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REGULAR ARTICLE

Copper tolerance in the cuprophyte *Haumaniastrum katangense* (S. Moore) P.A. Duvign. & Plancke

François K. Chipeng · Christian Hermans · Giles Colinet · Michel-Pierre Faucon · Michel Ngongo · Pierre Meerts · Nathalie Verbruggen

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Abstract Cu tolerance and accumulation have been studied in *Haumaniastrum katangense*, a cuprophyte from Katanga (DR Congo), previously described as a copper hyperaccumulator. *Nicotiana plumbaginifolia*, a well-known non-tolerant and non-accumulator species, was used as a control. The germination rate of *H. katangense* was enhanced by copper and fungicide addition, suggesting that fungal pathogens, which restrain germination in normal conditions, are limiting. In hydroponic culture in the Hoagland medium, *H. katangense* did not grow well, in contrast to *N. plumbaginifolia*. Better growth was achieved by adding fungicide or higher copper concentrations. The maximal non-effective concentration (NEC) was 12 µM $CuSO_4$ for *H. katangense* grown in hydroponics, i.e. 24 times greater than Cu concentration in the Hoagland medium. By comparison, copper concentrations greater than 0.5 µM had a negative effect on the growth of *N. plumbaginifolia*. EC_{50} (50% effective concentration) in hydroponics was 40 µM $CuSO_4$ for *H. katangense* and 6 µM $CuSO_4$ for *N. plumbaginifolia*. EC_{100} (100% effective concentration) was 100 µM $CuSO_4$ for *H. katangense* and 15 µM $CuSO_4$ for *N. plumbaginifolia.* In soil, growth was also stimulated by Cu addition up to 300 mg kg^{-1} $CuSO_4$. Surplus copper was also required for cultivating *H. katangense* in sterile conditions, suggesting that Cu excess may be necessary for needs other than pathogen defence. Cu accumulation in the shoot has been measured for *N. plumbaginifolia* and *H. katangense* at their respective NEC. Cu allocation in the two species showed a similar response to increasing Cu concentrations, i.e. root/shoot concentration ratio well above 1. In conclusion, *H. katangense* is highly tolerant to copper and has elevated copper requirement even in the absence of biotic interactions. Its accumulation pattern is typical of an excluder species.

Keywords Tolerance · Excluders · Copper · *Haumaniastrum katangense* · Heavy metal · *Nicotiana plumbaginifolia*

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F. K. Chipeng · C. Hermans · N. Verbruggen (✉)
Laboratoire de Physiologie et de Génétique Moléculaire des Plantes, Université Libre de Bruxelles,
Campus Plaine - CP242, Bld. du Triomphe,
Brussels 1050, Belgium
e-mail: nverbru@ulb.ac.be

G. Colinet
Laboratoire de Géopédologie,
Unité Sol-Ecologie-Territoire,
Faculté universitaire des Sciences Agronomiques de Gembloux,
Gembloux, Belgium

M.-P. Faucon · P. Meerts
Laboratoire d'Ecologie Végétale et Biogéochimie,
Université Libre de Bruxelles,
Campus plaine - CP244, Bld. du Triomphe,
Brussels 1050, Belgium

F. K. Chipeng · M. Ngongo
Faculté des Sciences Agronomiques,
Université de Lubumbashi,
Lubumbashi, DR, Congo

Introduction

The accumulation of toxic trace elements in soil, surface water and groundwater is a major environmental concern worldwide. Industrial activity related to exploitation of metal-rich minerals is causing major soil pollution (Baize and Tercé 2002). Copper caught our attention because it is one of the most exploited minerals in the Democratic Republic of the Congo, particularly in the Katanga province where bioavailable Cu soil concentrations above 30,000 mg kg^{-1} have been reported (Faucon et al. 2009; Gilles Colinet pers. comm.). Copper excess is particularly toxic for plants. High copper in the soil has triggered the evolution of highly specialised plant species, called cuprophytes (Duvigneaud and Denaeyer-De Smet 1963; Brooks et al. 1980, 1982; Baker et al. 2000), equipped with mechanisms of resistance to these particular environmental conditions (Baker 1981; Ye et al. 2003; Xia and Shen 2007). Some cuprophytes apparently have the ability to accumulate Cu and/or Co in their shoots, up to concentrations that have been considered as hyperaccumulation (>1,000 mg kg^{-1}) (Baker and Brooks 1989). However those reports are exclusively based on specimens collected in the field and these plants failed to display the phenomenon in the laboratory (Morrison et al. 1979). The most plausible explanation for this discrepancy is a failure to wash contaminated dust off the leaf surface (Faucon et al. 2007).

