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

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Abstract

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

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

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

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

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Abbreviations

MnOX	Manganese oxides
FeOx	Iron oxides
HM	Humic material
OM	Organic matter
-MnOx	Bound to manganese oxides
-FeOx	Bound to iron oxides

-OM   Bound to organic matter
SD   Standard deviation

Introduction

Soils contaminated by metals represent an important constraint for vegetation. Only a few species tolerate soil metal concentrations up to 1,000 times higher than the concentration usually found in normal soils (Ernst 1974; Reeves and Baker 2000). Some of these, called hyperaccumulators, are able to concentrate metal in their shoot up to extremely high concentrations (Baker 1981; Macnair 2003; Krämer 2010; Rascio and Navari-Izzo 2011). For two decades, hyperaccumulator species have attracted much attention because of their potential use as a phytoextraction strategy. According to the recent literature, species would be considered as Cu and Co hyperaccumulators when the metal concentration in shoots exceeds 300 mg.kg^{-1}, without toxicity symptoms and growth inhibition (Krämer 2010; Van der Ent et al. 2013). Although Macnair (2003) suggests that Cu hyperaccumulation has not yet been observed in control conditions, Küpper et al. (2009) found that *Crassula helmsii* growing in hydroponic conditions could accumulate more than 9,000 mg.kg^{-1} in shoot when the nutrient solution was enriched with less than 1 mg.kg^{-1} Cu2+. Only a few species around the world (mostly Brassicaceae), are considered as constitutive hyperaccumulators. These species show that all populations have very high foliar metal concentrations irrespective of metal concentration in soil, including *Noccaea caerulescens* (Dechamps et al. 2007), *Arabidopsis halleri* (Frerot et al. 2010) and *Gomphrena claussenii* (Villafort Carvalho et al. 2013) (zinc and cadmium hyperaccumulators); as well as several *Alyssum* species (nickel hyperaccumulator) (Brooks and Radford 1978; Brooks et al. 1979; Broadley et al. 2001; Hanikenne and Nouet 2011). Most of metallophytes are defined as facultative hyperaccumulators, which means that populations or individuals are hyperaccumulators and some are not (Pollard et al. 2002).

Plants which accumulate Cu and Co in their shoots are rare and most of them occur in Katanga (Dem. Rep of Congo) (Reeves and Baker 2000; Reeves 2006). In this region of South Central Africa, a species-rich vegetation is associated to natural outcrops of Cu and Co enriched bedrocks, including cuprophytes (Duvigneaud 1958; Duvigneaud and Denaeyer- De Smet 1963; Ernst 1974, 1990; Faucon et al. 2012a; Ilunga wa Ilunga et al. 2013) and cobaltophytes (Duvigneaud 1959; Faucon et al. 2010; Saad et al. 2012; Séleck et al. 2013). Ecology and evolution of copper and cobalt tolerance and accumulation in this vegetation is still misunderstood, due to high inter- and intraspecific variations of the Cu and Co shoot concentrations. Unusually high shoot Cu and Co concentrations reported in earlier studies may result from surface contamination by soil particles and, therefore, plant materials from metal-rich soils should be washed prior to analysis (Faucon et al. 2007; Van der Ent et al. 2013). Copper in shoots ranges from 80 to 1,400 mg.kg^{-1} and from 330 to 1,200 mg.kg^{-1} for *Crepidorhopalon perennis* and *Anisopappus chinensis*, respectively (Faucon et al. 2007, 2009). In the same study, for Co, both species had shoot concentrations that ranged from 61 to 1,105 mg.kg^{-1} and from 933 to 1,948 mg.kg^{-1}, respectively. Such variations have a genetic origin for Cu, especially due to genetic differentiations among populations, as demonstrated by cultivation in uniform conditions (Faucon et al. 2012b). However, high inter- and intra-population phenotypic variations in Cu and Co shoot concentration suggest a diversity of accumulation responses and an influence of edaphic parameters upon Cu and Co accumulation by plants. A good understanding of the Cu and Co accumulation variations would go through an accurate characterization of the soil-root interface properties and mechanisms, controlling metal availability. The influence of soil upon metal accumulation by plants can be studied in experimental conditions, by using a metal contamination gradient in the substrate, but this approach has limits and can be criticized due to differences with the in nature soil properties (e.g. Van der Ent et al. 2013). In such an approach, Cu hyperaccumulation is poorly expressed (Morrison et al. 1979, 1981; Chipeng et al. 2010; Faucon et al. 2012a, b) and variations could not be distinguished from substrate differences (Escarre et al. 2013). Another approach could identify the relationship between metal concentrations in soils and in shoots by studying populations from pedogeochemically contrasted sites. Ecological studies have commonly used total metal concentrations or extractable metal concentrations determined at a fixed pH value (AcNH4EDTA 1 M pH=4.65) (Brun et al. 1998; Faucon et al. 2009). In this way, pH variations are not considered, while it highly influences metal mobility (Alloway 1995; McLaren and Crawford 1973; Kabata-Pendias and Pendias 2001; Chaignon et al.

