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RESEARCH ARTICLE

May Rare Metallophytes Benefit from Disturbed Soils Following Mining Activity? The Case of the *Crepidorhopalon tenuis* in Katanga (D. R. Congo)

Michel-Pierre Faucon,[1,2,3] Ingrid Parmentier,[4] Gilles Colinet,[5] Grégory Mahy,[6] Michel Ngongo Luhembwe,[3] and Pierre Meerts[1]

Abstract

Cuprophytes are plants that mostly occur on Cu-rich soil. In South Central Africa, these species are threatened by intensive mining exploitation destroying their habitats. *Crepidorhopalon tenuis* (Scrophulariaceae) is a tiny annual cuprophyte endemic to the Zambesian center of endemism and is particularly abundant in the Lubumbashi area. We investigate here the ecological niche of *C. tenuis* through the analyses of its abundance and distribution in relation to soil factors, plant community composition, and anthropogenic perturbations. Soil and vegetation data were collected in seven sites (five metalliferous and two nonmetalliferous). The current study shows that *C. tenuis* has its ecological optimum on copper-rich soil and can be referred to as an elective pseudometallophyte. This species is rare in primary steppic savanna on natural metalliferous soil. Its frequency and abundance peak in pioneer communities on bare soil. In particular, the species showed a surprising ecological plasticity as it was able to benefit from anthropogenic disturbance and to colonize the large areas of bare, contaminated soil left over by mining activities. Our results strongly suggest that *C. tenuis* was a very rare species in natural metalliferous communities, restricted to patchy areas of open soil in steppic savanna. Recent anthropogenic habitats may have conservation value for some rare metallophytes with colonizing traits and low competitive ability.

Key words: **anthropogenic habitats, conservation, ecological niche, metallophyte, plant communities, restoration.**

Introduction

Soils with elevated concentrations of trace metals (metalliferous soils) are a good example of scattered habitats. Because of their phytotoxicity, metalliferous soils represent very restrictive habitats for plants. Such sites offer outstanding examples of microevolution and speciation processes due, on the one hand, to the severe selection pressure induced by trace metal and, on the other hand, to founder effect and genetic drift induced by the insularity of these habitats. As a result, metalliferous sites may host rare, ecologically endemic taxa adapted to large concentrations of trace metals (metallophytes). Metalliferous sites are highly significant to biodiversity conservation (Whiting et al. 2004).

The habitat of metallophytes is directly threatened by mining activities, particularly by extraction surface mining (e.g., Bradshaw 2000). However, mining activities may also create new metalliferous habitats due to surface deposition of residual ore deposits and overburdening, as well as aerial fallout from smelters and industrial processes (e.g., Allen & Sheppard 1971; Ginocchio et al. 2002; Batty 2005).

These habitats present a broad range of soil conditions and can be recolonized by species adapted to open habitats and metal toxicity (e.g., Bradshaw 1983; Ash et al. 1994). This may increase the number of suitable habitats for those plant species that are naturally metal tolerant (Morrey 1995; Krüger et al. 2002; Batty 2005; Bizoux et al. 2008). Several recent studies pointed to the possible conservation value of such recent anthropogenic habitats for edaphically restricted species (Krüger et al. 2002; Brock et al. 2007; Reisch 2007; Bizoux et al. 2008; Esfeld et al. 2008).

In South Central Africa (SC), particularly in the Katangan copper belt (Democratic Republic of Congo), natural copper outcrops bear a distinctive steppic vegetation. These outcrops are remarkable in the landscape in the form of grasslands, mostly developed on hills emerging from the medium plateau

[1] Laboratoire de Génétique et Ecologie végétales—Université Libre de Bruxelles 1850, chaussée de Wavre, B-1160 Bruxelles, Belgium
[2] Address correspondence to M.-P. Faucon, email mfaucon@ulb.ac.be
[3] Université de Lubumbashi, Faculté des Sciences Agronomiques, Lubumbashi, Democratic Republic of the Congo
[4] Laboratoire d'Eco-Ethologie Evolutive—Université Libre de Bruxelles 50 Av. F. Roosevelt CP 160/12, B-1050 Bruxelles, Belgium
[5] Laboratory of Geopedology, Gembloux Agricultural University, Passage des Déportés 2, 5030 Gembloux, Belgium
[6] Laboratory of Ecology, Gembloux Agricultural University, Passage des Déportés 2, 5030 Gembloux, Belgium

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covered with miombo woodland (Brooks & Malaisse 1985; Leteinturier 2002). Copper toxicity in the soil creates an ecological isolation of this habitat, and as most outcrops are not contiguous, copper hills are also geographically isolated across the Katangan copper belt. This may explain variation in the floristic composition of the copper hill flora (Duvigneaud 1958; Duvigneaud & Denaeyer-De Smet 1963). Katanga copper belt constitutes a hotspot for metallophyte species (Wild & Bradshaw 1977; Brooks & Malaisse 1985). The regional copper flora comprises some 600 taxa present on approximately 160 metalliferous sites. Some 40 species are endemic of the copper habitat (Leteinturier 2002; Whiting et al. 2004). Actions and researches aiming to preserve the metallophyte species in Katanga are imperative (Whiting et al. 2004). Indeed, intensive mining exploitation threatens the habitat of rare copper-tolerant species. A dozen sites are already completely destroyed and many others have been disturbed. At least four metal-tolerant taxa endemic to the regions are already extinct (Brooks et al. 1992). Katangan copper species have not been included in the IUCN Red List and this may seriously undermine conservation efforts (for a discussion of appropriate uses of the Red List, see Possingham et al. 2002; Lamoreaux et al. 2003).