While copper is an essential element, mechanisms of Cu homeostasis and tolerance by plants remain little known (Reeves 2006; Pilon et al. 2006, Kobayashi et al. 2008), notably in tropical cuprophytes (Morrison et al. 1979; Morrison et al. 1981; Baker et al. 1983).

Haumaniastrum katangense (Lamiaceae), the Katangan "copper flower" is such a cuprophyte. This annual species, which is a colonist of natural and man-polluted Cu-enriched soils (Malaisse and Brooks 1982, Leteinturier and Malaisse 2000), has been used as a biogeochemical indicator of Cu ore (Paton and Brooks 1996). Once considered as an absolute metallophyte (Duvigneaud and Denaeyer-De Smet 1963), the species has occasionally been found growing on normal soil in Zambia (Paton and Brooks 1996). The species has been reported to hyperaccumulate Cu, but in one of the very few published studies investigating the physiology of *H. katangense* in cultivation conditions, hyperaccumulation could not be reproduced (Morrison et al. 1979). The reason for the rarity of the species on Cu-poor soil is still unclear, but it has been suggested that susceptibility to pathogenic fungi might be involved (Malaisse and Brooks 1982; Brooks and Malaisse 1985; Paton and Brooks 1996).

In this paper, we optimized and conducted tests for Cu tolerance and accumulation in hydroponics, soil and in vitro conditions. The well-characterized non-tolerant and non-accumulator plant, *Nicotiana plumbaginifolia*, was used as a control for comparison.

Materials and methods

Site harvest and plant material

Haumaniastrum katangense seeds and whole plant samples (for analysis of Cu content) were collected in four contaminated sites in the vicinity of Lubumbashi, Katanga, Democratic Republic of Congo: "Tshondo", "Cimetière GCM", "Cercle Hippique" and "Jardin Zoologique"; 12°36'51,2" S 27° 28' 51,2" E, altitude 1,278 m; soils contain between 409 to 825 mg kg^{-1} Cu extracted in KCl 1 N. *Nicotiana plumbaginifolia* seeds were received from Michel Jacobs (VUB, Brussels).

Germination tests

Seeds were harvested in August 2006 and dried out at ambient temperature, and just after harvest seeds were dormant and unable to germinate. Some 3 months were necessary to break dormancy. Therefore, all germination tests were done on 3-month-old seeds. Germination tests were performed in Petri dishes on humidified filter paper at 25°C. There were 200 seeds per dish. Germinated seeds were scored upon appearance of the ridicule. Different treatments were tested in an effort to improve germination. 10 µM virolex® fungicide (carbendazim 4.7%, Protex, Wijnegem, B) was used to prevent possible inhibition of germination by pathogenic fungi. Other treatments include the application of a thermal shock, i.e. 60°C during 15 min; scarification (scrubbing of seed surface); Cu addition (10 mg l^{-1}=159.6 µM $CuSO_4.5H_2O$); and seed washing in running water. The factors were applied separately and in factorial combinations.

Tolerance tests

Tolerance tests have been performed in hydroponics, in vitro or in compost soil.

