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1 **Variation in copper and cobalt tolerance and accumulation among**
2 **six populations of the facultative metallophyte *Anisopappus***
3 ***chinensis* (Asteraceae)**

4
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20
21 **Highlights**

22
23 - Population variation in Co tolerance and translocation is demonstrated in *Anisopappus*
24 *chinensis*;

25 - Population variation in Co tolerance is correlated to Co concentrations in the native soil;

26 - Copper tolerance is weakly expressed in metallicolous populations grown in hydroponics;

27 - Cobalt tolerance may have evolved in response to directional selection in *Anisopappus*
28 *chinensis*.

29 **Abstract**

30

31 Advances on the ecology and evolution of adaptation to metal toxicity are based on
32 studying metallophytes that are not restricted to soils strongly enriched in trace elements. The
33 evolution of Cu and Co tolerance and accumulation, which principally occurs among the
34 Copperbelt of Central Africa, is poorly known. In this paper, we studied Cu and Co tolerance
35 and accumulation in a facultative metallophyte occupying a very broad ecological niche in
36 southeastern Democratic Republic of Congo: *Anisopappus chinensis* (Asteraceae). The
37 population variation in Cu and Co tolerance and accumulation was experimentally investigated
38 using four metallicolous and two non-metallicolous populations from contrasted habitats.
39 Surprisingly, Cu tolerance was poorly expressed in metallicolous populations grown in
40 hydroponics, suggesting that specific rhizosphere processes may account for the ability to grow
41 without toxicity symptoms under high Cu concentration on metalliferous soils. Population
42 variation in Co tolerance and accumulation was demonstrated, which positively correlated to
43 the concentration of Co in the native soil. Increased tolerance seems to have evolved in
44 populations originating from Co-enriched soils. Foliar Co accumulation above 300 mg kg⁻¹ dry
45 weight with increased translocation was observed in the most tolerant populations, possibly
46 making such populations promising materials to test for Co-phytomining applications.

47

48

49 **Keywords** Hyperaccumulation; Intraspecific variation; Metallicolous; Non-metallicolous;
50 Plant evolution

51 **1. Introduction**

52

53 Facultative metallophytes, *i.e.* metallophytes that are not restricted to soils strongly
54 enriched in trace elements (TEs), represent powerful models for studying evolution of
55 adaptation to metal toxicity (Baker, 1981; Pollard et al., 2014). Studies of the past decades have
56 mostly focused on species comprising populations adapted to Zn-, Cd-, Ni- and Cu-enriched
57 soils (Antonovics et al., 1971; Macnair et al., 1993; Bert et al., 2000; Escarré et al., 2000;
58 Dechamps et al., 2006; Pauwels et al., 2006; Meyer et al., 2010; Faucon et al., 2012;
59 Schwartzman et al., 2018). In rare facultative metallophytes, TEs tolerance is constitutive at the
60 level of the species; such is the case for the Zn- and Cd-tolerant *Arabidopsis halleri* and
61 *Noccaea caerulea* (Bert et al., 2002). Most often, increased tolerance to one or several TEs
62 has been found in metallicolous populations compared to their relatives originating from normal
63 soils (Gonnelli et al., 2001; Assunção et al., 2003). However, the plant response when
64 increasing soil TEs concentration can vary widely between species and populations, and Baker
65 (1981) proposed three main conceptual strategies of tolerance, including so-called “excluder”,
66 “indicator” and true “hyperaccumulator” plants (*i.e.* accumulation occurring from either low or
67 high available concentration in the growth medium).

68 Most often, facultative metallophytes from Cu and/or Co-enriched soils behaved as Cu
69 and/or Co excluders or indicators in culture, with limited metal translocation from the roots to
70 the leaves (Lange et al., 2017a). Foliar Cu and/or Co concentrations above 300 mg kg⁻¹ dry
71 weight (DW) may be due to passive transport at phytotoxic concentrations in the growth
72 medium (Clemens et al., 2002; Verbruggen et al., 2009). Plant populations established on
73 naturally Cu- and Co-enriched soils are almost restricted to southeastern Democratic Republic
74 of Congo (D.R. Congo) (Faucon et al., 2016; copperflora.org). These plants can thrive on soils
75 extremely enriched with Cu and/or Co (1 to 10 g kg⁻¹ inorganic Cu–Co) deriving from the

76 natural Cu- and Co-outcrops of the so-called “Katangan Copperbelt”. Some of the facultative
77 metallophytes from that region have been reported to accumulate Cu and Co above 300 mg kg⁻¹
78 DW in the leaves *in situ* (Lange et al., 2017a). Cobalt, with no physiological function
79 established so far in higher plants, is known to be more highly phytotoxic compared to Cu (Palit
80 et al., 1994; Morrissey et al., 2009; Pilon-Smits et al., 2009), which contrasts the rather accepted
81 Irving Williams theory on metal toxicity (Stumm and Morgan 1996). It has been hypothesized
82 that Co could be an important driver in the evolution of local adaptation in metallophytes
83 (Duvigneaud, 1959; Malaisse et al., 1983). However, large variations in shoot biomass and
84 foliar Cu and/or Co concentrations are observed between populations and plants (Faucon et al.,
85 2009; Lange et al., 2014; Delhayé et al., 2016). These variations could be accounted for by
86 variations in Cu and Co mobility in soil (varying widely among sites) (Pourret et al., 2016), but
87 also by variation in the capacity to tolerate and accumulate Cu and/or Co (Lange et al., 2014).
88 However, only few works have investigated variation in Cu and Co tolerance and accumulation
89 within the flora of Cu and Co-enriched soils.

90 Baker et al. (1983) observed a variation in Cu and Co tolerance between three *Silene*
91 taxa. Variation in Cu–Co tolerance was found in the *Silene burchellii* complex, correlated to the
92 Cu–Co status of native soils. More recently, increased tolerance to Cu was found in
93 metallicolous populations of *Crepidorhopalon tenuis* (Faucon et al., 2012) and *Haumaniastrum*
94 *katangense* (Peng et al., 2012). Most often, increased tolerance is coupled to decreased Cu
95 concentrations in shoots, as a result of specific tolerance mechanisms restricting Cu uptake
96 and/or translocation (Ouzounidou et al., 1994; Gonnelli et al., 2001). The plant response to Cu
97 treatment can vary widely depending on the concentration, the species and the growth medium
98 including growth inhibition, no effect on growth to growth stimulation (Jiang et al., 2004; Lou
99 et al., 2004; Lange et al. 2016). Increased requirements of Cu were found in *Crotalaria*
100 *cobalticola* (Boisson et al., 2016) and *Crepidorhopalon perennis* (Faucon et al., 2012) two strict

101 endemic species of Cu-enriched soils. In contrast to Cu, studies on intraspecific variation in Co
102 tolerance and accumulation are very scarce. Paliouris and Hutchinson (1991) found increased
103 tolerance to Co in a population of *Silene vulgaris* from mine tailings compared to a population
104 from uncontaminated site. In another study, a genetic variation in Co tolerance was shown
105 among clones of *Agrostis gigantea* (Hogan and Rauser, 1979). A more recent study has
106 demonstrated that Co tolerance may be associated with the translocation of Co from the root to
107 the shoot, and accumulation in *Haumaniastrum katangense* and *H. robertii*, two metallophytes
108 from the Katangan Copperbelt (Kabeya et al., 2018).

109 *Anisopappus chinensis* (Asteraceae) is a widespread facultative metallophyte in tropical
110 Africa. Compared to the few facultative metallophytes studied so far, *A. chinensis* appears to
111 have a broader niche, being very widespread on non-contaminated soils in tropical Africa
112 (Lange, 2016), and being particularly frequent on cobalt-rich soils in southeastern D.R. Congo
113 (Duvigneaud 1959). The ecology and evolution of Cu and Co tolerance and accumulation in
114 facultative metallophytes remains poorly studied, especially for Co and species that present a
115 high occurrence on non-metalliferous soils. Our working hypothesis is that differentiation in
116 Cu and Co tolerance and accumulation exists between populations of such species, and is
117 related to variations in the level of Cu and Co in the native soil.

