Copper tolerance and accumulation in two cuprophytes of South Central Africa :Crepidorhopalon perennis and C. tenuis (Linderniaceae)

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Michel-Pierre Faucon[a,b,*], François Chipeng[c,d], Nathalie Verbruggen[d], Grégory Mahy[e], Gilles Colinet[f], Mylor Shutcha[c], Olivier Pourret [b], Pierre Meerts[a]

[a] Laboratoire d'Ecologie végétale et Biogéochimie, Université Libre de Bruxelles, Boulevard du Triomphe. B-1050 Bruxelles, Belgium.

[b] Hydrogéochimie Interactions Sol-Environnement (HydrISE). Institut Polytechnique LaSalle Beauvais (IGAL-ISAB). 19 rue Pierre Waguet – BP 30313-F-60026 Beauvais, France

[c] Université de Lubumbashi. Faculté des Sciences Agronomiques, Lubumbashi, Democratic Republic of Congo.

[d] Laboratoire de Physiologie et de Génétique moléculaire des Plantes, Université Libre de Bruxelles, Campus Plaine – CP242, Bld. du Triomphe, B-1050 Brussels, Belgium;

[e] Laboratory of Ecology. Université de Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, B-5030 Gembloux, Belgium.

[f] Laboratory of Geopedology. Université de Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, B-5030 Gembloux, Belgium.

*corresponding author:

Michel-Pierre Faucon

E-mail address: michel-pierre.faucon@lasalle-beauvais.fr

ABSTRACT

Crepidorhopalon perennis is an endemic metallophyte restricted to only one site on Cu-rich soils in Katanga. *Crepidorhopalon tenuis* has a broader niche, from normal to Cu-rich soils, but has high affinity for cupriferous habitats. Both plants have been considered as Cu-Co accumulators. Cu tolerance and accumulation of *C. tenuis* were studied in axenic conditions *in vitro* in four metallicolous populations and one non-metallicolous population whereas for *C. perennis* only one population was investigated. Results showed a Cu tolerance of both *C. tenuis* and *C. perennis*. Variation of tolerance among metallicolous and non-metallicolous populations was also observed. The addition of Cu enhanced the growth of some metallicolous populations under sterile conditions, hence confirming the high needs in copper of metallicolous populations. This could represent a cost of tolerance which would explain the high affinity of species for cupriferous habitats. On the other hand, *Crepidorhopalon perennis* did not show the same features. Its restricted distribution may be explained by a cost of Cu tolerance. *Crepidorhopalon perennis* and *C. tenuis* are not Cu hyperaccumulators and seem to behave rather like excluder species.

Key words: copper tolerance, trace metals, copper accumulation, cost of tolerance

Introduction

Soil contamination by trace metals is a major environmental issue throughout the world (Alloway, 1995; Smith and Huyck, 1999; Baize and Tercé, 2002) in the last century as a consequence of the intensification of mining and mineral processing, which dispersed toxic trace metals leading to large contaminated areas. In this context, new types of metalliferous habitats, which did not exist or were rare before the industrial revolution, appeared on the Earth (Allen and Sheppard, 1971; Ginocchio et al., 2002). The soils of such habitats have concentrations of trace metals up to 1000 times higher than the "normal" soils, which hence gives them a high phytotoxicity (Ernst, 1974; Baker et al., 2000; Reeves and Baker, 2000). Only species that have adapted to these extreme soil conditions can grow in such contaminated habitats. The trace metals tolerance of plants is nowadays a research topic dealing mainly with the processes of adaptation to very restrictive environmental factors (Antonovics et al., 1971). Copper and zinc tolerance has been largely studied in some pseudometallophytes, hence contributing to a better understanding in terms of genetics and evolution of trace metals tolerance of such plants. Several studies on copper tolerance have highlighted the intensity of selection due to toxicity of trace metals (McNeilly and Bradshaw, 1968; Wu et al., 1975), heritability of tolerance (Macnair, 1981, 1983; Schat and Ten Bookum, 1992; Schat et al., 1993), cost of copper tolerance (Macnair et al., 2000), reproductive isolation (Macnair and Christie, 1983; Macnair and Gardner, 1998), and speciation (Macnair and Gardner, 1998).

