

Prediction of the edaphic factors influence upon the copper and cobalt accumulation in two metallophytes using copper and cobalt speciation in soils

Bastien Lange, Michel-Pierre Faucon, Pierre Meerts, Mylor Shutcha, Grégory Mahy, Olivier Pourret

► **To cite this version:**

Bastien Lange, Michel-Pierre Faucon, Pierre Meerts, Mylor Shutcha, Grégory Mahy, et al.. Prediction of the edaphic factors influence upon the copper and cobalt accumulation in two metallophytes using copper and cobalt speciation in soils. *Plant and Soil*, Springer Verlag, 2014, 379 (1-2), pp.275-287. 10.1007/s11104-014-2068-y . hal-02265597

HAL Id: hal-02265597

<https://hal.archives-ouvertes.fr/hal-02265597>

Submitted on 10 Aug 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Authors: Bastien Lange^{1,2,*} - Michel-Pierre Faucon¹ - Pierre Meerts² - Mylor Shutcha⁴ -**
2 **Grégory Mahy³ - Olivier Pourret¹**

3

4 **Prediction of the edaphic factors influence upon the copper and cobalt accumulation in**
5 **two metallophytes using copper and cobalt speciation in soils**

6

7 ¹HYdrISE (Hydrogeochemistry Interactions Soil Environment) unit, UP.2012.10.102, Institut
8 Polytechnique LaSalle Beauvais (ISAB-IGAL), 19 rue Pierre Waguët, FR-60026 Beauvais,
9 France

10 ²Laboratory of Plant Ecology and Biogeochemistry, Université Libre de Bruxelles, 50 Avenue
11 F. Roosevelt, BE-1150 Brussels, Belgium

12 ³Département of Forest, Nature and Landscape, Biodiversity and Landscape Unit, Gembloux
13 Agro-Bio Tech, University of Liège, 2 Passage des Déportés, BE-5030 Gembloux, Belgium

14 ⁴Université de Lubumbashi, Faculté des Sciences Agronomiques, Lubumbashi, Democratic
15 Republic of Congo

16

17 *Corresponding author: bastien.lange2@lasalle-beauvais.fr ; bastien.lange@ulb.ac.be

18

19

20

21

22

23

24

25

26

27 **Key-words** chemical speciation, copper, cobalt, hyperaccumulation, metal availability, WHAM 6.

28

29 **Abstract**

30 **Background and Aims** Among the unique flora on copper and cobalt rich soils, some species are able to
31 hyperaccumulate the Cu and Co in their shoots, however, the unexplained high variations of Cu and Co
32 concentrations in shoots have been highlighted. A good comprehension of the Cu and Co accumulation
33 variations would go through a characterization of the Cu and Co speciation in soils. We examined the
34 covariations of Cu and Co speciation in soils and Cu and Co concentrations in plants.

35 **Methods** Plant samples of two species and soil samples (n=146) were collected in seven pedogeochemically
36 contrasted sites. Cu and Co speciation in soils was modeled by WHAM 6.0.

37 **Results** Variation in copper accumulation in plant shoots were mostly influenced by Cu adsorbed by the Mn and
38 Fe oxides fractions, whereas Co accumulation variations were strongly influenced by Co free and Co adsorbed
39 by the OM and Fe fractions.

40 **Conclusions** Availability of Cu and Co seems to be species-specific and is not explained only by the free Cu and
41 Co content in the soil solution, but also strongly by the part linked to colloidal fractions. Availability of Cu and
42 Co is a complex mechanism, closely related to all the biogeochemical processes which occur in the rhizosphere.
43 Future work should perform experiments in controlled conditions to examine the soil parameters that influence
44 the Cu and Co availability.

45

46

47

48

49 **Abbreviations**

50

51 MnOX = manganese oxides

52 FeOx = iron oxides

53 HM = humic material

54 OM = organic matter

55 -MnOx = bound to manganese oxides

56 -FeOx = bound to iron oxides

57 -OM = bound to organic matter

58 SD = standard deviation

59

60

61

62

63

64 **Introduction**

65 Soils contaminated by metals represent an important constraint for vegetation. Only a few species
66 tolerate soil metal concentrations up to 1,000 times higher than the concentration usually found in normal soils
67 (Ernst 1974; Reeves and Baker 2000). Some of these, called hyperaccumulators, are able to concentrate metal in
68 their shoot up to extremely high concentrations (Baker 1981; Macnair 2003; Krämer 2010; Rascio and Navari-
69 Izzo 2011). For two decades, hyperaccumulator species have attracted much attention because of their potential
70 use as a phytoextraction strategy. According to the recent literature, species would be considered as Cu and Co
71 hyperaccumulators when the metal concentration in shoots exceeds 300 mg.kg^{-1} , without toxicity symptoms and
72 growth inhibition (Krämer 2010; Van der Ent et al. 2013). Although Macnair (2003) suggests that Cu
73 hyperaccumulation has not yet been observed in control conditions, Küpper et al. (2009) found that *Crassula*
74 *helmsii* growing in hydroponic conditions could accumulate more than $9,000 \text{ mg.kg}^{-1}$ in shoot when the nutrient
75 solution was enriched with less than $1 \text{ mg.kg}^{-1} \text{ Cu}^{2+}$. Only a few species around the world (mostly
76 Brassicaceae), are considered as constitutive hyperaccumulators. These species show that all populations have
77 very high foliar metal concentrations irrespective of metal concentration in soil, including *Noccaea caerulescens*
78 (Dechamps et al. 2007), *Arabidopsis halleri* (Frerot et al. 2010) and *Gomphrena claussenii* (Villafort Carvalho et
79 al. 2013) (zinc and cadmium hyperaccumulators); as well as several *Alyssum* species (nickel hyperaccumulator)
80 (Brooks and Radford 1978; Brooks et al. 1979; Broadley et al. 2001; Hanikenne and Nouet 2011). Most of
81 metallophytes are defined as facultative hyperaccumulators, which means that populations or individuals are
82 hyperaccumulators and some are not (Pollard et al. 2002).

83 Plants which accumulate Cu and Co in their shoots are rare and most of them occur in Katanga (Dem.
84 Rep of Congo) (Reeves and Baker 2000; Reeves 2006). In this region of South Central Africa, a species-rich
85 vegetation is associated to natural outcrops of Cu and Co enriched bedrocks, including cuprophytes (Duvigneaud
86 1958; Duvigneaud and Denaeyer-De Smet 1963; Ernst 1974; Ernst 1990; Faucon et al. 2012a; Ilunga wa Ilunga
87 et al. 2013) and cobaltophytes (Duvigneaud 1959; Faucon et al. 2010; Saad et al. 2012; Seleck et al. 2013).
88 Ecology and evolution of copper and cobalt tolerance and accumulation in this vegetation is still misunderstood,
89 due to high inter- and intraspecific variations of the Cu and Co shoot concentrations. Unusually high shoot Cu
90 and Co concentrations reported in earlier studies may result from surface contamination by soil particles and,
91 therefore, plant materials from metal-rich soils should be washed prior to analysis (Faucon et al. 2007; Van der
92 Ent et al. 2013). Copper in shoots ranges from 80 to $1,400 \text{ mg.kg}^{-1}$ and from 330 to $1,200 \text{ mg.kg}^{-1}$ for
93 *Crepidiorhodon perennis* and *Anisopappus chinensis*, respectively (Faucon et al. 2007; Faucon et al. 2009a). In

94 the same study, for Co, both species had shoot concentrations that ranged from 61 to 1,105 mg.kg⁻¹ and from 933
95 to 1,948 mg.kg⁻¹, respectively. Such variations have a genetic origin for Cu, especially due to genetic
96 differentiations among populations, as demonstrated by cultivation in uniform conditions (Faucon et al. 2012b).
97 However, high inter- and intra-population phenotypic variations in Cu and Co shoot concentration suggest a
98 diversity of accumulation responses and an influence of edaphic parameters upon Cu and Co accumulation by
99 plants. A good understanding of the Cu and Co accumulation variations would go through an accurate
100 characterization of the soil-root interface properties and mechanisms, controlling metal availability. The
101 influence of soil upon metal accumulation by plants can be studied in experimental conditions, by using a metal
102 contamination gradient in the substrate, but this approach has limits and can be criticized due to differences with
103 the in nature soil properties (e.g. Van der Ent et al. 2013). In such an approach, Cu hyperaccumulation is poorly
104 expressed (Morrison et al. 1979, 1981; Chipeng et al. 2010; Faucon et al. 2012) and variations could not be
105 distinguished from substrate differences (Escarre et al. 2013). Another approach could identify the relationship
106 between metal concentrations in soils and in shoots by studying populations from pedogeochemically contrasted
107 sites. Ecological studies have commonly used total metal concentrations or extractable metal concentrations
108 determined at a fixed pH value (AcNH₄EDTA 1M pH=4.65) (Brun et al. 1998; Faucon et al. 2009). In this way,
109 pH variations are not considered, while it highly influences metal mobility (Alloway 1995; McLaren and
110 Crawford 1973; Kabata-Pendias and Pendias 2001; Chaignon et al. 2002; Faucon et al. 2011). It is important to
111 include soil pH, due to the direct impact of the Cu and Co speciation upon the Cu and Co uptake by plant
112 (Poschenreider et al. 2001; Krishnamurti and Naidu 2002; Chaignon et al. 2002).