118 In this paper, we assessed Cu and Co tolerance and accumulation in four metallicolous
119 and two non-metallicolous populations of the facultative metallophyte *A. chinensis*. We
120 addressed the following questions: (i) Is *A. chinensis* constitutively tolerant to Cu and/or Co?
121 (ii) Does intraspecific variation in Cu and Co tolerance, if any, related to Cu and Co
122 concentrations in native soils? (iii) Does increased tolerance to Co, if any, rely on Co
123 translocation and foliar accumulation?

124

125 2. Materials and methods

126 2.1. Plant material

127 *Anisopappus chinensis* L. Hook.f. & Arn. (Asteraceae, subfam. Asteroideae, tribe
128 Anthemideae) is a perennial facultative metallophyte native of and widely distributed in tropical
129 central, austral, western and eastern Africa (but also extended to China and southeastern Asia)
130 (Wild, 1963). In south-eastern D.R. Congo, *A. chinensis* is widespread in grasslands of natural
131 Cu–Co outcrops and the surrounding wooded savannas (Séleck et al., 2013; Lange et al.,
132 2017b). Four metallicolous (M) and two non-metallicolous (NM) populations were sampled.
133 At each site, the seeds from 20 plants were harvested and pooled. The sites of harvest were:
134 Etoile (Et, M), Fungurume V (F5, M), Goma II (G2, M), Niamumenda (Nm, M), Mikembo (Mi,
135 NM) and Kiswishi (Ki, NM) (Table 1). In nature, M populations exhibit broad variations in leaf
136 Cu and Co concentrations: 3–1335 and 4–2820 mg kg⁻¹ DW, respectively (Lange et al., 2014).
137 *Leucanthemum superbum* D.H.Kent (Asteraceae, subfam. Asteroideae, tribe Anthemidae), an
138 ornamental perennial herb which has not evolved mechanisms to tolerate Cu–Co, was chosen
139 as control species (hereafter “Ctrl”). Seeds were commercially purchased (Vilmorin©).

140

141 **Table 1 Populations of *Anisopappus chinensis*: location and habitat description**

142

Sites	Status	Coordinates (GCSWGS84 DD)	Habitat	Soil inorganic Cu (mg kg ⁻¹)	Soil inorganic Co (mg kg ⁻¹)
Etoile (E)	M	11.38°S 27.35°E	Cu-Co hill disturbed by mining. Secondary vegetation on mine waste.	953 (2836)	1311 (2541)
Fungurume 5 (F5)	M	10.62°S 26.29°E	Natural Cu-Co hill not disturbed by mining. Grassland.	4774 (9070)	2558 (2685)

Goma 2 (G2)	M	10.60'S 26.14'E	Natural Cu-Co hill marginally disturbed by mining. Grassland.	8469 (13 263)	456 (236)
Niamumenda (Nm)	M	11,60'S 27,29'E	Natural Cu-Co hill marginally disturbed by mining. Grassland.	1950 (4735)	29 (34)
Mikembo (Mi)	NM	11.47'S 27.66'E	Miombo woodland with grassy herbaceous layer	0 (5e-4)	1 (0.02)
Kiswishi (Ki)	NM	11.53'S 27.46'E	Miombo woodland with grassy herbaceous layer	0 (3e-4)	44 (1.2)

143

144 M = metallicolous, NM = non-metallicolous. Inorganic concentrations of Cu and Co in the
 145 rooting zone (ionic forms) were obtained by Cu and Co speciation modelling (Lange et al.,
 146 2014; Pourret et al., 2016). Standard deviation is given in brackets.

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2.2. Experimental design

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A hydroponic experiment was conducted in a greenhouse from April to June 2015 (three months). Seeds were germinated in vermiculite watered with nutrient solution for three weeks before being transferred in hydroponic conditions. Three-week old seedlings were then grown for eight weeks in 5 L polyethylene pots filled with a nutrient solution composed of: 20 μM Fe-EDDHA (iron salt of ethylenediamine-di-o-hydroxyphenylactic acid), 0.88 mM K_2SO_4 , 0.25 mM KH_2PO_4 , 10 μM NaCl , 1000 μM $\text{Ca}(\text{NO}_3)_2$, 1000 μM MgSO_4 , 10 μM H_3BO_3 , 1 μM ZnSO_4 , 1 μM MnSO_4 , 0.5 μM CuSO_4 , 0.01 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (adapted from Meyer *et al.*, 2010). The nutrient solution was adjusted to pH 5.8 with KOH (0.01 M) and changed each week. Five treatments were performed using Cu (II) sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) or Co (II) sulfate ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$): Control (with no addition of Cu and Co), +10 μM Cu, +50 μM Cu, +5 μM Co, +20 μM Co. Plants of *A. chinensis* (one plant from each population) and one plant of *L. superbum* were grown for each treatment (one pot per treatment). Pots were replicated 10 times ($n = 10$) and randomized each week.

163 2.3. *Measurements*

164 After eight weeks in hydroponics, plants were harvested and separated into shoots and
165 roots. Shoots were washed with Alconox[®] 1% in deionized water, dried at 65 °C for 48 h and
166 weighed (Faucon et al., 2007). Roots were washed with 0.1 mM EDTA for 5 min, rinsed three
167 times for 5 min in deionized water before being dried at 65 °C for 48 h and weighed (Chipeng
168 et al., 2010). Leaf Cu–Co and root Cu–Co concentrations in *A. chinensis* and *L. superbum* were
169 determined using Inductively Coupled Plasma–Mass Spectrometry (Thermo Scientific
170 XSERIES2) after a dry ashing (Faucon et al., 2009). Briefly, 0.2 g (accurately weighed) of leaf
171 or root powder were ashed at 500 °C for 12 h in a muffle furnace. Then, ashes were dissolved
172 in a 32.5% HNO₃ solution (3 mL) at 80 °C for 5 min. Samples were then diluted to a 5% HNO₃
173 solution (50 mL) and stored at 4 °C until being analyzed. The precision and accuracy of analysis
174 were determined using the SRM 1547 (peach leaves, NIST); bias of ± 5% were obtained in this
175 case. At each treatment, average tolerance indices (TI) of populations were calculated: average
176 biomass on metal divided by average biomass on control.

177

178 2.4. *Statistical analysis*

179 Plant biomass, leaf Cu and leaf Co were analyzed by partially nested ANOVAs on log-
180 transformed data. The “edaphic group” (*i.e.* M vs NM) and “treatment” factors were fixed
181 factors. The “population” factor was a random factor nested in the “edaphic group” factor. The
182 “block” factor was tested as a random factor and did not have a significant effect on any
183 variable. Data for Cu and Co were tested independently. The population variation in leaf Cu,
184 root Cu, leaf Co, root Co and leaf/root Cu–Co ratio (Cu–Co translocation factor) were
185 investigated by one-way ANOVAs on log-transformed data. The control species was not
186 considered for the population variation analysis. Pearson’s correlations were used to test the

187 relationship between shoot and root biomass and between leaf and root Cu–Co. The population
188 variation in average TI was not tested statistically due to the lack of replication. Relationship
189 between population TI and native soil concentration or translocation factor was tested for each
190 treatment using Spearman’s rank correlation (corrected by Monte Carlo permutation test based
191 on 999 replications). The analyses were performed using R software 3.2.2 (www.R-
192 project.org).

