

Specialized edaphic niches of threatened copper endemic plant species in the D.R. Congo: implications for ex situ conservation

Sylvain Boisson · Michel-Pierre Faucon · Soizig Le Stradic · Bastien Lange · Nathalie Verbruggen · Olivier Garin · Axel Tshomba Wetschy · Maxime Séleck · Wilfried Masengo Kalengo · Mylor Ngoy Shutcha · Grégory Mahy

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Abstract

Background and aims Copper (Cu) rich soils derived from rocks of the Katangan Copperbelt in the south-eastern Democratic Republic of Congo (DRC) support a rich diversity of metallophytes including 550 heavy metal tolerant; 24 broad Cu soil endemic; and 33 strict

Cu soil endemic plant species. The majority of the plant species occur on prominent Cu hills scattered along the copperbelt. Heavy metal mining on the Katangan Copperbelt has resulted in extensive degradation and destruction of the Cu hill ecosystems. As a result, approximately 80 % of the strict Cu endemic plant species are classified as threatened according to IUCN criteria and represent a conservation priority. Little is known about the soil Cu tolerance optimum of the Cu endemic plant species. The purpose of this study was to quantify the soil Cu concentration (Cu edaphic niche) of four Cu endemic plant species to inform soil propagation conditions and microhabitat site selection for planting of the species in Cu hill ecosystem restoration.

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S. Boisson (✉) · S. Le Stradic · O. Garin · M. Séleck · G. Mahy

Biodiversity and Landscape Unit, BIOSE - Biosystem Engineering Department, Gembloux Agro-Bio Tech, University of Liege, 2 Passage des Déportés, 5030 Gembloux, Belgium
e-mail: sylvain.boisson@ulg.ac.be

M.-P. Faucon · B. Lange

Hydrogeochemical Interactions Soil-Environment (HydrISE) Unit, Polytechnic Institute LaSalle Beauvais (ISAB-IGAL), 15 rue Pierre Waguët, 60026 Beauvais, France

B. Lange

Laboratory of Plant Ecology and Biogeochemistry, Université Libre Bruxelles, CP 244, Boulevard du Triomphe, 1050 Bruxelles, Belgium

N. Verbruggen

Laboratory of Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, CP 244, Boulevard du Triomphe, 1050 Bruxelles, Belgium

A. Tshomba Wetschy · W. Masengo Kalengo ·

M. Ngoy Shutcha

Ecology, Restoration Ecology and Landscape Research Unit, Faculty of Agronomy, University of Lubumbashi, Route Kasapa, Campus Universitaire, 1825 Lubumbashi, Democratic Republic of Congo

Methods The soil Cu concentration tolerance of Cu endemic plant species was studied including *Crotalaria cobalticola* (CRCO); *Gladiolus ledoctei* (GLLE); *Diplophium marthozianum* (DIMA); and *Triumfetta welwitschii* var. *rogersii* (TRWE-RO). The in situ natural habitat distributions of the Cu endemic plant species with respect to soil Cu concentration (Cu edaphic niche) was calculated by means of a generalised additive model. Additionally, the seedling emergence and growth of the four Cu endemic plant species in three soil Cu concentrations was tested ex situ and the results were compared to that of the natural habitat soil Cu concentration optimum (Cu edaphic niche).

Results CRCO exhibited greater performance on the highest soil Cu concentration, consistent with its calculated Cu edaphic niche occurring at the highest soil Cu concentrations. In contrast, both DIMA and TRWE-RO exhibited greatest performance at the lowest soil Cu

concentration, despite the calculated Cu edaphic niche occurring at moderate soil Cu concentrations. GLE exhibited equal performances in the entire range of soil Cu concentrations.

Conclusions These results suggest that CRCO evolved via the edaphic specialization model where it is most competitive in Cu hill habitat with the highest soil Cu concentration. In comparison, DIMA and TRWE-RO appear to have evolved via the endemism refuge model, which indicates that the species were excluded into (i.e., took refuge in) the lower plant competition Cu hill habitat due to their inability to effectively compete with higher plant competition on normal soils. The soil Cu edaphic niche determined for the four species will be useful in conservation activities including informing soil propagation conditions and microhabitat site selection for planting of the species in Cu hill ecosystem restoration.

Keywords Copper soil · Heavy metal tolerance · Edaphic · Endemic · Niche · Generalised additive model · Endangered plant

Introduction

Metalliferous substrates including natural soils derived from metal-rich rocks and minerals, as well as anthropogenic mine tailings, present a chemically extreme environment for plant establishment and productivity (Shaw 1990; Brooks 1998). Due to the microbially-mediated oxidation of associated metal sulfides, the substrate pH is typically low (< 5) and the resulting bioavailable heavy metal availability is high. Metalliferous substrates (eg., mining complexes) are an uncommon and unusual habitat type that may be widely distributed and have an island-like distribution, subject to the selective processes that occur with respect to island biogeography (Kruckeberg 1986). Evolution of tolerance to the chemically extreme conditions of the metalliferous substrates, in combination with the isolating effects of island-like distribution of the habitat, promotes speciation and can be the origin of edaphic endemic plant species (Antonovics et al. 1971; Kruckeberg and Rabinowitz 1985; Kruckeberg 1986; Baker 1987; Kruckeberg and Kruckeberg 1990; Shaw 1990; Brooks 1998; Macnair and Gardner 1998; Van Rossum et al. 2004; Rajakaruna and Boyd 2008; Harrison and Rajakaruna 2011; Escarré et al. 2013).

