C
162 for 48 h (Faucon et al., 2007). The dried samples were then mineralised and solubilised in a
163 Tecator digester using a mixture of nitric and perchloric acid (1:1). Cu concentration was
164 measured by flame atomic absorption spectrometry FAAS (Varian 220).

165

166 *Statistical analysis*

167 ANOVA (analysis of variance) provided by Statistica 8 (Statsoft 2008) was used for
168 the statistical analysis. For the biomass analysis, ANOVA with two crossed factors:
169 « treatments » and « populations » (fixed factors) was used. For the analysis of the variation
170 of Cu concentration in plants one way ANOVA was performed. Significant differences in
171 mean biomass and Cu concentration among populations and treatments were tested by post-
172 hoc multiple comparison (Fisher's Least Significant Difference).

173

174

175

176

177 **Results**

178

179 *Biomass variation among populations of C. tenuis in response to Cu*

180 ANOVA showed significant effects of populations and Cu concentrations as well as for
181 population*treatment (Table 2). The response to copper varied greatly between populations.
182 Growth was better at 60 $\mu\text{mol L}^{-1}$ of CuSO_4 for metallicolous populations VK, Ru, Nm and
183 QG. On the contrary, the best growth was found in the medium without added CuSO_4 for the
184 non-metallicolous population Ky. At 60 $\mu\text{mol L}^{-1}$ of CuSO_4 , Ky had half that of the control
185 ($F_{1,47} = 13.25$; $P > 0.001$) (Figure 1). In addition, chlorosis was present for 5 individuals of Ky
186 on the CuSO_4 enriched medium. No significant differences were observed between
187 metallicolous populations when growing in the medium without added CuSO_4 . At 60 $\mu\text{mol L}^{-1}$
188 of CuSO_4 , significant difference of biomass existed between populations ($F_{5,21} = 8.8$;
189 $P > 0.001$) (Figure 1). Biomass of populations changed depending on the Cu concentration in
190 the medium. When medium without added CuSO_4 was used, $\text{VK} > \text{Ky} > \text{Nm} > \text{QG} > \text{Ru}$ whereas
191 on CuSO_4 rich medium, $\text{Ru} > \text{Nm} > \text{VK} > \text{QG} > \text{Ky}$. An unexpected result was the large decrease
192 of biomass in the non-metallicolous population Ky grown on CuSO_4 rich medium. In contrast,
193 three out of four metallicolous populations showed a significant increase in biomass in
194 response to Cu (Figure 1).

195

196 *Reaction norms of biomass in response to Cu in C. perennis*

197 *Crepidiorhpalon perennis* did not show any significant difference in biomass on medium
198 without added CuSO_4 (mean = 13.8 mg, SD ± 9.8) and CuSO_4 enriched medium (mean = 26.2
199 mg, SD ± 6.3). On medium without added CuSO_4 , *C. perennis* did not show any difference in
200 biomass compared to the non-metallicolous population of *C. tenuis* Ky (mean = 27 mg, SD
201 ± 20.5) ($F_{5,26} = 2.1$; ns).

202

203 *Variation of Cu concentration in shoot*

204 A significant difference in Cu concentration in plant shoots was shown among populations
205 ($F_{5,17} = 7.8$ ($P < 0.001$)) (Figure 2). The non-metallicolous population Ky showed the highest
206 value (mean = 115 $\mu\text{g mg}^{-1}$, SD ± 17.6), followed by Ru (mean = 80 $\mu\text{g mg}^{-1}$, SD ± 34) (Figure
207 2) whereas *C. perennis* showed Cu concentration below 40 $\mu\text{g mg}^{-1}$.

