

# Chemical soil factors influencing plant assemblages along copper-cobalt gradients: implications for conservation and restoration

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## Abstract

**Aims** Define the chemical factors structuring plant communities of three copper-cobalt outcrops (Tenke-Fungurume, Katangan Copperbelt, D. R. Congo) presenting extreme gradients.

**Methods** To discriminate plant communities, 172 vegetation records of all species percentage cover were classified based on NMDS and the Calinski criterion. Soil samples were analyzed for 13 chemical factors and means compared among communities with ANOVA. Partial canonical correspondence analysis

(pCCA) was used to determine amount of variation explained individually by each factor and site effect.

**Results** Seven communities were identified. Six of the studied communities were related to distinct sites. Site effect (6.0 % of global inertia) was identified as the most important factor related to plant communities' variation followed by *Cu* (5.5 %), *pH* (3.6 %) and *Co* (3.5 %). Unique contribution of site effect (3.8 %) was higher than that of *Cu* (1.1 %) and *Co* (1.0 %).

**Conclusions** In restoration, not only *Cu* and *Co* contents will be important to maintain vegetation diversity,

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attention should also be given to co-varying factors potentially limiting toxicity of metals: *pH*, organic matter, *Ca* and *Mn*. Physical parameters were also identified as important in the creation of adequate conditions for diverse communities. Further studies should focus on the effect of physical parameters and geology.

**Keywords** Endemics · Environmental gradients · Katanga (D. R. Congo) · Metallophyte · Mining · pCCA (Partial Canonical Correspondance analysis)

## Introduction

Metalliferous habitats are unique to biodiversity. They support highly distinctive plant communities (Bizoux et al. 2004; Brooks et al. 1992; Ernst 1974; Whiting et al. 2002, 2004) and host rare, ecologically specialized taxa adapted to elevated environmental stress including high concentration of metals (Chiarucci and Baker 2007; Chipeng et al. 2010; Faucon et al. 2009, 2007; Jacobi et al. 2007; Whiting et al. 2004; Wolf et al. 2000). Worldwide, metalliferous ecosystems are severely threatened by the growing of mining activities with high risks on metallophyte diversity (Kruckeberg 1984; Whiting et al. 2002, 2004).

The ICMM (International Council on Mining and Metals) recognized the need for sustainable operations and committed to contribute to the conservation of biodiversity (ICMM 2006; Whiting et al. 2002, 2004). Besides the conservation of representative areas of metalliferous ecosystems in untouched nature reserves, the most straightforward strategy to ensure the survival of metallophytes in mining concessions is to promote their use in ecological restoration and site rehabilitation at the point of mine closure (Faucon et al. 2012a, b; Saad et al. 2012; Whiting et al. 2004). Such a conservation strategy can only be reached through a deep understanding of patterns and processes of the plant community's variability (Whiting et al. 2004).

Influence of soil chemistry is rarely used in conservation strategies. In the case of metallophytes, it is however expected that soil chemistry would act as the main driver of metallophytes distribution along the contaminated gradients and the variation should be taken into account if we are to recreate adequate conditions for plants growth and survival. The province of Katanga, in Democratic Republic of Congo, hosts one of the most

important metallogenic areas of the world composed of some 160 hills scattered over more than 300 km (W-E) and forming the so-called Katangan Copperbelt (Cailteux et al. 2005; Duvigneaud and Denayer-De Smet 1963). It presents a high degree of geological and economic interest while being recognized as a hotspot for metallophytes (Brooks and Malaisse 1985; Duvigneaud and Denayer-De Smet 1963; Ernst 1974; Leteinturier 2002; Malaisse et al. 1983; Morrison et al. 1979; Wild and Bradshaw 1977). Mainly covered by Steppic savanna, copper outcrops contrast from the surrounding Miombo woodland (Duvigneaud 1958; Duvigneaud and Denayer-De Smet 1963) (Fig. 1). They are known to host more than 550 species (Leteinturier 2002). Of the world's copper ores, the Katangan outcrops are the only ones known to host primary vegetation presenting strict endemic species (Faucon et al. 2010). A recent revision of the list of Katangan copper endemic plant species has confirmed 32 strict endemics (i.e. absolute metallophytes: species that occur solely on Katangan copper sites) and 24 broad endemic taxa (i.e. with more than 75 % occurrence on copper sites) to occur on copper sites, and proposed IUCN (International Union for Conservation of Nature) status (Faucon et al. 2010). Human influence on these habitats has previously consisted of little artisanal ore extraction, on limited areas, and to an increased rate of burning due to propagation of fires at dry season from neighboring fields or fire-hunting in the surrounding forests. Large herbivores' grazing is not observed in actual times likely because of the general degradation of the megafauna in Katanga. The physiognomy of vegetation of copper outcrops follows a general pattern from top to bottom with a community of chasmophytic species on poorest in *Cu* and *Co* mineralized rocks, succeeded by a steppe in higher part of the outcrops, and a steppic savanna at the foothill. Because of metals toxicity, woody and shrubby species are excluded from the steppic savannas and steppes thus composed uniquely of an herbaceous layer (herbs and forbs) with some rare trees found at edges and in the upper parts, less contaminated areas of the hills. Copper hills are usually surrounded by a *Uapaca robynsii* (Euphorbiaceae) shrubby savanna belt (Brooks and Malaisse 1985; Duvigneaud 1958; Duvigneaud and Denayer-De Smet 1963).

The strong revival of the mining industry in Katanga makes no exception of copper outcrops. Faucon et al. (2010) reported that 12 sites had already been destroyed and more than 40 damaged by mining

activities, leading to the extinction of at least three endemics. In order to allow for conservation and/or restoration, metal-related plant communities are in need of a better scientific knowledge. In particular, previous studies have not fully assessed the relationships between plant community's variation and edaphic factors other than *Cu* and *Co* nor the potential differentiation of plant communities among sites. This information is crucial for future monitoring of reconstructed ecosystems (see <http://www.copperflora.org>: Conservation) and revegetation programs of the mining sites with local flora adapted to contamination in metals. The importance of endemic plant communities in ecological succession has already been demonstrated in previous studies (Kirmer et al. 2008; Tropek et al. 2010) and understanding chemical and physical drivers of communities of interest will help establishing revegetation programs.