113 By considering soil pH, organic matter content and total concentrations of several elements in soil,
114 chemical speciation modeling appears to be an interesting tool to investigate the relationships between soil
115 properties and metal accumulation in plants. Using speciation calculation codes including chemical constants
116 and equilibrium, these methods can assess metal fractionation in soils. Windermere Humic Aqueous Model
117 (WHAM) (Tipping 1998; Pourret et al. *submitted*) can be used to calculate metal fractionation, and thus, estimate
118 the bound to MnOx, FeOx and OM metal concentrations, as well as the free metal concentration (i.e. ionic form)
119 in a soil sample. To understand Cu and Co accumulation variations, inclusion of the essential soil parameters
120 controlling Cu and Co mobility in soils is necessary: pH, redox potential, OM quality and quantity, oxides, clays,
121 sulphides and carbonates (Kabata-Pendias and Pendias 2001). These factors may partly control Cu and Co
122 availability, by influencing the equilibrium between total and available metal concentration in soils. However,
123 predicting the availability of metals, especially in contaminated environments, is still very difficult (Hinsinger

124 and Courchesne 2008). Key role of root-induced processes on soil constituents' mobility (Harter and Naidu
125 2001; Hinsinger 2001; Adriano et al. 2004; Houben and Sonnet 2012) as well as rhizosphere chemistry and
126 microbiology (Wenzel 2009; Alford et al. 2010) increases the complexity of metal availability. Mobility and
127 availability of metals at the soil root-interface need to be investigated and to date, there is no universal method to
128 estimate the metal availability to plants and soil organisms (Nolan et al. 2003). It has however been established
129 that the metal concentration in the soil solution would be the only fraction directly available for plant uptake
130 (Fageria et al. 1991; Marschner 1995; Whitehead 2000).

131 In the present study, speciation modeling is used to investigate the relationships between Cu and Co
132 accumulation by plants and Cu and Co chemical forms in soil. Aims were to (i) examine variations of Cu and Co
133 speciation in soils and Cu and Co concentrations among plant populations, (ii) determine which edaphic factors
134 and Cu and Co fractions influence the Cu and Co accumulation in plants, and (iii) compare the responses for two
135 different species. Several hypotheses can be suggested: (i) variations in the Cu and Co speciation would explain
136 the Cu and Co shoot concentration variations; (ii) the mobile Cu and Co concentration in soils would explain
137 accumulation variations.

138

139 **Materials and methods**

140

141 *Plant populations*

142

143 Two model species have been selected in the study. *Crepidorhopalon tenuis* (S. Moore) Fischer (Linderniaceae)
144 is a pseudometallophyte, Cu and Co hyperaccumulator species (Faucon et al. 2009). It is an annual species
145 colonizing recently disturbed mine deposits. *Anisopappus chinensis* (L.) Hook.f. & Arn. (Asteraceae) is also a
146 pseudometallophyte. It is a short-lived perennial found in relatively closed steppic savanna on stabilised
147 contaminated substrates (Ilunga wa ilunga et al. 2013; Seleck et al. 2013). Large variations of Cu and Co
148 concentrations in shoots of this species have already been highlighted (Faucon et al. 2007). Four populations of
149 both species were selected in different sites, in the Katanga region (Dem. Rep. of Congo), as described in Table
150 1. Two populations of *C. tenuis* were sampled on the anthropogenically disturbed sites Ruashi (Ru)
151 (recolonization of mine deposits) and Vallée Karavia (VK) (Cu-Co atmospheric fall-out contamination) and two
152 naturally occurring populations in the Cu-Co hills locally disturbed by reworked substrate (artisanal mining):
153 Niamumenda (Nm) and Kalabi (Ka). The four populations of *A. chinensis* have been sampled on two natural Cu-

154 Co undisturbed hills from the Tenke-Fungurume region: Fungurume5 (F5) and Goma2 (G2), and two disturbed
155 Cu-Co hills in the Lubumbashi region: Mine de l'Etoile (E) and Niamumenda (Nm).

156

157 *Sampling and samples preparation*

158

159 In each site, 20 plants (whole shoot) and 20 soil samples from the rhizosphere of each plant (0-15 cm) were
160 collected. Study populations were carefully delimited and sampling was carried out systematically across the
161 sites, covering the soil heterogeneity in each site. Plants were collected at the same development stage. Sampling
162 of *C. tenuis* occurred in April 2006 and 2007 and sampling for *A. chinensis* occurred during March and April
163 2012. A total of 146 plant and soil samples were considered in this study.

164 After harvesting, plants were carefully brushed (whole shoots), washed with Alconox® 1% in demineralized
165 water, dried at 60°C for 48 h (Faucon et al. 2007) and weighted. Soil samples were dried at room temperature,
166 sieved (2 mm) and ground in a mill (RETSCH RM 200).

167

168 *Plant and soil analysis*

169

170 The *Crepidiorhopalon tenuis* samples were mineralized by dry ashing in a muffle furnace at 550°C for 12 h. Ash
171 was dissolved in HCl. The samples were then analyzed by Inductively Coupled Plasma Optical Emission
172 Spectrometry (ICP-OES) (Varian Vista MPX) to determine Cu-Co concentration in shoot. To determine Cu-Co
173 concentration in shoot of *A. chinensis* two steps were performed. A mass of shoot were digested using a mixture
174 of 8 mL HNO₃ and 2 mL HCl (Avula et al. 2010) with a low pressure mineralization (Lavilla et al. 2009).
175 Vessels containing the mix were placed in a Mars 5 microwave (Microwave Accelerated Reaction System –
176 CEM corporation, USA) according to the Avula et al. (2010) treatment. Then, Cu and Co concentrations in
177 samples were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific
178 XSERIES2). Quantitative analyses were carried out by external calibration (eight points) by using mono- and
179 multi-element standard solutions (Accu Trace Reference, USA). Indium was used as an internal standard at a
180 concentration of 100 µg.L⁻¹ in order to correct for instrumental drift and matrix effects. The measurement bias
181 for the determination of the concentration of Cu and Co was assessed by the analysis of the SRM1573a certified
182 reference material (tomato leaves: Gills 1995). Typical uncertainties including all error sources are below ±5%
183 for Cu and Co. The soil samples pH (water) was determined on a saturated soil-water paste and Organic Matter

184 (OM) content by loss on ignition (500°C for 8 h). Soil chemical analyses were performed by Acme Analytical
185 Laboratories Ltd. (Vancouver Canada), accredited under ISO 9002. The considered analyzed elements were Co,
186 Cu, Fe, Mn, Zn, K, Mg, Al, Ca. Soil samples were digested using a strong multi-acid method that dissolves most
187 minerals. Then, 0.25 g split was heated in HNO₃-HClO₄-HF to fuming and taken to dryness. The residue was
188 further dissolved in HCl and solutions were analyzed using ICP-MS.

189

190 *Chemical speciation calculations*

191

192 WHAM 6 (version 6.0.10) was used to calculate Cu and Co speciation. Predictions for the equilibrium metal
193 binding by environmental colloids made for the present study were done using the combined WHAM-SCAMP
194 speciation code. WHAM-SCAMP is able to provide a full description of solid-solution speciation by
195 incorporating two main codes: (i) the Windermere Humic Aqueous Model (WHAM) to calculate the equilibrium
196 solution speciation (Tipping 1994), and (ii) the Surface Chemistry Assemblage Model for Particles (SCAMP) to
197 calculate the binding of protons and metals by natural particulate matter (Lofts and Tipping 1998). The code for
198 the WHAM model incorporates a number of submodels: Humic Ion-Binding Model 6 and a description of
199 inorganic solution chemistry, cation exchange by clays, the precipitation of aluminium and iron oxyhydroxides,
200 and adsorption-desorption of fulvic acids. The SCAMP model consists of three submodels: (i) Humic Ion-
201 Binding Model 6, (ii) a SCM describing proton and metal binding to oxides (e.g., MnOx or FeOx), and (iii) a
202 model describing the electrostatic exchange of cations on clays. Three binding phases were examined: MnOx,
203 FeOx, and Humic Material (HM). The X-Ray diffraction revealed that MnOx and FeOx are respectively
204 pyrolusite and a mix hematite-goethite. The concentration of HM were derived from the experimental OM
205 measurements: 50% of the OM measured in the field samples was assumed to be HM, themselves being defined
206 as 100% fulvic acid. Saturation index and mineral precipitation were not considered; which could be a limitation
207 of this approach (Pourret et al. *submitted*).