The origins of edaphic endemic plant species can be determined by genetic analysis of biotic depletion, allopatric speciation or genetic differentiation of individuals occurring in populations (Kruckeberg and Rabinowitz 1985; Macnair and Gardner 1998; Rajakaruna 2004) and/or through ecological comparison (Gankin and Major 1964; Meyer 1986; Harrison and Rajakaruna 2011). There are three leading models regarding the origin of edaphic endemic plant species including 1) endemism refuge, 2) edaphic specialization, and 3) pathogen refuge (Gankin and Major 1964; Meyer 1986; Boyd 2007; Springer 2009; Fones et al. 2010; Peng et al. 2012; Faucon et al. 2012a; Boyd and Rajakaruna 2013; Anacker 2014). In the endemism refuge model, edaphic stress tolerant plant species are outcompeted on normal soils and displaced into (or take refuge in) metalliferous soil habitats where competitive pressure is lower (Gankin and Major 1964; Anacker 2014). In the edaphic specialization model, a plant species becomes specialized to metalliferous soil habitats because they outcompete other plant species in those habitats and/or have a high physiological requirement for the metal (Gankin and Major 1964; Boyd and Rajakaruna 2013; Anacker 2014). In the pathogen refuge model, the high bioavailability of heavy metal reduces pathogens (eg., pathogenic fungi) that typically adversely impact the plant species (Boyd 2007; Springer 2009; Fones et al. 2010; Strauss and Boyd 2011).

Copper (Cu) rich soils derived from rocks of the Katangan Copperbelt in the south-eastern D. R. Congo support a rich diversity of metallophytes including 550 heavy metal tolerant; 24 broad Cu soil endemic; and 33 strict Cu soil endemic plant species (Malaisse et al. 1999); Duvigneaud and Denaeyer-De Smet 1963; Cailteux et al. 2005). Soils on the hills of the Katangan Copperbelt present large differences in soil Cu concentration that varies by topographic position (Duvigneaud and Denaeyer-De Smet 1963; Cailteux et al. 2005). On the hill tops, plant-available soil Cu concentration ranges from 3500 to 10,000 mg kg⁻¹ and declines to 100–3500 mg kg⁻¹ on hillslopes and toeslopes (Duvigneaud and Denaeyer-De Smet 1963; Ilunga wa Ilunga et al. 2013; Séleck et al. 2013). Plant species endemic to the Cu hills exhibit niche segregation with some species occurring primarily on hill tops at the higher soil Cu concentration and other species occurring on hillslopes and toeslopes at the lower soil Cu concentration. Heterogeneity of heavy metal concentrations

within and among sites could lead to divergent selection in the population and thus contribute to ecotypic differentiation (Ilunga wa Ilunga et al. 2013). Studies on some of these Cu endemic plant species have identified a high degree of intraspecific variability with respect to heavy metal tolerance differences (Faucon et al. 2012a) and genetic structure among populations (Peng et al. 2012; Faucon et al. 2012a).

The recent revival of Cu and Co mining on the Katangan Copperbelt has led to the destruction and degradation of the unique and rare Cu hill ecosystems (Brooks and Malaisse 1990; Leteinturier et al. 1999; Saad et al. 2012). To date, approximately 80 % of Cu endemic plant species have been identified as threatened (i.e., critically endangered, endangered or vulnerable) according to IUCN criteria (Faucon et al. 2010), and represent a conservation priority. Although establishing preserves where mining is banned would be the most effective strategy to protect the Cu hill ecosystems (Leteinturier 2002; Saad et al. 2012), this appears unrealistic since Cu and Co mining comprises a large percentage of D. R. Congo's economic base. The lack of recognition of the Cu endemic plant species in environmental protection laws and regulations in the D. R. Congo further hampers conservation of the Cu hill ecosystems.

In addition to in situ species conservation, ex situ strategies such as the establishment of a Cu endemic plant species seed bank, plant propagation, and revegetation of lands degraded by mining is important in maintaining biological integrity of the Cu hill ecosystems (Faucon et al. 2011; Faucon et al. 2012b; Saad et al. 2012; Faucon et al. 2016). Greater knowledge of the ecology of a plant species including the abiotic (e.g., soil Cu concentration) and biotic factors that influence species distribution can improve propagation techniques and ecosystem restoration success. To date, most studies focusing on Cu endemic plant species of the Katangan Copperbelt have been performed under laboratory conditions that poorly match the natural conditions of Cu hill ecosystems (Chipeng et al. 2010; Faucon et al. 2012a; Godefroid et al. 2013; Boisson et al. 2016a; Boisson et al. 2016c). In this context, the purpose of this study was to determine the in situ natural Cu edaphic niche and ex situ growth response to a gradient of soil Cu concentration for the Cu endemic plant species *Crotalaria cobalticola*, *Diplolophium marthozianum*, *Gladiolus ledoctei* and *Triumfetta welwitschii* var. *rogersii*.