Natural *Cu* concentrations span a large range on Katangan copper hills (total *Cu* from ~100 mg.kg<sup>-1</sup> to values as high as 35 000 mg.kg<sup>-1</sup>, Saad et al. 2012) and largely exceed those of normal soils (total *Cu* <100 mg.kg<sup>-1</sup> in normal soils, Duvigneaud 1958; Faucon et al. 2011). Cobalt concentrations may also be as high as 1,000 mg.kg<sup>-1</sup>. Hence, it has generally been considered that strong gradients of *Cu* and *Co* are the most important factors driving the composition and diversity of plant communities on copper hills (Duvigneaud and Denayer-De Smet 1963; Malaisse 1995). However, recent studies (Faucon et al. 2011; Saad et al. 2012) point out that natural copper–cobalt soils differ from other metalliferous soils worldwide, by the fact that they present high concentrations in nutrients (*P*, *Ca* and *K*). The lack of these elements is regularly considered as exercising a high selective pressure, for example, on serpentine soils (Proctor and Woodell 1975). Saad et al. (2012) suggested that nutrient status may also be of prime importance in relation to plant community variability. It is also known that different pool of species occur in the different parts of the Katangan Copperbelt (Duvigneaud and Denayer-De Smet 1963) but little is known about the variability of plant communities located within a same regional pool of species.

The aim of this study is to provide a comprehensive analysis of the variation of copper plant communities within a regional pool of species. For the first time we take explicitly into account, the interactions across

strong environmental gradients within sites and potential flora differentiation among sites. We further aim to derive implications for conservation and restoration of these unique ecosystems.

## Materials and methods

Nomenclature: African Plant Database (Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute - Pretoria 2013)

### Site description

Our study took place in the Tenke-Fungurume region, in the centre of the Katangan Copperbelt (Katanga province, D. R. Congo). The region (1,000 km<sup>2</sup>) hosts more than 40 copper outcrops distributed along two parallel low ridges between the cities of Tenke (10.61°S; 26.12°E) and Fungurume (10.62°S; 26.32°E) (altitude around 1,300 m). The area represents a subtropical climate with a rainy (November to end of March) and a dry (May to September) season. Mean annual temperature is about 20 °C. Temperatures range from 15–17 °C, at the beginning of the dry season, to 31–33 °C, in September–October. Dry season lows can reach 5 °C at night.

The geology of the region is highly influenced by the Mines Series making up the mineralized zone of the Roan Series (Francois 1973). It includes calcareous rocks with dark minerals (CMN), dolomitic shales and schists (SDS and SDB), cellular siliceous rocks (RSC), foliated siliceous rocks (RSF), stratified dolomites (D-Strat) and talcose argillaceous rocks (RAT). The more resistant siliceous rock unit forms have led to the formation of prominent outcrops contrasting with the surrounding flat terrain. Geological succession appears as the main driver of altitudinal change on the sites and these changes can be used as a proxy of sites' geological variation.

Three sites were selected within the region (Fig. 1): Fungurume V North, Kavifwafwaulu I and Kazinyanga. A brief description of sites along with coordinates, altitudinal ranges and surfaces are presented in Table 1. Distances among sites range from 4 to 15 km. Those sites were selected because they were defined as the most species rich copper hills in the Tenke Fungurume region (unpublished data).



**Fig. 1** Studied sites. **a** Fungurume V North (slope on the right): a rocky crest surmounting a steep slope of steppe and steppic savanna becoming gentler at the base and surrounded by a *Uapaca robynsii* fringe and Miombo woodland. **b** Kavifwafwaulu I:

steppic savanna area with gentle slopes surmounted by a wooded rocky crest. **c** Kazinyanga: intermediary slope with a large steppic savanna surmounted by a steppe zone

## Data collection

Field surveys were carried out in March 2008 and 2009, corresponding to the vegetation biomass peak on the hills. For the purpose of the study, only herbaceous vegetation was surveyed, excluding vegetation of siliceous cellular rocks and shrubby edges that host vegetation not exclusive of copper sites (Faucon et al. 2010). For each site, 1 m<sup>2</sup> plots were laid on the nodes of a

systematic grid following six to eight parallel transects stretched from the bottom to the top of the hill. On each transect, the nodes were placed at every change of 5 m in altitude to cover the geological and directly influenced edaphic variability. This led to a total of 172\*1 m<sup>2</sup> vegetation samples plots. In each plot, percentage cover of all herbaceous plants were recorded. Identifications were based on Flore d’Afrique Centrale (Bamps 1973–1993), Flora Zambesiaca (Board of trustees Kew Royal

**Table 1** Studied sites description

Sites	Coordinates	Altitude(m)	Site description	n	Surface (ha)
Fungurume V North (Fung)	S10.61762° E26.28950°	1195–1270	Natural <i>Cu/Co</i> outcrop; strong altitudinal gradient with slopes ranging from 0° (bottom) to 30° (top); lower down an escarpment of the rocky outcrop.	83	3.3
Kavifwafwaulu I (Kavi)	S10.57909° E26.15389°	1425–1450	Natural <i>Cu/Co</i> outcrop; vegetation dominated by Steppic savanna vegetation; gentle slopes (~5°) forming a dome.	37	11.9
Kazinyanga (Kazi)	S10.62840° E26.25482°	1260–1300	Natural <i>Cu/Co</i> outcrop; morphology intermediary between those of Fungurume V North and Kavifwafwaulu I	52	5.8

Studied clearings, decimal degrees coordinates, brief morphological description and number of plots surveyed for the study (n)

Botanic Gardens 1960–2010) and Flora of Tropical East Africa (Kew Royal Botanical Gardens 1952–2008) completed with more recently published taxonomic literature for particular genera and species. Herbarium voucher samples were collected for species determination and *a posteriori* comparisons. Individuals that could not be identified to a species level were well individualized as morpho-species.