2002; Faucon et al. 2011). It is important to include soil pH, due to the direct impact of the Cu and Co speciation upon the Cu and Co uptake by plant (Poschenreider et al. 2001; Krishnamurti and Naidu 2002; Chaignon et al. 2002).

By considering soil pH, organic matter content and total concentrations of several elements in soil, chemical speciation modeling appears to be an interesting tool to investigate the relationships between soil properties and metal accumulation in plants. Using speciation calculation codes including chemical constants and equilibrium, these methods can assess metal fractionation in soils. Windermere Humic Aqueous Model (WHAM) (Tipping 1998; Pourret et al. *submitted*) can be used to calculate metal fractionation, and thus, estimate the bound to MnOx, FeOx and OM metal concentrations, as well as the free metal concentration (i.e. ionic form) in a soil sample. To understand Cu and Co accumulation variations, inclusion of the essential soil parameters controlling Cu and Co mobility in soils is necessary: pH, redox potential, OM quality and quantity, oxides, clays, sulphides and carbonates (Kabata-Pendias and Pendias 2001). These factors may partly control Cu and Co availability, by influencing the equilibrium between total and available metal concentration in soils. However, predicting the availability of metals, especially in contaminated environments, is still very difficult (Hinsinger and Courchesne 2008). Key role of root-induced processes on soil constituents' mobility (Harter and Naidu 2001; Hinsinger 2001; Adriano et al. 2004; Houben and Sonnet 2012) as well as rhizosphere chemistry and microbiology (Wenzel 2009; Alford et al. 2010) increases the complexity of metal availability. Mobility and availability of metals at the soil root-interface need to be investigated and to date, there is no universal method to estimate the metal availability to plants and soil organisms (Nolan et al. 2003). It has however been established that the metal concentration in the soil solution would be the only fraction directly available for plant uptake (Fageria et al. 1991; Marschner 1995; Whitehead 2000).

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

Materials and methods

Plant populations

Two model species have been selected in the study. *Crepidorhopalon tenuis* (S. Moore) Fischer (Linderniaceae) is a pseudometallophyte, Cu and Co hyperaccumulator species (Faucon et al. 2009). It is an annual species colonizing recently disturbed mine deposits. *Anisopappus chinensis* (L.) Hook.f. & Arn. (Asteraceae) is also a pseudometallophyte. It is a short-lived perennial found in relatively closed steppic savanna on stabilised contaminated substrates (Ilunga wa Ilunga et al. 2013; Séleck et al. 2013). Large variations of Cu and Co concentrations in shoots of this species have already been highlighted (Faucon et al. 2007). Four populations of both species were selected in different sites, in the Katanga region (Dem. Rep. of Congo), as described in Table 1. Two populations of *C. tenuis* were sampled on the anthropogenically disturbed sites Ruashi (Ru) (recolonization of mine deposits) and Vallée Karavia (VK) (Cu-Co atmospheric fall-out contamination) and two naturally occurring populations in the Cu-Co hills locally disturbed by reworked substrate (artisanal mining): Niamumenda (Nm) and Kalabi (Ka). The four populations of *A. chinensis* have been sampled on two natural Cu-Co undisturbed hills from the Tenke-Fungurume region: Fungurume5 (F5) and Goma2 (G2), and two disturbed Cu-Co hills in the Lubumbashi region: Mine de l'Etoile (E) and Niamumenda (Nm).