In addition to natural copper-enriched soil, industrial mining exploitations and metal smelters foundries have contaminated extensive areas in their surroundings. These anthropogenic sites have been colonized by annual species originating from naturally mineralized areas, for example, *Bulbostylis pseudoperennis*, *Haumaniastrum katangense*, and *Crepidorhopalon tenuis* (Paton 1997; Leteinturier et al. 1999). Therefore, although for most cuprophytes industrial mining has dramatically negative effects on population numbers, a few taxa may actually accommodate new habitats created by mining and are expanding their range. In view of the recent revival of mining industry in Katanga, the significance of secondary habitats to conservation strategies of cuprophytes should be urgently considered. Cuprophytes, that is, plant species that mostly occur on copper-rich soil, are poorly known plant species. The ecological niche of only few cuprophytes has been characterized: *Ocimum centraliafricanum* (*Becium homblei*) (Howard-Williams 1970), and *Haumaniastrum katangense* (Malaisse & Brooks 1982; Paton & Brooks 1996).

In this study, we examine the conservation of the cuprophyte *Crepidorhopalon tenuis* (S. Moore) Fischer (Syn.: *Lindernia damblonii* P.A. Duvign.) in relation with the creation of habitats through human activities. An ecogeographical survey and the characterization of the species' ecological niche are essential to infer the conservation status of a species (IUCN 2001) and to design appropriate conservation measures. First regarded as an absolute metallophyte restricted to the Lubumbashi area (Duvigneaud & Denaeyer-De Smet 1963), *C. tenuis* actually has a broader geographic range (Fischer 1999), being endemic to the Zambesian center of endemism (White 1983). However, as most populations occur in metal-rich soil, revival of mining activities raises concerns as to the future of this and other metallophytic species.

Here, we investigate the ecological niche of *C. tenuis* in Katanga through the analysis of its abundance and distribution in relation to soil factors and plant community structure.

Specifically, we address the following questions: (1) What variability exists in ecological conditions of *C. tenuis* sites and what are the soil factors most strongly correlated with the species abundance? (2) Are *C. tenuis* population occurrences restricted to primary plant communities on naturally Cu-rich soil or do they occur favorably on secondary habitats? (3) What is the significance of recent metalliferous anthropogenic habitats for the conservation of metallophyte?

Methods

Study Area

Crepidorhopalon tenuis (Scrophulariaceae) is a small annual species, with lilac-blue flowers. It has at least 24 populations, 6 of which are localized in Burundi, Tanzania, and Zambia and 18 populations occur in the Eastern "Katangan Copper Belt" (Fischer 1995, 1999; Leteinturier et al. 1999). Among the Katangan populations, 4 populations occur in nonmetalliferous sites and 14 in metalliferous sites. As *C. tenuis* is more frequent in metalliferous sites, the species is considered an elective metallophyte (Duvigneaud 1958). *Crepidorhopalon tenuis* germinates at the beginning of the rain season in November and blossoms in March and April. It is pollinated by at least eight species of Hymenoptera, one Diptera, and one Lepidoptera (Muding & Faucon, Lubumbashi University, personal observation). The seeds are dispersed from May to June. However, its life cycle was different in permanent moist habitat. Germination, growth, flowering, and fructification continue during different seasons and several cycles are made. It has been considered as Cu–Co accumulators (Faucon et al. 2007, 2009).

Seven sites were surveyed in the East of the "Katangan Copper Belt" in the Lubumbashi area, the region with most known locations for *C. tenuis* (Leteinturier et al. 1999). Climate is subtropical (altitude around 1,300 m) and characterized by one rainy season (November to the end of March), one dry season (May to September), and two transition months (October and April). Annual rainfall is approximately 1,300 mm of which 1,200 mm fall during the rainy season. At the beginning of the dry season, the herbaceous vegetation scorches, except in the permanent wetlands. Mean annual temperature is about 20°C. Temperature is the lowest at the beginning of the dry season (15–17°C). September and October are usually the warmest months with daily maxima of about 31–33°C. Temperature amplitude between day and night is low during the rainy season but is larger during the dry season when night temperature can fall to 5°C.

Floristic Data and Soil Sample Collection

Field surveys were carried out in March 2006 and March 2007, at the end of the rainy season that corresponds to the flowering period of *C. tenuis*. Prior to data collections, each site was mapped with a Global Positioning System (GPS) and its limits were digitized in ArcView 3.2. The area covered by the site was derived directly from the Geographic Information System (GIS). In each site, two parallel transects were established that cover the topographical variation of the site and extend over its longest axis. Four habitat types can be recognized (Table 1): (1) primary metalliferous habitat (Pr) with undisturbed vegetation on natural copper outcrops; (2) secondary metalliferous habitats (S1) with substrate (often mine debris) disturbed and reworked by mining activities; (3) secondary metalliferous habitat (S2) contaminated by atmospheric fallout from an ore-smelter; (4) nonmetalliferous habitat (NM), mostly corresponding to forest clearings on lateritic crusts. One site may host a combination of different habitats (Table 1).

To estimate the population size of *C. tenuis* at the scale of the site, we first recorded the density of individuals in 1-m^2 plots located systematically (each 15 m) within transects (10 plots/transect). The mean density on 1 m^2 was reported to the total area of the site to evaluate the total *C. tenuis* population size.

To characterize plant communities associated with *C. tenuis*, 30 plots of 1 m^2 were located in each site (expect in Quartier Gécamines [QG] site) along the transects with a stratified random method: 20 plots located randomly in areas where *C. tenuis* was present and 10 plots randomly in areas where *C. tenuis* was absent. The abundance of all higher plants (including *C. tenuis*) in the 1-m^2 plots was recorded with the point-quadrat method (Spedding & Large 1957).

Species identification was based on the Flore d'Afrique Centrale (Jardin Botanique National de Belgique 1972), Flora Zambesiaca (Board of Trustees of the Kew Royal Botanic Gardens 2004), and Flora of Tropical East Africa (Kew Royal Botanic Gardens 1952–2008), completed with more recently published taxonomic literature for particular genera and species. A few species that could not be identified to the species level were well individualized as separate morphospecies.