To assess the variation in chemical edaphic factors, 15 cm depth composite soil samples were collected with a dutch auger at the four corners of each plot. All samples were air-dried and sieved to 2 mm (*pH* and bioavailable elements analysis) and to 0.2 mm (*C* and *N* analysis). For every soil sample, *pH* in  $H_2O$  was recorded with a glass electrode in a 2:5 soil-solution ratio and with 2 h equilibration time. Total organic content was measured following the Springer-Klee method (Springer and Klee 1954): hot oxidation with  $K_2Cr_2O_7$  and titration of oxidant excess with  $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ . Total nitrogen was determined following the Kjeldahl method (Bremner and Mulvaney 1982): oxidation of soil organic matter by  $H_2SO_4$  (56 %) and *Se* reagent mixture (catalyst), conversion to ammonium, distillation, and titration with *HCl*. Bioavailability—the concentrations theoretically available for plants at the scale of a growing season—of *Cu*, *Co*, *Zn*, *Fe*, *Mn*, *K*, *Mg*, *Ca* and *P* were determined after an extraction by 1 N of  $CH_3COONH-EDTA$  (*pH*4.65) for 30 min (ratio soil:solution of 1:5) (Lakanen and Erviö 1971). Supernatant was filtered through an S&S 595 folded filter and analyzed using a flame atomic absorption spectrometer (Varian 220). Phosphorus content was determined with a Shimadzu UV-1205 spectrophotometer by coloration at 430 nm (Shimadzu Corporation).

Soil profiles were described following the characterization of plant assemblages, with a dutch auger sampling in order to determine soil physical conditions for the identified communities. The following data were collected according to Delecour and Kindermans (1977): slope, rock type, rooting depth, depth of humiferous horizons, rockiness at and below roots level, texture at and below roots level. The method uses the quantitative data for slope and rooting depths, semi-quantitative scales for rockiness (1: absence; 2: <15 %; 3: 15–50 %; 4: >50 %) while texture is defined by the relative abundance of clays, loams and sands.

## Data analysis

Prior to analysis, species cover data were  $\log(x+1)$  transformed to equalize the relative importance of common and rare species (McCune et al. 2002). Analyses were performed with the Vegan package of the R statistical environment (Oksanen et al. 2011; R Development Core Team 2009) and the IndVal software (Dufrêne and Legendre 1997).

## Factors influencing species composition

An exploratory Detrended Correspondence Analysis (DCA) determined a gradient length of 4.4 for the first canonical axis, we thus opted for the Canonical Correspondence Analysis (CCA, unimodal model) (ter Braak and Šmilauer 2002). CCA (ter Braak 1986) and partial CCA (pCCA) were run in Vegan (Oksanen et al. 2011). pCCA provides the ability to relate responses to a unique environmental factor by removing the effect of others variables (ter Braak 1988).

A first CCA was conducted with all edaphic chemicals parameters and Site (identity of the hill, hereafter referred as site effect) to relate the floristic dataset with available environmental factors. Site effect encompasses the potential other factors influencing plant assemblages: i) niche-based ecological filtering due to remaining soil conditions, i.e. physical parameters, imposed on species and ii) chance arrival of propagules from the regional species pool enforced by the insular nature of metalliferous habitats.

In order to assess the relative contribution of measured chemical factors and site effect in explaining species composition, variation partitioning was computed using the approach of Borcard et al. (1992). The proportion of constrained inertias from total inertia ( $100 \times \text{Eigenvalue of constrained axis} / \sum \text{eigenvalues (Global Inertia)}$ ) was assessed to determine the contribution to global inertia of Site and chemical edaphic factors as well as their unique contribution. A randomization test with 999 permutations was performed to establish the pseudo F-values and the significances of all (p)CCA models (methodology inspired by Kalusova et al. 2009).

Forward selection was implemented through the ‘step’ command in R in order to build a model for the species data (Oksanen 2010). Permutations (steps = 999) were implemented at each step to test for significance of variables included in the model.

## Plant assemblages' characterization

To discriminate plant assemblages, a two dimensional Non-Metric Multidimensional scaling (NMDS) (Kruskal 1964a, b; McCune et al. 2002; Shepard 1962a, b) ordination was run on the Bray-Curtis similarity matrix, and the coordinates of the plots were submitted to a K-means clustering (command 'cascadeKM' in Vegan (Legendre and Legendre 1998; Oksanen 2010; Oksanen et al. 2011)). The ordination was evaluated by calculating correlations between fitted vectors and ordination values ( $R^2$ ) using the 'stressplot' command of Vegan. The most appropriate number of clusters was then determined using the Calinski and Harabasz (1974) stopping rule (Milligan and Cooper 1985; Oksanen et al. 2011). The fitted edaphic vectors and centroids were overlain using the 'envfit' command of Vegan to allow for a better visualization of relations between plant assemblages and chemical edaphic factors.

Diagnostic species for the partitions were determined with the IndVal method (IndVal 2.0). The method calculates the Indicator Value (IV, function of fidelity and species abundance) for each species in each predefined plant community (Dufrière and Legendre 1997; Tichy and Chytrý 2006). Statistical significance (at the 1 % level) of the IV's obtained was computed with a 999 Monte Carlo randomization procedure (Dufrière and Legendre 1997).

Analyses of variance followed by post hoc pair wise comparisons ('TukeyHSD' in R) were used to test for differences among plant assemblages in soils conditions, mean species richness.m<sup>-2</sup>, mean number of endemics.m<sup>-2</sup> (i.e. absolute metallophytes: species that occur solely on Katangan copper sites) and mean number broad endemics.m<sup>-2</sup> (i.e. with more than 75 % occurrence on copper sites) (Faucon et al. 2010). Square root (for *C*, *N* and *Fe*) and log (for *pH*, *C:N*, *Cu*, *Co*, *Zn*, *Mn*, *K*, *Mg*, *Ca*, *P*, species richness and endemics richness) transformations were used to improve the normality and homoscedasticity of variables.

Three soil profiles were compiled and reported to the corresponding plant assemblages identified.

## Results

A total of 183 taxa of herbaceous plants, of which five are strict endemics and nine broad endemics (sensu

Faucon et al. 2010, detailed in Appendix S1), were encountered in the 172 plots surveyed.