Hydroponics was performed in a growth chamber at 20°C, with a relative humidity of 66% and light intensity of 100 µE, in the Hoagland nutrient solution which contains 0,5 µM $CuSO_4$. We tested the effects of 0 – 100 µM $CuSO_4$ addition. Three-week-old *H. katangense* plants, resulting from seed germination in commercial soil containing 50 mg kg^{-1} $CuSO_4$ and sprayed with 10 µM virolex® fungicide, measuring 3 to 5 cm in length, with an average of 600 mg FW per plant and 4 to 6 leaves were transferred to hydroponics in the standard Hoagland nutrient solution and adapted during one week. Without these pre-treatments survival of plantlets was very weak. Wild type *Nicotiana plubaginifolia* P2 seeds (from an inbreeding line obtained from the tobacco company SEITA; Geert Angenon pers. comm.) were germinated on commercial soil. Two-week-old *N. plumbaginifolia* plants, measuring 4 to 7 cm in length, with an average of 800 mg fresh weight (FW) per plant and 4 to 6 leaves were transferred in the standard Hoagland nutrient solution and adapted during one week. Contamination with Cu was carried out the following week. The fresh weight of each plant was measured before the transfer to hydroponics, and every week onwards for one month at the time of nutrient solution renewal (corresponding to 1, 2, 3 weeks Cu surplus treatments). Non-effective concentration (NEC), 50 % effective concentration (EC_{50} decreasing maximal growth by 50%) and 100 % effective concentration (EC_{100} complete inhibition concentration) were determined for *H. katangense* and *N. plumbaginifolia* after 3 weeks of Cu treatment.

In vitro culture was performed in a growth chamber at 20°C, with light intensity of 30 µE, on Murashige & Skoog (MS) medium, which contains 0.15 µM $CuSO_4$ (Murashige and Skoog 1962). Seeds were immersed in 70% ethanol for 5 min, followed by a 10 min soak in 5% bleach 0.1% SDS. Seeds were rinsed 3 times in sterile water. Seedlings were germinated on MS, and after 10 days were transferred on MS enriched with up to 100 µM $CuSO_4$. Observations were made during three weeks. The protocol of sterilization was controlled by plating an extract of *H. katangense* seeds (2 mg seeds crushed in 1 ml sterile water) on Sabouraud medium, a rich medium used to isolate fungi. The negative control was an extract of seeds sterilized at 120°C for 20 min, and the positive control was an extract of unsterilized seeds. Observations were made after two weeks and we did not observe any fungal growth on our extracts from sterilized seeds (after ethanol/bleach water treatment or after the 120°C treatment). On the contrary substantial fungal growth was observed on extracts from unsterilized seeds (data not shown).

In soil (universal potting soil, Compo Sana® soil for balcony plants), growth was performed in a greenhouse at 25°C with 50% relative humidity, copper was spiked as sulphate ($CuSO_4.5H_2O$) 10 days before the transfer of the young seedlings germinated under the same conditions as in hydroponics, the addition of Cu was from 0 to 2,000 mg kg^{-1} $CuSO_4$. The plants were grown on contaminated soil in pots for 7 weeks. Fresh weight, length of stem and root, number of surviving plants, dry weight (DW) were measured at the end of the experiment.

Mineral element analysis

After harvest the plants were washed with 0.1 mM EDTA for 5 min, rinsed 3 times for 5 min with demineralised water and dried before mineralization. Copper concentration was analysed by flame atomic absorption spectrophotometry (Hermans and Verbruggen 2005).

Statistical analysis

The results of germination tests were subjected to ANOVA (analysis of variance) with 4 factors crossed and post-hoc multiple comparison tests were applied (Fisher LSD) after log-transformation. Growth and accumulation measurements were subjected to one-way ANOVA. All analyses were performed with Statistica 6 (Statsoft 2005).

Results

Germination tests

Germination of *Haumaniastrum katangense* was studied three months after seed harvest. The impact of thermal shock, scarification, copper addition, fungicide (virolex®) application and seed washing were tested (Fig. 1). Germination percentage was low (<15 %) for the control (untreated seeds) and washing or heat shock treatments alone did not improve it. A beneficial impact on germination was observed upon copper addition or fungicide treatments. In combination with washing and heat exposure, copper or fungicide addition brought germination rate above 80%. Addition of copper to the fungicide treatment did not significantly improve germination.