Methods

Study site

The study was performed at sites between the towns of Tenke (Lat. -10.603889, Lon. 26.121343 and Fungurume (Lat. -10.617539, Lon. 26.309615 in the far south-eastern D. R. Congo in the Katangan Copperbelt. In the Katangan Copperbelt, scattered Cu hills were formed during the weathering of the most resistant siliceous rock of the Roan Series (Duvigneaud and Denaeyer-De Smet 1963, François 1973, Cailteux et al. 2005). Altitude of the area ranges from 1200 to 1400 m. This region has a humid subtropical climate (Cwa; Köppen climate) with warm, rainy summers (November to March) and pleasant, dry winters (May to September). Autumn (April) and spring (October) are transition periods between the two seasons.

The dominant vegetation types is Miombo (*Brachystegia*) woodland and savannah. Two major plant communities have been identified along the Cu hill topographic gradient, including steppe (grassland) on the hill tops, gradating into savannah on the hillslopes and the toeslopes (Séleck et al. 2013). Soils on hill tops contains bioavailable Cu concentrations ranging from 3500 mg kg⁻¹ to 10,000 mg kg⁻¹, whereas soil plant-available Cu concentration is substantially lower on hillslopes and toeslopes, with lows of 100 mg kg⁻¹ (Duvigneaud and Denaeyer-De Smet 1963; Ilunga wa Ilunga et al. 2013; Séleck et al. 2013).

Study plant species

Our study focused on four plant species endemic to the Katanga Copperbelt, including *Crotalaria cobalticola* P.A. Duvign. & Plancke (CRCO; Fabaceae); *Diplolophium marthozianum* P.A. Duvign. (DIMA; Apiaceae); *Gladiolus ledoctei* P.A. Duvign. & Van Bockstal (GLLE; Iridaceae); and *Triumfetta welwitschii* var. *rogersii* (N.E. Br.) Brummitt & Seyani (TRWE-RO; Malvaceae). CRCO is a strict Cu endemic herbaceous annual. It is primary distributed in the steppe (grassland) on Cu hills. Both DIMA and GLLE are broad Cu endemic herbaceous perennials. Both species occur in savannah on Cu hills, but GLLE is more common in the steppe. TRWE-RO is a strict Cu endemic herbaceous perennial. It is primarily present in savannah on Cu hills. CRCO, DIMA, and GLLE are all proposed endangered

(EN) IUCN status (Faucon et al. 2010). TRWE-RO has no proposed IUCN status due to taxonomic data deficiency (DD).

In situ modelling of plant species Cu edaphic niches

The in situ Cu edaphic niches of the plant species CRCO, DIMA, GLLE, and TRWE-RO were modelled using the species presence-absence database from Séleck et al. (2013). This database contains phytosociological data of 172 square-metre plots surveyed along systematic grids on three Cu hills between the towns of Tenke and Fungurume. Data from each vegetation plot was linked with bioavailable (EDTA-extractable) soil Cu concentration in soil from the database of Séleck et al. (2013). Edaphic niches along the natural Cu gradient for each plant species were then modelled with generalised additive models (GAM), using a binomial likelihood (Hastie and Tibshirani 1986). The resulting species response curves represent the probability of occurrence of the taxon along the Cu gradient. For each taxon, the best fit model among three degrees of smoothing (3, 4 or 5) was selected, based on the Akaike Information Criterion (AIC; Akaike 1987). The optimum soil Cu concentration of each taxa was then estimated by identifying the highest probability of occurrence. The borders of the Cu edaphic niche widths (or amplitudes) were determined by the lower and upper limits of 80 % of the area under the species response curves (Gégout and Pierrat 1998) followed by the calculation of the Cu edaphic niche widths as the difference between the upper and the lower limits.

Plant species response to Cu in ex situ conditions

Seed collection

Seeds of CRCO, DIMA, GLLE, and TRWE-RO were collected from Cu hills located between Tenke and Fungurume (Table 1). For each species, seeds were collected on a minimum of 50 individuals from distinct populations from three separate hill tops (three populations). Seed samples were stored in ultra-dry (relative humidity <5 %) conditions at room temperature.