193

194 **3. Results**

195 *3.1. Response of shoot biomass and leaf copper and cobalt concentrations to Cu and Co in*
196 *the growth medium*

197 *3.1.1. Response to Cu*

198 In all tested plants (including the Ctrl plants), except those from the M population F5,
199 shoot biomass decreased dramatically with increasing Cu to +10 μM (Fig. 1). Growth was very
200 poor at +50 μM Cu in all populations and plants from Mi (NM) did not survive at this
201 concentration (Fig. 1). In contrast to other populations, leaf Cu of F5 plants did not increase
202 significantly with an increase of Cu to +10 μM , whereas a steady increase was observed in all
203 populations, up to 250–500 mg kg^{-1} DW in leaves, with increasing Cu from +10 to +50 μM .

204 Tolerance indices for Cu were consistently very low in NM populations at +10 and +50
205 μM Cu ($\text{TI} \leq 0.07$), while they showed extensive variation among M populations (TI : 0.12–
206 0.51 and 0.08–0.29 at +10 and +50 μM Cu, respectively). The ranking of population TI differed
207 between +10 and +50 μM Cu and did not match the ranking in inorganic Cu concentration in
208 the native soil (rank correlation not significant).

209 Shoot and root biomass were highly correlated at +10 and +50 μM Cu ($r = 0.96$ and $r =$
210 0.76 ; $p < 0.001$, respectively). ANOVA on plant biomass did not show a significant effect of the
211 edaphic group (*i.e.* M vs NM) (Table S1). Otherwise all factors and interactions were

212 significant. Patterns of variation in plant biomass varied between +10 and +50 μM Cu (data not
213 showed), in accordance with the highly significant interaction population within edaphic
214 group*treatment.

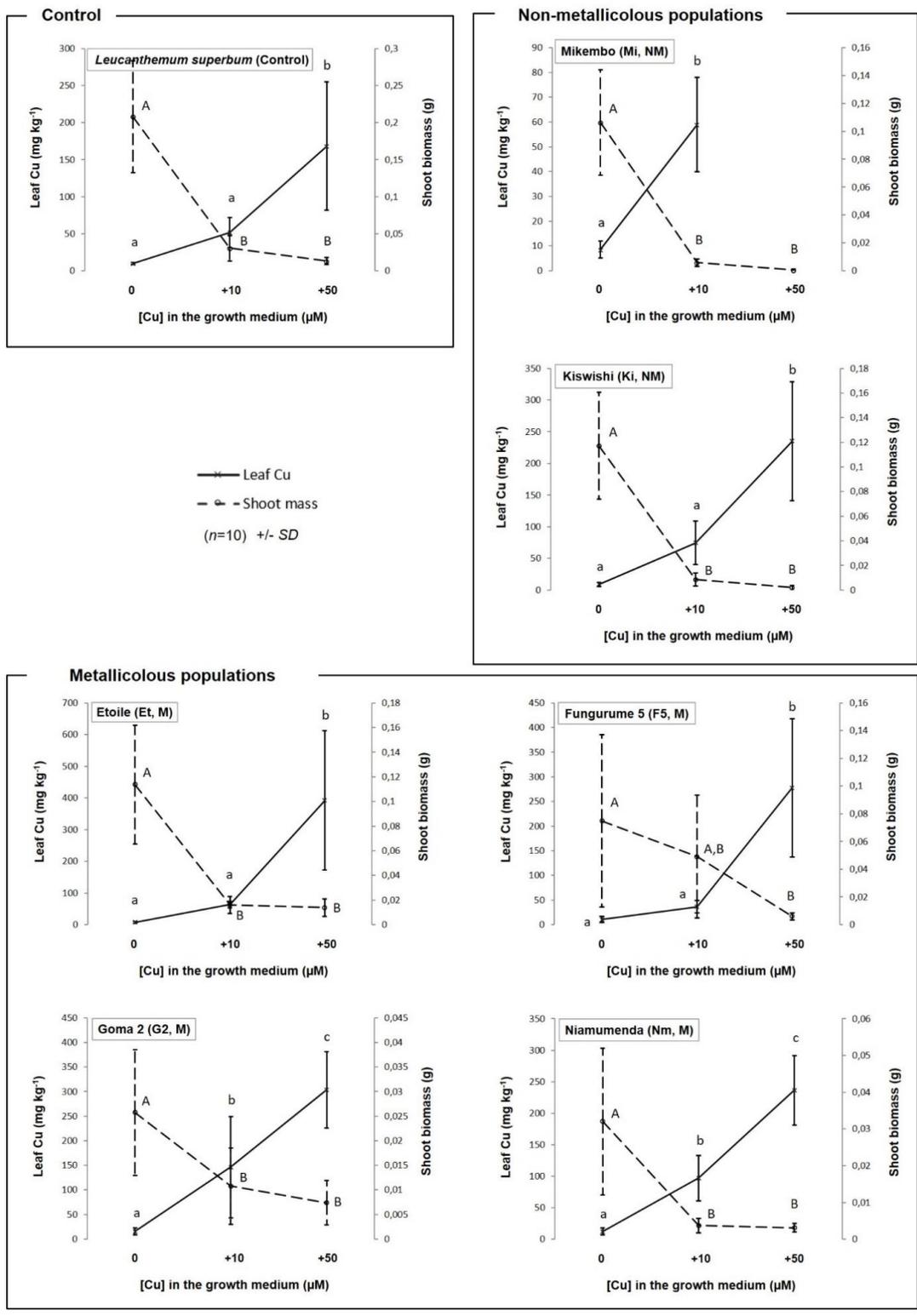
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216 3.1.2. Response to Co

217 The response of shoot biomass to the increase of the concentration of Co in the nutrient
218 solution varied widely among populations. The growth of M populations Et, F5 and G2 was not
219 significantly affected, and leaf Co concentrations increased progressively up to $>300 \text{ mg kg}^{-1}$
220 DW (Fig. 2). For the M population Nm, increasing Co to +5 μM had no significant effect on
221 shoot biomass, and leaf Co increased only slightly (+25 mg kg^{-1}). However, increasing Co from
222 +5 to +20 μM led to a significant decrease in shoot biomass and a steady leaf Co increase in
223 these plants (from 25 to 250 mg kg^{-1}). In both NM populations Mi and Ki and the Ctrl species,
224 increasing Co in the growth medium had a strong negative impact on shoot biomass, coupled
225 to dramatic increase in the leaf Co ($>400 \text{ mg kg}^{-1}$).