To characterize soil conditions, samples of 100 g soil were collected in the upper soil layer, that is, in the rooting depth of *C. tenuis* (0–10 cm) in the center of each floristic relevé. Additional quadrats were sampled in the QG site, where vegetation was not studied. When a vegetation relevé was realized, compaction was measured with a penetrometer and slope, surface cover of rocks, and percentage soil surface occupied by bare soil were measured and water presence was noted. In addition to *C. tenuis* sites, for comparison, soil samples were also collected in three stands of miombo woodland, the dominant spontaneous vegetation in Katanga, where *C. tenuis* does not grow. In each stand, one composite soil sample was collected, composed of four soil cores from the upper soil layer (0–10 cm). Soil cores were collected randomly in the vicinity of the corners of a 0.25-ha square area and were thoroughly mixed.

All soil samples were air dried and sieved to 2 mm. The percentage of stones greater than 2 mm was measured. Water pH was measured with a glass electrode. Organic matter (OM) content was measured by loss on ignition at 550°C during 12 hours. Mineral elements were extracted with 1M ammonium acetate–ethylenediaminetetraacetic acid (pH 4.65) for 30 minutes (5 g dry soil in 50 mL) (Cottenie et al. 1982).

Table 1. Location and habitats description of study sites and population size of *Crepidorhopalon tenuis*.

Sites	*Habitat Type*	*Habitat Description*	*Altitude (m)*	*Co-ordinates*	*Site Area (m^2)*	*Population Size*
Niamumenda (Nm)	Pr + S1	Natural copper hill; substrate locally disturbed by mining	1,340	S11,60492° E27,29400°	15,000	10^5
Kalabi (Ka)	Pr + S1	Natural copper hill; substrate locally disturbed by mining: open pit	1,200	S10,78168° E26,74053°	12,000	10^5
Ruashi (Ru)	S1	Anthropic site: recolonization of mine deposits	1,300	S11,62645° E27,56328°	14,000	10^6
Quartier Gécamines (QG)	S2	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter moist environment	1,220	S11,70760° E27,42985°	20,000	10^5
Vallée Karavia (VK)	S2	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter, moist environment	1,230	S11,67270° E27,43091°	50,000	10^7
Baya (Ba)	NM	Natural site, forest clearing (miombo) on yellow compact clay	1,320	S11,90051° E27,45393°	600	10^3
Kyembe (Ky)	NM	Natural site, forest clearing (miombo) on lateritic gravel	1,190	S11,11269° E27,25825°	2,000	10^5

Pr, primary metalliferous habitats; S1, secondary metalliferous habitat with substrate locally disturbed by mining; S2, secondary metalliferous habitat contaminated by atmospheric fallout from ore-smelter; NM, nonmetalliferous habitat.

Supernatant was filtered and analyzed by ICP-OES (Varian Vista MPX) for Ca, Cu, Co, Fe, Mg, Mn, K, P, and Zn.

Data Analyses

Relationships with Edaphic Conditions. The variation of edaphic conditions in the sites colonized by *C. tenuis* was analyzed with a principal component analysis (PCA) based on the 15 chemical and physical soil properties measured in 200 soil samples collected in plots for vegetation analyses (soils samples collected in the QG site). All the plots were included in the analyses regardless of whether *C. tenuis* was present. Significant differences in mean edaphic factors among the four types of habitats (P, S1, S2, NM) were tested by one-way analysis of variance (ANOVA) followed by post hoc multiple comparison (Fisher's Least Significant Difference [LSD] tests). ANOVA p-value was corrected by Bonferonni adjustment ($p < 0.0033$).

We used the data collected in plots used for vegetation analyses to model the response curve of the abundance of *C. tenuis* in relation to edaphic variables. The relationships between the abundance of the species (cover estimated by the point-quadrat method) and the edaphic variables measured in the same plots were tested with a generalized additive modeling (GAM) regression (Hastie & Tibshirani 1990) with a cubic smooth spline function. All the plots were included in the analyses regardless of whether *C. tenuis* was present. These analyses were performed with Canoco 4.5 (Ter Braak & Smilauer 2002). The GAM regression has been used in many studies of species–environment relationships (e.g., Bio et al. 1998; Austin 1999; Guisan & Zimmermann 2000; Vetaas 2002). It does not assume any general shape of the response prior to the estimation (Austin & Meyers 1996). Because response data are relative abundance, a Poisson distribution was assumed with a logistic link function. The model was tested with different degrees of smoothing. The optimum degree of smoothing (i.e., giving the best p-value for the deviance-based test) was 2.

Identification of Associated Plant Communities. To identify the plant communities (com) associated with *C. tenuis*, a cluster analysis computed with MVSP 3.1 (Kovach Computing Services 2004) was run on all floristic relevés, including quadrats from which *C. tenuis* was absent (UPGMA, Bray Curtis distance, Legendre & Legendre 2003). *Crepidorhopalon tenuis* was removed from the list of species when it was present. Plant communities were defined from the resulting hierarchical tree (Bray Curtis dissimilarity level = 0.8). Species characteristic of each level of the hierarchical classification were identified with IndVal, the indicator method of Dufrene and Legendre (1997). This method calculates an indicator value (IV) for each predefined group of plots: it is an integrated measure for the relative mean abundance and the relative frequency of the species in each group. Only species that have a high mean abundance and are present in the majority of floristic samples in a group will score a high IV for that particular group. To test whether the observed IV of a species in a group was higher than expected based on a random distribution of individuals over the locations, the observed IV was compared with 999 randomly generated IVs (Dufrene & Legendre 1997).

To visualize the floristic variation of plant communities associated with *C. tenuis* and their relationships with edaphic factors, we realized a detrended correspondence analysis (DCA, with CANOCO) where part of the measured edaphic variables were added as passive variables. These edaphic variables were first selected with a randomization test in the forward selection process of a canonical analysis. Abundance data were transformed before analyses (square root of the species abundance).