Copper tolerance of *Haumaniastrum katangense*

Tolerance to copper was studied in hydroponics with a first range of 0–100 µM $CuSO_4$ concentrations. Treatment was applied to 4-week-old *H. katangense* and 3-week-old *N. plumbaginifolia* plantlets (4– to 6–leaves stage). Growth was measured every week for 3 weeks (Fig. 2). Growth of *N. plumbaginifolia* was completely inhibited by the addition of 20 µM $CuSO_4$ after 1 week treatment. Maximal relative growth was observed at 0.5 µM and 10 µM $CuSO_4$ for *N. plumbaginifolia* and *H. katangense* respectively (Fig. 2a, b). Stem length and root elongation were also measured in both species and the same trend as in Fig. 2 was observed (data not shown). It is noteworthy that the maximal relative growth of *N. plumbaginifolia* was the double of that of *H. katangense*. Further tests on a second range of copper addition ([0–24] µM $CuSO_4$) during 3 weeks allowed determination of the non-effective concentration (NEC) of *H. katangense* at 12 µM $CuSO_4$, at which growth rate was the highest (Fig. 2c). Without copper addition to the control solution, relative growth of *H. katangense* was only one third of its maximal value (Fig. 2c). The concentrations at which 50% growth was observed, EC_{50}, were 40 µM for *H. katangense* and 6 µM for *N. plumbaginifolia*. Total inhibition of growth (EC_{100}) after 3 weeks was observed around 100 µM $CuSO_4$ for *H. katangense* and 15 µM for *N. plumbaginifolia* (Fig. 2a, c). Figure 3 shows a picture of the two species at their respective NEC.

Growth of *H. katangense* and *N. plumbaginifolia* was also compared in vitro by sowing seeds on vertical plates containing Murashige & Skoog medium supplemented with 0, 5, 50 or 100 µM $CuSO_4$. Figure 4 shows plants three weeks after sowing on extreme tested concentrations. *H. katangense* growth was inhibited upon no $CuSO_4$ addition to the MS medium, and enhanced upon $CuSO_4$ surplus. The highest concentration tested still improved the growth of *H. katangense*. Opposite observations were made for *N. plumbaginifolia* (Fig. 4c).

In soil (commercial compost), the survival rate and growth of *H. katangense* were highly dependent on copper addition. Without $CuSO_4$ addition, the survival rate 7 weeks after sowing was only 40% (Fig. 5a) and plants were frail (Fig. 5b). Maximal *H. katangense* survival (Fig. 5a) and growth (Fig. 5b) were observed at 300 mg kg^{-1} $CuSO_4$. After 7 weeks complete growth inhibition was not observed on the highest tested concentration (2,000 mg kg^{-1} $CuSO_4$ added) but survival was as low as 7%. The high organic matter in the commercial soil is thought to have decrease copper bioavailability. Nevertheless, growth and survival of *N. plumbaginifolia* were maximal in non-treated soil and were severely affected by the

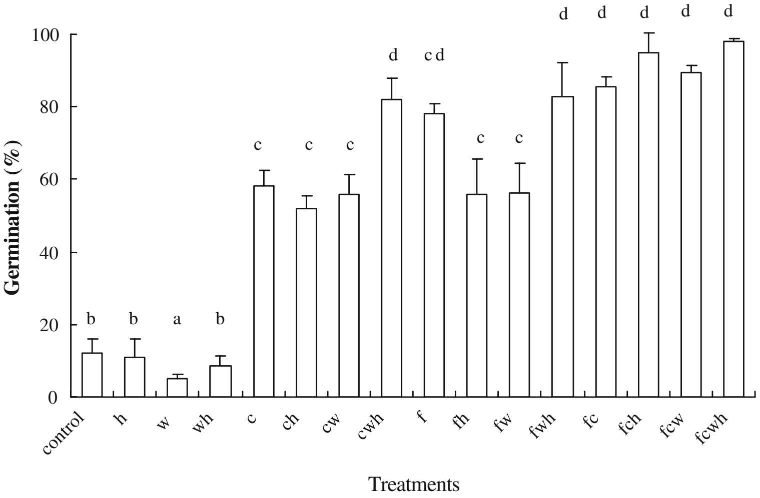

Fig. 1 Influence of various treatments on germination rate of *Haumaniastrum katangense.* Seeds (n=200) of *Haumaniastrum katangense* were germinated in Petri dishes on Whatman paper imbibed with water. The different treatments are h (heat shock); w (washing); f (fungicide); c ($CuSO_4$). Letters above bars indicate significant differences ($P<0.05$) after ANOVA