## Relation between plant assemblages and environmental parameters at plot scale

Table 2 presents percentages of the variability in plant species assemblage explained by chemical edaphic factors and site effect. It is divided between raw variation, and variation unique to each factor.

Total inertia of the dataset was 9.48. The sum of all canonical eigenvalues of the CCA including chemical edaphic factors and site effect was 2.14 (i.e., contribution to global inertia: 22.6 %). Site effect (6.0 %) had the largest gross influence on the dataset with a similar contribution as *Cu* (5.5 %) and an elevated unique contribution (3.8 %). The set of edaphic factors contributed to 18.77 % of total inertia with largest unique contributions for *Mn* (1.3 %), *Cu* (1.1 %), *Co* (1.0 %) and *K* (0.8 %). Shared variation between site effect and chemistry was 2.2 % of total inertia. Low unique contribution values can be attributed to important co-correlation patterns in the edaphic set (Appendix S2).

The regression model ran on chemical edaphic factors by forward selection included, in the following order: *Cu* (contribution to global inertia when included in the model: 5.5 %), Site (5.8 %), *Mg* (2.2 %), *Mn* (1.6 %), *Co* (1.5 %) and *K* (1.1 %). The model accounted for 17.5 % of global inertia. All factors included in the model were significant ( $p$ -value <0.001).

## Plant assemblages and indicator species

The two dimensional solution of the NMDS had a final stress of 20.5 after seven iterations ( $R^2=0.96$ ). The first axis of the NMDS ordination was correlated to a gradient of *Cu*, *C* and *N* contents, while the second was related to the trophic status (*Mn* and *Mg*) of the soil and to acidity (Fig. 2).

Two peaks in the value of the Calinski criteria were detected within the first ten partitions. The first partition distinguished two groups (Calinski: 180) (Table 3). Indicator species for the first group were *Cryptosepalum maraviense*, *Loudetia simplex* and *Scleria bulbifera*. The vegetation corresponds to Steppic savannas. The second group was characterized by *Xerophyta equisetoides*, *Anisopappus davyi* and *Ascolepis metallorum* and corresponds to Steppe vegetation. Plots from the Fungurume V North site are evenly distributed between both groups (43

**Table 2** Variance partitioning among measured chemical edaphic factors and site effect

Factor	Variation explained				Unique variation		
	X		Pseudo-F	p-value	X   other factors	Pseudo-F	p-value
Edaphic	18.77	-	2.81	*	16.63	2.58	*
Site	5.95	(5.75)	5.34	*	3.81	3.84	*
Cu	<b>5.46</b>	(5.46)	9.81	*	1.05	2.11	*
pH	3.55		6.25	*	0.75	1.52	0.003
Co	<b>3.47</b>	(1.48)	6.11	*	1.03	2.06	*
N	3.23		5.68	*	0.37	0.74	0.932
C	3.08		5.41	*	0.42	0.85	0.808
Ca	2.95		5.16	*	0.75	1.52	0.010
Zn	2.66		4.64	*	0.68	1.37	0.033
Fe	2.65		4.63	*	0.66	1.33	0.033
Mn	<b>2.37</b>	(1.55)	4.12	*	1.25	2.52	*
Mg	<b>2.33</b>	(2.20)	4.06	*	0.68	1.38	0.104
P	1.96		3.41	*	0.37	0.74	0.654
K	<b>1.88</b>	(1.05)	3.25	*	0.83	1.67	0.004
C:N	1.16		2.00	*	0.51	1.02	0.468
Global inertia						9.48	
Percentage of global inertia explained by the entire dataset						22.57	
Percentage of global inertia explained by the model						17.49	

Variation Explained (X) = (100\*eigenvalue of constrained axis:  $\sum$  eigenvalues) (Global inertia). Unique variation (X | other factors) = X after removing the shared variation with all other factors. Variables included by forward selection (P-value<0.001) in boldface; increase in variation explained by inclusion of a variable is given in brackets

\*p-value<0.001

and 40 plots) while Kavifwafwaulu I and Kazinyanga are dominated by Steppic savannas (84 % and 71 %). Species richness per 1 m<sup>2</sup> was highest for Steppic savannas (mean (SD): 16.9 (4.2)) than for Steppes (11.3 (3.4)), but the second exhibited a higher mean number of endemics per 1 m<sup>2</sup> (2.4 (1.3) versus 0.5 (0.8) for Steppic savannas) (Table 4).

The second partition distinguished seven communities (1 to 7; Calinski: 181.3). Communities 1, 2 and 3, belonging to Steppic savannas, host a wider diversity of species. Communities 5, 6 and 7, belonging to Steppes, are dominated by broad and strict endemics (i.e. absolute metallophytes) of Katangan *Cu*-rich soils such as *Haumaniastrum robertii* and *Ascolepis metallorum* (Table 3). Community 4 is an intermediary between those two groups with five plots belonging to Steppic savannas group and four to Steppes group.

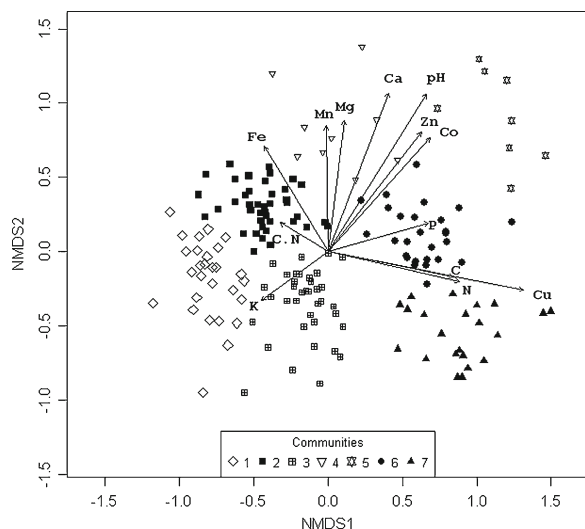
The partition in seven communities showed an aggregation among sites and communities. Community 1

(Steppic savanna) occurred mainly on Kavifwafwaulu I, community 2 (Steppic savanna), 4 (intermediary), 5 (Steppe) and 6 (Steppe) mainly occurred on Fungurume V North, community 3 (Steppic savanna) on Kazinyanga, and community 7 (Steppe) was evenly split between Kavifwafwaulu I and Kazinyanga.