Copper tolerance is little known particularly in species that grow only or show a frequency and/or high abundance on copper soils (Brooks and Malaisse, 1990; Harper et al. 1997; 1998), i.e. the cuprophytes (Duvigneaud and Denaeyer-De Smet, 1963; Ernst, 1974, 1990). Tolerance to extreme Cu concentrations in soils (1-50 g kg^{-1}) was demonstrated experimentally only for a few cuprophytes such as *Haumaniastrum katangense*, *H. robertii*, *Aeolanthus biformifolius* (Lamiaceae) (Morrison et al., 1979; Chipeng et al., 2010; Peng et al., in press); *Mimulus guttatus* (Scrophulariaceae) (Allen and Sheppard, 1971; Macnair, 1983); *Silene cobalticola* (Caryophyllaceae) (Baker et al., 1983) and *Elsholtzia haichowensis* (Lou et al., 2004). The limited number of experimental studies in these cuprophytes, under the intrinsic value of biological material, may be partly explained by difficulty of cultivation in soils with low Cu concentrations (Malaisse and Brooks, 1982; Paton and Brooks, 1996). In fact, cuprophytes are known almost exclusively on copper soils of southern central Africa (Duvigneaud and Denaeyer-De Smet, 1963; Brooks and Malaisse, 1985). Among these, *Crepidorhopalon tenuis* (S. Moore) Eb. Fisch. (syn.: *Lindernia damblonii* P.A. Duvign. and *L. tenuis* S. Moore) (Linderniaceae) grows on soils with a wide range of Cu concentrations, including occasionally non-metalliferous soils (Faucon et al., 2009, 2011a). In contrast, *Crepidorhopalon perennis* (P.A. Duvign.) Eb. Fisch. (syn.: *Lindernia perennis* (P.A. Duvign.) is endemic to a single metalliferous site (Faucon et al., 2010) and occupies a narrow niche, growing on soils with extremely high concentrations of Cu and Co (Faucon et al., 2011a; Faucon et al., in press). In this context, the *C. perennis*-*C. tenuis* species pair appears to be a very promising model to investigate the relationship between the degree of tolerance of plant populations, copper bioavalaible content of sites and the ecological isolation of an endemic species.

In *Crepidorholon perennis* and *C. tenuis,* genuine cuprophily was assessed by examining the relationship between the concentration of copper in soil and plant biomass (Faucon et al., 2009, 2011a). It appeared that copper-tolerant populations showed lower fitness on non-metalliferous soil than non-tolerant populations and *vice versa* on copper soil. It seems that needs in essential metals are higher for tolerant populations than for non-tolerant plants (Macnair et al., 2000). Strict endemism on copper-rich soils could be explained by a "cost of copper tolerance" (Harper et al., 1997; Macnair et al., 2000; Tadros, 1957; Kazakou et al., 2008). However, the existence of a cost of copper tolerance was demonstrated only in *Mimulus guttatus* and remains highly controversial. Another hypothesis is that cuprophytes are very sensitive to pathogens of soil (Malaisse and Brooks, 1982; Paton and Brooks, 1996; Chipeng et al., 2010).

Thirty-two cuprophytes apparently have the capacity to accumulate copper in their top growth at concentrations above the threshold of Cu hyperaccumulation (>1000 mg kg^{-1}, Baker and Brooks, 1989) (Reeves and Baker, 2000; Reeves, 2006). Nevertheless, There has been extensive discussion of the reliability of earlier data on Cu and Co accumulation, the wide variation of these element concentrations in a number of the African species, and the effects of contamination by dusts of secondary minerals of elements such as Cu, e.g. in Reeves and Baker (2000). The validity of these concerns was demonstrated in the work of Faucon et al. (2007). Macnair (2000) states that Cu hyperaccumulation has not yet been observed in culture, but Küpper et al. (2009) found that *Crassula helmsii* could accumulate more 9000 mg kg^{-1} in its shoot at 10 μM (=0.6 ppm) Cu^{2+} in the nutrient solution. A large inter and intra-specific variation of foliar concentrations of Cu was observed in the supposed hyperaccumulators (Malaisse and Grégoire, 1978; Brooks et al., 1987; Malaisse et al., 1979, 1994, 1999; Paton and Brooks, 1996; Faucon et al., 2007; Faucon et al., 2009). In *Crepidorhopalon perennis* and *C. tenuis*, the range of Cu concentrations in leaf was respectively, 500 to 1500 mg kg^{-1} and 100 to 980 mg kg^{-1} (Morrison, 1980; Faucon et al., 2007; Faucon et al., 2009).