208 Input data for the Cu and Co speciation determination were total concentrations of Cu, Co, Mg, Ca, Mn, Fe, K,
209 Zn, pH of soils and OM content. A speciation calculation was achieved for the 160 soil samples. In this study,
210 the term free was used to qualify the Cu and Co modeling output mobile fraction, considered as the ionic
211 fraction. Sulphates and carbonates were not considered.

212

213 *Statistical analysis*

214

215 Descriptive statistics were performed on total soil analysis and normality of data and homogeneity of variances
216 were verified. One way ANOVA (Analysis of Variance) tested differences in Cu and Co concentrations in shoots
217 among populations and differences in Cu and Co fractionation among sites. Significance was defined and
218 represented as follow: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS=non-significant. Post-hoc multiple comparisons
219 (Tukey HSD) have been applied to compare populations from each other and Cu-Co fractions resulting from
220 speciation modeling from each other. The relationship between element concentration in plants and edaphic
221 factors (concentrations of Cu, Co, Mn, Fe, Mg, Ca, OM (%), pH and the binding phases from speciation
222 modeling results) were characterized with Pearson correlations.

223

224 **Results**

225

226 *Cu and Co fractionation in soils*

227

228 Results of Cu and Co fractionation are presented in Table 2. Total Cu and Co concentrations showed differences
229 between sites ($F_{(6,147)} = 7.65$, $p < 0.001$ and $F_{(6,147)} = 14.26$, $p < 0.001$, respectively). There were significant
230 differences between Cu fractions for each site (Tab. 2). For Cu, the two largest fractions were Cu bound to OM
231 (Cu-OM) and to FeOx (Cu-FeOx). The free Cu concentration varied from 0.7% to 18.8% of the total Cu
232 concentration. Copper was mainly bound to FeOx and OM. Significant differences for each Cu fraction existed
233 between sites (Tab. 2). The highest concentrations of Cu-FeOx were found in Nm and E (69% of total Cu) where
234 mean total Fe concentration reached 48,500 mg.kg⁻¹ and 42,300 mg.kg⁻¹, respectively. Samples from VK were
235 characterized by low total Fe and Mn contents (respectively mean = 19,610 ± 9,654 mg.kg⁻¹; and mean = 65 ± 37
236 mg.kg⁻¹) and high OM content (mean = 9.38% ± 4.2%). At this site, the fraction of Cu bound to FeOx was the
237 lowest while the fraction of Cu bound to OM was the highest (respectively mean = 15% and 81.8% of total Cu).
238 There were significant differences between Co fractions for each site (except samples from E) (Tab. 2). Cobalt
239 was predominantly free and bound to MnOx (Co-MnOx) in soils. The free Co fraction was higher than the free
240 Cu one for each site (meanly from 28.1 to 69.4% of total Co). Significant differences for each Co fraction
241 existed between sites. Nm was characterized by the highest Mn content in soil (mean = 5,700 mg.kg⁻¹). At this
242 site, the Co-MnOx concentration was the highest (mean = 71.1% of total Co). At VK, site where Mn

243 concentration was the lowest, Co was mainly bound to OM (meanly 53.8% of total Co). At this site, the Co-
244 MnOx concentration was the lowest (mean = 5.8% of total Co).

245

246 *Cu and Co accumulation in plants*

247

248 Results of Cu and Co concentrations in shoots showed large variations between species and populations
249 (Fig. 1). For Cu, 22 of 79 samples of *A. chinensis* and 25 of 67 samples of *C. tenuis* accumulated more than 300
250 mg.kg⁻¹ (range: 4 - 2,821 mg.kg⁻¹ and 34 - 2,524 mg.kg⁻¹ for *A. chinensis* and *C. tenuis*, respectively). For Co, 26
251 of 79 samples of *A. chinensis* and 2 of 67 samples of *C. tenuis* accumulated more than 300 mg.kg⁻¹ (range: 3 -
252 1,334 mg.kg⁻¹ and 8 - 605 mg.kg⁻¹ for *A. chinensis* and *C. tenuis*, respectively).

253 Large Cu and Co accumulation variations existed among populations of both species (for Cu, $F_{(7,145)} =$
254 7.1, $p < 0.001$; for Co, $F_{(7,145)} = 17.6$, $p < 0.001$). Population F5 of *A. chinensis* had the highest Co shoot
255 concentration (mean = 1,089 ± 768 mg.kg⁻¹) ($F_{(7,145)} = 19.2$, $p < 0.001$). Strikingly low concentrations of Co in
256 plant shoots (<20 mg.kg⁻¹) were found in the Nm site for both species. On the contrary, that site yielded the
257 highest shoot Cu accumulation for both species (*A. chinensis*, range: 104 - 1,335 mg.kg⁻¹, mean = 455 mg.kg⁻¹
258 and *C. tenuis*, range: 84 - 2,525 mg.kg⁻¹, mean = 673 mg.kg⁻¹) ($F_{(7,145)} = 125.4$, $p < 0.001$).

259

260 *Relationships between edaphic factors and Cu and Co accumulation in shoot*

261

262 Correlations between soil fractions and shoot accumulation were element- and species- dependent (Tab.
263 3, Fig. 2), however, some general patterns emerged. Correlations between total Cu soil content and shoot Cu
264 concentration were positive for *C. tenuis* ($r = 0.51$, $p < 0.001$) and non-significant for *A. chinensis* (Fig. 2a). Total
265 Co soil content was positively correlated with shoot Co concentration for *A. chinensis* and *C. tenuis* ($r = 0.77$,
266 $p < 0.001$ and $r = 0.45$, $p < 0.001$, respectively) (Fig. 2b). No correlations occurred between pH and shoot Co
267 concentration for both species. On the contrary, pH was positively correlated with Cu concentration in shoots for
268 *C. tenuis* ($r = 0.40$, $p < 0.001$) but not significant for *A. chinensis*. Organic matter soil content was negatively
269 correlated with shoot Cu concentration for both species. Positive correlation have been observed between OM
270 soil content and shoot Co concentration for *A. chinensis* ($r = 0.57$, $p < 0.001$) but not significant for *C. tenuis*. Shoot
271 Cu concentration was correlated with total Mn soil content for *A. chinensis* and *C. tenuis* ($r = 0.51$, $p < 0.001$ and
272 $r = 0.65$, $p < 0.001$, respectively). However, the strong negative correlation between total Mn soil content and shoot

273 Co concentration for *A. chinensis* ($r = -0.65$, $p < 0.001$) was not verified for *C. tenuis*. A positive correlation was
274 established between total Fe soil content and shoot Cu concentration for *A. chinensis* ($r = 0.42$, $p < 0.001$), but not
275 for *C. tenuis*. A negative correlation occurred between total Fe soil content and Co concentration in shoot for *A.*
276 *chinensis* ($r = 0.5$, $p < 0.001$). This relationship was not verified for *C. tenuis*.

277 A positive correlation was observed between free Cu concentration and shoot Cu concentration for *C.*
278 *tenuis* ($r = 0.50$, $p < 0.001$), but not for *A. chinensis* ($r = 0.49$, $p < 0.05$) (Fig. 2c). Free Co concentration was
279 positively correlated with shoot Co concentration for *A. chinensis* and *C. tenuis* ($r = 0.8$, $p < 0.001$ and $r = 0.57$,
280 $p < 0.001$, respectively) (Fig. 2d). For Cu, the highest correlation was observed between Cu bound to MnOx (Cu-
281 MnOx) soil concentration and shoot Cu concentration for *A. chinensis* and *C. tenuis* ($r = 0.39$, $p < 0.001$ and
282 $r = 0.76$, $p < 0.001$, respectively) (Fig. 2e). However, Cu-FeOx soil concentration was also positively correlated
283 with shoot Cu concentration for both species. This latter concentration was negatively correlated with shoot Co
284 concentration for *A. chinensis*. For Co, the highest correlation was observed between Co bound to OM (Co-OM)
285 soil concentration and shoot Co concentration for *A. chinensis* and *C. tenuis* ($r = 0.9$, $p < 0.001$ and $r = 0.64$,
286 $p < 0.001$, respectively) (Fig. 2f). A positive correlation was also observed between Co bound to FeOx (Co-FeOx)
287 soil concentration and shoot Co concentration for both species.