Soil treatment preparation and analyses

Three soil Cu level treatments were created using a homogeneous arable soil (ferralsol; oxisol) as the base substrate and additions of Cu in the form of copper

sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) powder. The base ferralsol soil has a very low natural bioavailable Cu concentration (27.2 mg kg^{-1} ; see Results). Additionally, bioavailable macronutrient content is N_{tot} : $0.22 \pm 0.02 \text{ g } 100 \text{ g}^{-1}$, P: $0.031 \pm 0.006 \text{ mg } 100 \text{ g}^{-1}$, K: $56.0 \pm 2.4 \text{ mg } 100 \text{ g}^{-1}$, Ca: $92.0 \pm 5.9 \text{ mg } 100 \text{ g}^{-1}$, Mg: $23.8 \pm 1.5 \text{ mg } 100 \text{ g}^{-1}$, $\pm 5.9 \text{ mg } 100 \text{ g}^{-1}$. Soil texture of this soil has been identified as sandy-clay (Mukalay et al. 2008). Total nitrogen (N) content was measured by combustion of organic matter to NO_x and N_2 at $1050 \text{ }^\circ\text{C}$ in a flow of oxygen and measured by Thermal Conductivity Detection (Margesin and Schinner 2005). Phosphorus (P) content was determined with a Shimadzu UV-1205 spectrophotometer (Shimadzu Corporation) at 430 nm after the development of blue coloration. Bioavailable soil Ca, Cu, Mg, K concentration were analysed by ammonium acetate–EDTA ($\text{CH}_3\text{COONH}_4$ –EDTA) 0.5 N extraction (Lakanen and Erviö 1971). The soil:solution ratio was 1:5 and the pH of the extraction solution was buffered at 4.65. The supernatant was filtered through a S&S 595 folded filter and analysed using a flame atomic absorption spectrometer (Varian 220), following the norm NF X 31–120.

For the control (low Cu “treatment”), no Cu was added. For the medium Cu treatment, 100 mg kg^{-1} soil Cu was added and thoroughly mixed (theoretical total 127.2 mg kg^{-1} Cu). For the high Cu treatment, 1000 mg kg^{-1} soil of Cu was added and thoroughly mixed (theoretical total $1027.2 \text{ mg kg}^{-1}$ Cu).

Study establishment

This study was conducted outdoors at ambient climate conditions in the experimental botanical garden of the University of Lubumbashi (Faculty of Agronomy; Lat. -11.610005 , Lon. 27.480011), 180 km southeast of Tenke and Fungurume. Climatic conditions of the experimental garden closely match that of the plant populations original sites.

Plastic nursery bags were filled with 1.5 kg (c. 20 cm height) of the different substrates for $n = 10$ of each soil Cu treatment per plant species. Bags were perforated to facilitate water drainage. For each combination of species and population, five seeds were sown per bag in early December, at the beginning of the rainy season. The bags were then fully randomized and placed under a shade structure and watered to field capacity to initiate seed germination. During the study, all bags were

Table 1 Plant species seed collected at each site.

Site name	Site ID	Latitude	Longitude	Plant species collected			
				CRCO	GLLE	DIMA	TRWE-RO
Fungurume V	A	-10.618049	26.290399	X	X	X	
Fungurume VIII [1]	A	-10.601051	26.311085				X
Kakalawe [1]	B	-10.613342	26.133768			X	
Shimbidi	B	-10.592873	26.142197		X		
Kabwelunono [1]	B	-10.596726	26.133318	X			X
Kavifwafwaulu [1]	C	-10.579099	26.153847		X		
Kavifwafwaulu [4]	C	-10.582313	26.152612	X		X	X

watered to field capacity every two days and fully re-randomized every two days.

Seedling emergence for each bag was recorded weekly. After all of the seedlings had established after two months in January, the number of seedlings per bag was reduced to a single individual and allowed to grow for another five months until termination of the study at the end of April. At termination of the study, the maximum height of each plant was measured. Additionally, for CRCO only, the number of main stems, the total number of flowers, and the maximum root length was measured. For DIMA and TRWE-RO only, the number of leaves was counted.

Post-plant growth soil analyses

At termination of the study, soils were randomly collected from 12 bags representing each plant species \times soil Cu treatment, in order to have four replicates of soil treatment. Soil samples were air dried and sieved to 2 mm prior to the analyses. Soil pH was measured in potassium chloride (KCl) 1 N with a glass electrode in a 2:5 soil:solution ratio, after a 2 h equilibration time. Bioavailable soil Cu concentration was measured using the flame atomic absorption spectrometer on the supernatant, following the norm NF X 31–120, as detailed previously.

Data analysis

The percentage of seedling emergence per bag was compared using a two-way ANOVA (plant population \times soil Cu concentration; R statistical software). The growth parameters (i.e., height, number of leaves or main stems and length of the root system) and the number of flowers per individual were also compared by a two-way ANOVA

(population \times soil Cu concentration). Prior to analysis, normality and homoscedasticity of the data were checked. Log transformations were required for the number of flowers of CRCO and number of leaves for TRWE-RO. Means were structured via the Tukey HSD test, with an error rate of 5 %. Soil compounds were compared with a Kruskal-Wallis test followed by a Bonferroni mean adjustment comparison. Spearman correlation tests were performed between height and number of leaves (or stems), to identify the relationship between these responses. All analyses were performed using the R statistical software (R Development Core Team 2010).

Results

Soil Cu concentration

As expected, soil analyses revealed that the EDTA-extractable Cu concentrations were significantly higher in the 1000 mg kg⁻¹ Cu-enriched soils than the two other soils (control and 100 mg kg⁻¹ Cu-enriched soils), ($X^2 = 8.346$; p -value < 0.05 ; Table 2). The pH values were inversely proportional to the Cu concentrations added in soils with a significantly lower pH in 1000 mg kg⁻¹ Cu-enriched soils than in the control and the 100 mg kg⁻¹ ($X^2 = 7.731$; p -value < 0.05 , Table 2). The means stayed between 5.0 ± 0.3 .