In this paper, we examine the tolerance and accumulation of copper in controlled conditions *in vitro* of four metallicolous populations and one non-metallicollous of *C. tenuis* and in the single population of *C. perennis*. To our knowledge, this is the first study comparing the Cu tolerance among metallicolous and non-metallicolous in cuprophytes from Katanga (Democratic Republic of Congo). We specifically address the following questions:(i) is there inter-populational variation in the ability to tolerate Cu in *C. tenuis*? (ii) do both cuprophytes show an increase in their performance in response to high Cu concentrations? (iii) can the restricted endemism of *C. perennis* be explained by a cost of tolerance? and (iv) are *C. perennis* et *C. tenuis* guenine Cu hyperaccumulators and is there any inter-populational variation of the ability to accumulate Cu in *C. tenuis*?

Material and methods

Site harvest and plant material

Seeds were harvested in six populations in Upper-Katanga (Dem. Rep. of Congo) in April 2008 (Table 1) and a pool of representative seeds of the morphological variability of each population was established. Seeds of *Crepidorhopalon perennis* (CP) were collected in its single population located at the Mine de l'Etoile, 10 km northeast of Lubumbashi. This endemic cuprophyte is a pioneer species of mining debris or reworked mining substrates and grows on soils having concentrations of Cu higher than the habitats occupied by the populations of *C. tenuis* (CTC) (range of Cu, for CP = 2161-55300 mg kg^{-1}, CTC=201-12557 mg kg^{-1}) (Faucon et al. 2009, 2011a). *Crepidorhopalon tenuis* has a much broader geographical range, sometimes occurring on nonmetalliferous soils (Brooks and Malaisse, 1985; Fischer, 1999). *Crepidorhopalon tenuis* has its ecological optimum in Cu and Co rich soils contaminated by metallic atmospheric fallout from ore-smelter or substrate (often mine debris) disturbed and reworked by mining activities (Faucon et al., 2011a). The non-metallicolous population considered in this study is one of the few known in the plant communities of woodland clearings (miombo) on laterite soil in Katanga.

Method of culture

In vitro culture was performed in a growth chamber at 20°C on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). MS medium contained EDTA that can reduce bioavailability of copper (Christensen et al., 2008). Seeds were immersed in 70% ethanol for 5 min, followed by a 10 min soak in solution of 5% bleach and 0.1% SDS (sodium dodecyl sulfate). After three successive washes with sterile ultrapure water, seeds (approximately 30 per dish) were sown on 10 mL of MS spiked with $CuSO_4$ at a concentration of 60 µmol L^{-1} (this concentration was chosen based on the results of Lequeux et al. (2010) and Chipeng et al. (2010). A control test (no added $CuSO_4$) at 0.1 µmol L^{-1} $CuSO_4$ was also processed in parallel. After one month of incubation, 12 individuals per population were transplanted into pots of 50 mL of MS, six without added $CuSO_4$ and six containing 60 µmol L^{-1} $CuSO_4$ (n total individuals = 72). After eight weeks, the dry mass of shoot plants was measured. The biomass was used as a rough estimate of fitness because it was correlated with the number of seeds ($r = 0.78$, $n = 123$ ind. x three fruits, $P < 0.05$).

Mineral analysis

After eight weeks of pot culture, plants were harvested, washed with 1% Alconox solution (Alconox Inc.) during 2 min, rinsed with demineralised water and then dried at 60°C for 48 h (Faucon et al., 2007). The dried samples were then mineralised and solubilised in a Tecator digestor using a mixture of nitric and perchloric acid (1:1). Cu concentration was measured by flame atomic absorption spectrometry FAAS (Varian 220).

Statistical analysis

ANOVA (analysis of variance) provided by Statistica 8 (Statsoft 2008) was used for the statistical analysis. For the biomass analysis, ANOVA with two crossed factors: « treatments » and « populations » (fixed factors) was used. For the analysis of the variation of Cu concentration in plants one way ANOVA was performed. Significant differences in mean biomass and Cu concentration among populations and treatments were tested by post-hoc multiple comparison (Fisher's Least Significant Difference).