288

289 **Discussion**

290

291 *Cu and Co speciation in soils*

292

293 Copper appeared to have a strong affinity for FeOx and OM which would explain the low free Cu
294 concentrations in soils compared to other Cu fractions. In normal soils, copper co-adsorbs with Mn and Fe
295 oxides (McLaren and Crawford 1973). Copper was fixed by OM when total Fe content was low (VK). An
296 increase in the total Fe content (E, Nm, Ka) resulted in an increase of the Cu-FeOx concentration. This suggests
297 that Fe oxides are potentially major competitors of OM for Cu speciation.

298 Our results showed that cobalt had a particular affinity with Mn oxides (Tab. 2). This is in agreement
299 with the literature which highlights that cobalt is adsorbed at the surface of Mn oxides (Childs 1975; Li et al.
300 2004; Tontgavee et al. 2005; Luo et al. 2010). Sites with the lowest Mn soil content exhibited major Co free and
301 Co-OM fractions, suggesting an important role of Mn in the Co speciation.

302 Samples from VK, with a high OM content compared to other sites and the lowest total Mn
303 concentration in soils showed the highest Co-OM concentration. Organic matter appears to be a major
304 competitor of Mn oxides for Co speciation (Collins and Kinsela 2010, Collins and Kinsela 2011). Free Co
305 concentration is higher, in percentage, than free Cu concentration, relative to the other Co or Cu fractions (Tab.
306 2). This could be explained by the stronger capacity to complex with colloids for Cu, compared to Co. Indeed,
307 the sequence of complex stability of transition metals, known as the Irving Williams series, is typically
308 $\text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} < \text{Zn}^{2+}$ (Stumm and Morgan 1996). Organic matter content seems to have a strong influence upon
309 metal fractionation, which confirms results obtained by Pourret et al. (2007). It can be assumed that mobile and
310 non-mobile Cu and Co fractions vary among sites and seem to be closely related to the MnOx and FeOx
311 concentrations, the OM content and the soil pH. High variability of Cu and Co speciation in soils has been
312 observed, which would involve variations of the Cu and Co availability.

313

314 *Variations of Cu and Co concentrations in shoots among populations*

315

316 Results confirm that *A. chinensis* and *C. tenuis* are facultative Cu and Co hyperaccumulators (Faucon et
317 al. 2009), characterized by a high variation of Cu and Co shoot concentrations. For Cu and Co respectively, 17%
318 and 21% of plants of *A. chinensis* and 17% and 2% of plants of *C. tenuis* have been recorded with a
319 concentration in shoots of up to 300 mg.kg^{-1} , i.e. the hyperaccumulation threshold (Van der Ent et al. 2013),
320 without showing any toxicity symptoms or growth inhibition.

321 The phenotypic accumulation variations would be associated with specific soil conditions.
322 Interpopulation variations of Cu and Co accumulation in plant shoots would not only be explained by variations
323 of the total and free Cu and Co soil content. The variation of edaphic factors, and especially the OM and total Fe
324 content, could explain the Cu accumulation variability among populations of both species. The high Cu-OM and
325 Cu-FeOx concentration seem to limit the Cu uptake in populations of both species (Fig. 1, Tab. 2). For Co, the
326 high Co-MnOx concentration at Nm compared to the other Co fractions could explain the lowest Co
327 accumulation observed for the two species (Fig. 1, Tab. 2). In this context, variations of Cu and Co accumulation
328 among populations of *A. chinensis* and *C. tenuis* would correspond, not only to the genetic capacity to
329 accumulate metal in shoots (Faucon et al. 2012), but also in part to edaphic context variations, especially Cu and
330 Co speciation variations in the rhizosphere. Results showed the importance of relating Cu and Co concentrations

331 in shoots with Cu and Co fractions in soils, to explain the Cu and Co shoot concentration variations observed *in*
332 *natura*.

333

334 *Relationships between Cu and Co speciation variations in soils and Cu and Co shoot accumulation variations*

335

336 Results confirm the strong synergistic influence of soil total Mn content on Cu accumulation in cuprophytes
337 and its antagonist effect on Co accumulation as shown by Faucon et al. (2009). Interestingly, total Fe content in
338 soils seems to be a positive predictor of Cu accumulation for *A. chinensis*, the opposite effect seen in regrass, a
339 metal non-tolerant species (Li et al. 2004). The Cu mobility in soil is known to be negatively correlated with pH
340 (Kabata-Pendias and Pendias 2001). Therefore, the positive correlation between Cu accumulation in *C. tenuis*
341 and soil pH in this study may be surprising. However, the range in pH values in our study is relatively restricted
342 for *C. tenuis* soils (from 4.4 to 6.2) and it also appears that the correlation is driven mostly by one site (VK)
343 which has relatively low pH and relatively low Cu accumulation in plants.

344 The differences of Cu and Co accumulation in shoot plants with respect to the variability of Cu and Co
345 speciation in soils and following edaphic factors, pH, OM, total Mn and total Fe contents exist between both
346 species. These differences may also suggest species-specific mechanisms of the Cu and Co availability, uptake
347 and transport in the plant, and confirms the complexity of soil-plant processes (Hinsinger and Courchesne 2008).

348 The results showed the influence of Cu and Co speciation on the variability of Cu and Co accumulation in
349 both species (Tab. 3). Copper accumulation in plant shoots were positively influenced by Cu-MnOx and Cu-
350 FeOx concentrations, whereas Co accumulation variations were strongly influenced by Co free and Co-OM and
351 Co-FeOx concentrations. The Co-OM soil fraction seems to appear to be the most available Co fraction, in those
352 soils for both species. Similarly, both Li et al. (2004) and McLaren et al. (1987) highlighted that organically-
353 bound Co influenced Co availability to plants. Availability of Cu and Co does not seem to be strictly a result of
354 the free Cu and Co concentrations in soils. Even if ionic species metal concentrations in soils would appear, by
355 hypothesis, to be a good predictor of metal uptake concentration by plants, because of their direct availability
356 (Fageria et al. 1991; Marschner 1995; Whitehead 2000), the bound to solid-phase fractions have to be considered
357 for availability assessment. Indeed, some plants are able to mobilize and take up elements from the non-mobile
358 solid fraction (Knight et al. 1997; Hinsinger et al. 2005). These significant influences of the solid phases upon
359 the accumulation for both species could be explained by root-induced processes which could be responsible to
360 pH changes (Marschner 1995; Hinsinger et al. 2003). Indeed, the soil solution acidification by root activity, may

361 causes desorption of metals (Marschner 1995; Harter and Naidu 2001; Hinsinger et al. 2003) and thereby,
362 increase the ionic form metal concentration. This study confirms that the availability of metals is closely related
363 to all the biogeochemical processes which occur in the rhizosphere. Estimate of the available metal concentration
364 in soils seem to be complex but the present study is the first attempt of Cu and Co availability assessment in
365 metalliferous soils. Undertaking a laboratory experiment which simulates the field conditions would help unravel
366 some of this complexity. For this, both species could be cultivated in growth-chamber, on homogeneous soil
367 contaminated by defined Cu and Co concentrations, in which chemical factors influencing Cu and Co
368 availability (pH, OM, MnOx, FeOx) would vary in controlled conditions (Chaignon and Hinsinger 2003).
369 However, as microbial activities would also influence the availability of chemical elements in soil (Hinsinger et
370 al. 2005) and the patterns of metal accumulation in plant species (Fomina et al. 2005; Toler et al. 2005; Barzanti
371 et al. 2007; Kabagale et al. 2010) incorporating microbial activities in an experimental setting to test their effect
372 on the variation of Cu and Co accumulation in plants would prove fruitful. Perspectives would be to integrate the
373 study of influence of microbial activities on the variation of Cu and Co accumulation in plants.

374 Comprehension of the Cu and Co accumulation variations in plant shoots *in situ* and methods of
375 phytoremediation of Cu and Co contaminated soils need therefore to be reassessed in the light of the present
376 results.

377

378 **Acknowledgements**

379
380 The Polytechnic Institute LaSalle Beauvais (IPLB, Fr) and the Belgian Fund for Scientific Research (FRS-
381 FNRS) are acknowledged for financial support to Bastien Lange, who is a research fellow of the Fonds pour la
382 Recherche dans l'Industrie et l'Agriculture (FRIA, Belgium). Chemaf society, Kalumine society, Tenke
383 Fungurume Mining S.a.r.l. permitted us the plants and soils collection. We are grateful to Serge Ngoy and Jean-
384 Jacques Lunzanga for their help in the plants and soils sampling. We are grateful to Petru Jitaru from HydrISE
385 unit (IPLB, France) for his help in the ICP-MS analysis.