Sampling and samples preparation

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

Table 1 Location and habitat description of study sites

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	*A. chinensis*
Goma 2 (G2)	Natural Cu-Co hill locally disturbed by mining. Sampled on grassland.	1,300	S 10,59966 E 26,13894	*A. chinensis*
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	*A. chinensis*
Niamumenda (Nm)	Natural Cu-Co hill locally disturbed by mining. Sampled on grassland locally disturbed.	1,340	S 11,60492 E 27,29400	*A. chinensis* *C. tenuis*
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	*C. tenuis*
Vallée Karavia (VK)	Anthropogenic site: soil contaminated by atmospheric fallout from ore-smelter, moist.	1,230	S 11,67270 E 27,43091	*C. tenuis*
Ruashi (Ru)	Anthropic site: recolonization of Mine deposits. Sampled on remaining substratum.	1,300	S 11,62645 E 27,56328	*C. tenuis*

April 2012. A total of 146 plant and soil samples were considered in this study.

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

Plant and soil analysis

The *Crepidorhopalon tenuis* samples were mineralized by dry ashing in a muffle furnace at 550 °C for 12 h. Ash was dissolved in HCl. The samples were then analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Varian Vista MPX) to determine Cu-Co concentration in shoot. To determine Cu-Co concentration in shoot of *A. chinensis* two steps were performed. A mass of shoot were digested using a mixture of 8 mL HNO_3 and 2 mL HCl (Avula et al. 2010) with a low pressure mineralization (Lavilla et al. 1999). Vessels containing the mix were placed in a Mars 5 microwave (Microwave Accelerated Reaction System – CEM corporation, USA) according to the Avula et al. (2010) treatment. Then, Cu and Co concentrations in samples were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific XSERIES2). Quantitative analyses were carried out by external calibration (eight points) by using mono- and multi-element standard solutions (Accu Trace Reference, USA). Indium was used as an internal

standard at a concentration of 100 $\mu g.L^{-1}$ in order to correct for instrumental drift and matrix effects. The measurement bias for the determination of the concentration of Cu and Co was assessed by the analysis of the SRM1573a certified reference material (tomato leaves: Gills 1995). Typical uncertainties including all error sources are below ±5 % for Cu and Co. The soil samples pH (water) was determined on a saturated soil-water paste and Organic Matter (OM) content by loss on ignition (500 °C for 8 h). Soil chemical analyses were performed by Acme Analytical Laboratories Ltd. (Vancouver Canada), accredited under ISO 9002. The considered analyzed elements were Co, Cu, Fe, Mn, Zn, K, Mg, Al, Ca. Soil samples were digested using a strong multi-acid method that dissolves most minerals. Then, 0.25 g split was heated in HNO_3- $HClO_4$-HF to fuming and taken to dryness. The residue was further dissolved in HCl and solutions were analyzed using ICP-MS.

Chemical speciation calculations

WHAM 6 (version 6.0.10) was used to calculate Cu and Co speciation. Predictions for the equilibrium metal binding by environmental colloids made for the present study were done using the combined WHAM-SCAMP speciation code. WHAM-SCAMP is able to provide a full description of solid-solution speciation by incorporating two main codes: (i) the Windermere Humic Aqueous Model (WHAM) to calculate the equilibrium solution speciation (Tipping 1994), and (ii) the Surface

Chemistry Assemblage Model for Particles (SCAMP) to calculate the binding of protons and metals by natural particulate matter (Lofts and Tipping 1998). The code for the WHAM model incorporates a number of submodels: Humic Ion-Binding Model 6 and a description of inorganic solution chemistry, cation exchange by clays, the precipitation of aluminium and iron oxyhydroxides, and adsorption-desorption of fulvic acids. The SCAMP model consists of three submodels: (i) Humic Ion-Binding Model 6, (ii) a SCM describing proton and metal binding to oxides (e.g., MnOx or FeOx), and (iii) a model describing the electrostatic exchange of cations on clays. Three binding phases were examined: MnOx, FeOx, and Humic Material (HM). The X-Ray diffraction revealed that MnOx and FeOx are respectively pyrolusite and a mix hematite-goethite. The concentration of HM were derived from the experimental OM measurements: 50 % of the OM measured in the field samples was assumed to be HM, themselves being defined as 100 % fulvic acid. Saturation index and mineral precipitation were not considered; which could be a limitation of this approach (Pourret et al. *submitted*).