Plant species response to soil Cu concentration

Seedling emergence

Global means of the percentages of emergence differed according to the species. GLLE and CRCO presented

Table 2 Physico-chemical conditions (Mean \pm SD) including the EDTA-extractable copper (Cu) of the three substrates of the test ($n = 4$)

Physico-chemical parameters	Soil Cu treatment (mg kg ⁻¹)			Kruskal-Wallis test	
	Control (0)	100	1000	X^2	p -value
Cu (mg kg ⁻¹)	96.7 \pm 12.4 ^b	128.1 \pm 38.1 ^b	1280.9 \pm 322.4 ^a	8.346	0.015
pH KCl	5.2 \pm 0.1 ^b	5.1 \pm 0.1 ^b	4.7 \pm 0.0 ^a	7.731	0.021

the highest percentage of emergence, respectively 49.6 \pm 3.0 % and 44.4 \pm 4.8 % (Mean \pm SE), in comparison to TRWE-RO and DIMA, which had percentages of emergence of 13.1 \pm 2.1 % and 11.7 \pm 1.5 %, respectively. Our results suggest that the percentage of emergence of the four plant species was not affected by Cu concentration (Table 3). In contrast, the percentage of emergence for the population of TRWE-RO from Fungurume VIII (24.7 \pm 4.5 %) was significantly higher than the populations from Kavifwafwaulu [4] (10.7 \pm 3.4 %) and Kabwelunono [1] (4.0 \pm 1.8 %, $F = 9.323$, p -value < 0.001). For CRCO and TRWE-RO, no interaction between the population and Cu concentrations were identified for the percentages of emergence (CRCO: $F = 0.334$; p -value = 0.855; TRWE-RO: $F = 0.608$, p -value = 0.657). Interactions between both factors affected the percentages of emergence for GLLE ($F = 3.665$, p -value < 0.01) and DIMA ($F = 2.500$, p -value = 0.049), but no significant difference between the combination of the population and the Cu concentrations were evident in the Tukey HSD test.

Plant species growth response

There was no significant interaction between the different populations within a species and the soil Cu concentration on the growth for the four plant species. Effects of each factor are thus highlighted independently. Considering that the individual plant height and the number of main stems of individuals of CRCO were correlated and presented the same pattern of variation (Spearman coefficient correlation = 0.80, p -value < 0.001), only the number of stems is presented in the results. The number of main stems was not significantly different between the populations within a species, but was significantly different between soil Cu concentrations. Individuals grown on control soils had significantly fewer stems (9.8 \pm 2.1 stems) than the 100 mg kg⁻¹ (17.8 \pm 1.8 stems) and the 1000 mg kg⁻¹ Cu treatment

soils (21.5 \pm 2.0 stems; Fig. 1; Table 3). The same pattern of response to soil Cu concentration was also observed for the length of the root system, with significantly longer roots observed in individuals growing on 1000 mg kg⁻¹ (5.5 \pm 0.6 cm) and on 100 mg kg⁻¹ Cu treatment soils (5.3 \pm 0.8 cm), than on the control soils (2.6 \pm 0.7 cm; Table 3). While the number of flowers per individual did not significantly vary between soil Cu concentrations (Table 3), the percentage of flowering individuals was higher on those on the 100 and 1000 mg kg⁻¹ Cu treatment soils (respectively 36 % and 44 %) than on the control soils (25 %).

The individuals of DIMA presented a distinctly different pattern in relation to Cu concentrations (Fig. 1; Table 3). Considering that the number of leaves was significantly correlated with the height ($r = 0.73$, p -value < 0.001), the number of leaves was chosen for the comparisons. The number of individuals' leaves was greater on the control soils (4.6 \pm 0.6 leaves) and in the 100 mg kg⁻¹ Cu treatment soils (4.9 \pm 0.6 leaves), than on the 1000 mg kg⁻¹ Cu treatment soils (2.8 \pm 0.4 leaves, Fig. 1; Table 3). Additionally, an effect of population was observed, in which the number of leaves of individuals from the Kakalalwe [1] population (4.9 \pm 0.5 leaves) was significantly higher than the mean number of leaves of individuals from the Fungurume V (2.9 \pm 0.3 leaves) and Kavifwafwaulu [4] (3.3 \pm 0.3 leaves, Table 3) populations. For GLLE the height of individuals and the number of the individuals' leaves were not significantly different, either between populations (Table 3) or soil Cu concentrations (Fig. 1; Table 3).