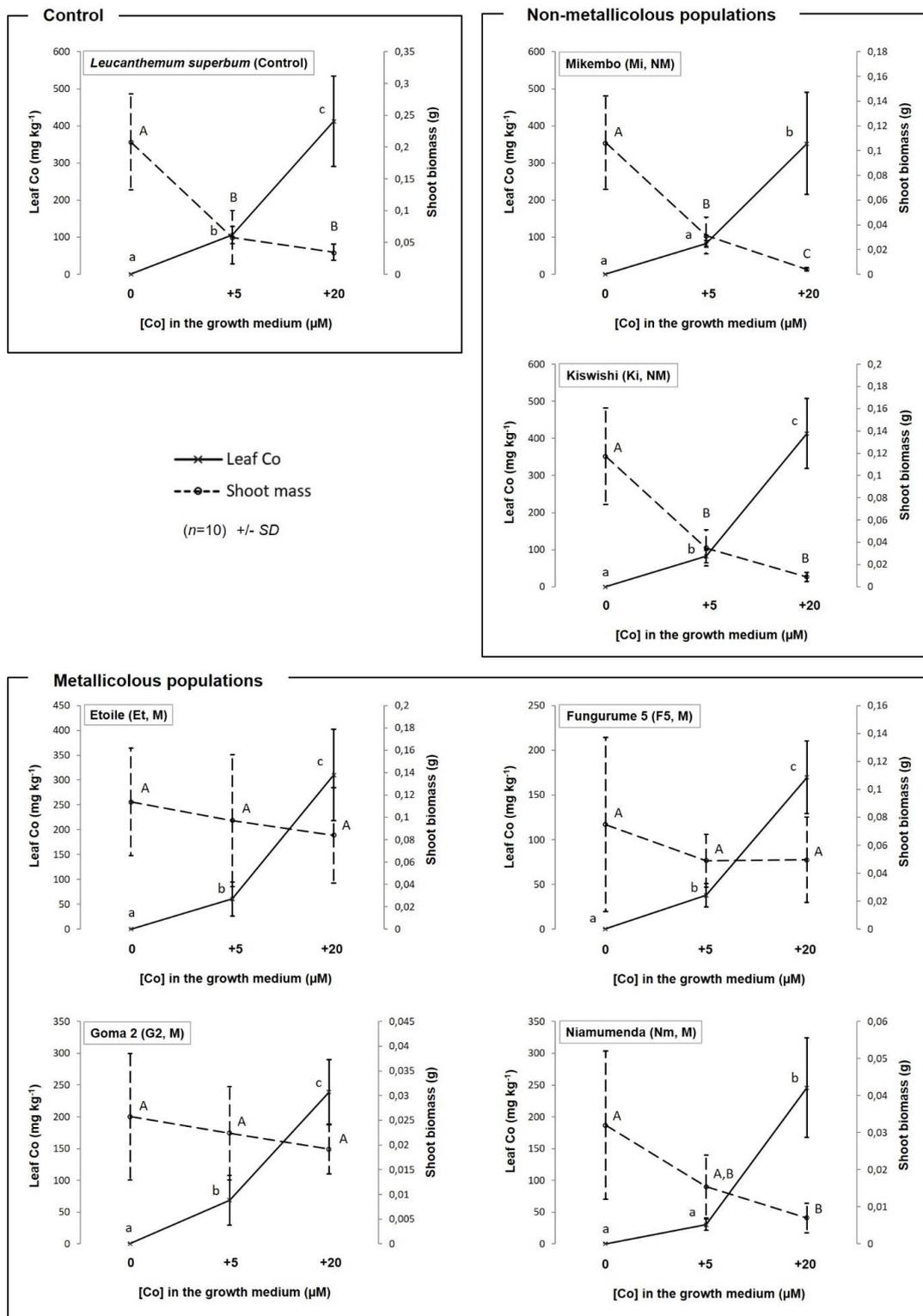
226 Tolerance indices were consistently lower in both NM populations and the Ctrl species
227 at +5 and +20 μM Co compared to M populations. Large variations of TI were observed among
228 populations at +20 μM Co (from 0.03 to 0.75). The ranking of populations for TI did not differ
229 between +5 and +20 μM Co and matched remarkably the ranking of native soils for Co
230 concentration: TI = 0.03 (Mi) < 0.08 (Ki) < 0.22 (Nm) < 0.66 (G2) < 0.74 (Et) < 0.75 (F5) (r_s
231 = 0.89; $p=0.015$ and $r_s = 0.94$; $p=0.005$ at +5 and +20 μM Co, respectively).

232 Shoot and root biomass were highly correlated at +5 and +20 μM Co ($r = 0.92$ and $r =$
233 0.94 ; $p<0.001$, respectively). Similarly to Cu, ANOVA on plant biomass did not show a
234 significant effect of the edaphic group, while all factor and interaction effects were significant
235 (Table S2). The interaction population within edaphic group*treatment was slightly significant.



236

237 **Figure 1** Shoot mass and leaf Cu as a function of increasing Cu in the nutrient solution in
 238 **four metallicolous and two non-metallicolous populations of *Anisopappus chinensis*.**
 239 *Leucanthemum superbum* was used as a control. Three Cu treatments: Control, +10 μM Cu,
 240 +50 μM Cu. Mean ± standard deviation (n = 10). Mean values with the same superscript are
 241 not significantly different (one-way ANOVAs on log-transformed data followed by post-hoc
 242 multiple comparison, Tukey HSD test).



243

244 **Figure 2** Shoot mass and leaf Co as a function of increasing Co in the nutrient solution in
 245 **four metallicolous and two non-metallicolous populations of *Anisopappus chinensis*.**
 246 *Leucanthemum superbum* was used as a control. Three Co treatments: Control, +5 μM Co, +20
 247 μM Co. Mean ± standard deviation (n = 10). Mean values with the same superscript are not
 248 significantly different (one-way ANOVAs on log-transformed data followed by post-hoc
 249 multiple comparison, Tukey HSD test).

250 *3.2. Population variation in leaf and root Cu and Co concentration*

251 *3.2.1. Leaf and root Cu concentration*

252 Leaf Cu ranged from 16 to 338 mg kg⁻¹ and from 76 to 761 mg kg⁻¹ at +10 and +50 μM
253 Cu, respectively. Root Cu ranged from 828 to 6813 mg kg⁻¹ and from 4347 to 81 088 mg kg⁻¹
254 at +10 and +50 μM Cu, respectively. Leaf Cu and root Cu were not significantly correlated.

255 ANOVAs on leaf and root concentrations showed a significant effect of all factors and
256 interactions, except the interaction edaphic group*treatment interaction (Table S3). The few
257 variations of leaf and root Cu observed at +10 μM Cu did not match TI (rank correlation not
258 significant) (Fig. 3a). Leaf Cu did not vary significantly between populations at +50 μM Cu
259 (Fig. 3b).

260 Leaf/root Cu ratio was very low in all populations and the Ctrl, and varied weakly at
261 +10 μM (from 0.01 [Ctrl] to 0.05 [G2, M]) and at +50 μM (from 0.00 [Ctrl] to 0.04 [Ki]) (Fig.
262 3). Variations in leaf/root Cu ratio did not match TI (rank correlation not significant).