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

Statistical analysis

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

Results

Cu and Co fractionation in soils

Results of Cu and Co fractionation are presented in Table 2. Total Cu and Co concentrations showed differences between sites (F $_{(6,147)}$=7.65, $p<0.001$ and F $_{(6,147)}$=14.26, $p<0.001$, respectively). There were significant differences between Cu fractions for each site (Table 2). For Cu, the two largest fractions were Cu bound to OM (Cu-OM) and to FeOx (Cu-FeOx). The free Cu concentration varied from 0.7 to 18.8 % of the total Cu concentration. Copper was mainly bound to FeOx and OM. Significant differences for each Cu fraction existed between sites (Table 2). The highest concentrations of Cu-FeOx were found in Nm and E (69 % of total Cu) where mean total Fe concentration reached 48,500 mg.kg^{-1} and 42,300 mg.kg^{-1}, respectively. Samples from VK were characterized by low total Fe and Mn contents (respectively mean=19,610± 9,654 mg.kg^{-1}; and mean=65±37 mg.kg^{-1}) and high OM content (mean=9.38 %±4.2 %). At this site, the fraction of Cu bound to FeOx was the lowest while the fraction of Cu bound to OM was the highest (respectively mean=15 % and 81.8 % of total Cu). There were significant differences between Co fractions for each site (except samples from E) (Table 2). Cobalt was predominantly free and bound to MnOx (Co-MnOx) in soils. The free Co fraction was higher than the free Cu one for each site (meanly from 28.1 to 69.4 % of total Co). Significant differences for each Co fraction existed between sites. Nm was characterized by the highest Mn content in soil (mean=5,700 mg.kg^{-1}). At this site, the Co-MnOx concentration was the highest (mean=71.1 % of total Co). At VK, site where Mn concentration was the lowest, Co was mainly bound to OM (meanly 53.8 % of total Co). At this site, the Co-MnOx concentration was the lowest (mean=5.8 % of total Co).
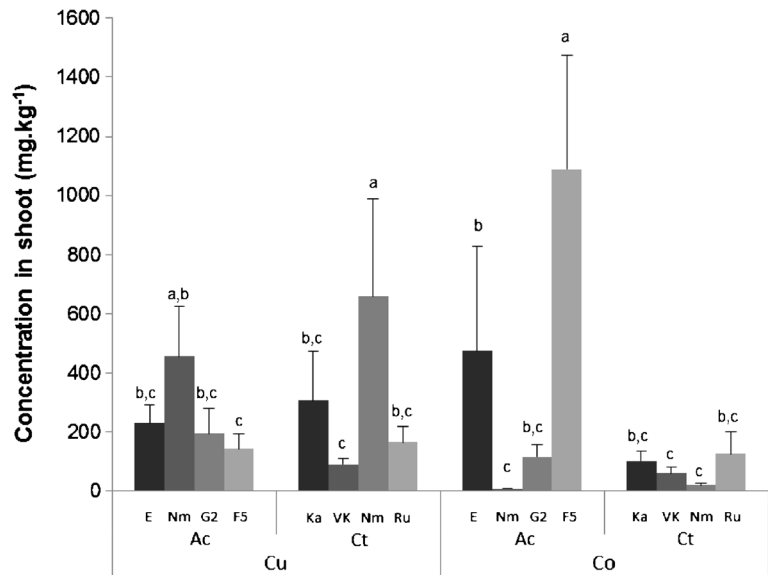
Cu and Co accumulation in plants

Results of Cu and Co concentrations in shoots showed large variations between species and populations (Fig. 1). For Cu, 22 of 79 samples of *A. chinensis* and 25 of 67 samples of *C. tenuis* accumulated more than 300 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 of 79 samples of *A. chinensis* and 2 of 67 samples of *C. tenuis*

Table 2 Cu/Co mean fractionation among seven sites (%) according to the total mean Cu/Co concentration in soil (mg.kg^{-1})

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

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

Fig. 1 Copper and cobalt concentrations accumulated in shoots of four populations of *Anisopappus chinensis* and four populations of *Crepidorhopalon tenuis*. Sites are designated as follow: *E* Etoile; *Nm* Niamumenda; *G2* Goma2; *F5* Fungurume 5; *Ka* Kalabi; *VK* Vallée Karavia; *Ru* Ruashi. *Ac* *Anisopappus chinensis*; $n=79$; *Ct* *Crepidorhopalon tenuis*; $n=67$. Error bars = standard deviation. For each species, there are no significant differences between populations with the same letter (results of one-way ANOVA followed by post-hoc multiple comparison, Tukey HSD test)

accumulated more than 300 mg.kg^{-1} (range: 3–1,334 mg.kg^{-1} and 8–605 mg.kg^{-1} for *A. chinensis* and *C. tenuis*, respectively).