Results

Biomass variation among populations of C. tenuis in response to Cu

ANOVA showed significant effects of populations and Cu concentrations as well as for population*treatment (Table 2). The response to copper varied greatly between populations. Growth was better at 60 µmol L^{-1} of $CuSO_4$ for metallicolous populations VK, Ru, Nm and QG. On the contrary, the best growth was found in the medium without added $CuSO_4$ for the non-metallicolous population Ky. At 60 µmol L^{-1} of $CuSO_4$, Ky had half that of the control ($F_{1, 47}$ = 13.25; P>0.001) (Figure 1). In addition, chlorosis was present for 5 individuals of Ky on the $CuSO_4$ enriched medium. No significant differences were observed between metallicolous populations when growing in the medium without added $CuSO_4$. At 60 µmol L^{-1} of $CuSO_4$, significant difference of biomass existed between populations ($F_{5, 21}$ = 8.8; P>0.001) (Figure 1). Biomass of populations changed depending on the Cu concentration in the medium. When medium without added $CuSO_4$ was used, VK>Ky>Nm>QG>Ru whereas on $CuSO_4$ rich medium, Ru>Nm>VK>QG>Ky. An unexpected result was the large decrease of biomass in the non-metallicolous population Ky grown on $CuSO_4$ rich medium. In contrast, three out of four metallicolous populations showed a significant increase in biomass in response to Cu (Figure 1).

Reaction norms of biomass in response to Cu in C. perennis

Crepidorhopalon perennis did not show any significant difference in biomass on medium without added $CuSO_4$ (mean = 13.8 mg, SD ±9.8) and $CuSO_4$ enriched medium (mean = 26.2 mg, SD ±6.3). On medium without added $CuSO_4$, *C. perennis* did not show any difference in biomass compared to the non-metallicolous population of *C. tenuis* Ky (mean = 27 mg, SD ±20.5) ($F_{5, 26}$ = 2.1; ns).

Variation of Cu concentration in shoot

A significant difference in Cu concentration in plant shoots was shown among populations ($F_{5, 17}$ = 7.8 (P<0.001)) (Figure 2). The non-metallicolous population Ky showed the highest value (mean = 115 µg mg^{-1}, SD ±17.6), followed by Ru (mean = 80 µg mg^{-1}, SD ±34) (Figure 2) whereas *C. perennis* showed Cu concentration below 40 µg mg^{-1}.

Discussion

Between population variation of copper tolerance

Crepidorhopalon perennis and all populations of *C. tenuis* from metal rich soil grew as well or better in the $CuSO_4$ enriched medium compared to the control treatment, indicating that they are highly tolerant to copper. In the same experimental conditions, growth of non tolerant species was negatively affected at concentrations as low as 5 µmol L^{-1} of $CuSO_4$ e.g. in *Nicotiana plumbaginifolia* (Chipeng et al., 2010) and *Arabidopsis thaliana* (Lequeux et al., 2010). Conversely, the population from normal soil (Kyembe) is significantly less tolerant, and strongly affected by 60 µmol L^{-1} of $CuSO_4$. This suggests that *C. tenuis* is not constitutively tolerant to copper. Instead, the broad ecological amplitude of the species in Katanga may be due to its genetic variation of copper tolerance. The results suggest that metal tolerance in population from metal-rich soil has evolved in response to natural selection. This pattern of variation has been found in a number of pseudometallophytes, in which copper-tolerant ecotypes have been reported (Gregory and Bradshaw, 1965; Antonovics et al., 1971; Kruckeberg and Wu, 1992; Gonnelli et al., 2001; Ginocchio et al., 2002; Nicholls and McNeilly, 1985; Nordal et al., 1999; Macnair et al., 2000). Our results represent the first confirmation of the existence of such ecotypes in metallophytes from Katanga. However, local adaptation remains to be formally confirmed by reciprocal transplantation.

However, variation in tolerance among populations from metal-rich soil does not appear to be correlated to copper concentration in their native soil (Table 3). Thus, Ru population, with the highest degree of tolerance, originates from the site with the lowest Cu concentration in the soil. However, copper bioavailability and toxicity is strongly influenced by physical and chemical properties of soil, including pH and Mn concentration (e.g. Faucon et al., 2009, 2011b). Therefore, other elements in the soil may mitigate Cu toxicity and relax selection pressure for enhanced tolerance. Other factors can also explain variation in tolerance among populations, including population age (Wu et al., 1975). *Crepidorhopalon tenuis* appears to have strongly expanded its range in Katanga in response to mining activities and many populations may be of relatively recent origin and not yet in equilibrium with local soil conditions.