386 We gratefully thank David Houben (IPLB) and Jean-Paul Reynoird (IPLB) for the manuscript pre-review and
387 Kristine French (University of Wollongong, Au), native speaker, for English reviewing.

388

389

390

391 **References**

- 392
393 Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural remediation in
394 environmental cleanup. *Geoderma* 122:121-142
395
396 Alford ER, Pilon-Smits EA, Paschke MW (2010) Metallophytes – a view from the rhizosphere. *Plant Soil* 321(1-
397 2):33-50
398
399 Alloway BJ (1995) *Heavy Metals in Soils*. 2nd Ed. Blackie Academic and Professional, London. pp 368
400
401 Avula B, Wan YH, Smillie TJ, Duzgoren-Aydin N, Khan TJ (2010) Quantitative Determination of Multiple
402 Elements in Botanicals and Dietary Supplements Using ICP-MS. *Jour Agri Food Chem* 58:8887-8894
403
404 Baker AJM (1981) Accumulators and excluders – Strategies in the response of plants to heavy metals. *J Plant*
405 *Nutr* 3:643-654
406
407 Barzanti R, Ozino F, Bazzicalupo M, Gabbrielli R, Galardi F, Gonnelli C, Mengoni A (2007) Isolation and
408 characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. *Microb Ecol*
409 53:306-316
410
411 Brun LA, Maillet J, Richarte J, Herrmann P, Remy JC (1998) Relationships between extractable copper, soil
412 properties and copper uptake by wild plants in vineyard soils. *Environ Pollut* 102:151-161
413
414 Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ (2001) Phylogenetic variation in heavy
415 metal accumulation in angiosperms. *New Phytol* 152:9-27
416
417 Brooks RR, Morrison RS, Reeves RD, Dudley TR, Akman Y (1979) Hyperaccumulation of nickel by *Alyssum*
418 *linaeus* (Cruciferae). *Proc Roy Soc Lond* 203:387-403
419
420 Brooks RR, Radford CC (1978) Nickel accumulation by European species of the genus *Alyssum*. *Proc Roy Soc*
421 *Lond* 200:217-224
422
423 Chaignon V, Bedin F, Hinsinger P (2002) Copper bioavailability and rhizosphere pH changes as affected by
424 nitrogen supply for tomato and oilseed rape cropped on an acidic and calcareous soil. *Plant Soil* 243:219-228
425
426 Chaignon V, Hinsinger P (2003) A Biotest for Evaluating Copper Bioavailability to Plants in a Contaminated
427 Soil. *J Environ Qual* 32:824-833
428
429 Childs CW (1975) Composition of iron-manganese concretions from some New Zealand soils. *Geoderma*
430 13:141-152
431
432 Chipeng FK, Hermans C, Colinet G, Faucon MP, NgongoLuhembwe M, Meerts P, Verbruggen N (2010) Copper
433 tolerance in the cuprophyte *Haumaniastrum katangense* (S. Moore) P.A. Duvign. & Plancke. *Plant Soil* 328:235-
434 244
435
436 Collins RN, Kinsela AS (2010) The aqueous phase speciation and chemistry of cobalt in terrestrial
437 environments. *Chemosphere* 79:763-771
438
439 Collins RN, Kinsela AS (2011) Pedogenic factors and measurements of the plant uptake of cobalt. *Plant Soil*
440 339:499-512
441
442 Dechamps C, Lefèbvre C, Noret N, Meerts P (2007) Reaction norms of life history traits in response to zinc in
443 *Thlaspi caerulescens* from metalliferous and non metalliferous sites. *New Phytol* 173:191-198
444
445 Duvigneaud P (1958) The vegetation of Katanga and its metalliferous soils. *Bull Soc Roy Bot Belg* 90:127-286
446
447 Duvigneaud P (1959) Plantes cobaltophytes dans le Haut Katanga. *Bull Soc Roy Bot Belg* 91:111-134
448
449 Duvigneaud P, Denaeyer- De Smet S (1963) Cuivre et vegetation au Katanga. *Bull Soc Roy Bot Belg* 96:92-231
450

451 Ernst W (1974) *Schwermetallvegetation der Erde*. G. Fisher Verlag, Stuttgart.

452

453 Ernst W (1990) Mine vegetation in Europe. In: Shaw JA (ed) *Heavy metal tolerance in plants: evolutionary*

454 *aspects* vol 18. CRC, New York, 21-38

455

456 Escarre J, Lefebvre C, Frerot H, Mahieu S, Noret N (2013) Metal concentration and metal mass of metallicolous,

457 non metallicolous and serpentine *Noccaea caerulescens* populations, cultivated in different growth media. *Plant*

458 *Soil* 370:197-221

459

460 Fageria NK, Wright RJ, Baligar VC, Sousa CMR (1991) Characterization of physical and chemical properties of

461 varzea soils of Goias State of Brazil. *Commun Soil Sci Plant Anal* 22:1631-1646

462

463 Faucon MP, Colinet G, Jitaru P, Verbruggen N, Shutcha M, Mahy G, Meerts P, Pourret O (2011) Relation

464 between Cobalt Fractionation and its Accumulation in Metallophytes from South Central Africa. *Mineral Mag*

465 75:832

466

467 Faucon MP, Colinet G, Mahy G, NgongoLuhembwe M, Verbruggen N, Meerts P (2009) Soil influence on Cu

468 and Co uptake and plant size in the cuprophytes *Crepidorrhopalon perennis* and *C. tenuis* (Scrophulariaceae) in

469 SC Africa. *Plant Soil* 317:201-212

470

471 Faucon MP, Chipeng F, Verbruggen N, Mahy G, Colinet G, Shutcha M, Pourret O, Meerts P (2012) Copper

472 tolerance and accumulation in two cuprophytes of South Central Africa: *Crepidorrhopalon perennis* and *C. tenuis*

473 (Linderniaceae). *Environ Exp Bot* 84:11-16

474

475 Faucon MP, Meersseman A, Shutcha MN, Mahy G, Luhembwe MN, Malaisse F, Meerts P (2010) Copper

476 endemism in the Congolese flora: a database of copper affinity and conservational value of cuprophytes. *Plant*

477 *Ecol Evol* 143:5-18

478

479 Faucon MP, Shutcha M, Meerts P (2007) Revisiting copper and cobalt concentrations in supposed

480 hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil. *Plant Soil* 301:29-36

481

482 Faucon MP, Tshilong BM, Rossum F, Meerts P, Decocq G, Mahy G (2012) Ecology and Hybridization Potential

483 of Two Sympatric Metallophytes, the Narrow Endemic *Crepidorrhopalon perennis* (Linderniaceae) and its More