Large Cu and Co accumulation variations existed among populations of both species (for Cu, $F_{(7,145)}=$ 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 concentration (mean$=1,089\pm768$ mg.kg^{-1}) ($F_{(7,145)}=$ 19.2, $p<0.001$). Strikingly low concentrations of Co in plant shoots (<20 mg.kg^{-1}) were found in the Nm site for both species. On the contrary, that site yielded the highest shoot Cu accumulation for both species (*A. chinensis*, range: 104–1,335 mg.kg^{-1}, mean$=$ 455 mg.kg^{-1} and *C. tenuis*, range: 84–2,525 mg.kg^{-1}, mean$=673$ mg.kg^{-1}) ($F_{(7,145)}=125.4$, $p<0.001$).

Relationships between edaphic factors and Cu and Co accumulation in shoot

Correlations between soil fractions and shoot accumulation were element- and species- dependent (Table 3, Fig. 2), however, some general patterns emerged. Correlations between total Cu soil content and shoot Cu concentration were positive for *C. tenuis* ($r=0.51$, $p<0.001$) and non-significant for *A. chinensis* (Fig. 2a). Total Co soil content was positively correlated with shoot Co concentration for *A. chinensis* and *C. tenuis* ($r=0.77$, $p<0.001$ and $r=0.45$, $p<0.001$, respectively) (Fig. 2b). No correlations occurred between pH and shoot Co concentration for both species. On the contrary, pH was positively correlated with Cu

concentration in shoots for *C. tenuis* ($r=0.40$, $p<0.001$) but not significant for *A. chinensis*. Organic matter soil content was negatively correlated with shoot Cu concentration for both species. Positive correlation have been observed between OM soil content and shoot Co concentration for *A. chinensis* ($r=0.57$, $p<0.001$) but not significant for *C. tenuis*. Shoot Cu concentration was correlated with total Mn soil content for *A. chinensis* and *C. tenuis* ($r=0.51$, $p<0.001$ and $r=0.65$, $p<0.001$, respectively). However, the strong negative correlation between total Mn soil content and shoot Co concentration for *A. chinensis* ($r=-0.65$, $p<0.001$) was not verified for *C. tenuis*. A positive correlation was established between total Fe soil content and shoot Cu concentration for *A. chinensis* ($r=0.42$, $p<0.001$), but not for *C. tenuis*. A negative correlation occurred between total Fe soil content and Co concentration in shoot for *A. chinensis* ($r=0.5$, $p<0.001$). This relationship was not verified for *C. tenuis*.

A positive correlation was observed between free Cu concentration and shoot Cu concentration for *C. tenuis* ($r=0.50$, $p<0.001$), but not for *A. chinensis* ($r=0.49$, $p<0.05$) (Fig. 2c). Free Co concentration was positively correlated with shoot Co concentration for *A. chinensis* and *C. tenuis* ($r=0.8$, $p<0.001$ and $r=0.57$, $p<0.001$, respectively) (Fig. 2d). For Cu, the highest correlation was observed between Cu bound to MnOx (Cu-MnOx) soil concentration and shoot Cu concentration for *A. chinensis* and *C. tenuis* ($r=0.39$, $p<0.001$ and $r=$ 0.76, $p<0.001$, respectively) (Fig. 2e). However, Cu-FeOx soil concentration was also positively correlated

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

| | A. chinensis | | C. tenuis | |
	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

***p<0.001, **p<0.01, *p<0.05, *NS* non-significant

with shoot Cu concentration for both species. This latter concentration was negatively correlated with shoot Co concentration for *A. chinensis*. For Co, the highest correlation was observed between Co bound to OM (Co-OM) soil concentration and shoot Co concentration for *A. chinensis* and *C. tenuis* (*r*=0.9, *p*<0.001 and *r*=0.64, *p*<0.001, respectively) (Fig. 2f). A positive correlation was also observed between Co bound to FeOx (Co-FeOx) soil concentration and shoot Co concentration for both species.