208

209

210 **Discussion**

211

212 *Between population variation of copper tolerance*

213 *Crepidorrhopalon perennis* and all populations of *C. tenuis* from metal rich soil grew as well
214 or better in the CuSO₄ enriched medium compared to the control treatment, indicating that
215 they are highly tolerant to copper. In the same experimental conditions, growth of non tolerant
216 species was negatively affected at concentrations as low as 5 μmol L⁻¹ of CuSO₄ e.g. in
217 *Nicotiana plumbaginifolia* (Chipeng et al., 2010) and *Arabidopsis thaliana* (Lequeux et al.,
218 2010). Conversely, the population from normal soil (Kyembe) is significantly less tolerant,
219 and strongly affected by 60 μmol L⁻¹ of CuSO₄. This suggests that *C. tenuis* is not
220 constitutively tolerant to copper. Instead, the broad ecological amplitude of the species in
221 Katanga may be due to its genetic variation of copper tolerance. The results suggest that metal
222 tolerance in population from metal-rich soil has evolved in response to natural selection. This
223 pattern of variation has been found in a number of pseudometallophytes, in which copper-
224 tolerant ecotypes have been reported (Gregory and Bradshaw, 1965; Antonovics et al., 1971;
225 Kruckeberg and Wu, 1992; Gonnelli et al., 2001; Ginocchio et al., 2002; Nicholls and
226 McNeilly, 1985; Nordal et al., 1999; Macnair et al., 2000). Our results represent the first
227 confirmation of the existence of such ecotypes in metallophytes from Katanga. However,
228 local adaptation remains to be formally confirmed by reciprocal transplantation.

229 However, variation in tolerance among populations from metal-rich soil does not appear to be
230 correlated to copper concentration in their native soil (Table 3). Thus, Ru population, with the
231 highest degree of tolerance, originates from the site with the lowest Cu concentration in the
232 soil. However, copper bioavailability and toxicity is strongly influenced by physical and
233 chemical properties of soil, including pH and Mn concentration (e.g. Faucon et al., 2009,
234 2011b). Therefore, other elements in the soil may mitigate Cu toxicity and relax selection
235 pressure for enhanced tolerance. Other factors can also explain variation in tolerance among
236 populations, including population age (Wu et al., 1975). *Crepidorrhopalon tenuis* appears to
237 have strongly expanded its range in Katanga in response to mining activities and many
238 populations may be of relatively recent origin and not yet in equilibrium with local soil
239 conditions.

240

241 *Cost of copper tolerance and growth stimulation*

242 An interesting result is the enhanced growth at 60 $\mu\text{mol L}^{-1}$ of CuSO_4 compared to the
243 control, which was observed in 4 out of 5 populations from metal-rich soil. This finding is in
244 agreement with the field observations indicating that plant size and population density are
245 much larger on metal rich soil compared to normal soil (Faucon et al., 2009, 2011a). Because
246 the plants studied here have been grown in axenic conditions, a protective effect against
247 pathogens cannot be invoked, which indicates that *C. tenuis* and *C. perennis* actually have
248 elevated requirements of Cu. Growth stimulation by elevated concentrations of Cu has only
249 rarely been reported, for example in the copper-moss *Scopelophila cataractae* (Shaw, 1994),
250 the Katangan cuprophyte (*Haumaniastrum katangense* (Chipeng et al., 2010) and a couple of
251 *Elsholtzia* species in China (Lou et al., 2004; Jiang et al., 2008).

252 These results can also be interpreted as evidences for cost of metal tolerance, i.e. on “normal”
253 medium, performance of Cu-tolerant populations is lower than the non-tolerant population in
254 *C. tenuis*. Such cost has only been demonstrated in experimental conditions in few species,
255 e.g. *Mimulus guttatus* for the Cu tolerance (Allen and Sheppard, 1971; Macnair et al., 2000).
256 Other works failed to reveal such fitness costs (e.g. Nicholls and McNeilly, 1985; Dechamps
257 et al., 2006).