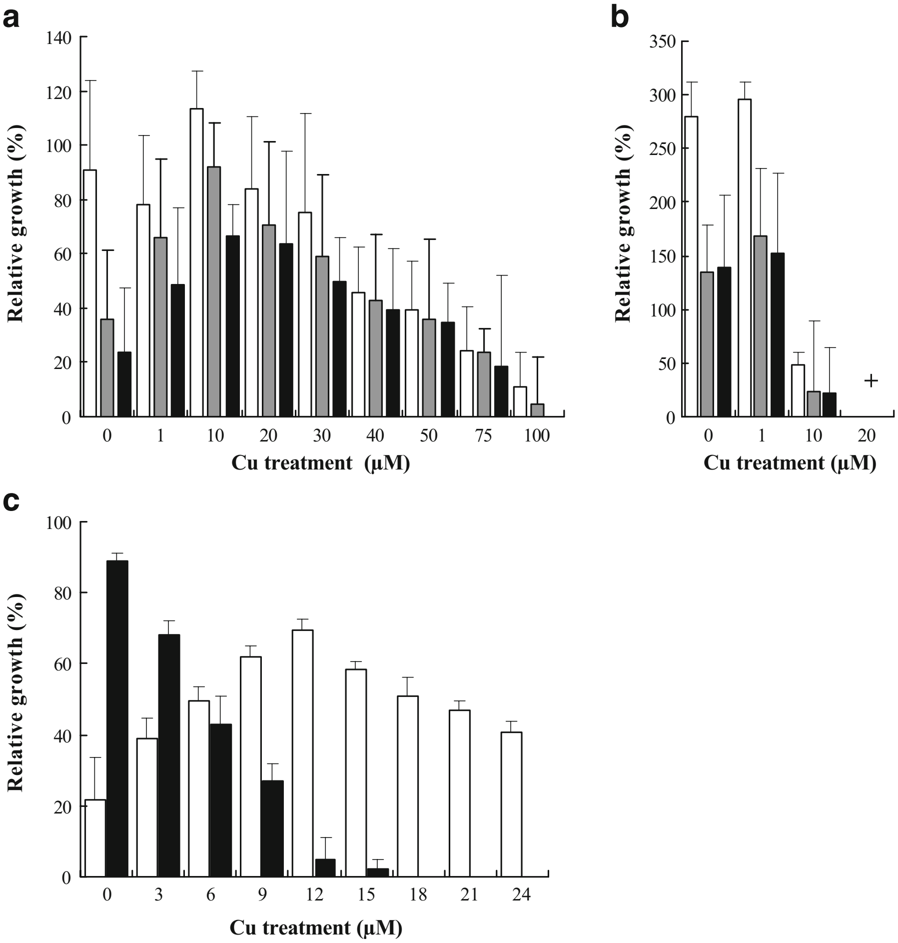

Fig. 2 Growth in hydroponics of *Haumaniastrum katangense* and *Nicotiana plumbaginifolia* in the presence of increasing copper content. Added $CuSO_4$ concentrations to the Hoagland solution are mentioned on X axis. Relative growth (RG) is defined as: $RG = (FW_{x+1} - FW_x)/FW_x \times 100$, where FW: fresh weight; x represents weeks 1 (white), 2 (grey), 3 (black). **a** *Haumaniastrum katangense* (n=27); **b** *Nicotiana plumbaginifolia*; (n=9); **c** Determination of the copper non-effective concentration (NEC) after 3 weeks treatment; white columns: *H. katangense*; black columns: *N. plumbaginifolia*

addition of $CuSO_4$. There was no survival of tobacco plants on soil treated with concentrations above 400 mg kg^{-1}.

Mineral analysis

Cu accumulation of *H. katangense* and *N. plumbaginifolia* were compared in hydroponic culture after 3 weeks $CuSO_4$ treatment (Fig. 6). During the same treatments, *H. katangense* always accumulated higher Cu concentrations both in roots and in shoots than *N. plumbaginifolia*. At its NEC (12 µM), *H. katangense* accumulated 1,402 mg kg^{-1} DW in roots and 305 mg kg^{-1} DW in shoots (Table 1). In comparison, at its NEC (0.5 µM) *N. plumbaginifolia* accumulated 41 mg kg^{-1} DW in roots and 11 mg kg^{-1} DW in shoots. At those concentrations root/shoot Cu concentration ratios were 4.6 and 3.7 for *H. katangense* and *N. plumbaginifolia* respectively. At higher Cu concentrations, ratios were always above 1 and did not significantly differ between the two species.