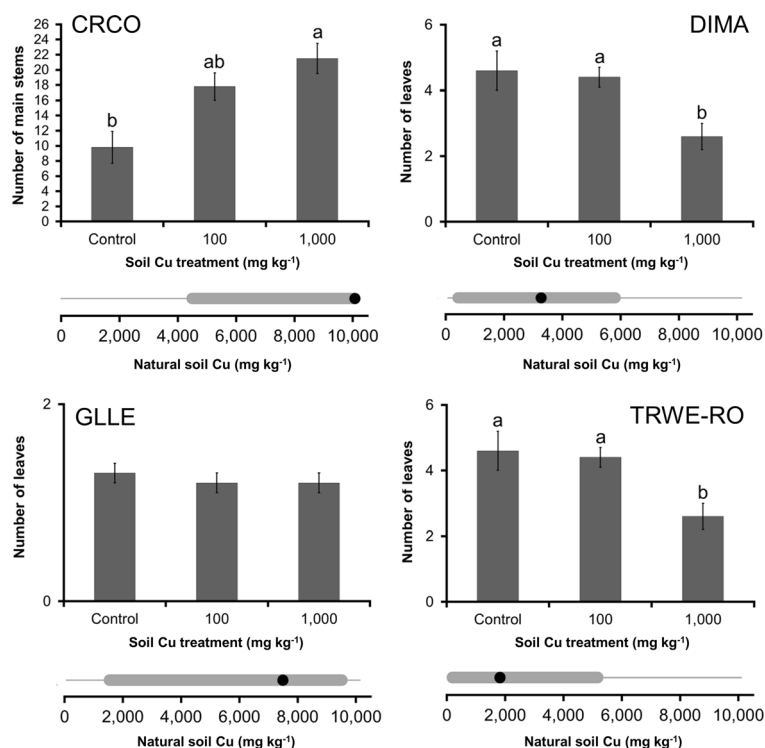
Since a significant positive correlation was observed between the number of leaves and the height of TRWE-RO ($r = 0.56$, p -value < 0.001), only the results for the number of leaves according to the soil Cu concentrations were presented. The number of leaves for plants that were grown on the control soils (16.3 \pm 1.6 leaves) and on 100 mg kg⁻¹ Cu treatment soils (15.1 \pm 1.6

Table 3 Percentage of emergence and growth trait variations by soil Cu concentration for CRCO, GLLLE, DIMA, and TRWE-RO. For Site ID, refer to Table 1

Traits	Soil Cu treatment (mg kg ⁻¹)						F	p-value	Site ID			F	p-value
	Control (0)	100	1000	10000	A	B			C				
CRCO													
Percentage of emergence (%)	42.7 ± 5.2	44.7 ± 5.0	46.00 ± 4.1	0.115	0.891	43.3 ± 4.6	46.0 ± 4.9	44.0 ± 4.9	0.079	0.924			
Number of main stems	9.8 ± 2.1 ^b	17.8 ± 1.8 ^a	21.5 ± 2.0 ^a	6.337	0.003	15.7 ± 2.4	19.5 ± 2.3	18.2 ± 2.1	0.824	0.444			
Maximum root length (cm)	2.6 ± 0.7 ^b	5.3 ± 0.8 ^{ab}	5.5 ± 0.6 ^a	5.955	0.004	3.8 ± 0.7	5.2 ± 0.7	5.1 ± 0.9	1.905	0.157			
Number of flowers	2.7 ± 1.7	5.6 ± 2.2	5.1 ± 1.3	0.507	0.612	2.5 ± 0.7	6.1 ± 1.9	5.6 ± 2.0	1.415	0.274			
DIMA													
Percentage of emergence (%)*	13.3 ± 2.7	12.7 ± 2.6	9.3 ± 2.4	0.721	0.489	14.0 ± 2.9	8.0 ± 2.5	13.3 ± 2.4	1.698	0.190			
Number of leaves	4.6 ± 0.6 ^a	4.4 ± 0.3 ^a	2.6 ± 0.4 ^b	4.971	0.012	2.9 ± 0.3 ^b	4.9 ± 0.5 ^a	3.3 ± 0.3 ^b	8.948	<0.001			
GLLLE													
Percentage of emergence (%)*	52.7 ± 5.4	46.0 ± 4.9	50.0 ± 5.3	0.467	0.628	49.3 ± 5.6	44.7 ± 4.8	54.7 ± 5.1	1.038	0.359			
Height (cm)	9.4 ± 0.6	10.1 ± 0.5	9.2 ± 0.6	0.753	0.475	9.2 ± 0.5	9.5 ± 0.5	10.0 ± 0.6	0.607	0.548			
Number of leaves	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.098	0.339	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.067	0.935			
TRWE-RO													
Percentage of emergence (%)	12.0 ± 4.2	16.0 ± 4.0	11.3 ± 2.8	0.534	0.588	24.7 ± 4.5 ^a	10.7 ± 3.4 ^b	4.0 ± 1.8 ^c	9.323	<0.001			
Number of leaves	16.3 ± 1.6 ^a	15.1 ± 1.6 ^a	8.3 ± 1.4 ^b	10.192	<0.001	14.0 ± 1.2	11.0 ± 3.0	13.8 ± 1.9	1.020	0.370			

*Significant interaction, Tukey HSD test was performed when the *p*-value was lower than 0.05.