Species richness per 1 m<sup>2</sup> was the highest for community 2 with a mean number of 20.2 (SD: 3) species while it was the poorest for community 5 (7.8 (SD: 2)). The number of endemics per 1 m<sup>2</sup> presented the same trends as for the partition in Steppic savannas and Steppes, with communities pertaining to Steppes (5, 6 and 7) presenting higher mean numbers of endemics per 1 m<sup>2</sup> (1.9 to 2.7 versus 0.4 to 0.6 for Steppic savannas) (Table 4).

#### Related edaphic factors

All chemical factors but *Mn* and *Mg* were significantly different between Steppic savanna group and Steppe



**Fig. 2** NMDS ordination diagram of the plots ( $n=172$ ) distributed in the seven communities. Fitted edaphic vectors are overlain by using the `envfit` command of `Vegan`. Steppic savannas: 1: on Kavifwafwaulu; 2: on Fungurume V North; 3: on Kazinyanga. Steppes: 4: transition on Fungurume V North; 5, 6: Steppe on Fungurume V North; 7: Steppe on Kavifwafwaulu and Kazinyanga

group (Table 4). Steppic savannas presented higher  $C:N$  ratio and mean concentrations of  $K$  and  $Fe$  than

Steppes. Steppe soils presented higher mean concentrations of extractable  $Cu$  and  $Co$  than those from Steppic savannas. The  $pH$  of Steppe soils were also closer to neutrality, contained higher organic  $C$  and  $N$  and were richer in  $Ca$  and  $P$ .

For the subdivision in seven communities, all chemical edaphic factors presented at least one significant difference. Communities 1, 2 and 3 followed the trends of Steppic savannas, and 5, 6 and 7 the ones of Steppes. Community 4 mean values of chemical edaphic factors were intermediary between both groups.

Communities 1, 2 and 3 were not well distinguished in terms of soil chemistry. Community 3 presented similar soils as encountered in community 1 with only small distinctions for  $K$  (lower contents for community 2),  $Co$ ,  $Mn$ ,  $Fe$ , and  $Ca$  (higher contents for community 2).

For Steppes, community 5 presented the highest mean values for  $pH$ ,  $Co$ ,  $Zn$ ,  $Mn$ ,  $Fe$ ,  $Mg$ ,  $Ca$ . It presented low contents of organic matter content ( $C$  and  $N$ ) compared to community 6 and 7. Community 7 was the overall richest in extractable  $Cu$  with a mean value of  $5,881 \text{ mg.kg}^{-1}$ .

**Table 3** Distribution of communities among sites and first three indicating species

Community	Plots membership (hill's plots percentage)			Indicator species
	Fung	Kavi	Kazi	
Steppic savannas	43 (52)	31 (84)	37 (71)	<i>Cryptosepalum maraviense</i> (Caesalpiniaceae); <i>Loudetia simplex</i> (Poaceae); <i>Scleria bulbifera</i> (Cyperaceae)
Steppes	40 (48)	6 (16)	15 (29)	<i>Xerophyta equisetoides</i> (Velloziaceae); <i>Anisopappus davyi</i> (Asteraceae); <i>Ascolepis metallorum</i> (Cyperaceae)
1		26 (70)	2 (4)	<i>Tristachya bequaertii</i> (Poaceae); <i>Crotalaria argenteotomentosa</i> (Fabaceae); <i>Droogmansia pteropus</i> var. <i>pteropus</i> (Fabaceae)
2	38 (46)	2 (5)	1 (2)	<i>Endostemon dissitifolius</i> (Lamiaceae); <i>Tristachya</i> sp.1 (Poaceae); <i>Rhytachne rottboellioides</i> (Poaceae)
3	1 (1)	2 (5)	34 (65)	<i>Cyanotis</i> aff. <i>cupricola</i> (Commelinaceae); <i>Monocymbium ceresiiforme</i> (Poaceae); <i>Loudetia simplex</i> (Poaceae)
4	8 (10)	1 (3)		<i>Hyparrhenia diplandra</i> var. <i>diplandra</i> (Poaceae); <i>Schizachyrium brevifolium</i> (Poaceae); <i>Justicia elegantula</i> (Acanthaceae)
5	8 (10)			<i>Michrochloa altera</i> (Poaceae); <i>Xerophyta equisetoides</i> (Velloziaceae); <i>Bulbostylis cupricola</i> (Cyperaceae)
6	27 (33)			<i>Pandiaka carsonii</i> (Amaranthaceae); <i>Ascolepis metallorum</i> (Cyperaceae); <i>Anisopappus davyi</i> (Asteraceae)
7	1 (1)	6 (16)	15 (29)	<i>Eragrostis racemosa</i> (Poaceae); <i>Sporobolus congoensis</i> (Poaceae); <i>Gladiolus ledoctei</i> (Iridaceae)

Numbers correspond to the number of  $1 \text{ m}^2$  plots from the site present in the community, along with the percentages of the site it represents. Fung: Fungurume V North; Kavi: Kavifwafwaulu I; Kazi: Kazinyanga



**Table 4** Ranges, means and standard errors of chemical edaphic factors and species per plot for the partitions in two and seven communities