484 Widespread Congener *C. tenuis*. *Biotropica*44:454-462

485

486 Frerot H, Faucon MP, Willems G, Godé C, Courseaux A, Darracq A, Verbruggen N, Saumitou-Laprade P (2010)

487 Genetic architecture of zinc hyperaccumulation in *Arabidopsis halleri*: the essential role of QTL × environment

488 interactions. *New Phytol* 187:355–367

489

490 Fomina MA, Alexander IJ, Colpaert JV, Gadd GM (2005) Solubilization of toxic metal minerals and metal

491 tolerance of mycorrhizal fungi. *Soil Biol Biochem* 37:851-866

492

493 Gills TE (1995) Standard reference material 1537a – Tomatoe leaves – Certificate of Analysis. National Institute

494 of Standards and Technology pp 5

495

496 Harter RD, Naidu R (2001) An assessment of environmental and solution parameter impact on trace-metal

497 sorption by soils. *Soil Sci Soc Am J* 65:597-612

498

499 Hanikenne M, Nouet C (2011) Metal hyperaccumulation and hypertolerance: a model for plant evolutionary

500 genomics. *Curr Opin Plant Biol* 14:252-259

501

502 Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical

503 changes: a review. *Plant Soil* 237:173-195

504

505 Hinsinger P, Courchesne F (2008) Biogeochemistry of metals and metalloids at the soil-root interface 268-312.

506 In: *Biophysico-Chemical Processes of Heavy Metals and Metalloids in Soil Environment*. Edited by A Violante,

507 PM Huang, GM Gadd (2008) John Wiley and Sons, Inc

508

509 Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from

510 root-mediated physical and chemical processes. *New Phytol* 168:293-303

511
512 Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-induced pH changes in the rhizosphere and
513 their responses to environmental constraints: a review. *Plant Soil* 248:43-59
514
515 Houben D, Sonnet Ph (2012) Zinc mineral weathering as affected by plant roots. *Appl Geochem* 27:1587-1592
516
517 Ilunga wa Ilunga E, Seleck M, Colinet G, Faucon MP, Meerts P, Mahy G (2013) Small-scale diversity of plant
518 communities and distribution of species niches on a copper rock outcrop in Upper Katanga, DR Congo. *Plant*
519 *Ecol Evol* 146:173-182
520
521 Kabagale AC, Cornu B, van Vliet F, Meyer CL, Mergeay M, LumbuSimbi JB, Droogmans L, Vander Wauven
522 C, Verbruggen N (2010) Diversity of endophytic bacteria from the cuprophytes *Haumaniastrum katangense* and
523 *Crepidorhopalon tenuis*. *Plant Soil* 334:461-474
524
525 Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants. 3rd ed. CRC Press. Boca Raton, London,
526 New-York, Washington D.C. pp 403
527
528 Knight B, Zaho FJ, McGrath SP, Shen ZG (1997) Zinc and cadmium uptake by the hyperaccumulator *Thlaspi*
529 *caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil
530 solution. *Plant Soil* 197:71-78
531
532 Krämer U (2010) Metal hyperaccumulation in plants. *Annu Rev Plant Biol* 61:517-534
533
534 Krishnamurti GSR, Naidu R (2002) Solid-solution speciation and phytoavailability of copper and zinc in soils.
535 *Environ Sci Technol* 36:2645-2651
536
537 Küpper H, Götz B, Mijovilovich A, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper
538 in higher plants: I. Characterization of copper accumulation, speciation, and toxicity in *Crassula helmsii* as a
539 new copper accumulator. *Plant Physiol* 151:702–714
540
541 Lavilla I, Filgueiras AV, Bendicho C (1999) Comparison of Digestion Methods for Determination of Trace and
542 Minor Metals in Plant Samples. *J Agr Food Chem* 47:5072-5077
543
544 Li Z, McLaren RG, Metherell AK (2004) The availability of native and applied soil cobalt to ryegrass in relation
545 to soil cobalt and manganese status and other soil properties. *New Zeal J Agr Res* 47:33-43
546
547 Lofts S, Tipping E (1998) An assemblage model for cation binding by natural particulate matter. *Geochim*
548 *Cosmochim Acta* 62:2609-2625
549
550 Luo D, Zheng H, Chen Y, Wang G, Fenghua D (2010) Transfer characteristics of cobalt from soil to crops in the
551 suburban areas of Fujian Province, southeast China. *J Environ Manage* 91:2248-2253
552
553 Macnair MR (2003) The hyperaccumulation of metal by plants. *Adv Bot Res* 40:63-105
554
555 Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press International, San Diego, CA, USA
556
557 McLaren RG, Crawford DV (1973) Studies on soil copper. I. The fractionation of copper in soils. *J Soil Sci*
558 24:172-181.
559
560 McLaren RG, Lawson DM, Swift RS (1987) The availability to pasture plants of native and applied soil cobalt in
561 relation to extractable soil cobalt and other soil properties. *J Sci Food Agric* 39:101–112
562
563 Morrison RS, Brooks RR, Reeves RD, Malaisse F (1979) Copper and Cobalt uptake by metallophytes from
564 Zaïre. *Plant Soil* 53:535-539
565
566 Nolan AL, Lombi E, McLaughlin MJ (2003) Metal bioaccumulation and toxicity in soils – why bother with
567 speciation? *Aust J Chem* 56:77-91
568
569 Pollard AJ, Powell KD, Harperf A, Smith JAC (2002) The Genetic Basis of Metal Hyperaccumulation in Plants.
570 *Plant Sci* 21:539-566.

571
572 Poschenreider C, Bech J, Llugany M, Pace A, Fenes E, Barcelo J (2001) Copper in plant species in a copper
573 gradient in Catalonia (North East Spain) and their potential for phytoremediation. *Plant Soil* 230:247-256
574
575 Pourret O, Dia A, Davranche M, Gruau G, Henin O, Angee M (2007) Organo-colloidal control on major- and
576 trace-element partitioning in shallow groundwaters: Confronting ultrafiltration and modeling. *App Geochem*
577 22:1568-1582
578
579 Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what
580 makes them so interesting? *Plant Sci* 180:169-181
581
582 Reeves RD (2006) Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N
583 (eds) *Phytoremediation of Metal-Contaminated Soils*. NATO Science series: IV: earth and environmental
584 sciences vol 68. Springer, New York 193-221
585
586 Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) *Phytoremediation of*
587 *toxic metals*. Wiley, New York 193-221
588
589 Saad L, Parmentier I, Colinet G, Malaisse F, Faucon MP, Meerts P, Mahy G (2012) Investigating the vegetation–
590 soil relationships on the copper–cobalt rock outcrops of Katanga (DR Congo), an essential step in a biodiversity
591 conservation plan. *Restor Ecol* 20:405-415
592
593 Séleck M, Bizoux JP, Colinet G, Faucon MP, Guillaume A, Meerts P, Piqueray J, Mahy G (2013) Chemical soil
594 factors influencing plant assemblages along copper-cobalt gradients: implications for conservation and
595 restoration. *Plant Soil* 373:455-469
596
597 Stumm W, Morgan JJ (1996) *Aquatic chemistry, Chemical Equilibria and Rates in Natural Waters*, 3rd ed. John
598 Wiley & Sons, Inc, New York pp1022
599
600 Tipping E (1994) WHAM – a chemical equilibrium model and computer code for waters, sediments and soils
601 incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Comp Geosci* 20:973-1023
602
603 Tipping E (1998) Humic ion-binding model VI: an improved description of the interactions of protons and metal
604 ions with humic substances. *Aquat Geochem* 4:3-48
605
606 Toler HD, Morton JB, Cumming JR (2005) Growth and metal accumulation of Mycorrhizal sorghum exposed to
607 elevated copper and zinc. *Water Air Soil Poll* 164:155-172
608
609 Tongtavee N, Shiwatana J, McLaren RG, Buanam J (2005) Evaluation of distribution and chemical
610 associations between cobalt and manganese in soils by continuous-flow sequential extraction. *Commun Soil Sci*
611 *Plan* 36:2839-2855
612
613 Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid
614 trace elements: Facts and fiction. *Plant Soil* 362:319-334
615
616 Villafort Carvalho M, Amaral DC, Guilheme LRG, Aarts MGM (2013) *Gomphrena claussenii*, the first South
617 American metallophyte species with indicator-like Zn and Cd accumulation and extreme metal tolerance. *Front*
618 *Plant Sci*. 4: pp10 DOI 10.3389/fpls.2013.00180
619
620 Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation
621 (phytoremediation) of soils. *Plant Soil* 321:385-408
622
623 Whitehead DC (2000) *Nutrient elements in grassland. Soil–plant–animal relationships*. CAB International,
624 Wallingford, UK, 369 pp
625
626
627
628
629
630

631 **Table 1 Location and habitat description of study sites**

632

633 **Table 2 Cu/Co mean fractionation among seven sites (%) according to the total mean Cu/Co**
634 **concentration in soil (mg.kg⁻¹)**

635 F5: n= 20; G2: n =20; E: n=20; Nm: n=35; Ka: n=17; VK: n=16; Ru: n=18. ***: p<0.001, **: p<0.01, *: p<0.05,
636 NS=non-significant. For columns, variables with the same letter above are not significantly different. For lines,
637 Cu and Co fractions (considered separately) with the same letter below are not significantly different (results of
638 post-hoc multiple comparison, Tukey HSD test). Free = ionic form, -MnOx = bound to manganese oxides, -
639 FeOx = bound to iron oxides, -OM = bound to organic matter, SD= standard deviation

640

641 **Table 3 Correlation between soil factors and Cu and Co concentrations in shoots of *Anisopappus chinensis***
642 **(n = 79) and *Crepidorhopalon tenuis* (n = 67): Pearson correlation coefficients using log-transformed data**

643 ***: p<0.001, **: p<0.01, *: p<0.05, NS=non-significant.