Cost of copper tolerance and growth stimulation

An interesting result is the enhanced growth at 60 µmol L^{-1} of $CuSO_4$ compared to the control, which was observed in 4 out of 5 populations from metal-rich soil. This finding is in agreement with the field observations indicating that plant size and population density are much larger on metal rich soil compared to normal soil (Faucon et al., 2009, 2011a). Because the plants studied here have been grown in axenic conditions, a protective effect against pathogens cannot be invoked, which indicates that *C. tenuis* and *C. perennis* actually have elevated requirements of Cu. Growth stimulation by elevated concentrations of Cu has only rarely been reported, for example in the copper-moss *Scopelophila cataractae* (Shaw, 1994), the Katangan cuprophyte (*Haumaniastrum katangense* (Chipeng et al., 2010) and a couple of *Elsholtzia* species in China (Lou et al., 2004; Jiang et al., 2008).

These results can also be interpreted as evidences for cost of metal tolerance, i.e. on "normal" medium, performance of Cu-tolerant populations is lower than the non-tolerant population in *C. tenuis*. Such cost has only been demonstrated in experimental conditions in few species, e.g. *Mimulus guttatus* for the Cu tolerance (Allen and Sheppard, 1971; Macnair et al., 2000). Other works failed to reveal such fitness costs (e.g. Nicholls and McNeilly, 1985; Dechamps et al., 2006).

Crepidorhopalon perennis, an absolute metallophyte

Absolute metallophytes, i.e. species that are currently known only from metal rich soil, may not be able to grow on normal soil (Tadros, 1957). Based on this assumption, the elevated cost of tolerance would account for the restricted niche. The cost of tolerance means that metal-tolerant populations show lower fitness on non-metalliferous soil than non-tolerant populations and *vice versa* on copper soil. It seems that essential needs in metals are more important for tolerant populations than for non-tolerant plants (Macnair et al., 2000). Our failure to cultivate *C. perennis* on normal soil, in spite of repeated trials, indicates that such cost might indeed be involved (Faucon, 2009). However, in this work in axenic conditions, *C. perennis* did not grow significantly less well in normal soil. This result does not support the hypothesis of a high cost of tolerance in this species and hence it should be taken with caution. Firstly, the sample size was relatively low (n= 6 individuals per population and per treatment); secondly, there was only one contaminated treatment in this study and it is not impossible that the physiological optimum of *C. perennis* is actually higher than the concentration of Cu (60 µmol L^{-1}). Furthermore, apart from copper, tolerance of other metals might be involved, most notably of cobalt, which is present at high concentration at the Mine de l'Etoile, the native site of *C. perennis*. Limited dispersal ability is unlikely, as newly formed malachite deposits at the Mine de l'Etoile are readily colonised by this species. *Crepidorhopalon* species have tiny seeds, which are probably easily transported by the wind. Finally, the narrow realised niche may be related to limiting biotic factors. Cuprophytes appear specifically to be susceptible to soil pathogenic fungi (Malaisse and Brooks, 1982; Paton and Brooks, 1996; Chipeng et al., 2010), which may be explained by the relaxed pressure of pathogenic fungi on metal-rich soil (Tadros, 1957).

Copper accumulation or exclusion?

Crepidorhopalon perennis and *C. tenuis* have been reported as Cu/Co hyperaccumulators (Duvigneaud, 1958; Malaisse and Grégoire, 1978; Brooks et al., 1987). In this study, the copper concentrations in shoot were always below the conventional hyperaccumulation threshold (1000 mg kg^{-1}). This result is in agreement with other studies in which cuprophytes did not show hyperaccumulation when cultivated in controlled conditions (Morrison et al., 1979, 1981; Chipeng et al., 2010; Peng et al., in press). They suggest that these species are excluders rather than hyperaccumulators. The high foliar concentrations of Cu found in leaves collected in the field (Duvigneaud, 1958; Malaisse and Grégoire, 1978; Brooks et al., 1987) may in part be due to surface contamination by dust (Faucon et al., 2007). However, foliar concentrations in this study are much lower than those found in carefully cleaned samples on the field (Faucon et al., 2007, 2009) (Table 3), which indicates that copper accumulation may be expressed only under specific conditions that are difficult to mimic in cultivation as in this study. Variation of copper concentrations in shoots of these species should be studied in controlled conditions with Cu contaminated substrate considering physical conditions and fertility relatives of soils where these species grow.