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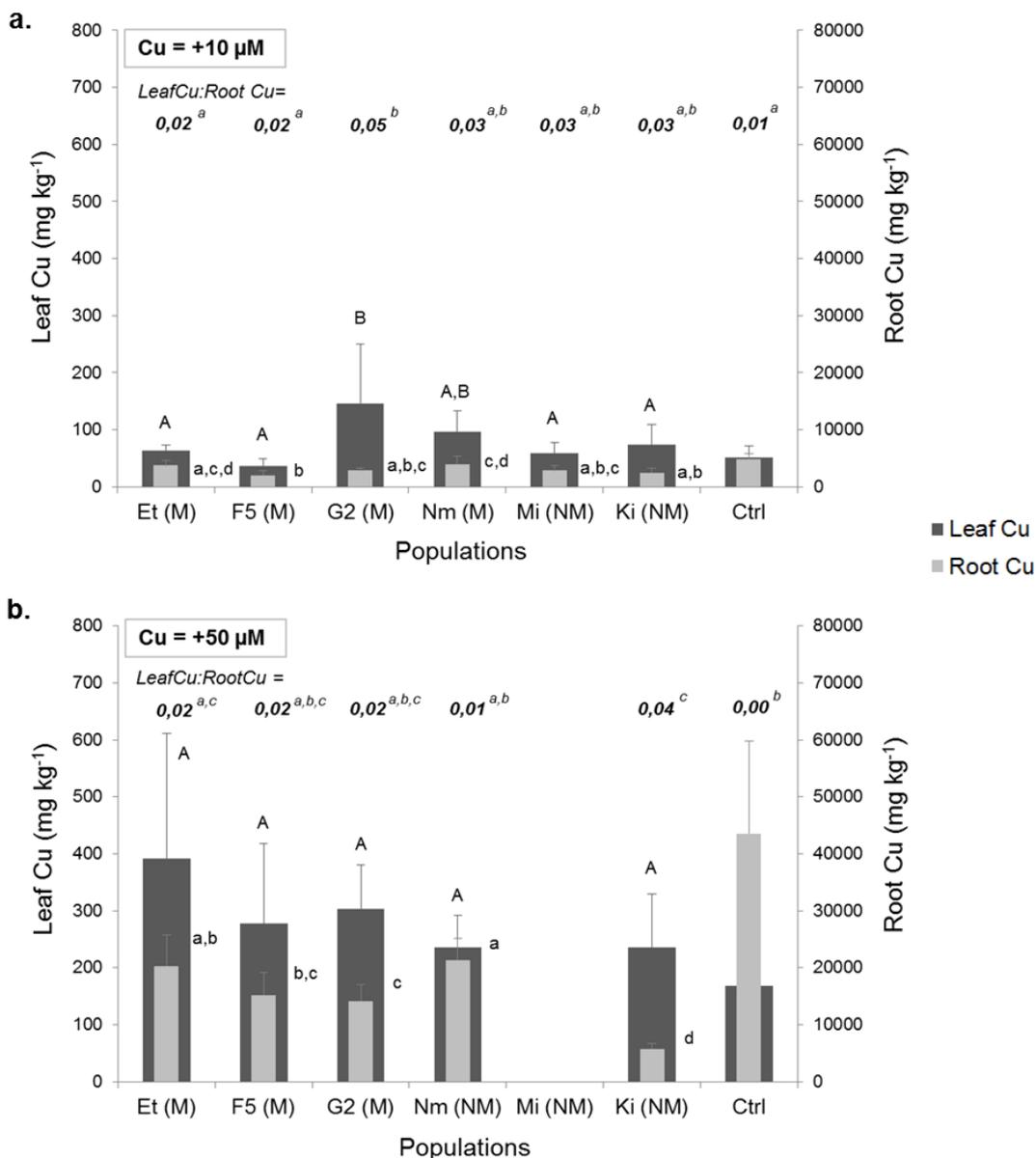
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272 **Figure 3 Leaf and root Cu concentrations in four metallicolous and two non-metallicolous**
 273 **populations of *Anisopappus chinensis* at two concentrations of Cu in the nutrient solution**
 274 **a. +10 µM Cu in the nutrient solution b. +50 µM Cu in the nutrient solution.**
 275 Et (Etoile), F5 (Fungurume V), G2 (Goma II) and Nm (Niamumenda) are metallicolous (M)
 276 populations. Mi (Mikembo) and Ki (Kiswishi) are non-metallicolous (NM) populations. Ctrl is
 277 the control species: *Leucanthemum superbum*. Shoot mass and root mass, as well as leaf Cu
 278 and root Cu were tested independently, without the Ctrl. Translocation factors (leaf/root Cu
 279 ratios), including the Ctrl, were also calculated. Mean ± standard deviation (n = 10) with the
 280 same superscript are not significantly different (results of one-way ANOVAs on log-
 281 transformed data followed by post-hoc multiple comparison, Tukey HSD test).

282 3.2.2. Leaf and root Co concentration

283 At +5 μM Co, leaf Co ranged from 19 to 149 mg kg^{-1} in M populations and from 49 to
284 116 mg kg^{-1} in NM populations; and root Co ranged from 204 to 1186 mg kg^{-1} in M populations
285 and from 1729 to 6577 mg kg^{-1} in NM populations. At +20 μM Co, leaf Co ranged from 110 to
286 504 mg kg^{-1} in M populations and from 89 to 620 in NM populations; and root Co ranged from
287 485 to 2690 mg kg^{-1} in M populations and from 2995 to 7794 mg kg^{-1} in NM populations. Leaf
288 Co and root Co concentration were positively correlated ($r = 0.51$, $p < 0.001$).

289 ANOVA on leaf and root Co showed a significant effect of all factors and interactions,
290 except the population effect for leaf Co (Table S4). In contrast to Cu, leaf and root Co varied
291 among populations at the intermediate and highest treatments (Fig. 4a,b). At +5 μM Co, root
292 Co was much lower in M populations ($442 \pm 120 \text{ mg kg}^{-1}$) compared to NM populations (3967
293 $\pm 1318 \text{ mg kg}^{-1}$) and the control species ($2198 \pm 575 \text{ mg kg}^{-1}$). A similar pattern was observed
294 at +20 μM Co. Variations in root Co among populations matched variations in TI at +20 μM
295 Co, with higher root Co concentrations in populations having lower TI (Mi and Ki) ($r_s = -0.94$;
296 $p < 0.05$).

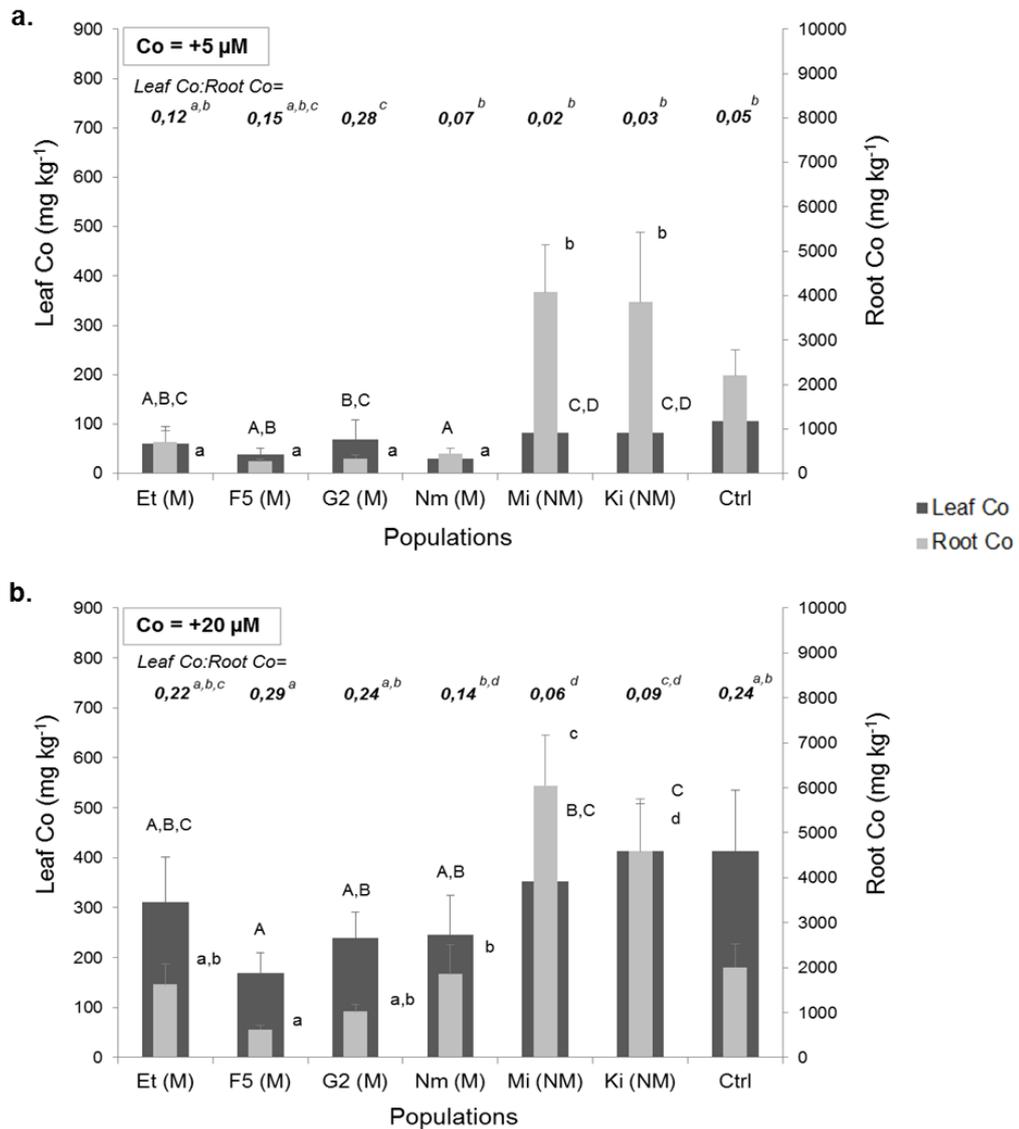
297 Leaf/root Co ratio varied weakly at +5 μM Co (Fig. 4a). At +20 μM Co, leaf/root Co
298 ratio was significantly higher in the M populations F5 (0.29) and G2 (0.24) compared to the
299 NM populations Mi (0.06) and Ki (0.09) (Fig. 4b). A positive correlation was observed between
300 leaf/root Co and TI at +20 μM Co ($r_s = 0.94$; $p < 0.01$).