H. katangense shoot concentrations values were far below the threshold value of Cu hyperaccumulation (i.e. 1,000 mg kg^{-1}). *H. katangense* accumulated more than 1,000 mg Cu kg^{-1} shoot DW only when plants were grown in solution supplemented with damaging Cu concentrations (above 75 µM $CuSO_4$ which inhibits growth at NEC by 75%).

Accumulation of Cu by *H. katangense* in hydroponics was compared to field growing plants. Three-month-old vegetative plants were harvested on four contaminated sites in Lubumbashi and copper concentration was determined in the shoot of the field specimens. Cu accumulation in shoots was not significantly different in the four sites. On average, shoots contained 67 mg kg^{-1} DW copper.

Fig. 3 Growth of *Haumaniastrum katangense* and *Nicotiana plumbaginifolia* in hydroponics. The picture of a representative container was taken after 3 weeks Cu treatment at *H. k.* and *N. p.* respective NEC. Standard nutrient solution (**a**) Control (no $CuSO_4$ surplus); (**b**) addition of 12 µM $CuSO_4$. Scale: 4 cm

Discussion

This study is the first to compare response to copper in the cuprophyte *H. katangense* with a non-tolerant control species. The results highlight the extraordinarily high Cu tolerance of *H. katangense*, and point to beneficial effects of copper on germination, growth and survival, in different experimental conditions. These results will be discussed regarding to the ecological range of *H. katangense*.