To identify optimal habitat and optimal associated plant community, we tested for significant differences in mean abundance of *C. tenuis* among habitats where the species was present (S1, S2, NM, see Results) and plant communities where the species was present (four plant communities), with one-way ANOVA followed by post hoc multiple comparisons (Fisher LSD tests).

To test if *C. tenuis* is a colonizing species, we calculated the correlation between the abundance (% cover) of *C. tenuis* and the coverage percentage of perennial species. The perennial or annual character of all the species in the dataset was obtained from the Flore d'Afrique Centrale (Bamps 1973–1993) completed with personal observations. All univariate analyses were conducted on arcsin (for %) or log-transformed data for all variables. Analyses were performed with Statistica 8 (Statsoft 2008).

Results

Edaphic Conditions in the Four Habitats

The first two axes of the PCA on 15 chemical and physical soil properties explained 42% of total variation (Fig. 1). Variables best correlated with PC1 were P, Co, Cu, Zn, and Fe and variables best correlated with PC2 were rock cover, slope, and stone percentage (negative correlation) and Ca, Zn, and organic matter (positive correlation). Nonmetalliferous plots formed a distinct group with negative values on PC1 (Fig. 1). Metalliferous plots were much more variable (both within and among sites) on both axes. S2 habitat (contaminated by atmospheric fallout) had positive values on PC2. S1 and P habitats were not clearly separated.

Comparisons of mean values of soil variables among the four types of habitats are given in Table 2. As expected, the nonmetalliferous (NM) habitat had significantly smaller mean concentrations of trace metals, but also of several nutrients notably Ca, Mg, and P and a smaller percentage of organic matter than metalliferous habitats (Table 2). Within metalliferous habitats, anthropogenic habitat contaminated by atmospheric fallout (S2) differed from primary metalliferous habitats (Pr), and secondary habitats disturbed by mining (S1) with greater Fe and Zn concentrations and lower pH, and less percentage rock cover. Pr had greater Mn and K concentration and smaller Co concentration than S1.

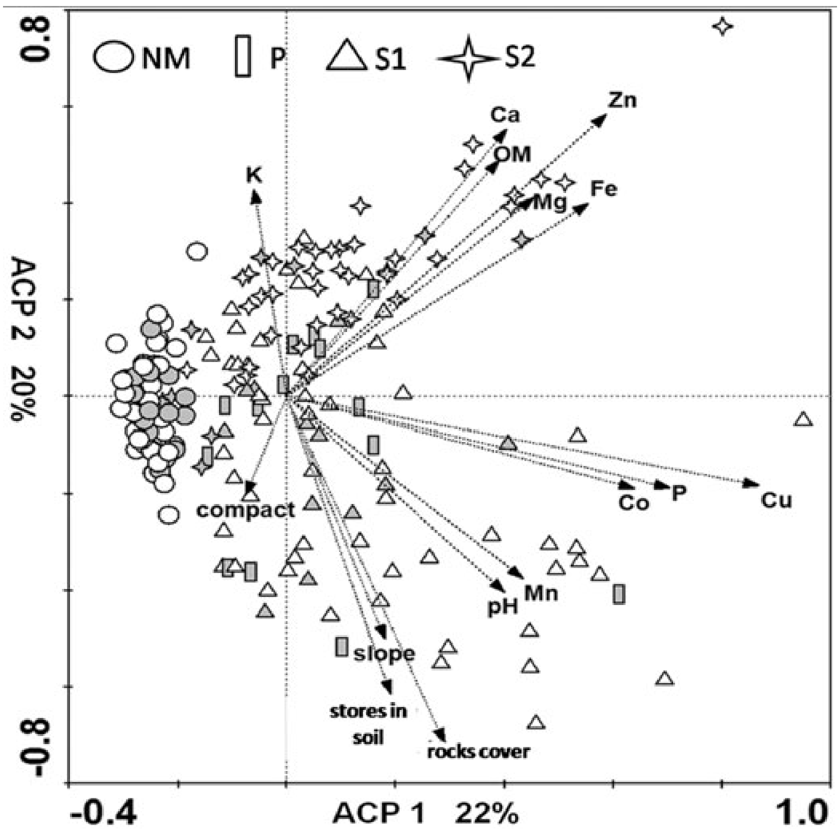

Figure 1. Principal components analysis of 15 edaphic variables in seven sites with *Crepidorhopalon tenuis* (30 plots per site: 10 without *C. tenuis* and 20 including *C. tenuis*). PC 1 accounts for 22% of variance, and PC 2 for 20%. Pr, primary metalliferous habitats; S1, secondary metalliferous habitat with substrate locally disturbed by mining; S2, secondary metalliferous habitat contamined by atmospheric fallout from ore-smelter; NM, nonmetalliferous habitat; Gray, plot without *C. tenuis* and empty with *C. tenuis*.

Table 2. Edaphic factors in four habitats present in sites of *Crepidorhopalon tenuis* (mean and error type) ($n = 7$ sites × 30 samples).