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302

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306 **Figure 4 Leaf and root Co concentrations in four metallicolous and two non-metallicolous**
 307 **populations of *Anisopappus chinensis* at two concentrations of Co in the nutrient solution**
 308 **a. +5 µM Co in the nutrient solution b. +20 µM Co in the nutrient solution.**

309 Et (Etoile), F5 (Fungurume V), G2 (Goma II) and Nm (Niamumenda) are metallicolous (M)
 310 populations. Mi (Mikembo) and Ki (Kiswishi) are non-metallicolous (NM) populations. Ctrl
 311 is the control species: *Leucanthemum superbum*. Shoot mass and root mass, as well as leaf Co
 312 and root Co were tested independently, without the Ctrl. Translocation factors (leaf/root Co
 313 ratios), including the Ctrl, were also calculated. Mean ± standard deviation (n = 10) with the
 314 same superscript are not significantly different (results of one-way ANOVAs on log-
 315 transformed data followed by post-hoc multiple comparison, Tukey HSD test).

316 **4. Discussion**

317 *4.1. Low expression of Cu tolerance in metallicolous populations grown in hydroponics*

318 *Anisopappus chinensis* is a facultative metallophyte occupying a broad niche including non-
319 metalliferous habitats and soils enriched in Cu and Co in southeastern D.R. Congo. The latter
320 species presents a high occurrence on non-metalliferous soils, as opposed to species from the
321 Copperbelt studied so far (Lange, 2016). Results of growth under Cu stress for the control
322 species and *A. chinensis* plants demonstrated that tolerance to Cu is not constitutive in the
323 species. Surprisingly, a very poor expression of Cu tolerance was observed in hydroponics for
324 metallicolous populations originating from soils extremely concentrated in Cu (Fig. 1, Table
325 1). We expected higher tolerance to Cu in metallicolous compared to non-metallicolous
326 populations, with respect to the metallic status of their native soils. Despite somewhat higher
327 tolerance indices in metallicolous populations, *A. chinensis* contrasts with other facultative
328 metallophytes such as *C. tenuis* and *H. katangense*. The latter species consistently showed a
329 strong increased tolerance to Cu in metallicolous compared to non-metallicolous populations
330 (Faucon et al., 2012; Peng et al., 2012). For *C. tenuis*, increasing Cu in the growth medium even
331 showed a growth stimulation. Contrast in the realized ecological niche between *A. chinensis*
332 and species that present a higher occurrence on metalliferous soils may account for contrast in
333 the degree of tolerance and response to Cu of M populations.

334 Elevated tolerance to Cu was demonstrated in metallicolous plants of *H. katangense* and *C.*
335 *tenuis* growing in hydroponics or MS medium (Chipeng et al., 2010; Faucon et al., 2012). At
336 an equivalent concentration, metallicolous plants of *A. chinensis* suffered from Cu toxicity.
337 Metallicolous populations of *A. chinensis* may have evolved contrasting tolerance to Cu, which
338 could be the result of specific soil-plant interactions not expressed in hydroponics. For instance,
339 specific rhizosphere processes involving the microbial communities might account for the
340 ability to grow under high Cu concentration on metalliferous soils. Among them, metal-tolerant

341 arbuscular mycorrhizal fungi (AMF) and endophytic bacteria can have a beneficial effect on
342 Cu tolerance by plants (Cornejo et al., 2008; Sun et al., 2010; Ma et al., 2011; Lenoir et al.,
343 2016). Future work should test the presence of AMF and endophytic bacteria in M populations
344 of *A. chinensis* and, if any, explore possible relationships with the ability to grow under high
345 soil Cu levels.

346

347 *4.2. Increased tolerance to Co in populations from Co-enriched soils as a result of local*
348 *adaptation*

349 Results showed that tolerance to Co is not constitutive in *A. chinensis*. Non-
350 metallicolous populations of *A. chinensis* have not evolved mechanisms to tolerate
351 concentrations of Co that are toxic for nontolerant plants such as *L. superbum*. In contrast to
352 Cu, large intraspecific variation in the response to increasing Co in the growth medium was
353 observed in *A. chinensis*. This study is only the second one to report intraspecific variation in
354 tolerance to Co in a metallophyte. Paliouris and Hutchinson (1991) demonstrated an increased
355 tolerance to Co in *Silene vulgaris* from a Co-contaminated soil compared to its relative on
356 uncontaminated soil. Interestingly, these authors did not find any relationship between tolerance
357 indices and Co concentrations in the native soils (water extract). Our work is the first to report
358 population differentiation in Co tolerance related to the level of Co in the native soil. Tolerance
359 indices at each Co treatment matched remarkably well the ranking of native soils for Co
360 concentration. Selective pressure stemming from Co toxicity in highly contaminated soils may
361 have induced local adaptation and enhanced tolerance to Co in the populations Et, F5 and G2.
362 Therefore, the broad ecological niche of *A. chinensis* in southeastern D.R. Congo might be
363 ascribed to within species genetic variation in Co tolerance. A similar conclusion was drawn
364 by Faucon et al. (2012), for the facultative metallophyte *Crepidorhapon tenuis* for Cu. The

365 existence of such Co-tolerant ecotypes suggests that Co tolerance can explain the broad
366 ecological niche of facultative metallophytes growing on Cu-enriched soils.

367

368 *4.3. High accumulation of Co and increased translocation in populations adapted to*
369 *extreme Co-levels*

370 Relatively high foliar concentrations of Cu and Co have been reported from field
371 growing plants of *A. chinensis* among M populations. *Anisopappus chinensis* from M
372 populations was classified by Lange et al. (2014) as a Cu-Co hyperaccumulator based on
373 operationally defined threshold values proposed by van der Ent et al. (2013) for foliar Cu and
374 Co (>300 mg kg⁻¹ DW). In our experimental conditions, the foliar concentration increase up to
375 >300 mg kg⁻¹ DW in all population with increasing Cu to +50 μM might be explained by passive
376 absorption (Baker and Walker, 1989; Verkleij and Schat, 1989). This study is the first
377 demonstration of a high degree of tolerance to Co associated with foliar Co accumulation up to
378 >300 mg kg⁻¹ DW in a facultative metallophyte largely distributed among non-metalliferous
379 soils. Interestingly, Co tolerance was positively correlated to Co translocation to the leaves at
380 elevated concentration, implying that the strategy of Co tolerance in *A. chinensis* does not rely
381 on Co exclusion. The lower root Co concentration in the most tolerant compared to non-tolerant
382 populations seems to confirm this hypothesis. Our results suggest that *A. chinensis* from soils
383 highly enriched in available Co would behave as Co indicator rather than true hyperaccumulator
384 *sensu* Baker (*i.e.* accumulation occurring from either low or high available concentration in the
385 growth medium). The latter hypothesis should be verified by looking at the response of
386 metallophilous plants grown on a natural metalliferous soil of reference with different levels of
387 Co.