258

259 *Crepidorrhodon perennis, an absolute metallophyte*

260 Absolute metallophytes, i.e. species that are currently known only from metal rich soil, may
261 not be able to grow on normal soil (Tadros, 1957). Based on this assumption, the elevated
262 cost of tolerance would account for the restricted niche. The cost of tolerance means that
263 metal-tolerant populations show lower fitness on non-metalliferous soil than non-tolerant
264 populations and *vice versa* on copper soil. It seems that essential needs in metals are more
265 important for tolerant populations than for non-tolerant plants (Macnair et al., 2000). Our
266 failure to cultivate *C. perennis* on normal soil, in spite of repeated trials, indicates that such
267 cost might indeed be involved (Faucon, 2009). However, in this work in axenic conditions, *C.*
268 *perennis* did not grow significantly less well in normal soil. This result does not support the
269 hypothesis of a high cost of tolerance in this species and hence it should be taken with

270 caution. Firstly, the sample size was relatively low (n= 6 individuals per population and per
271 treatment); secondly, there was only one contaminated treatment in this study and it is not
272 impossible that the physiological optimum of *C. perennis* is actually higher than the
273 concentration of Cu ($60 \mu\text{mol L}^{-1}$). Furthermore, apart from copper, tolerance of other metals
274 might be involved, most notably of cobalt, which is present at high concentration at the Mine
275 de l'Etoile, the native site of *C. perennis*. Limited dispersal ability is unlikely, as newly
276 formed malachite deposits at the Mine de l'Etoile are readily colonised by this species.
277 *Crepidorhopalon* species have tiny seeds, which are probably easily transported by the wind.
278 Finally, the narrow realised niche may be related to limiting biotic factors. Cuprophytes
279 appear specifically to be susceptible to soil pathogenic fungi (Malaisse and Brooks, 1982;
280 Paton and Brooks, 1996; Chipeng et al., 2010), which may be explained by the relaxed
281 pressure of pathogenic fungi on metal-rich soil (Tadros, 1957).

282

283 *Copper accumulation or exclusion?*

284 *Crepidorhopalon perennis* and *C. tenuis* have been reported as Cu/Co hyperaccumulators
285 (Duvigneaud, 1958; Malaisse and Grégoire, 1978; Brooks et al., 1987). In this study, the
286 copper concentrations in shoot were always below the conventional hyperaccumulation
287 threshold (1000 mg kg^{-1}). This result is in agreement with other studies in which cuprophytes
288 did not show hyperaccumulation when cultivated in controlled conditions (Morrison et al.,
289 1979, 1981; Chipeng et al., 2010; Peng et al., in press). They suggest that these species are
290 excluders rather than hyperaccumulators. The high foliar concentrations of Cu found in leaves
291 collected in the field (Duvigneaud, 1958; Malaisse and Grégoire, 1978; Brooks et al., 1987)
292 may in part be due to surface contamination by dust (Faucon et al., 2007). However, foliar
293 concentrations in this study are much lower than those found in carefully cleaned samples on
294 the field (Faucon et al., 2007, 2009) (Table 3), which indicates that copper accumulation may
295 be expressed only under specific conditions that are difficult to mimic in cultivation as in this
296 study. Variation of copper concentrations in shoots of these species should be studied in
297 controlled conditions with Cu contaminated substrate considering physical conditions and
298 fertility relatives of soils where these species grow.

299 We also have shown that competition with Co and Mn strongly influences the patterns of Cu
300 accumulation (Faucon et al., 2009), interactions with microorganisms might also be involved

301 (Fomina et al., 2005; Toler et al., 2005) and explain the absence of high Cu concentrations in
302 shoot in these culture conditions. The pH is likely to be the most important physicochemical
303 parameter controlling metal fractionation and mobility. Even though the stability of colloids
304 (i.e. either organic or inorganic, which are microscopic metals adsorbent phases and therefore
305 govern, via their mobilization in soils, their fate and/or bioavailability) according to pH is
306 well documented, few studies were dedicated to the understanding of colloids and associated
307 metal release from soils and water with regard to pH change and thus mobility/bioavailability
308 leading to accumulation in plants (e.g. Pourret et al., 2010). In the future, it would be of
309 interest to examine concentration of bioavailable Cu in relation with Cu accumulation in
310 plants to understand the high Cu concentrations and their variation in *C. tenuis* and *C.*
311 *perennis* observed *in natura* (Table 3).