	Pr (n = 16)	*S1 (n = 72)*	*S2 (n = 50)*	*NM (n = 58)*	*Woodland Miombo*	$F_{3,192}$
Slope (°)	9.6 (2.5)^{a}	5.8 (0.9)^{a}	1.6 (0.3)^{c}	0 (0)^{c}		20.1*
Cover rocks (%)	22.1 (8.5)^{a}	27.9 (3.7)^{a}	0.0 (0)^{b}	4.6 (0.8)^{b}		21.6*
Compaction	3.1 (0.3)^{a}	3.0 (0.1)^{a}	1.6 (0.1)^{b}	2.2 (0.2)^{c}		13.9*
Stones in soil (%)	19.6 (3.1)^{a}	22.8 (1.6)^{a}	8.3 (2.2)^{b}	8.0 (1.1)^{b}		19.5*
pH	5.5 (0.1)^{a}	5.5 (0.05)^{b}	5.0 (0.04)^{c}	5.3 (0.05)^{b}	4.9 (0.2)	18.8*
% OM	9.2 (1.0)^{a}	7.7 (0.5)^{a}	9.5 (1.4)^{a}	3.9 (0.2)^{b}	7.6 (3.4)	9.5*
Fe	85.2 (9.6)^{ac}	132 (23.6)^{a}	495 (43.0)^{b}	69.0 (8.5)^{c}	90 (21)	43.9
Mn	382.5 (103.5)^{a}	735 (181.1)^{b}	17.5 (3.1)^{a}	5.9 (1.2)^{a}	17 (10)	8.5*
K	75.7 (10.1)^{a}	55.7 (3.4)^{b}	46.1 (1.8)^{b}	53.8 (6.8)^{b}	111 (83)	2.8
Mg	84 (10.4)^{ab}	96.5 (6.0)^{a}	79.2 (7.2)^{b}	24.9 (3.8)^{c}	163 (119)	29.0*
Ca	399 (75.9)^{a}	316.1 (32.4)^{a}	309 (29.9)^{a}	88.6 (18.1)^{c}	80 (76)	15.0*
P	37.9 (11.5)^{a}	68.2 (12.3)^{ab}	78.6 (8.4)^{b}	2.6 (0.2)^{c}	3 (0.9)	12.9*
Cu	2807 (1116.6)^{a}	3186 (3518)^{a}	2559 (336.7)^{a}	6.5 (0.9)^{c}	16 (6.6)	17.0*
Co	22.2 (4.1)^{a}	54.8 (7.6)^{b}	14.5 (1.5)^{ac}	0.4 (0.2)^{c}	0.7 (0.5)	21.9*
Zn	25.9 (4.7)^{a}	21.1 (2.2)^{a}	81.5 (7.9)^{b}	2.2 (0.3)^{c}	2.6 (1.7)	64.4

Edaphic variables in woodland miombo are given as reference values for normal soil in the region ($n = 3$ sites × 1 composite sample). Mineral element concentration (1M Ac EDTA, pH 4.65 in mg/kg) and penetration resistance in soil (kg/cm^2).
Superscript letters (a, b, c) indicate homogeneous groups (Fisher LSD test after a one-way ANOVA).
Analyses were conducted on arcsin (for %) or log-transformed data for all variables.
Pr, primary metalliferous habitats; S1, secondary metalliferous habitat with substrate locally disturbed by mining; S2, secondary metalliferous habitat contaminated by atmospheric fallout from ore-smelter; NM, nonmetalliferous habitat; OM, organic matter.
*Significant differences in mean edaphic factors among habitats after a Bonferonni adjustment: $p < 0.0033$.

Compared with miombo woodland soils, soils in NM sites occupied by *Crepidorhopalon tenuis* displayed smaller concentrations of Mg, K, and organic matter (Table 2). Soil miombo woodland would be much less rich in Co, Cu, and P than metalliferous soils.

Relationships Between *C. tenuis* and Habitat Edaphic Conditions

Within the habitats where *C. tenuis* was found (S1, S2, and NM; *C. tenuis* was absent from primary habitats), the ordination suggested no clear differences among quadrats where *C. tenuis* was present and quadrats where it was absent. The two groups of quadrats were intermingled in the ordination graph (Fig. 1).

However, the mean abundance of *C. tenuis* differed among the three habitats where the species was found (S1, S2, and NM) ($F_{2,116} = 68.3$; $p < 0.001$). Anthropogenic metalliferous habitat contaminated by atmospheric fallout (S2) had the greatest mean *C. tenuis* abundance (S1: mean = 11.3% cover, SD = 10.1; S2: mean = 54.1% cover, SD = 29.6) (Fisher LSD, $p < 0.01$). *Crepidorhopalon tenuis* was locally (1 m^2) more abundant in M habitats (mean = 22.7% cover, SD = 25.9) than in NM (mean = 6.8% cover, SD = 12.1).

The general additive model (GAM) demonstrated significant relationships among *C. tenuis* abundance and Cu ($F = 1.19$; $p < 0.05$), Ca ($F = 9.17$; $p < 0.01$), Fe ($F = 10.48$; $p < 0.001$) and Zn ($F = 29.7$; $p < 0.001$). The shape of all relationships was Gaussian and the optimum was 4,000–5,000 mg/kg for Cu, 500–600 mg/kg for Ca, 500–1,000 mg/kg for Fe, and 100–150 mg/kg for Zn. For all these variables, the optimum corresponded to the upper range of concentrations in our samples. For another group of variables, we found also a significant Gaussian relationship, with the optimum in the smaller range of values: Mn ($F = 3.37$; $p < 0.01$; optimum: 0–100 mg/kg); pH ($F = 5.31$; $p < 0.01$; optimum: 4–5); compaction ($F = 12.6$; $p < 0.001$; optimum: 0–1 kg/cm^2); rocks SC ($F = 17.21$; $p < 0.001$; optimum: 0–10%); and slope ($F = 5.21$; $p < 0.01$; optimum: 0–2°).

Plant Communities

A total of 94 species was found over all relevés. Cluster analyses of vegetation plots (not shown) identified five plant communities. Communities 1 and 2 were located only in nonmetalliferous sites, Ky and Ba, respectively. Communities 3, 4, and 5 were located in metalliferous sites. The physiognomic of communities 3, 4, and 5 differed (Table 3). Community 3 consisted of low grassland dominated by hemicryptophyte species and developed on disturbed stony soil. Community 4 consisted of open low grassland dominated by therophytes species developing on anthropogenic soils. Community 5 was a dense perennial steppic savanna developing on nondisturbed areas of primary copper outcrops.

The ordination analysis (DCA) mostly confirmed the interpretation based on the grouping method: groups of relevés derived from the cluster analysis were relatively well separated on the ordination diagram (Fig. 2). The two first axes of

Table 3. Characteristic species of five plant communities associated with *Crepidorhopalon tenuis* (6 sites, 30 vegetation plots per site, IndVal method level IV).