388 Intraspecific variation in leaf and root Co accumulation was shown for the first time in
389 a facultative metallophyte. Interesting work could be to test the possible use of the most tolerant
390 populations of *A. chinensis*, which translocate and accumulate Co above 300 mg kg⁻¹ DW in
391 leaves, in phytomining applications, for the production and valorization of a Co bio-ore (Faucon
392 et al., 2018). Interestingly, the latter populations that also present the lowest root Co
393 concentrations in our study, originate from soils with the highest level of inorganic Co.
394 Selective pressure stemming from Co toxicity in the soil of origin may have induced a particular
395 ability in (i) limiting Co storage in the root system and (ii) translocating Co to the leaves in
396 plants originating from Co-enriched soils. The ecophysiology of Co tolerance and accumulation
397 and interaction between is still poorly understood and particular adaptation mechanisms are
398 expected.

399

400 **5. Conclusion**

401

402 Tolerance to Cu and Co are not constitutive in *Anisopappus chinensis*. The contrast
403 between Cu toxicity in our study and field observations on Cu-enriched soils needs to be
404 clarified. For instance, rhizospheric processes and/or biotic interactions which can have a
405 beneficial effect on metal tolerance by plants but cannot be expressed in hydroponics, such as
406 associations with mycorrhizal fungi and/or endophytic bacteria, should be investigated. Such
407 processes might also account for the high concentrations of Cu in field growing plants. In
408 contrast, populations from Co-enriched soils did express increased tolerance to Co. Variation
409 in the degree of tolerance among these populations was related to the concentration of Co in
410 the native soil, strongly suggesting that Co tolerance has evolved in response to directional
411 selection. This study is the first to report population differentiation in Co tolerance in a
412 facultative metallophyte depending on Co soil status. Interestingly, Co tolerance was positively

413 correlated to Co translocation to the leaves up to $>300 \text{ mg kg}^{-1}$ dry weight at phytotoxic
414 concentration in the nutritive solution ($+20 \text{ } \mu\text{M Co}$). This suggests that *A. chinensis* from soils
415 highly enriched in available Co would behave as Co indicator *sensu* Baker (1981) and that
416 increased capacity to translocate Co may have evolved in the most tolerant populations.

417

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419

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429

430 **7. Author contributions**

431

432 BL, GD, SB, NV, MPF and PM designed the experiment. BL conducted the experiment
433 with the participation of GD. BL did the mineral and statistical analyses. BL wrote the
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435 manuscript and gave their final approval.

- 437 **Antonovics J, Bradshaw AD, Turner RG. 1971.** Heavy metal tolerance in plants. *Advances*
438 *in Ecological Research* **7**: 2–85.
- 439 **Assunção AG, Bookum WM, Nelissen HJ, Vooijs R, Schat H, Ernst WH. 2003.** Differential
440 metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations
441 originating from different soil types. *New Phytologist* **159**: 411–419.
- 442 **Baker AJM. 1981.** Accumulators and excluders-strategies in the response of plants to heavy
443 metals. *Journal of Plant Nutrition* **3**: 643–654.
- 444 **Baker AJM, Brooks RR, Pease AJ, Malaisse F. 1983.** Studies on copper and cobalt tolerance
445 in three closely-related taxa within the genus *Silene* L. (Caryophyllaceae) from Zaire. *Plant and*
446 *Soil* **73**: 377–385.
- 447 **Baker AJM, Walker PL. 1989.** Ecophysiology of metal uptake by tolerant plants, in: Shaw J,
448 eds *Heavy metal tolerance in plants: evolutionary aspects*. CRC Press, 155–177.
- 449 **Bert V, Bonin I, Saumitou-Laprade P, de Laguerie P, Petit D. 2002.** Do *Arabidopsis halleri*
450 from nonmetallicolous populations accumulate zinc and cadmium more effectively than those
451 from metallicolous populations? *New Phytologist* **155**: 47–57.
- 452 **Bert V, Macnair MR, De Laguerie P, Saumitou-Laprade P, Petit D. 2000.** Zinc tolerance
453 and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri*.
454 *New Phytologist* **146**: 225–233.
- 455 **Boisson S, Le Stradic S, Commans M, Dumont A, Leclerc N, Thomas C, Mahy G. 2016.**
456 Copper tolerance of three *Crotalaria* species from southeastern D.R. Congo at the early
457 development stage. *Biotechnologie, Agronomie, Société et Environnement* **20**: 151–160.
- 458 **Chipeng KF, Hermans C, Colinet G, Faucon MP, Ngongo Luhembwe M, Meerts P,**
459 **Verbruggen N. 2010.** Copper tolerance in the cuprophyte *Haumaniastrum katangense* (S.
460 Moore) P.A. Duvign. and Plancke. *Plant and Soil* **328**: 235–244.
- 461 **Clemens S, Palmgren MG, Krämer U. 2002.** A long way ahead: understanding and
462 engineering plant metal accumulation. *Trends in Plant Science* **7**: 309–315.

463 **Cornejo P, Meier S, Borie G, Rillig MC, Borie F. 2008.** Glomalin-related soil protein in a
464 Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn
465 sequestration. *Science of the Total Environment* **406**: 154–160.

466 **Dechamps C, Lefèbvre C, Noret N, Meerts P. 2006.** Reaction norms of life history traits in
467 response to Zn in *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites. *New*
468 *Phytologist* **173**: 191–198.

469 **Delhaye G, Violle C, Séleck M, Ilunga wa Ilunga E, Daubie I, Mahy G, Meerts P. 2016.**
470 Community variation in plant traits along copper and cobalt gradients. *Journal of Vegetation*
471 *Science* **27**: 854–864.

472 **Duvigneaud P. 1959.** Plantes cobaltophytes dans le Haut Katanga. *Bulletin de la Société Royale*
473 *de Botanique de Belgique* **91**: 111–134.

474 **Escarré J, Lefèbvre C, Gruber W, Leblanc M, Lepart J, Rivière Y, Delay B. 2000.** Zinc
475 and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and
476 nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New*
477 *Phytologist* **145**: 429-437.

478 **Faucon MP, Chipeng F, Verbruggen N, Mahy G, Colinet G, Shutcha M, Pourret O,**
479 **Meerts P. 2012.** Copper tolerance and accumulation in two cuprophytes of South Central
480 Africa: *Crepidorhopalon perennis* and *C. tenuis* (Linderniaceae). *Environmental and*
481 *Experimental Botany* **84**: 11–16.

482 **Faucon MP, Colinet G, Mahy G, Ngongo Luhembwe M, Verbruggen N, Meerts. P. 2009.**
483 Soil influence on Cu and Co uptake and plant size in the cuprophytes *Crepidorhopalon perennis*
484 and *C. tenuis* (Scrophulariaceae) in SC Africa. *Plant and Soil* **317**: 201–212.

485 **Faucon MP, Le Stradic S, Boisson S, Ilunga wa Ilunga E, Séleck M, Lange B, Delhaye G,**
486 **Shutcha M, Pourret O, Meerts P, Mahy G. 2016.** Implication of plant-soil relationships for
487 conservation and restoration of copper-cobalt ecosystems. *Plant and Soil* **403**: 153–165.

488 **Faucon MP, Pourret O, Lange B. 2018.** Element case studies: copper and cobalt. In:
489 Echevarria G, van der Ent A, Morel JL, Baker AJM (eds) *Agromining: farming for metals:*
490 *extracting unconventional resources using plants*. Springer, 233–239.

491 **Faucon MP, Shutcha MN, Meerts P. 2007.** Revisiting copper and cobalt concentrations in
492 supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations
493 in soil. *Plant and Soil* **301**: 29–36.

494 **Gonnelli C, Galardi F, Gabrielli R. 2001.** Nickel and copper tolerance and toxicity in three
495 Tuscan populations of *Silene paradoxa*. *Physiologia Plantarum* **113**: 507–514.

496 **Hogan GD, Rauser WE. 1979.** Tolerance and toxicity of cobalt, copper, nickel and zinc in
497 clones of *Agrostis gigantea*. *New Phytologist* **83**: 665–670.