312 Even though copper concentrations in leaves are low, they appear to be related to tolerance;
313 the non tolerant population of *C. tenuis* (Kyembe) has the highest accumulation in leaves.
314 This may indicate that elevated tolerance in populations from metal-rich soils is achieved
315 through enhanced capacity to restrict Cu uptake and/or translocation to shoot. Similar results
316 have been obtained in copper tolerant ecotypes of other species (Kruckeberg and Wu, 1992;
317 Schat et al., 1993; Ouzounidou et al., 1994; Gonnelli et al., 2001; Weng et al., 2005, but see
318 Macnair, 1981)

319

320

321 **Conclusion**

322

323 *Crepidorrhopalon perennis* and *C. tenuis*, two species with high affinity for Cu-rich soil in
324 Katanga, have tolerance to Cu, which is probably associated to a copper exclusion strategy.
325 The pattern of variation in tolerance in *C. tenuis*, suggests that populations from metal rich
326 soil have been subjected to directional selection for increased tolerance. Based on
327 experimental data, we can conclude that *C. tenuis* and *C. perennis*, together with
328 *Haumaniastrum katangense* can be considered as promising study models for the evolution of
329 copper tolerance in the flora of SC Africa.

330

331

332

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334

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342

343

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530 **Table 1 Location and habitats description of studied populations.** CP = *Crepidorhopalon*
531 *perennis*; CTC = *C. tenuis* from copper soil and CTN = *C. tenuis* from normal soil.

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533 **Table 2 Biomass variation of *C. tenuis* populations (four metallicolous populations and**
534 **one non-metallicolous) and single population of *C. perennis* on *in vitro* culture with two**
535 **treatments (0 and 60 $\mu\text{mol L}^{-1}$ of CuSO_4)**

536

537 **Table 3 Copper and cobalt levels of plants and soils *in natura* in *Crepidorhopalon tenuis***
538 **from normal soil (CTN), *C. tenuis* from copper soils (CTC) and *C. perennis* (CP)**
539 **(Faucon et al. 2009) (mean and min-max). Cu and Co concentrations in soil with**
540 **ammonium acetate -EDTA 1M (pH 4.65) extraction.**

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555 **Table 1**

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Sites	Type	Habitat description	Altitude (m)	Co-ordinates
Etoile (E)	CP	Natural copper hill; substrate locally disturbed by mining	1280	S11,63562° E27,58448°
Niamumenda (Nm)	CTC	Natural copper hill; substrate locally disturbed by mining	1340	S11,60492° E27,29400°
Ruashi (Ru)	CTC	Anthropic site: recolonization of mine deposits	1300	S11,62645° E27,56328°
Quartier Gécamines (QG)	CTC	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter, moist environment	1220	S11,70760° E27,42985°
Vallée Karavia (VK)	CTC	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter, moist environment	1230	S11,67270° E27,43091°
Kyembe (Ky)	CTN	Natural site: forest clearing (miombo) on lateritic gravel	1190	S 11,11269° E 27,25825°

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Table 2

Sites	Df	F	P
Population	5	3.1	P<0.05
Treatment	1	13.3	P<0.001
Treatment*population	5	6.4	P<0.001
Error	47		

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Table 3

Sites	Populations	Co $\mu\text{g g}^{-1}$ soil	Co $\mu\text{g g}^{-1}$ plant	Cu $\mu\text{g g}^{-1}$ soil	Cu $\mu\text{g g}^{-1}$ plant
E	CP	275 (98-572)	397 (61-1105)	11278 (2161-55300)	394 (80-1380)
Nm	CTC	23 (2-98)	19 (8 – 58)	3428 (382-18144)	660 (84-2524)
Ru	CTC	54 (9-278)	130 (24-605)	991 (71-5869)	190 (59-412)
QG	CTC	6 (0,8-16)	47 (24-99)	1082 (224-2727)	100 (41-338)
VK	CTC	20 (7-44)	58 (14-176)	3544 (362-8189)	100 (34-211)
Ky	CTN	< 1	< 5	1.6 (0.8-2.1)	22 (8-93)

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590 **Figure 1 Biomass of metallicolous populations (Nm, QG, VK and Ru) and non-**
591 **metallicolous (Ky) in *Crepidorhopalon tenuis* and in *C. perennis* (CP) at 0 et 60 $\mu\text{mol L}^{-1}$**
592 **of CuSO_4 .** N = 6 per populations and treatment; error bars = standard deviation. For each
593 population at 60 $\mu\text{mol L}^{-1}$ of CuSO_4 , means with the same letters are not significant (LSD
594 Fisher test). The two treatments per population were compared One way anova *** =
595 $P < 0.001$; ** = $P < 0.01$; * $P < 0.05$ between the treatments for the same population.