Community	*Indicator Species*	*Mean of Abundance C. tenuis (%)*	*Mean Cover of Perennial Species (%)*
1 (NM)	*Tristachya superba* (Poaceae), *Andropogon chinensis* (Poaceae), *Fabaceae* sp., *Antherotoma naudinii* (Melastomataceae), *Scleria pergracilis* (Cyperaceae), *Otiophora pycnostachys* (Rubiaceae), *Cassia mimosoïdes* (Caesalpiniaceae)	11.2 (15.5)[a]	30.6 (22.2)[a]
2 (NM)	*Hyparrhenia diplandra* (Poaceae), *Zonotriche inamoena* (Poaceae), *Panicum* sp. (Poaceae), *Vernonia perrottetii* (Asteraceae), *Crotalaria* sp. (Fabaceae), *Commelina africanum* (Commelinaceae)	2.5 (0.7)[a]	26.8 (25.8)[a]
3 (S1 + S2)	*Ascolepis metallorum* (Cyperaceae), *Eragrostis racemosa* (Poaceae) , *Diheteropogon grandiflorus* (Poaceae), *Justicia elegantula* (Acanthaceae), *Digitaria nitens* (Poaceae)	9.2 (4.5)[a]	34.9 (12.7)[a]
4 (S1 + S2)	*Rendlia altera* (Poaceae), *Crepidorhopalon tenuis* (Scrophulariaceae), *Bulbostylis pseudoperennis* (Cyperaceae), *Haumaniastrum katangense* (Lamiaceae)	28.8 (28.9)[b]	8.8 (13.0)[b]
5 (Pr)	*Heteropogon contortus* (Poaceae), *Loudetia simplex* (Poaceae), *Crotalaria peschiana* (Fabaceae), *Brachiaria serrata* (Poaceae), *Cryptosepalum maraviense* (Caesalpiniaceae), *Adenodolichos rhomboïdeus* (Fabaceae), *Tristachya bequaertii* (Poaceae), *Aeschynomene pygmaea* (Fabaceae)	0 (0)	61.0 (24.2)[c]
		($F_{3,116} = 13.6$; $p < 0.001$)	($F_{4,114} = 14.4$; $p < 0.05$)

Mean (SD) of *C. tenuis* abundance and mean (SD) cover of perennial species for each community.
Superscript letters (a, b, c) indicate significant differences between communities (Fisher LSD test following a significant one-way ANOVA, $p < 0.05$).
Pr, primary metalliferous habitats; S1, secondary metalliferous habitat with substrate disturbed by mining; S2, secondary metalliferous habitat contaminated by atmospheric fallout from ore-smelter; NM, nonmetalliferous habitat.

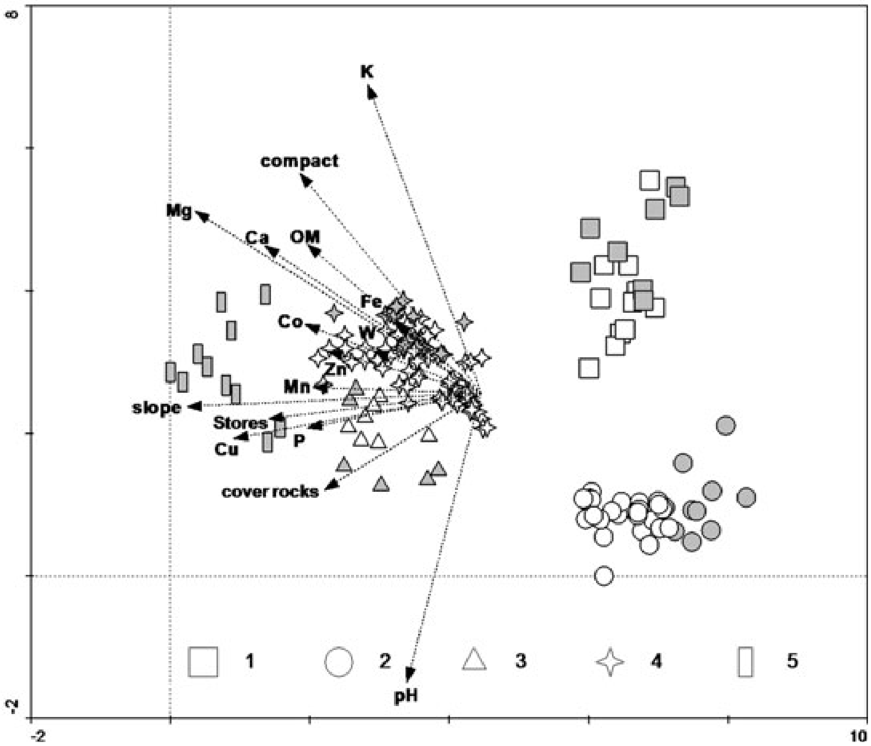

Figure 2. Detrended correspondence analysis of vegetation data collected on four metalliferous sites and two nonmetalliferous sites where *C. tenuis* is present (30 plots per site: 10 without *C. tenuis* and 20 including *C. tenuis*). Gray: plot without *C. tenuis* and empty with *C. tenuis*. Plant communities (1 to 5) were determined by cluster analysis (see Table 3 for details). Axes 1 and 2 represent 9.9% of total variation. Edaphic variables were passive in the analysis. W, presence of water in beginning dry season.

the DCA represented 10.6% of the variability in the species data (Fig. 2). The first axis was highly negatively correlated with available concentrations of all trace metals, with Mg and Mn concentrations, and with soil physical characteristics. Relevés from nonmetalliferous communities were clearly separated from relevés from the other communities along this axis. Within metalliferous habitat, there was also a separation along axis 1 among relevés from community 5 (Pr habitats) and relevés from communities 3 (S1 + S2 habitats) and 4 (S1 + S2 habitats). In contrast, there was no separation of the two nonmetalliferous communities (communities 1 and 2) along axis 1. Relevés from these two nonmetalliferous communities were clearly discriminated along axis 2, which was highly positively correlated with K concentration and negatively correlated with pH.