Discussion

Cu and Co speciation in soils

Copper appeared to have a strong affinity for FeOx and OM which would explain the low free Cu concentrations

in soils compared to other Cu fractions. In normal soils, copper co-adsorbs with Mn and Fe oxides (McLaren and Crawford 1973). Copper was fixed by OM when total Fe content was low (VK). An increase in the total Fe content (E, Nm, Ka) resulted in an increase of the Cu-FeOx concentration. This suggests that Fe oxides are potentially major competitors of OM for Cu speciation.

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

Samples from VK, with a high OM content compared to other sites and the lowest total Mn concentration in soils showed the highest Co-OM concentration.
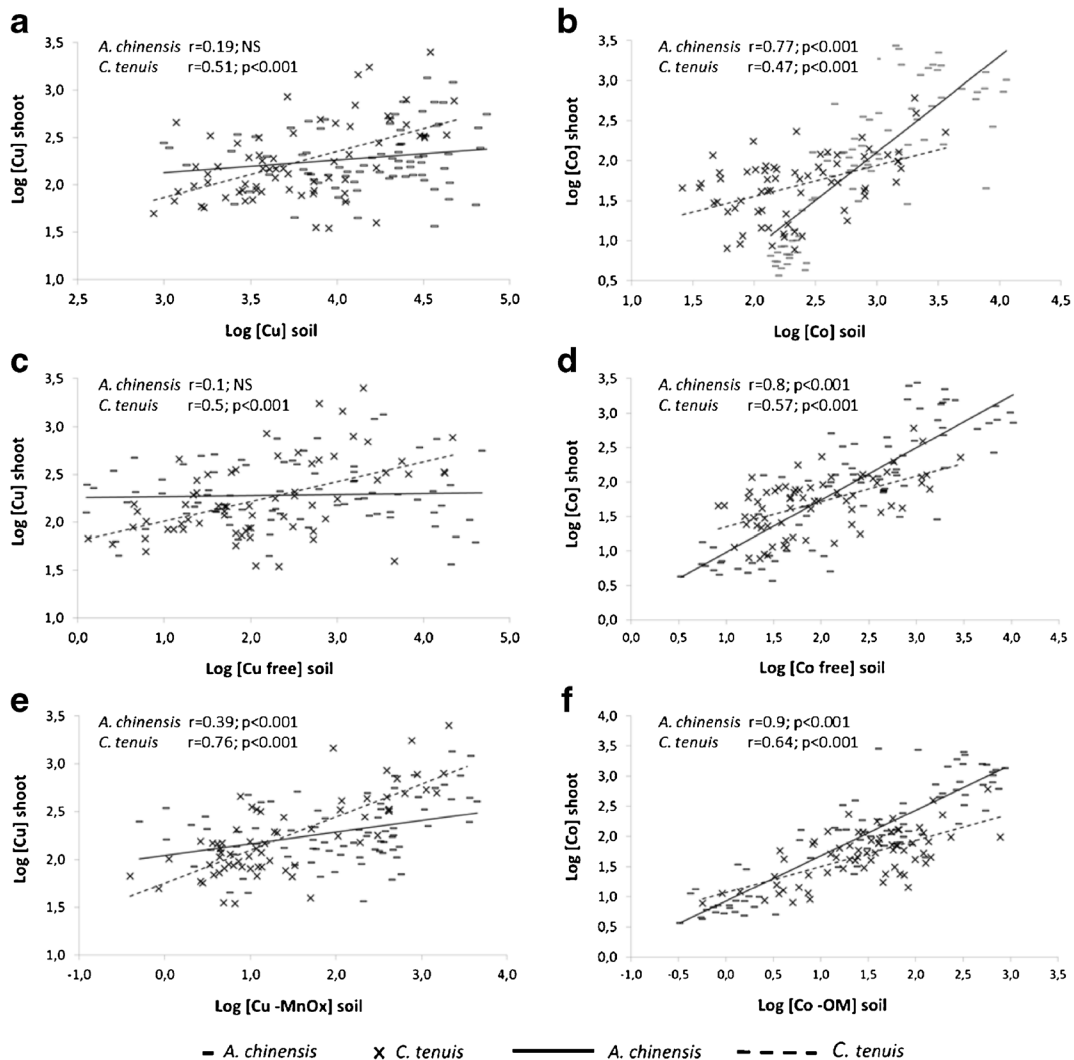
Fig. 2 Relationships between element concentrations in plants and in soils. **a** Cu in plant and total Cu in soil **b** Co in plant and total Co in soil **c** Cu in plant and free Cu in soil **d** Co in plant and free Co in soil **e** Cu in plant and bound to MnOx Cu **f** Co in plant and bound to OM Co (*A. chinensis*: *n*=79; *C. tenuis*: *n*=67)