We also have shown that competition with Co and Mn strongly influences the patterns of Cu accumulation (Faucon et al., 2009), interactions with microorganisms might also be involved

(Fomina et al., 2005; Toler et al., 2005) and explain the absence of high Cu concentrations in shoot in these culture conditions. The pH is likely to be the most important physicochemical parameter controlling metal fractionation and mobility. Even though the stability of colloids (i.e. either organic or inorganic, which are microscopic metals adsorbent phases and therefore govern, via their mobilization in soils, their fate and/or bioavailability) according to pH is well documented, few studies were dedicated to the understanding of colloids and associated metal release from soils and water with regard to pH change and thus mobility/bioavailability leading to accumulation in plants (e.g. Pourret et al., 2010). In the future, it would be of interest to examine concentration of bioavailable Cu in relation with Cu accumulation in plants to understand the high Cu concentrations and their variation in *C. tenuis* and *C. perennis* observed *in natura* (Table 3).

Even though copper concentrations in leaves are low, they appear to be related to tolerance; the non tolerant population of *C. tenuis* (Kyembe) has the highest accumulation in leaves. This may indicate that elevated tolerance in populations from metal-rich soils is achieved through enhanced capacity to restrict Cu uptake and/or translocation to shoot. Similar results have been obtained in copper tolerant ecotypes of other species (Kruckeberg and Wu, 1992; Schat et al., 1993; Ouzounidou et al., 1994; Gonnelli et al., 2001; Weng et al., 2005, but see Macnair, 1981)

Conclusion

Crepidorhopalon perennis and *C. tenuis*, two species with high affinity for Cu-rich soil in Katanga, have tolerance to Cu, which is probably associated to a copper exclusion strategy. The pattern of variation in tolerance in *C. tenuis*, suggests that populations from metal rich soil have been subjected to directional selection for increased tolerance. Based on experimental data, we can conclude that *C. tenuis* and *C. perennis*, together with *Haumaniastrum katangense* can be considered as promising study models for the evolution of copper tolerance in the flora of SC Africa.

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Table 1 Location and habitats description of studied populations. CP = *Crepidorhopalon perennis*; CTC = *C. tenuis* from copper soil and CTN = *C. tenuis* from normal soil.

Table 2 Biomass variation of *C. tenuis* populations (four metallicolous populations and one non-metallicolous) and single population of *C. perennis on in vitro* culture with two treatments (0 and 60 µmol L^{-1} of $CuSO_4$)

Table 3 Copper and cobalt levels of plants and soils *in natura* in *Crepidorhopalon tenuis* from normal soil (CTN), *C. tenuis* from copper soils (CTC) and *C. perennis* (CP) (Faucon et al. 2009) (mean and min-max). Cu and Co concentrations in soil with ammonium acetate -EDTA 1M (pH 4.65) extraction.

Table 1

Sites	Type	Habitat description	Altitude (m)	Co-ordinates
Etoile (E)	CP	Natural copper hill; substrate locally disturbed by mining	1280	S11,63562° E27,58448°
Niamumenda (Nm)	CTC	Natural copper hill; substrate locally disturbed by mining	1340	S11,60492° E27,29400°
Ruashi (Ru)	CTC	Anthropic site: recolonization of mine deposits	1300	S11,62645° E27,56328°
Quartier Gécamines (QG)	CTC	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter, moist environment	1220	S11,70760° E27,42985°
Vallée Karavia (VK)	CTC	Anthropic site: normal soil contaminated by atmospheric fallout from ore-smelter, moist environment	1230	S11,67270° E27,43091°
Kyembe (Ky)	CTN	Natural site: forest clearing (miombo) on lateritic gravel	1190	S 11,11269° E 27,25825°

Table 2

Sites	**Df**	**F**	**P**
Population	5	3.1	P<0.05
Treatment	1	13.3	P<0.001
Treatment*population	5	6.4	P<0.001
Error	47		