The pattern of arrangement of samples in the DCA of vegetation data differed markedly from the patterns shown in PCA investigated for environmental parameters. Contrary to PCA, DCA Pr samples (com 5) differed from other samples. Difference between NM (com 1 and 2) and M (com 3, 4, and 5) samples was more pronounced in DCA analyses (Fig. 2).

Crepidorhopalon tenuis was absent from the metalliferous community 5, which was mainly composed of perennial grasses and shrubby chamaephytes species (Table 3). The abundance of *C. tenuis* was significantly different among the four plant communities where the species was found ($F_{3,116} = 13.16$; $p < 0.001$) (Table 3). Following pair-wise comparisons, community 4 presented a significantly different (greater) mean abundance than the three other communities, which were not different from each other. Community 4 that had the greater mean *C. tenuis* abundance was mainly composed of annual species common on reworked soils in mining areas and contaminated soils around mining industries (Table 3). Mean cover of perennial species was significantly different among communities (Table 2, $F_{4,114} = 14.4$; $p < 0.05$) and was inversely correlated with the abundance of *C. tenuis* (Fig. 3, partial regression coefficient: $r = -0.47$, $p < 0.01$).

Discussion

Crepidorhopalon tenuis, a Cuprophile Species?

Our results highlight the wide ecological amplitude of *C. tenuis* with respect to soil conditions. The high variation of trace metal concentration in soils of *C. tenuis* habitat confirms that *C. tenuis* is a pseudometallophyte. However, the species abundance varies dramatically in relation to soil chemistry. A remarkable result is that populations on metalliferous sites exhibit greater local abundance compared with normal soil. Precisely, the response curve of the species indicated that *C. tenuis* meets its ecological optimum on soils with the greatest copper concentrations.

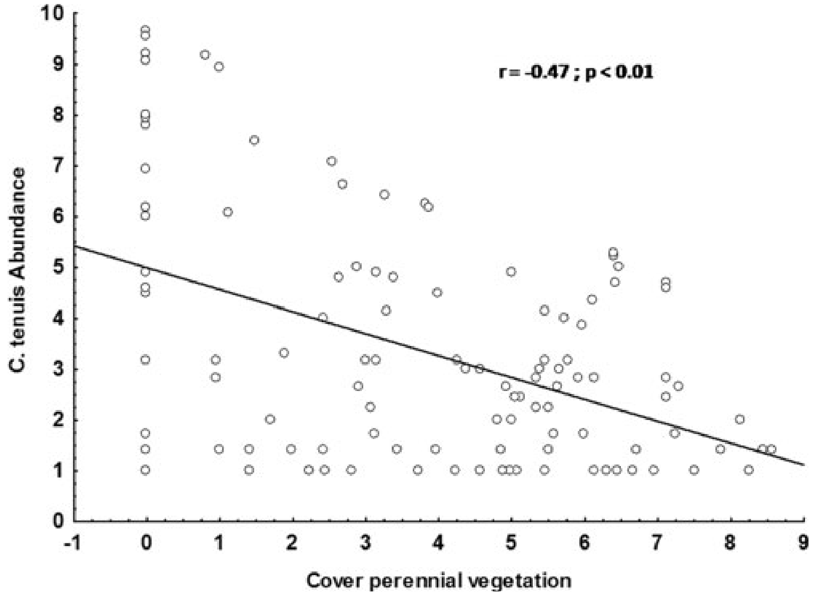

Figure 3. Relationship between *C. tenuis* abundance and cover of perennial vegetation. $n = 100$ (1×1–m plots from six sites), square root transformation of data.

In a previous study, we demonstrated that *C. tenuis* plant size was strongly correlated with the concentration of Co and Cu in the soil (Faucon et al. 2009). Together, these results strongly suggest that *C. tenuis* is genuinely cuprophilous. Could the lower abundance of *C. tenuis* on NM habitats be the consequence of the species having large Cu requirements? Experimental cultivation is needed to answer this question. There have been few attempts to cultivate cuprophytes with varying Cu concentrations in the substrate. There was no clear evidence for elevated Cu requirements neither for pseudometallophytes (Hogan & Rauser 1979; Schat & Ten Bookum 1992; Macnair et al. 1993; Lou et al. 2004) or Cu specialists (Baker et al. 1983). Only for the so-called *copper mosses* was there evidence for elevated copper requirements and growth stimulation in contaminated substrates (Shaw 1993, 1994).

The positive correlation between soil Cu concentrations and *C. tenuis* abundance might also result from the indirect effect of relaxed negative biotic interactions. Fungi, for instance, are highly susceptible to copper toxicity. Lower occurrence of pathogenic fungi in copper soil may conceivably relax selection pressure of defenses against pathogenic fungi in cuprophytes. Some cuprophytes, like *Haumaniastrum katangense*, are indeed known to be very susceptible to pathogenic fungi (Brooks & Malaisse 1985; Paton & Brooks 1996). Plant competition on metalliferous soils can also be less stringent compared with nonmetalliferous soils (McNeilly 1968).

Low nutrient in the soil is often considered as one of the most stringent selective forces for plants growing in metalliferous sites, most notably serpentine (Vlamy & Jenny 1948; Proctor & Woodell 1975; Brooks 1987). Interestingly, in this study, Cu-sites actually had greater concentrations of most nutrients in the soil. Indeed, in comparison with woodland miombo and M habitats, NM habitats of *C. tenuis* are oligotrophic habitats and have soil parent material (lateritic crust or compact yellow clay) characterized by low nutrient content at this early formation stage (Sys & Schmitz 1959).

Crepidorhopalon tenuis's Expansion due to Mining Disturbance

Crepidorhopalon tenuis has its ecological optimum in metalliferous habitats especially in areas where substrate was recently disturbed due to mining activities, with large Cu content, and where perennial species have not yet established. *Crepidorhopalon tenuis* has its greatest abundance in the open, therophytic grassland with *Rendlia altera* and *Haumaniastrum katangense* (4). The characteristic species of this community are pioneers of disturbed soil and are able to grow on highly Cu-contaminated sites (Leteinturier et al. 1999).