498 **Jiang LY, Yang XE, He ZL. 2004.** Growth response and phytoextraction of copper at different
499 levels in soils by *Elsholtzia splendens*. *Chemosphere* **55**: 1179–1187.

500 **Kabeya FI, Pongrac P, Lange B, Faucon MP, van Elteren JT, Šala M, Šelih VS, Vanden**
501 **Eeckhoudta E, Verbruggen N. 2018.** Tolerance and accumulation of cobalt in three species
502 of *Haumaniastrum* and the influence of copper. *Environmental and Experimental Botany* **149**:
503 27-33.

504 **Lange B. 2016.** Tolérance et accumulation du cuivre et du cobalt chez les métallobytes
505 facultatives d’Afrique tropicale. PhD dissertation, Université Libre de Bruxelles (Brussels,
506 Belgium), Institut UniLaSalle Beauvais (Beauvais, France), 196 pp.

507 **Lange B, Faucon MP, Delhaye G, Hamiti N and Meerts P. 2017b.** Functional traits of a
508 facultative metallophyte form tropical Africa: population variation and plasticity in response to
509 cobalt. *Environmental and Experimental Botany* **136**: 1–8.

510 **Lange B, Faucon MP, Meerts P, Shutcha M, Mahy G, Pourret O. 2014.** Prediction of the
511 edaphic factors influence upon the copper and cobalt accumulation in two metallophytes using
512 copper and cobalt speciation in soils. *Plant and Soil* **379**: 275–287.

513 **Lange B, Pourret O, Meerts P, Jitaru P, Cancès B, Grison C, Faucon MP. 2016.** Copper
514 and cobalt mobility in soil and accumulation in a metallophyte as influenced by experimental
515 manipulation of soil chemical factors. *Chemosphere* **146**: 75–84.

516 **Lange B, van der Ent A, Baker AJM, Echevarria G, Mahy G, Malaisse F, Meerts P,**
517 **Pourret O, Verbruggen N, Faucon MP. 2017a.** Copper and cobalt accumulation in plants: a
518 critical assessment of the current state of knowledge. *New Phytologist* **213**: 537–551.

519 **Lenoir I, Fontaine J, Sahraoui ALH. 2016.** Arbuscular mycorrhizal fungal responses to
520 abiotic stresses: A review. *Phytochemistry* **123**: 4–15.

521 **Lou LQ, Shen ZG, Li XD. 2004.** The copper tolerance mechanism of *Elsholtzia haichowensis*,
522 a plant from copper-enriched soils. *Environmental and Experimental Botany* **51**: 111–120.

523 **Ma Y, Prasad MNV, Rajkumar M, Freitas H. 2011.** Plant growth promoting rhizobacteria
524 and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnology advances* **29**:
525 248–258.

526 **Macnair MR, Smith SE, Cumbes QJ. 1993.** Heritability and distribution of variation in
527 degree of copper tolerance in *Mimulus guttatus* at Copperopolis, California. *Heredity* **71**: 445–
528 445.

529 **Malaisse F, Colonval-Elenkov E, Brooks RR. 1983.** The impact of copper and cobalt
530 orebodies upon the evolution of some plant species from Upper Shaba, Zaïre. *Plant Systematics*
531 *and Evolution* **142**: 207–221.

532 **Meyer CL, Kostecka AA, Saumitou-Laprade P, Créach A, Castric V, Pauwels M, Frérot**
533 **H. 2010.** Variability of zinc tolerance among and within populations of the pseudometallophyte
534 species *Arabidopsis halleri* and possible role of directional selection. *New Phytologist* **185**:
535 130–142.

536 **Morrissey J, Baxter IR, Lee J, Li L, Lahner B, Grotz N, Kaplan J, Salt DE, Guerinot ML.**
537 **2009.** The ferroportin metal efflux proteins function in iron and cobalt homeostasis in
538 *Arabidopsis*. *Plant Cell* **21**: 3326–3338.

539 **Ouzounidou G, Symeonidis L, Babalonas D, Karataglis S. 1994.** Comparative responses of
540 a copper-tolerant and a copper-sensitive population of *Minuartia hirsuta* to copper toxicity.
541 *Journal of Plant Physiology* **144**: 109–115.

542 **Paliouris G, Hutchinson TC. 1991.** Arsenic, cobalt and nickel tolerances in two populations
543 of *Silene vulgaris* (Moench) Garcke from Ontario, Canada. *New Phytologist* **117**: 449–459.

544 **Palit S, Sharma A, Talukder G. 1994.** Effects of cobalt on plants. *The Botanical Review* **60**:
545 149–181.

546 **Pauwels M, Frérot H, Bonin I, Saumitou-Laprade P. 2006.** A broad-scale analysis of
547 population differentiation for Zn tolerance in an emerging model species for tolerance study:
548 *Arabidopsis halleri* (Brassicaceae). *Journal of Evolutionary Biology* **19**: 1838–1850.

549 **Peng H, Wang-Müller Q, Witt T, Malaisse F, Küpper H. 2012.** Differences in copper
550 accumulation and copper stress between eight populations of *Haumaniastrum katangense*.
551 *Environmental and Experimental Botany* **79**: 58–65.

552 **Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M. 2009.** Physiological
553 functions of beneficial elements. *Current opinion in Plant Biology* **12**: 267–274.

554 **Pollard AJ, Reeves RD, Baker AJM. 2014.** Facultative hyperaccumulation of heavy metals
555 and metalloids. *Plant Science* **217**: 8–17.

556 **Pourret O, Lange B, Bonhoure J, Colinet G, Decrée S, Mahy G, Séleck M, Shutcha M,
557 Faucon M-P. 2016.** Assessment of soil metal distribution and environmental impact of mining
558 in Katanga (Democratic Republic of Congo). *Applied Geochemistry* **64**: 43–55.

559 **Pourret O, Lange B, Houben D, Colinet G, Shutcha M, Faucon MP. 2015.** Modeling of
560 cobalt and copper speciation in metalliferous soils from Katanga (Democratic Republic of
561 Congo). *Journal of Geochemical Exploration* **149**: 87–96.

562 **Schvartzman M, Corso M, Fataftah N, Scheepers M, Nouet C, Bosman B, Carnol M,
563 Motte P, Verbruggen N, Hanikenne M. 2018.** Adaptation to high zinc depends on distinct
564 mechanisms in metallicolous populations of *Arabidopsis halleri*. *New Phytologist* **218**: 269–
565 282.

566 **Séleck M, Bizoux JP, Colinet G, Faucon MP, Guillaume A, Meerts P, Piqueray J, Mahy
567 G. 2013.** Chemical soil factors influencing plant assemblages along copper-cobalt gradients:
568 implications for conservation and restoration. *Plant and Soil* **373**: 455–469.

569 **Stumm W, Morgan JJ. 1996.** Aquatic chemistry, chemical equilibria and rates in natural
570 waters, Wiley, New York, 1022 pp.

571 **Sun LN, Zhang YF, He LY, Chen ZJ, Wang QY, Qian M, Sheng XF. 2010.** Genetic
572 diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-
573 tolerant plant species on copper mine wasteland. *Bioresource Technology* **101**: 501–509.

574 **van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. 2013.** Hyperaccumulators of
575 metal and metalloid trace elements: facts and fiction. *Plant and Soil* **362**: 319–333.

- 576 **Verbruggen N, Hermans C, Schat H. 2009.** Molecular mechanisms of metal
577 hyperaccumulation in plants. *New Phytologist* **181**: 759–776.
- 578 **Verkleij JAC, Schat H. 1990.** Mechanisms of metal tolerance in higher plants. In: Shaw J, eds.
579 *Heavy metal tolerance in plants: evolutionary aspects*. CRC Press, 179–201.
- 580 **Wild H. 1963.** A revision of the genus *Anisopappus* Hook. & Arn. (Compositae). *Kirkia* **4**: 45–
581 73.