596

597 **Figure 2 Copper concentrations in plant shoot of metallicolous populations (Nm, QG,**
598 **VK and Ru) and non-metallicolous (Ky) in *Crepidorhopalon tenuis* and in *C. perennis***
599 **(CP) on growing medium enriched at 60 $\mu\text{mol L}^{-1}$ of CuSO_4 .** N = 6 per population ; error
600 bars = standard deviation. The means with the same letters are not significant (LSD Fisher
601 test).

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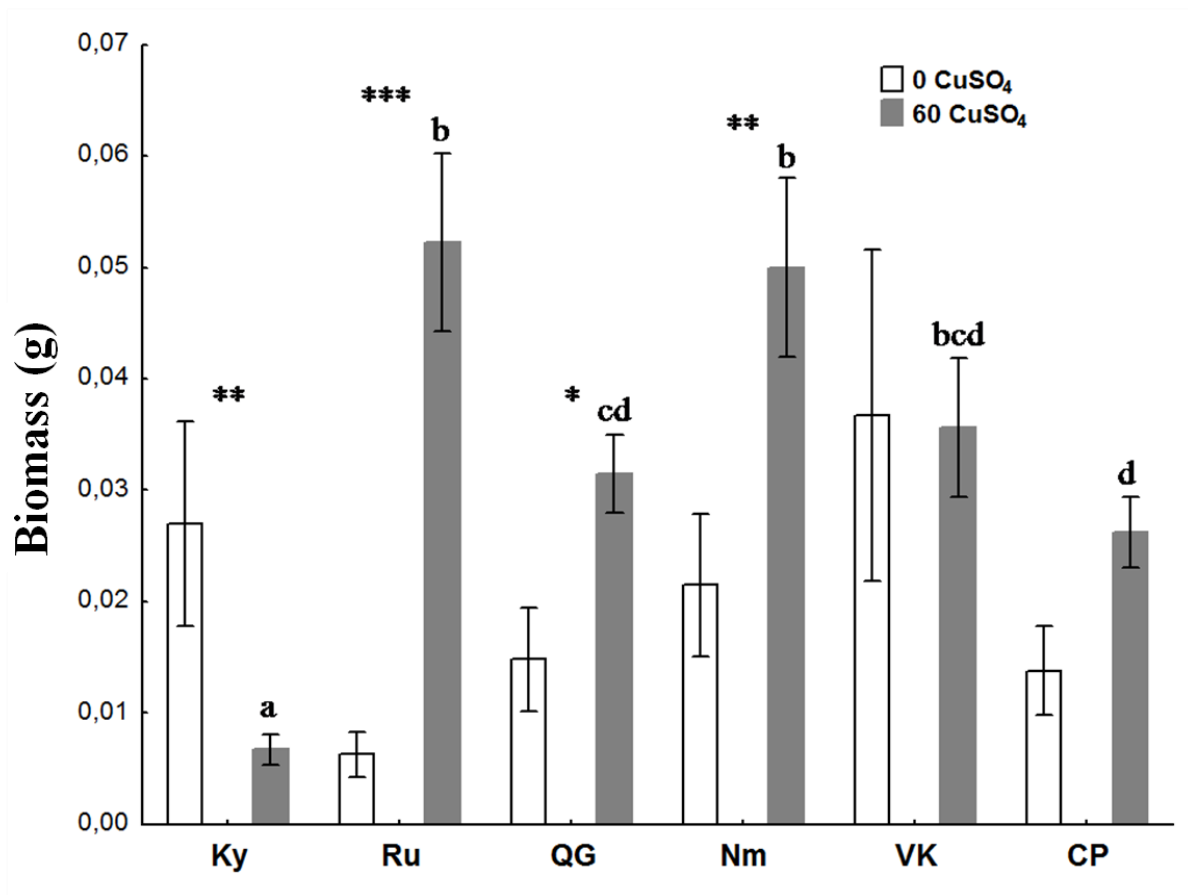
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616 **Figure 1**