Crepidorhopalon tenuis is absent in primary vegetation on natural metalliferous soil, that is, the steppic savanna community with *Loudetia simplex* and *Heteropogon contortus* (5). This is not due to dispersal limitation because this community is often situated in close proximity with the other plant communities where *C. tenuis* is present. This community consists of a relatively dense herbaceous layer of perennial grass species. A permanent litter is covering the soil. We believe that the tiny annual *C. tenuis* is excluded by competition for light and lack of regeneration niche in the primary steppic community. Relaxed competition appears to explain the greater abundance of *C. tenuis* on substrate recently disturbed. However, our data do not allow us to rule out the possibility that some other factor is limiting establishment of the annual in the soil of the primary steppic community.

Our results suggest that, prior to the development of industrial mining in the beginning of the 20th century, *C. tenuis* was an extremely rare species, mostly restricted to scattered patches of bare soil on lateritic crusts. In primary metalliferous sites, it may have been restricted to small patches of naturally disturbed soils for instance in steep slopes where soil is unstable. As industrial mining developed, large areas of open, metalliferous soil were created, which were open to colonization by therophytic species. Experimental investigations are needed to investigate whether populations of *C. tenuis* on nonmetalliferous soil have some degree of constitutive metal tolerance. It is intriguing that *C. tenuis* is so rare in the open plant communities that exist on lateritic crusts in upper Katanga, in spite of relaxed competition. This, we believe, points to a possible role of pathogens to explain the greater abundance of *C. tenuis* on copper soil. Experiments are necessary to test this hypothesis.

Thus, although mining activities are the main causes of metallophyte extinction, *C. tenuis* stands out as possible exception. Malaisse and Brooks (1982) already emphasized the surprising ecological versatility of another annual cuprophyte, *Haumaniastrum katangense*. This can be explained by the ecology of the species: its colonizing character and its tolerance of a broad range of Cu concentrations. The pollution originating from the Gecamine ore-smelter, build in 1953 in Lubumbashi, gives a good example of such recent colonization of a metalliferous habitat of anthropogenic origin by *C. tenuis*. Atmospheric fallout from this ore-smelter resulted in a 15-km-large contaminated area, with a decreasing East-West copper concentration gradient in the direction of the prevailing wind (Mbenza et al. 1989). This widespread contamination was accompanied by a demographic boom of *C. tenuis* in the area. Along the 15-km gradient, the variability of habitats was already quite high (e.g., soil types, humidity gradient, and topography). The contamination added a variability of metal concentrations, most notably in Cu and Zn. *Crepidorhopalon tenuis* had the opportunity to colonize the most convenient portion of these environmental and edaphic gradients according to its ecological optimum. Mining activities have created large areas of barren toxic soil, a habitat that is hardly represented on natural copper outcrops.

Consequences for Conservation of *C. tenuis*

On a worldwide scale, mining is the first cause of habitat destruction of most metallophytes (Whiting et al. 2004). *Crepidorhopalon tenuis* used to be a rare species in Katanga. However, because of its surprising ability to adapt easily to habitats perturbed by mining activities, it has steadily increased in range and abundance. It is thus not threatened by the strong revival of mining activities in Katanga. Recent metalliferous anthropogenic habitats actually have conservation value for *C. tenuis*. They currently host the largest populations of *C. tenuis* in Katanga. Other cuprophytes in Katanga may have similar ecological history, especially annual colonizing species (*Haumaniastrum katangense, Bulbostylis* div. sp.) (Malaisse & Brooks 1982). These few species may prove extremely useful to reclaim the huge areas of barren soil that the current revival of mining industry in Katanga may create. However, *C. tenuis* requires specific soil conditions, that is, high concentrations of Cu and organic matter. Therefore, low-grade deposits may not be suitable and organic matter amendments may be required.

Conclusion

Crepidorhopalon tenuis is one of the very few documented cases of a plant species with greater abundance and population size in copper-rich soil. Such restricted edaphic specialists might conceivably have low adaptability to changing environments. However, our results suggest that this is not necessarily true for some species. *Crepidorhopalon tenuis* offers a striking example of recent, rapid range expansion and ecological versatility in response to large-scale human disturbance through mining activities. This example illustrates that vulnerability status of some species of Katanga copper flora may rapidly change as landscape is modified by mining industry.

Our data also demonstrate that man-made secondary habitats should not be ignored for the conservation of rare species when these are adapted to extreme environments (Krüger et al. 2002; Batty 2005; Bröring & Wiegleb 2005; Brock et al. 2007; Bizoux et al. 2008; Esfeld et al. 2008). *Crepidorhopalon tenuis* and other cuprophytes with similar range expansion are also interesting materials to investigate the evolutionary ecology of copper tolerance. In particular, a phylogeographic approach using neutral markers is necessary to test which populations (either metallicolous or not) have served as the colonization source of recently founded populations.

Implications for Practice

- New metalliferous habitats created by mining industry may play a role in the conservation of *Crepidorhopalon tenuis*. These habitats that have no economic interest to the mineral industry could be utilized in conservation strategies for rare metallophytic species. Introduction and ex situ conservation could be attempted in these habitats, providing a source material for habitat restoration.
- In open habitats created by mining disturbance, *C. tenuis* peaks in abundance in a rather specific range of soil conditions, including high Cu concentration, and high organic matter content. Selection of suitable sites for conservation must consider the niche requirement of the species. Low-grade deposits may not be suitable and organic matter amendments may be required for successful establishment.
- Annual metallophytes with high ecological plasticity may prove interesting materials for restoration of recent metalliferous habitats and ecosystem reconstruction in the huge areas of barren soil that are being created by mining industry in Katanga and elsewhere in the world.

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