Community (number of plots)		1 (n=28)	2 (n=41)	3 (n=37)
Factor	Range (min. – max.)	Steppic savannas (n=111)	Steppes (n=61)	p-value
pH (H <sub>2</sub> O)	[4.6–7.8]	5.6 (0.4) <sup>a</sup>	6.3 (0.6) <sup>b</sup>	*
% C	[1–10.1]	2.7 (0.9) <sup>a</sup>	5 (1.9) <sup>b</sup>	*
% N	[0.07–0.59]	0.16 (0.06) <sup>a</sup>	0.32 (0.12) <sup>b</sup>	*
C:N	[12.9–22.3]	16.7 (1.5) <sup>b</sup>	15.7 (1.7) <sup>a</sup>	*
Cu (mg.kg <sup>-1</sup> )	[29–10136]	514 (451) <sup>a</sup>	4718 (2025) <sup>b</sup>	*
Co (mg.kg <sup>-1</sup> )	[2–927]	21 (26) <sup>a</sup>	153 (208) <sup>b</sup>	*
Zn (mg.kg <sup>-1</sup> )	[0.31–7.79]	1.27 (0.69) <sup>a</sup>	2.4 (1.61) <sup>b</sup>	*
Mn (mg.100 g <sup>-1</sup> )	[0.2–25.5]	5.4 (4.6)	5.1 (4.2)	0.476
Fe (mg.100 g <sup>-1</sup> )	[1–14.9]	6.6 (1.9) <sup>b</sup>	4.5 (2.8) <sup>a</sup>	*
K (mg.100 g <sup>-1</sup> )	[0.6–21]	7.8 (4) <sup>b</sup>	5.1 (2.5) <sup>a</sup>	*
Mg (mg.100 g <sup>-1</sup> )	[1.1–117.5]	14 (8.8)	16.8 (19.7)	0.679
Ca (mg.100 g <sup>-1</sup> )	[1–268]	21 (25) <sup>a</sup>	57 (64) <sup>b</sup>	*
P (mg.100 g <sup>-1</sup> )	[0.17–49.91]	1.62 (3.72) <sup>a</sup>	7.13 (6.55) <sup>b</sup>	*
sp.richness.m <sup>-2</sup>	[5–29]	16.9 (4.2) <sup>b</sup>	11.3 (3.4) <sup>a</sup>	*
Endemics. m <sup>-2</sup>	[0–6]	0.52 (0.8) <sup>a</sup>	2.38 (1.3) <sup>b</sup>	*
				Slope (%)
				Rock type - Minerals - Substrate
				Rooting Depth (cm)
				Depth of humiferous horizons
				Rockiness at roots level
				Rockiness below roots level
				Texture at roots level
				Texture below roots level
				C. - S.Si.
				S.Si. - Si.
				C.S - Si.S.

Community (number of plots)		4 (n=9)	5 (n=8)	6 (n=27)	7 (n=22)	p-value	Miombo
pH (H <sub>2</sub> O)	6.5 (0.5) <sup>c</sup>	7.3 (0.4) <sup>f</sup>	6.1 (0.4) <sup>de</sup>	6.0 (0.2) <sup>cd</sup>		*	[4.6–5.1]
% C	3.9 (1.1) <sup>b,c</sup>	3.1 (1.7) <sup>ab</sup>	5.6 (1.6) <sup>d</sup>	4.9 (2.1) <sup>cd</sup>		*	[2.8–6.6]
% N	0.25 (0.1) <sup>b,c</sup>	0.2 (0.11) <sup>ab</sup>	0.35 (0.1) <sup>c</sup>	0.32 (0.14) <sup>c</sup>		*	–
C:N	16.4 (2.5) <sup>ab</sup>	15 (1.5) <sup>a</sup>	16.3 (1.9) <sup>ab</sup>	15.5 (1.5) <sup>a</sup>		*	–
Cu (mg.kg <sup>-1</sup> )	1745 (1300) <sup>b</sup>	3977 (1792) <sup>b,c</sup>	4287 (1744) <sup>c</sup>	5881 (2010) <sup>c</sup>		*	[10–23]
Co (mg.kg <sup>-1</sup> )	63 (74) <sup>b</sup>	504 (305) <sup>d</sup>	149 (157) <sup>c</sup>	48 (42) <sup>b</sup>		*	[0.3–1.4]

Table 4 (continued)

Community (number of plots)	4 (n=9)	5 (n=8)	6 (n=27)	7 (n=22)	p-value	Miombo
Zn (mg.kg <sup>-1</sup> )	2.12 (1.17) <sup>b,c</sup>	4.85 (1.73) <sup>d</sup>	2.35 (1.44) <sup>c</sup>	1.56 (0.82) <sup>a,b,c</sup>	*	[1.50–4.50]
Mn (mg.100 g <sup>-1</sup> )	4.9 (1.8) <sup>b,c,d</sup>	13 (6.1) <sup>d</sup>	4.6 (1.8) <sup>b,c</sup>	3.1 (2.2) <sup>a,b</sup>	*	[0.6–2.5]
Fe (mg.100 g <sup>-1</sup> )	5.3 (1.7) <sup>b,c</sup>	9.3 (3.4) <sup>d</sup>	4.5 (1.8) <sup>b</sup>	2.5 (1.1) <sup>a</sup>	*	[7.6–11.5]
K (mg.100 g <sup>-1</sup> )	6 (1.2) <sup>a,b</sup>	3.8 (1.6) <sup>a</sup>	5.3 (2.1) <sup>a</sup>	5.1 (3.1) <sup>a</sup>	*	[6.1–20.8]
Mg (mg.100 g <sup>-1</sup> )	26.3 (12.2) <sup>c,d</sup>	49.5 (33.2) <sup>d</sup>	13.6 (10.2) <sup>b,c</sup>	6.3 (3.8) <sup>a</sup>	*	[5.8–29.2]
Ca (mg.100 g <sup>-1</sup> )	104 (70) <sup>d,e</sup>	154 (73) <sup>e</sup>	52 (48) <sup>c,d</sup>	16 (8) <sup>a,b</sup>	*	[3–17]
P (mg.100 g <sup>-1</sup> )	3.23 (2.86) <sup>b</sup>	11.5 (3.53) <sup>d</sup>	7.62 (8.85) <sup>c,d</sup>	5.41 (2.94) <sup>c</sup>	*	[0.19–0.36]
sp.richness.m <sup>-2</sup>	12.6 (3.4) <sup>b,c</sup>	7.8 (2) <sup>a</sup>	13.5 (2.8) <sup>c</sup>	10 (2.6) <sup>a,b</sup>	*	
Endemics. m <sup>-2</sup>	0.4 (0.5) <sup>a</sup>	1.9 (1.1) <sup>b</sup>	2.7 (1.3) <sup>b</sup>	2.5 (1.3) <sup>b</sup>	*	
Physical soil properties						
20–25	30		25–30	5–50		
Q.; S.	Mal.; Q.; S.		RSF; S./RAT	Mal.; Q.; S.		
>15	>55		30–60	15–30		
5–10	5–45		15–60	15		
3	2–3		2–3	2–4		
n.a.	n.a.		2	3–4		
S.Si.	S - S.Si.		S - Si.S.	Si.S.		
n.a.	n.a.		C.Si. - C.S.Si.	Si.S.		