Table 3

Sites	**Populations**	**Co μg g^{-1} soil**	**Co μg g^{-1} plant**	**Cu μg g^{-1} soil**	**Cu μg g^{-1} plant**
E	CP	275 (98-572)	397 (61-1105)	11278 (2161-55300)	394 (80-1380)
Nm	CTC	23 (2-98)	19 (8 – 58)	3428 (382-18144)	660 (84-2524)
Ru	CTC	54 (9-278)	130 (24-605)	991 (71-5869)	190 (59-412)
QG	CTC	6 (0,8-16)	47 (24-99)	1082 (224-2727)	100 (41-338)
VK	CTC	20 (7-44)	58 (14-176)	3544 (362-8189)	100 (34-211)
Ky	CTN	< 1	< 5	1.6 (0.8-2.1)	22 (8-93)

Figure 1 Biomass of metallicolous populations (Nm, QG, VK and Ru) and non-metallicolous (Ky) in *Crepidorhopalon tenuis* and in *C. perennis* (CP) at 0 et 60 µmol L^{-1} of $CuSO_4$. N = 6 per populations and treatment; error bars = standard deviation. For each population at 60 µmol L^{-1} of $CuSO_4$, means with the same letters are not significant (LSD Fisher test). The two treatments per population were compared One way anova *** = P<0.001 ; ** = P<0.01 ; * P<0.05 between the treatments for the same population.

Figure 2 Copper concentrations in plant shoot of metallicolous populations (Nm, QG, VK and Ru) and non-metallicolous (Ky) in *Crepidorhopalon tenuis* and in *C. perennis* (CP) on growing medium enriched at 60 µmol L^{-1} of $CuSO_4$. N = 6 per population ; error bars = standard deviation. The means with the same letters are not significant (LSD Fisher test).