644

645

646

647

648

649

650

651

652

653

654

655

656

657

658

659

660

661

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691 **Table 1**

Site	Habitat description	Altitude (m)	Coordinates (GCSWGS84 DD)	Species sampled
Fungurume 5 (F5)	Natural Cu-Co hill not disturbed by mining. Sampled on grassland.	1,300	S 10,61777 E 26, 29112	<i>A. chinensis</i>
Goma 2 (G2)	Natural Cu-Co hill locally disturbed by mining. Sampled on grassland.	1,300	S 10,59966 E 26,13894	<i>A. chinensis</i>
Etoile (E)	Former natural Cu-Co hill completely disturbed by mining. Open pit. Sampled on remaining substratum.	1,280	S 11,63562 E 27,58449	<i>A. chinensis</i>
Niamumenda (Nm)	Natural Cu-Co hill locally disturbed by mining. Sampled on grassland locally disturbed.	1,340	S 11,60492 E 27,29400	<i>A. chinensis</i> <i>C. tenuis</i>
Kalabi (Ka)	Natural Cu-Co hill locally disturbed by mining. Open pit. Sampled on grassland locally disturbed.	1,200	S 10,78168 E 26,74053	<i>C. tenuis</i>
Vallée Karavia (VK)	Anthropogenic site: soil contaminated by atmospheric fallout from ore-smelter, moist.	1,230	S 11,67270 E 27,43091	<i>C. tenuis</i>
Ruashi (Ru)	Anthropic site: recolonization of Mine deposits. Sampled on remaining substratum.	1,300	S 11,62645 E 27,56328	<i>C. tenuis</i>

723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744

745
746

Table 2

Sites	Cu						Co					
	Total (mg.kg ⁻¹)	Free (%)	-MnOx (%)	-FeOx (%)	-OM (%)	One-way ANOVA (among Cu fractions)	Total (mg.kg ⁻¹)	Free (%)	-MnOx (%)	-FeOx (%)	-OM (%)	One-way ANOVA (among Co fractions)
F5 (SD)	18 298 ^{a,b} (13 285)	14 ^b _{a,b}	0.3 ^b _b	45.7 ^{a,c} _a	39.9 ^a _a	F _(3,75) = 5.775 *	3 451 ^a (2 614)	62.2 ^a _a	11.3 ^c _b	13.7 ^{a,b} _b	12.8 ^a _b	F _(3,75) = 12.31 ***
G2 (SD)	27 793 ^b (17 117)	18.8 ^a _a	1.1 ^b _b	52.7 ^{b,c} _a	27.4 ^a _a	F _(3,79) =9.023 ***	714 ^b (247)	61.2 ^b _a	27.7 ^{b,c} _b	4.5 ^b _c	6.6 ^{b,c} _c	F _(3,79) =38.87 ***
E (SD)	12 566 ^a (12 502)	2.6 ^b _a	0.4 ^b _a	69.2 ^{b,c} _b	27.8 ^b _a	F _(3,79) =16.11 ***	2 732 ^a (3 336)	28 ^{a,b} _a	33.4 ^a _a	30 ^a _a	8.6 ^c _a	F _(3,79) =2.027 NS
Nm (SD)	20 833 ^{a,b} (17 899)	5.5 ^b _a	4.8 ^a _a	69.1 ^b _b	20.6 ^b _a	F _(3,136) = 33.4 ***	168 ^b (54)	23 ^b _b	71.1 ^{b,c} _c	3.3 ^b _a	2.6 ^b _a	F _(3,136) =97.02 ***
Ka (SD)	18 624 ^{a,b} (13 392)	15.7 ^b _a	0.8 ^b _a	58.7 ^{b,c} _b	24.9 ^b _a	F _(3,71) =12.55 ***	911 ^b (809)	69.4 ^b _a	16.4 ^{b,c} _b	4.8 ^b _b	9.4 ^{b,c} _b	F _(3,71) =16.47 ***
VK (SD)	5 444 ^c (3 315)	3.1 ^b _a	0.1 ^b _a	15 ^a _a	81.9 ^{a,b} _b	F _(3,67) =38.62 ***	79 ^b (39)	39.3 ^b _a	5.8 ^b _b	1 ^b _b	53.9 ^{b,c} _a	F _(3,67) =24.62 ***
Ru (SD)	3 556 ^c (2 407)	0.7 ^b _a	0.3 ^b _a	36.9 ^a _b	62 ^b _b	F _(3,67) =20.62 ***	657 ^b (677)	28.2 ^b _a	38 ^c _a	11.4 ^b _a	22.5 ^c _a	F _(3,67) =1.57 ***
One-way ANOVA (among sites)	F _(6,147) = 7.652 ***	F _(6,147) = 3.883 *	F _(6,147) = 14.43 ***	F _(6,147) = 10.66 ***	F _(6,147) = 11.18 ***	/	F _(6,147) = 14.26 ***	F _(6,147) = 8.684 ***	F _(6,147) = 14.34 ***	F _(6,147) = 5.025 ***	F _(6,147) = 16.47 ***	/

747
748
749
750
751
752
753
754
755

756 Table 3

	<i>A. chinensis</i>		<i>C. tenuis</i>	
	Cu (shoot)	Co (shoot)	Cu (shoot)	Co (shoot)
[Cu] total	0.188 NS	- 0.192 NS	0.513 ***	0.092 NS
[Cu] free	0.111 NS	0.071 NS	0.497 ***	0.075 NS
[Cu-MnOx]	0.395 ***	- 0.535 ***	0.761 ***	- 0.195 NS
[Cu] free+ [Cu-MnOx]	0.207 NS	- 0.135 NS	0.566 ***	- 0.007 NS
[Cu-FeOx]	0.282 *	- 0.440 ***	0.603 ***	0.071 NS
[Cu] free + [Cu-FeOx]	0.204 NS	- 0.313 **	0.592 ***	0.085 NS
[Cu-OM]	-0.138 NS	0.404 ***	0.086 NS	0.295 *
[Cu] free + [Cu-OM]	-0.087 NS	0.314 **	0.232 NS	0.268 *
[Co] total	- 0.339 **	0.772 ***	0.245 *	0.475 ***
[Co] free	- 0.311 **	0.804 ***	0.219 NS	0.567 ***
[Co-MnOx]	-0.073 NS	0.280 *	0.452 ***	0.137 NS
[Co] free + [Co-MnOx]	-0.270 *	0.725 ***	0.374 **	0.392 **
[Co-FeOx]	-0.313 **	0.464 ***	0.055 NS	0.379 **
[Co] free + [Co-FeOx]	-0.376 ***	0.812 ***	0.183 NS	0.568 ***
[Co-OM]	-0.405 ***	0.899 ***	- 0.405 ***	0.644 ***
[Co] free + [Co-OM]	-0.333 **	0.836 ***	0.055 NS	0.638 ***
pH	-0.069 NS	- 0.101 NS	0.401 ***	0.202 NS
OM content	-0.336 **	0.567 ***	- 0.395 ***	0.219 NS
[Mn] total	0.507 ***	- 0.646 ***	0.652 ***	- 0.054 NS
[Fe] total	0.419 ***	- 0.554 ***	0.236 NS	- 0.053 NS
[Ca] total	-0.333 **	0.465 ***	- 0.443 **	0.244 *
[Mg] total	0.114 NS	- 0.390 **	0.530 ***	- 0.116 NS

757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781

782 **Fig.1 Copper and cobalt concentrations accumulated in shoots of four populations of *Anisopappus***
783 ***chinensis* and four populations of *Crepidorhopalon tenuis***

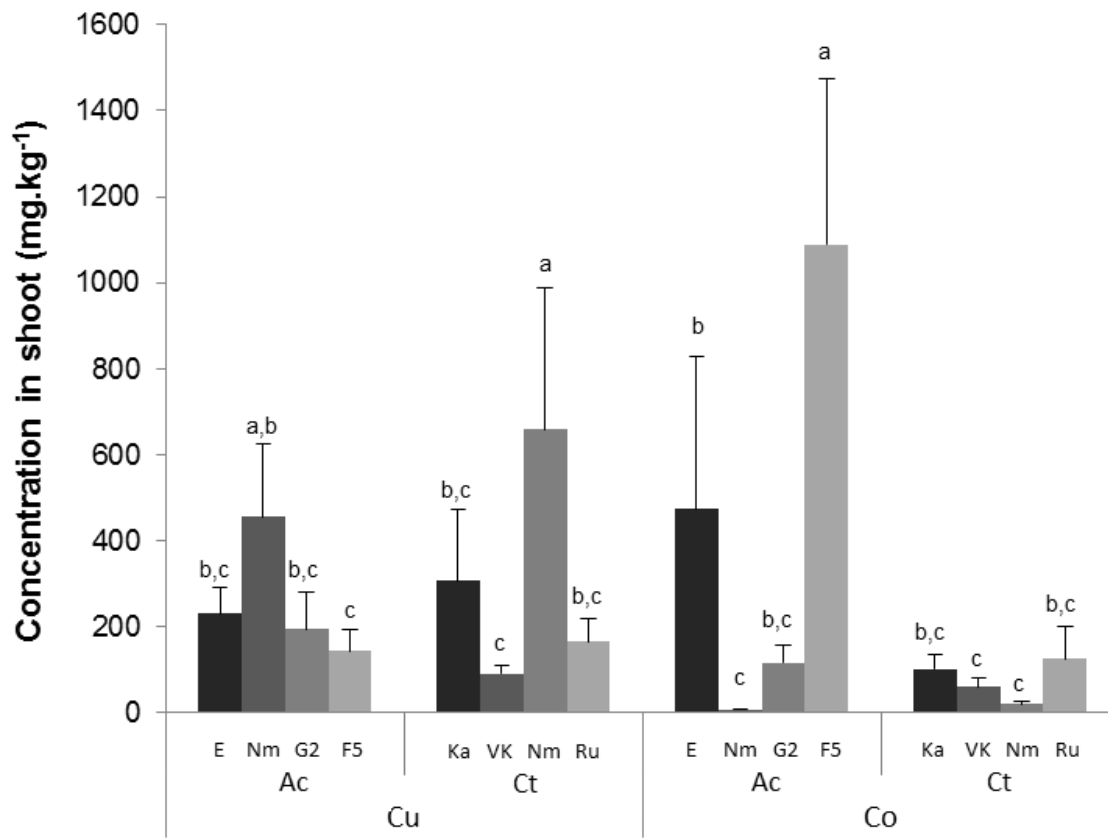
784 Sites are designated as follow: E=Etoile; Nm=Niamumenda; G2=Goma2; F5=Fungurume 5; Ka=Kalabi;
785 VK=Vallée Karavia; Ru=Ruashi. Ac=*Anisopappus chinensis*; n=79; Ct= *Crepidorhopalon tenuis*; n=67. Error
786 bars=standard deviation. For each species, there are no significant differences between populations with the
787 same letter (results of one-way ANOVA followed by post-hoc multiple comparison, Tukey HSD test).
788