Organic matter (Springer-Klee C and Kjeldahl N), mineral elements concentration (1 N of CH3COONH EDTA, *pH*4.65). Significant differences in mean factors are represented by different letters. Analysis of variance followed by Tukey HSD post hoc pair-wise comparisons. Physical properties compiled from three soil profiles are shown for further characterization

\*; *p*-value<0.001. Data for miombo woodland are given as reference values for normal soil in the region (*n*=3 sites × 1 composite sample) (Faucon et al. 2011). Physical properties were obtained through soil profile realized with a dutch auger. Legend: Rock type - Minerals - Substrate: Mal.: Malachite; Q.: Quartz; RAT: Talcoose Argillaceous Rocks; RSF: Foliated Siliceous Rocks; RSC: Cellular Siliceous Rocks; S.: Sandstone;/: above. Rockiness levels: 1: absence; 2: <15 %; 3: 15–50 %; 4: >50 %. Textures: C: Clayey; Si: Silty; S: Sandy; n.a. not accessed (limit of soils)

## Physical parameters

The semi-quantitative and qualitative description of soil profiles allows to further characterize communities observed. Communities 4, 5, 6 and 7 (intermediary and Steppes) are generally present in steeper areas than communities 1, 2 and 3 (Steppic savannas; +30 % vs. +5 %) (Table 4). Profiles of communities 5 and 7 stand out with the presence of malachite ( $CuCO_3$ ) and RSF, the rock layer associated with the largest concentrations of *Cu*.

Rooting depths are the deepest for community 2 (>50–120 cm) while roots are impeded by a compact clayey horizon in communities 1 and 3 (Steppic savannas; clayey texture below roots level), by soil rockiness in communities 4, 5 and 7 (intermediary and Steppes; rocks levels between 2 and 4 at roots level) or by a combination of both for community 6 (Steppes; clayey texture below roots level combined to rocks levels of 2–3 at roots level).

Both communities 3 (Steppic savannas) and 6 (Steppes) appear as occurring on RAT substrate buried under layers of exogenous rocks: RSF or RSC.

## Discussion

### Influencing factors on plant assemblages

As expected for sites with a high gradient in soil chemical properties, chemical edaphic factors studied explain a large share of the variation in plant assemblages on copper hills (contribution to global inertia: gross: 18.8 %; removing site effect: 16.6 %). From the variables that can possibly be considered responsible for toxicity effects, extractable (acetate-EDTA) *Cu* explains the highest proportion of variation among plant assemblages (contribution to global inertia: gross: 5.5 %; unique: 1.1 %). This is in accordance with hypotheses of previous studies (Duvigneaud and Denayer-De Smet 1963). However, our results support the idea that variation in other factors should also be considered if we are to understand patterns of plant assemblages in order to maintain or recreate adequate conditions for the conservation of copper flora. Toxicity is dependent of the nature of trace metals as well as on their bioavailability (Kabata-Pendias and Pendias 2001). Factors such as *pH* (3.6 %; 0.8 %), *C* (3.1 %; 0.4 %), *Ca* (3.0 %; 0.8 %) and *Mn* (2.4 %,

1.3 %), contributing to a large share of variation in plant assemblages of our study sites, can influence the availability of metals in soils. Párraga-Aguado et al. (2013) have showed that metal(oid) concentrations accounted for a minor role in the plant distribution of mine tailings from a semiarid area while low *pH* and salinity played a major role. In our study, because they are associated to carbonate (malachite) in the outcrop, *Cu* and *Co* concentrations are positively correlated to *pH*. Toxicity is thus likely mitigated by higher *pH* values. *Mn*-oxides and carbonates associated with *Ca*, another parameter with higher values for major concentrations in *Cu* and *Co*, present a strong affinity for *Co* and could thus decrease its bioavailability and toxicity (Collins and Kinsela 2011; Faucon et al. 2011b, 2009; Li et al. 2001; 2004; Robinson et al. 1999). High values of *Fe*-oxides and organic matter can also limit *Cu* bioavailability and thus its toxicity (Kabala and Singh 2001). Concentration of extractable (acetate-EDTA) *Co* (3.5 %; 1.0 %) is also a major factor to take into account due to its own toxicity and its potential interactions with *Cu*. *Cu* is known to have antagonist effects on plants *Co* uptake (Faucon et al. 2009) and soils with high levels of *Co* and lower levels of *Cu* (as in community 5) could thus be more toxic to plants. Correlations between extractable (acetate-EDTA) *Cu*, *Co* and *Zn* and their total values has previously shown (Saad et al. 2012) to be high (c. 0.8). The use of total concentration would therefore add low additional valuable information to the analysis.

Besides general influence of soil chemistry on plant assemblages, the most important result regarding development of conservation strategies comes from the identification of a strong site effect. Site effect (6.0 %; 3.8 %), encompassing the physical parameters variability and the chance arrival of propagules, appears to be similar to that of *Cu* but with a larger share being unique to it. Since the three different sites are situated in the same region and at similar altitudes, site effect are unlikely related to variations in global climatic conditions. Two non-exclusive hypotheses may support this pattern. First, the geographical isolation of copper outcrops could be responsible for random colonization from a regional species pool. Low alpha (local) and higher beta (among sites) diversity have already been observed at regional scales in isolated patches of Californian and Greek serpentine grasslands (see Harrison 1997, 1999; Tsiripidis et al. 2010). On copper hills, random site effect is supported

by the limited distribution of the broad endemics *Triumfetta likasiensis* and *Sopubia neptunii* which, in our three studied sites, are only known to occur on Kavifwafwaulu I. Alternatively, differences in plant assemblages among sites may also result from differences in environmental factors not considered in this study. Soil profiles support an influence of variation in physical factors such as rockiness and soil texture correlated to variation in geology and topography among sites. On Kavifwafwaulu I, the morphology of the hill, with a large share of gentle slopes, could lead to less cover of the most abundant substrate (RAT) by RSC or RSF rocks and thus lead to a differentiation in vegetation not observed for the two others sites. Fungurume V North presents steeper slopes combined to a well-marked geology exposing *Cu-Co* rich geological layers where only superficial soil has developed and where rocks regularly cover lower areas thus covering areas of RAT. The variation of the width of surface outcrops, depending on the dip of the geological layers, also contributes to differences in soil type representations and thus in changes in vegetation occurrences.