Fig. 1 Growth response (number of stems or number of leaves; mean \pm SE) of CRCO, DIMA, GLE and TRWE-RO to soil Cu concentration (ex situ; mg kg^{-1} ; vertical bar graphs) as compared to soil Cu concentration from natural populations (in situ; mg kg^{-1} ; slider bar graphs). Means with the same letters are not significantly different, according to the Tukey HSD test. Natural soil Cu concentrations (Cu edaphic niches) of the species were modelled by generalised additive models (GAM). Dots are the niche optima of taxa determined by the gradient value of the highest probability of occurrence. Thick and straight lines are the niche widths (or amplitudes) calculated by the difference between the borders of area of 80 % under the species response curves



leaves) were significantly greater than the number of leaves for plants sown on 1000 mg kg^{-1} Cu treatment soils (8.3 ± 1.4 leaves, Fig. 1; Table 3). Population of origin did not affect the number of leaves (Table 3).

In situ Cu edaphic niches of plant species

The four plant species have distinct, however overlapping, in situ Cu edaphic niches (Fig. 1). CRCO's optimum soil Cu concentration was 10,136 mg kg^{-1} Cu, with a truncated response curve, and a lower limit of its Cu edaphic niche, at 4547 mg kg^{-1} Cu (Fig. 1). CRCO could be considered the most Cu tolerant of our study. GLE occurred in a range of 1525 to 9499 mg kg^{-1} Cu, with a Cu optimum at 7468 mg kg^{-1} Cu (Fig. 1). Its response curve was skewed, with a longer tail toward the lowest soil Cu concentrations. DIMA's optimum soil Cu concentration was 3243 mg kg^{-1} Cu, with a symmetrical niche width (5387 mg kg^{-1} Cu, Fig. 1). TRWE-RO's Cu edaphic niche optimum was at a soil Cu concentration of 1838 mg kg^{-1} . Its Cu niche width extended from 201 up to 5194 mg kg^{-1} Cu, and had a skewed response with a tail toward the highest soil Cu concentrations (Fig. 1).

Discussion

In ex situ conditions, soil Cu concentrations did not significantly influence seedling emergence for any of the four plant species. It supports the observations of other studies on CRCO and DIMA in in vitro conditions showing that growing Cu concentration in substrate did not affect the percentage of germination (Boisson et al. 2016a; Boisson et al. 2016c). These results and those of other studies (Di Salvatore et al. 2008) suggest that the seed coat protects the plant embryo during the germination process from soil heavy metals.

Although no significant difference in seedling emergence rate of CRCO, GLE and DIMA was found between populations, the outcome is consistent with previous studies on metallophytes populations of *Spartina densiflora* (Mateos-Naranjo et al. 2011) and *Viola calaminaria* (Bizoux et al. 2008). Only the seedling emergence of TRWE-RO was significantly affected by the interaction of the population's origin and the Cu concentration, suggesting ecotypic differentiation. While our study did not highlight this effect at the growth stage for tested species, the adaptation of metallophyte showing ecotypical differentiation has been already observed in *Collinsia sparsiflora* (Wright

et al. 2006) or *Helianthus exilis* (Sambatti and Rice 2006) from serpentine soils. Large variation in biomass per plant were also shown for distinct populations of *Noccea caeruleascens* (syn. *Thlaspi caeruleascens*, Europa), *Crepidorhapon perennis* (D.R. Congo) and *Haumanisatrum katangense* (D.R. Congo) cultivated on distinct metal concentrations (Roosens et al. 2003; Peng et al. 2012; Faucon et al. 2012a). The intraspecific variations correspond to a combination of environmentally-induced and genetic differences between populations (Mengoni et al. 2000; Van Rossum et al. 2004; Yang et al. 2005), implying an opportunity to choose appropriated ecotypes according to the purposes of the conservation strategies. The importance of the edaphic and the climatic tolerance is often neglected but some endemic species from the Katanga Copperbelt could benefit from a wide degree of tolerance presenting more optimized ecotypes for restoration strategies at mine closure or for phytostabilisation strategies of polluted soils (Boisson et al. 2015; Shutcha et al. 2015; Boisson et al. 2016b). Some more tolerant ecotypes of TRWE-RO may be more appropriate for restoration of soils with higher Cu concentrations and inversely, highlighting the importance of preserving a large variation of the ecotypes diversity.

Three different growth patterns in terms of increasing ex situ soil Cu concentration responses are represented by the four plant species including positive (CRCO), neutral (GLLE), and negative (DIMA; TRWE-RO). There was a positive correlation of plant size for CRCO to increasing soil Cu concentration with the largest individuals occurring in the 1000 mg kg⁻¹ Cu treatment soil, suggesting that this species requires high level of Cu for optimal development (Baker 1987). There was no correlation, however of soil Cu concentration with flower number in the species. Enhanced growth of some metallophytes in the presence of growing metal concentrations is rarely reported in plants. It was supposed on *H. katangense* and *C. perennis* (Chipeng et al. 2010; Faucon et al. 2012a), both occurring in the steppes of the Cu hills, as well as, other hyperaccumulators occurring on serpentine soils (Millie Burrell et al. 2012; Ghasemi et al. 2014). Enhanced plant growth in metal-polluted soil could be explained by plant growth promoting bacterias (PGPB) in the natural population (Khan 2005; Pereira et al. 2015). However, soils from natural sites (i.e., natural steppe) were not experienced in this study, excluding the hypothesis of PGPB. As well, mineral analysis of