Figure 1

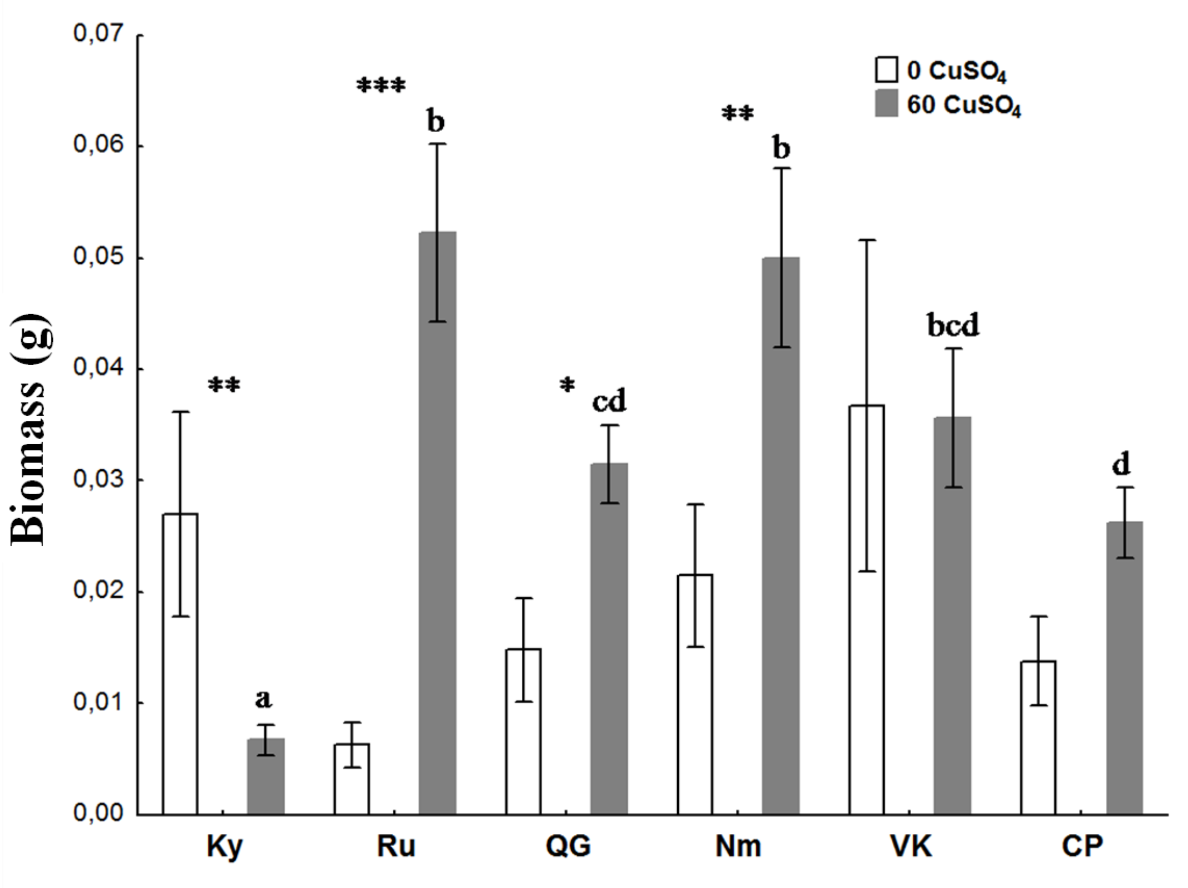
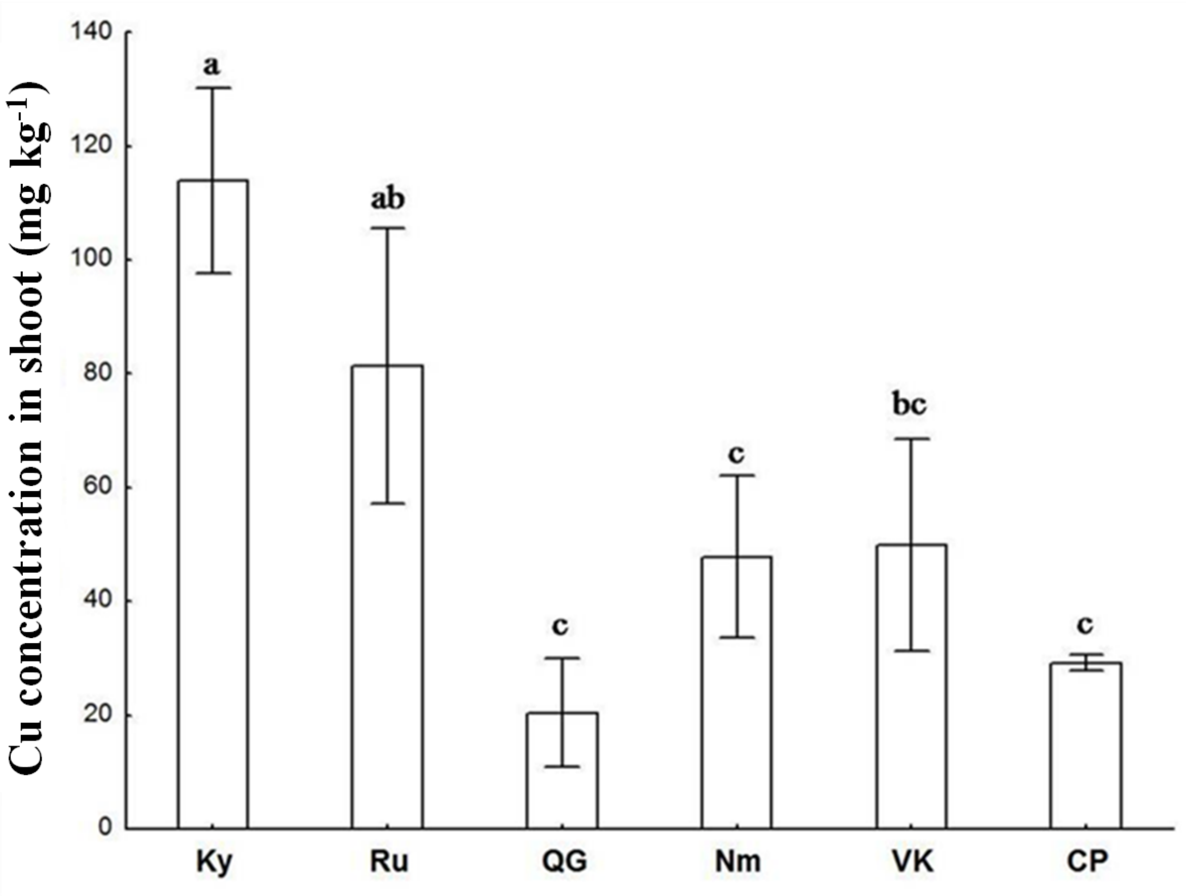

0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
Biomass (g)
0 CuSO4
60 CuSO4
**
a
***
b
*
cd
**
b
bcd
d
Ky
Ru
QG
Nm
VK
CP

Figure 2



Cu concentration in shoot (mg kg-1)
140
120
100
80
60
40
20
0
a
ab
c
c
bc
c
Ky
Ru
QG
Nm
VK
CP