#### Diversity of plant communities

Altogether, variation in soil edaphic properties and a strong site effect generate a high diversity of plant communities over limited surfaces. The definition of plant communities and related soil chemistry allows for a better comprehension of factors associated with characteristic vegetations of Katangan copper outcrops. A partition in two larger groups establishes the well-known distinction between *Cu-Co* poor Steppic savannas and highly contaminated Steppes (Duvigneaud and Denayer-De Smet 1963; Malaisse 1995; Saad et al. 2012). In accordance to Saad et al. (2012), established in the same area, not only *Cu* and *Co* are significantly different between both groups. Steppes present higher values for *pH*, *C*, *N* and extractable (acetate-EDTA) *Zn*, *Ca* and *P* and lower values for the *C:N* ratio and extractable (acetate-EDTA) *Fe* and *K* than Steppic savannas. As observed by Saad et al. (2012), trophic level does not appear as being restrictive for the vegetation: levels of the essential macronutrients are similar to that of normal soils in Katanga. Similar patterns are observed in eastern parts of the Katangan Copperbelt (unpublished data). This, however, differs from serpentine soils

where deficiency in *NPK* and *Ca* has been suggested as a potential reason for the limited plant productivity (Kruckeberg 1984; O'Dell et al. 2006; Proctor 1971; Whittaker 1954). While poorer in number of species per 1 m<sup>2</sup>, Steppes host a larger number of endemics with higher occurrences per 1 m<sup>2</sup>. This can be attributed to the nature of endemics on those outcrops: essentially species adapted to high levels of soil contamination (Faucon et al. 2009). The larger diversity on Steppic savannas can be attributed to the important number of species overstepping from the miombo woodland which can tolerate limited concentrations of *Cu* and *Co* in the soil but not the levels encountered in the Steppes area.

The partition in seven communities reveals the important differentiation of communities among sites, confirming that site related factors have an effect on plant assemblage.

This shows particularly for the Steppic savanna communities (1, 2 and 3), clearly distinguishing Kavifwafwaulu I for community 1, Fungurume V North for community 2 and Kazinyanga for community 3. All three communities present low metal contents in soils and only little distinction in edaphic chemistry factors that do not appear as being ecologically meaningful. However, community 1 tends to occur on deeper soils, hosting denser vegetation, while community 3 occurs on shallow and permeable soils (field observations). Community 5, 6 and 7 also confirmed a site effect with communities 5 and 6 limited to Fungurume V and community 7 occurring mainly on Kazinyanga and Kavifwafwaulu. Community 5 presents a low diversity per 1 m<sup>2</sup>, typical of areas highly contaminated by *Cu* and/or *Co*. This vegetation is similar to that of reworked mining areas with indicators such as the highly *Cu*-tolerant *Michrochloa altera* (Shutcha et al. 2010), the colonizing strict endemic *Haumaniastrum robertii* and broad endemics *Bulbostylis cupricola* and *B. pseudoperennis*. It illustrates the importance of local factors in filtering species diversity, as already observed by Gough and Grace (1999). Soils of Steppe communities are differentiated by different combinations of *Cu* and *Co* concentrations with community 5 exhibiting high levels of both *Cu* and *Co* while community 7 exhibited high concentration of *Cu* and only moderate concentrations of *Co*. Additional environmental factor such as soil texture and slope, with its influence on the presence of rocky materials, also appear to play a role.

Even if they present the same type of chemical properties, communities 1 and 2 are differentiated by the presence of a compact and impermeable substrate (RAT) for community 1 that likely maintains a higher level of humidity but is also a limit to roots. RAT seems to play a similar role in the differentiation of community 6 and 7: soil chemical parameters are undifferentiated, with high level of bioavailable *Cu* and *Co*, but community 6 occurs on a buried layer of RAT that is likely to influence the humidity of the soil. The highly contaminated soils on Fungurume V North, below an escarpment, would thus be present on more humid conditions. In contrast, with tussock grasses as indicators (*Sporobolus congoensis*, *Eragrostis racemosa*), community 7 can be related to more xeric conditions, created through the rapid drainage of water by the rocky substrate, and shallower soils rooting depths than community 6. Both communities host an interesting variety of endemics and should be prioritized in conservation plans.

#### Implications for conservation and future research

In order to conserve the diversity of the copper flora, plant communities will need to be restored after mining activities, preferentially in relation to site rehabilitation (Whiting et al. 2004). In a previous study, Saad et al. (2012) concluded that despite large variations in soil properties, only a limited number of plant communities occurred on copper hills in the same region. The current study based on a hierarchical sampling strategy challenges this conclusion. The importance of the variation in plant assemblages observed among hills indicates that, in order to conserve the diversity of copper communities, different restoration plans would need to be implemented for individual outcrops.

Within site, the importance of endemics observed on highly mineralized areas would imply to pay particular attention to maintain areas with a high level of soil contamination in the restored ecosystem. Edaphic compaction and texture should also be taken into account in order to recreate adequate conditions for a diversity of communities. Attention should also be given to factors potentially limiting the toxicity of metals such as *pH*, organic matter, *Ca* and soil fertility (*NPK*). However, a limited number of endemics such as *Lopholaena deltombei*, *Triumfetta likasiensis* and *Basananthe kisimbae* occurring on soils with lower

metal contents and their low frequency would imply the conservation of large areas of steppic savannas.

Our results also point to the need for further research elucidating the cause of site differences in plant communities of copper outcrops. We suggest that a functional approach of copper plant communities based on examination of functional traits will help to disentangle the effects of environmental factors from the effects of random colonization of sites.

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