CRCO plant after experiment showed that Cu concentrations in plant were constant whatever the Cu concentration in soil ($16.9 \pm 3.6 \mu\text{g g}^{-1}$ Cu, unpublished data), suggesting that potential defense against biotic stresses or plant osmoregulation would not be greater at high metal concentrations (Whiting et al. 2003; Hörger et al. 2013). In most cases, these explanations are argued in case of hyperaccumulators (Noret et al. 2005; Fones et al. 2010; Krämer 2010). Because Cu acts as a fungicide, enhanced growth on Cu-rich soil could result from a lower pathogen pressure than in normal soil, indicating a relaxed selection for resistance mechanisms (Chipeng et al. 2010; Faucon et al. 2012a). In contrast to CRCO's significant response to increasing soil Cu concentration, DIMA and TRWE-RO exhibited a significantly negative response. For DIMA and TRWE-RO, the largest individuals occurred in the lowest soil Cu treatment (100 mg kg⁻¹) and the control. GLLE displayed an indifferent response to the range of soil Cu concentrations.

The patterns of response to soil Cu concentration by the four plant species generally reflect their patterns of distribution with respect to natural soil Cu concentration on the Cu hills (Parish and Bazzaz 1985; Eriksson 2002; Miriti 2006). CRCO exhibited positive growth response to increasing soil Cu concentration and displayed highest productivity at the highest soil Cu concentration. This species response supports the finding of Cu edaphic niche optimum at 10,139 mg kg⁻¹ Cu, the highest of the four species. Conversely, DIMA and TRWE-RO shared the same pattern, with higher productivity in the lowest soil Cu treatment, since they had intermediate Cu niche widths between 0 and 6000 mg kg⁻¹ Cu. Finally, GLLE had no significant response to soil Cu concentration in ex-situ conditions, exhibiting the broadest Cu edaphic niche with an optimum between DIMA and CRCO.

The indifferent response of GLLE to increasing soil Cu concentration, contrasting to its very high natural habitat soil Cu concentration (i.e., Cu edaphic niche), is best explained by the refuge endemism model (Gankin and Major 1964; Kruckeberg and Rabinowitz 1985). The refuge endemism model proposes that plant species with low interspecific competitive ability are competitively excluded from highly productive and competitive habitats in favor of stressful, lowly productive and competitive habitats to which they are tolerant, such as Cu hills. In the case of GLLE, in the absence of competition such as the ex situ study, the species performs equally well in very low to very high soil Cu concentration.

Under natural habitat conditions however, the species most commonly occupies soils having the highest Cu concentration, possibly due to the effects of competitive exclusion from the lower and intermediate soil Cu concentrations. In contrast to GLLE, CRCO exhibits a strong positive response to increasing soil Cu concentration, best explained by the specialist endemism model (Boisson et al. 2016c).

Edaphic endemic specialist plant species are more competitive in their specialized habitat type than non-specialists or tolerators (refuge endemics). The tolerance to the highest Cu concentrations usually comes at a physiological cost (Macnair 1993; Macnair et al. 2000; Chipeng et al. 2010; Faucon et al. 2012a) due to the presence of cellular mechanisms involved in Cu detoxification (Yruela 2005; Furini 2012). These strategies include the reduction of metal uptake, the stimulation of efflux, the chelation, the compartmentation and the sequestration (Yruela 2005). This strategy implies that individuals could show lower performances on non-metal enriched soils than other species.

This study contributed to the quantification of the Cu edaphic niche of four endemic species, indicating that propagation conditions and the seed collection effort should depend on the species. Furthermore, it highlights that the restoration of Cu plant communities may be complex due to the various plant soil interaction within species level, as observed for TRWE-RO presenting various response to Cu concentrations according to the original population. The diversity of edaphic patterns observed for these species suggests that endemic species present opportunity for soil reclamation of degraded landscapes in south-eastern DRC. Notably, CRCO and GLLE could be used for the revegetation of highest Cu-rich soils while DIMA and TRWE-RO would be more adapted for intermediate Cu-rich substrates.

Conclusion

In the conservation of strict edaphic endemic plant species, it is important to characterize their optimal habitat conditions. Quantified and well-understood niche parameters of a species increase the success of in situ population conservation, in situ habitat restoration, and ex situ propagation. Additional study of the numerous diverse Cu endemic plant species is needed to improve holistic conservation and restoration of the Cu hill ecosystems. These approaches would allow to determine

the level of ecotypic and genetic variation within species, helping the choice of targeted site and the size of sampling. Cu and Co mining on the Katangan Copperbelt is a harsh disturbance that removes most of the local ecosystem function including organisms and soil, and causing a greater isolation than in the natural ecosystem. The barren, acidic heavy metal mine tailings represent an extreme habitat type to which the local Cu endemic plant species could be the most adapted for revegetation and reclamation purposes.

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