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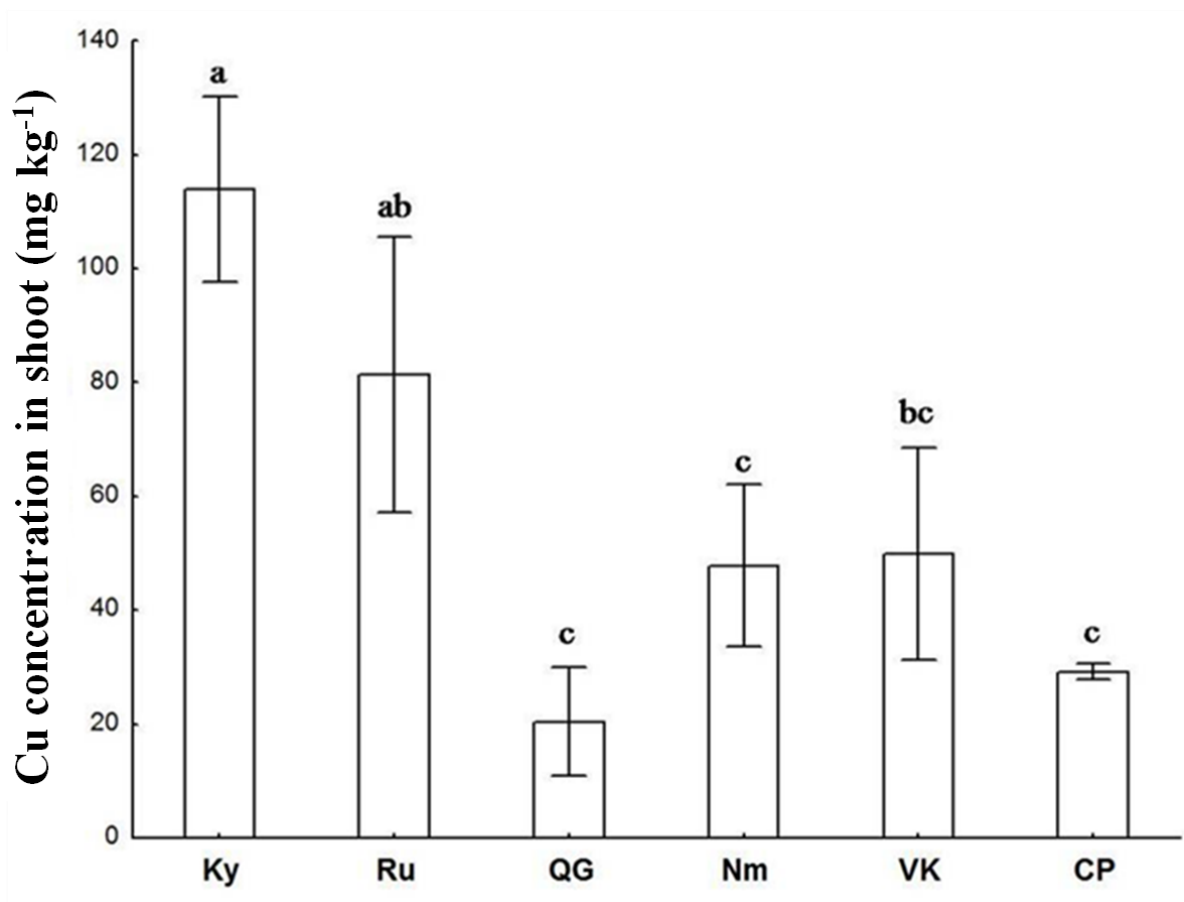
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633 **Figure 2**

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