789 **Fig.2 Relationships between element concentrations in plants and in soils**

790 **a** Cu in plant and total Cu in soil **b** Co in plant and total Co in soil **c** Cu in plant and free Cu in soil **d** Co in plant
791 and free Co in soil **e** Cu in plant and bound to MnOx Cu **f** Co in plant and bound to OM Co (*A. chinensis*: n=79;
792 *C. tenuis*: n=67)
793

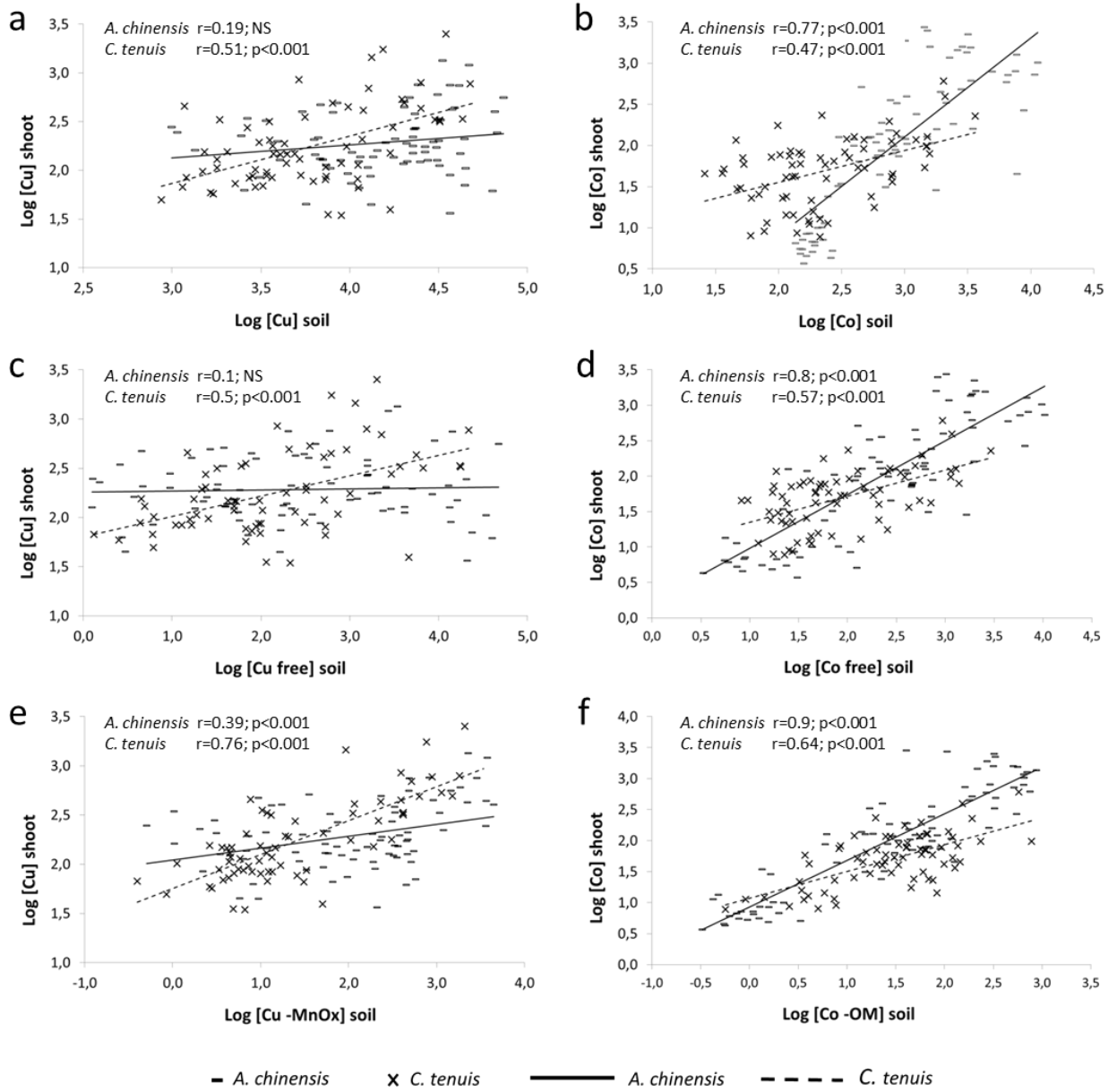
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841

842 Fig.1



843
 844
 845
 846
 847
 848
 849
 850
 851
 852
 853
 854
 855
 856
 857
 858
 859
 860
 861
 862
 863
 864
 865
 866
 867
 868
 869
 870
 871

872 Fig.2



873
874
875
876

Table S1 Total concentrations of elements, pH and organic matter in soils for seven metalliferous sites in Katanga (Dem. Rep. Congo). F5, Fungurume 5 (n= 20); G2, Goma 2 (n =20); E, Etoile (n=20); Nm, Niamumenda (n=35); Ka, Kalabi (n=17); VK, Vallée Karavia (n=16); Ru, Ruashi (n=18).

Mean	E	Nm	G2	F5	Ka	VK	Ru
Cu (mg.kg ⁻¹)	12,566	20,833	27,793	18,298	18,624	5,444	3,556
(SD)	(12,502)	(17,899)	(17,117)	(13,285)	(13,392)	(3,315)	(2,407)
Co (mg.kg ⁻¹)	2,732	168	714	3,451	911	79	657
(SD)	(3,336)	(54)	(247)	(2,614)	(809)	(39)	(677)
pH	6.37	5.70	6.04	6.18	5.60	4.99	5.19
(SD)	(0.64)	(0.57)	(0.17)	(0.58)	(0.31)	(0.23)	(0.25)
OM (%)	6,26	4,57	8,60	11,44	5,28	9,38	12,73
(SD)	(2,75)	(2,05)	(2,68)	(5,30)	(1,93)	(4,25)	(3,41)
Mn (mg.kg ⁻¹)	2,625	5,710	1,600	1,047	1,070	64	1,023
(SD)	(1,852)	(4,697)	(449)	(223)	(806)	(37)	(926)
Fe (mg.kg ⁻¹)	42,305	48,534	27,745	21,315	35,250	19,610	51,060
(SD)	(11,460)	(21,237)	(5,753)	(9,387)	(11,309)	(9,654)	(9,128)
Zn (mg.kg ⁻¹)	123	144	56	27	190	388	152
(SD)	(129)	(91)	(24)	(10)	(121)	(195)	(67)
Mg (mg.kg ⁻¹)	49,510	48,147	41,595	36,875	22,700	2,084	27,591
(SD)	(44,430)	(20,448)	(7,260)	(22,029)	(25,755)	(470)	(21,195)
Ca (mg.kg ⁻¹)	642	416	595	1,945	694	578	736
(SD)	(275)	(138)	(160)	(1,710)	(302)	(151)	(295)
K (mg.kg ⁻¹)	12,940	7,395	6,155	6,085	13,300	5,734	6,766
(SD)	(10,375)	(6,411)	(3,431)	(7,973)	(13,577)	(1,776)	(3,363)