Organic matter appears to be a major competitor of Mn oxides for Co speciation (Collins and Kinsela 2010, 2011). Free Co concentration is higher, in percentage, than free Cu concentration, relative to the other Co or Cu fractions (Table 2). This could be explained by the stronger capacity to complex with colloids for Cu, compared to Co. Indeed, the sequence of complex stability of transition metals, known as the Irving Williams series, is typically $Co^{2+}<Ni^{2+}<Cu^{2+}<Zn^{2+}$ (Stumm and Morgan 1996). Organic matter content seems to have a strong influence upon metal fractionation, which confirms results obtained by Pourret et al. (2007). It can be assumed that mobile and non-mobile Cu and Co

fractions vary among sites and seem to be closely related to the MnOx and FeOx concentrations, the OM content and the soil pH. High variability of Cu and Co speciation in soils has been observed, which would involve variations of the Cu and Co availability.

Variations of Cu and Co concentrations in shoots among populations

Results confirm that *A. chinensis* and *C. tenuis* are facultative Cu and Co hyperaccumulators (Faucon et al. 2009), characterized by a high variation of Cu and Co shoot concentrations. For Cu and Co respectively, 17 % and

21 % of plants of *A. chinensis* and 17 % and 2 % of plants of *C. tenuis* have been recorded with a concentration in shoots of up to 300 mg.kg^{-1}, i.e. the hyperaccumulation threshold (Van der Ent et al. 2013), without showing any toxicity symptoms or growth inhibition.

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

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

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

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

The results showed the influence of Cu and Co speciation on the variability of Cu and Co accumulation in both species (Table 3). Copper accumulation in plant shoots were positively influenced by Cu-MnOx and Cu-FeOx concentrations, whereas Co accumulation variations were strongly influenced by Co free and Co-OM and Co-FeOx concentrations. The Co-OM soil fraction seems to appear to be the most available Co fraction, in those soils for both species. Similarly, both Li et al. (2004) and McLaren et al. (1987) highlighted that organically-bound Co influenced Co availability to plants. Availability of Cu and Co does not seem to be strictly a result of the free Cu and Co concentrations in soils. Even if ionic species metal concentrations in soils would appear, by hypothesis, to be a good predictor of metal uptake concentration by plants, because of their direct availability (Fageria et al. 1991; Marschner 1995; Whitehead 2000), the bound to solid-phase fractions have to be considered for availability assessment. Indeed, some plants are able to mobilize and take up elements from the non-mobile solid fraction (Knight et al. 1997; Hinsinger et al. 2005). These significant influences of the solid phases upon the accumulation for both species could be explained by root-induced processes which could be responsible to pH changes (Marschner 1995; Hinsinger et al. 2003). Indeed, the soil solution acidification by root activity, may causes desorption of metals (Marschner 1995; Harter and Naidu 2001; Hinsinger et al. 2003) and thereby, increase the ionic form metal concentration. This study confirms that the availability of metals is closely related to all the biogeochemical processes which occur in the rhizosphere. Estimate of the available metal concentration in soils seem to be complex but the present study is the first attempt of Cu and Co availability assessment in metalliferous soils. Undertaking a laboratory experiment which simulates the field conditions would help unravel some of this complexity. For this, both species could be cultivated in growth-chamber, on homogeneous soil contaminated by defined Cu and Co concentrations, in which chemical

factors influencing Cu and Co availability (pH, OM, MnOx, FeOx) would vary in controlled conditions (Chaignon and Hinsinger 2003). However, as microbial activities would also influence the availability of chemical elements in soil (Hinsinger et al. 2005) and the patterns of metal accumulation in plant species (Fomina et al. 2005; Toler et al. 2005; Barzanti et al. 2007; Kabagale et al. 2010) incorporating microbial activities in an experimental setting to test their effect on the variation of Cu and Co accumulation in pants would prove fruitful. Perspectives would be to integrate the study of influence of microbial activities on the variation of Cu and Co accumulation in plants.

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

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