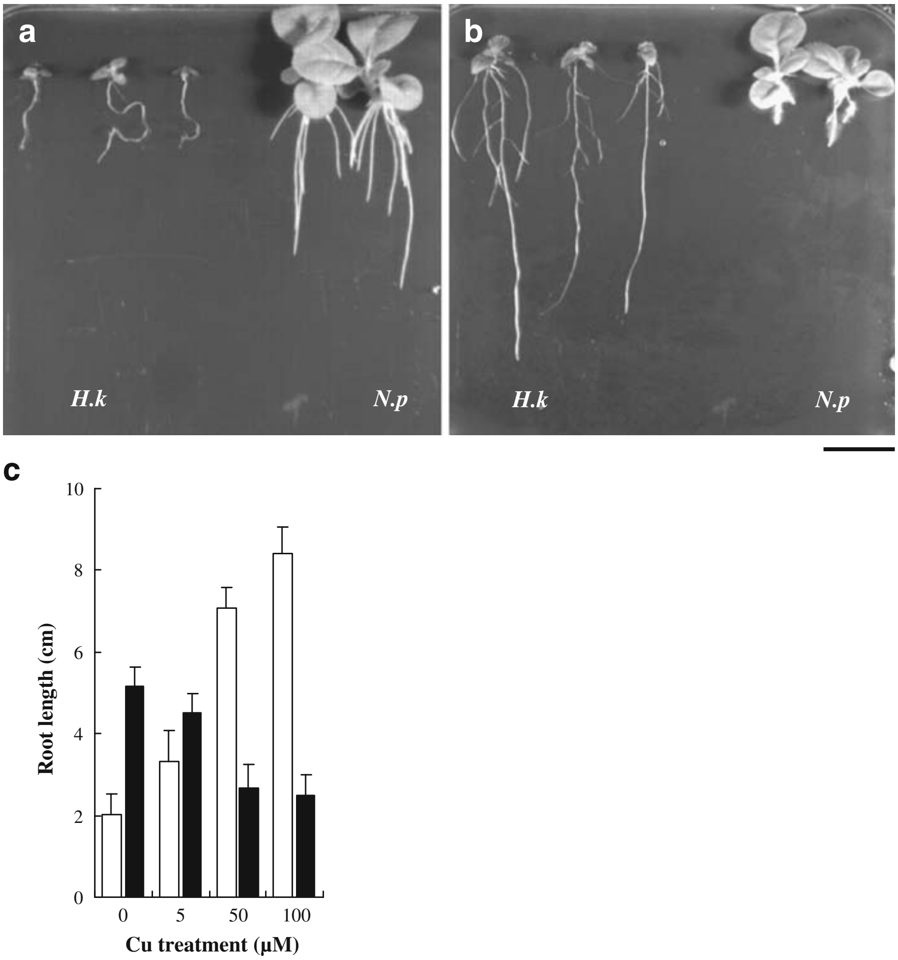

Fig. 4 Growth of *Haumaniastrum katangense* and *Nicotiana plumbaginifolia* in vitro. Seeds were sown on vertical plates containing Murashige & Skoog medium supplemented with $CuSO_4$. Pictures of plants 3 weeks after sowing on MS **a** or MS + 100 µM $CuSO_4$ **b**. Primary root length on MS supplemented with 0, 5, 50 or 100 µM $CuSO_4$ **c**; White columns *(H. katangense)*; black columns *(N. plumbagonifolia)*. Scale: 2 cm

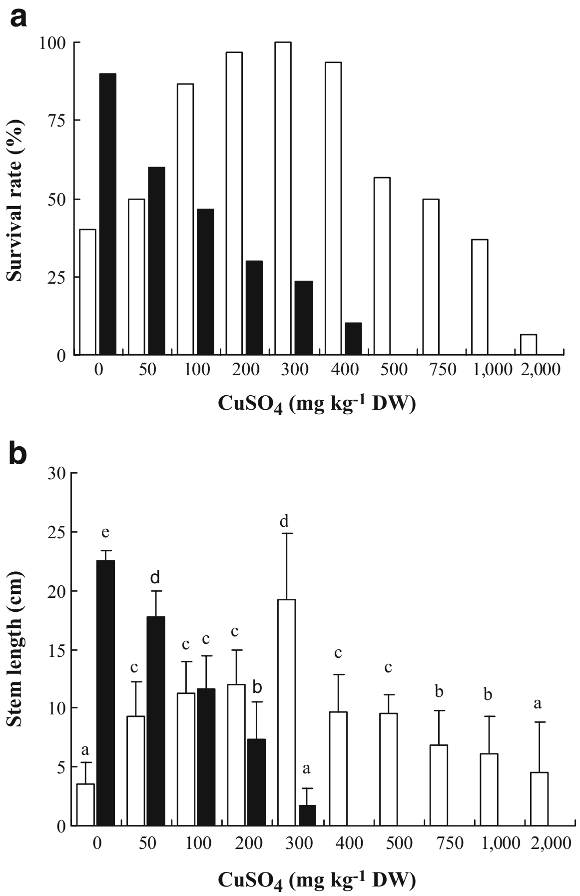

Fig. 5 Impact of copper addition on survival and growth of *Haumaniastrum katangense* and *Nicotiana plumbaginifolia* in soil. **a** Survival rate (%); **b** Stem length (cm). White columns *H. katangense*; black columns *N. plumbaginifolia*, observations were 7 weeks after sowing; n=30. Different letters above bars indicate significant differences ($P<0.05$) after ANOVA

H. katangense has a strong affinity for copper-rich soil and is a typical component of vegetations established on the most heavily Cu-contaminated soil in Katanga, either in natural habitats or in secondary habitats contaminated by industry (Malaisse and Brooks 1982). A first explanation to account for its rarity on normal soil invokes susceptibility to pathogenic fungi (Malaisse and Brooks 1982; Brooks and Malaisse 1985; Paton and Brooks 1996). Copper is a well-known broad-spectrum fungicide. Therefore, copper-rich soil may have lower pathogen loads compared to normal soil. This may have relaxed selection for resistance mechanisms. It is striking that both copper and the fungicide virolex® had a strong positive effect on germination. This result supports the hypothesis that pathogenic fungi limit establishment of *H. katangense* on normal soil.

However in sterile growth conditions, high copper addition to the medium was also required to optimize growth (Fig. 4). In hydroponics maximal growth was achieved at Cu treatments of 12 µM vs. 0.5 µM in *N. plumbaginifolia.* This effect was clearly independent from that of the fungicide and supports the hypothesis of a high copper requirement in *H. katangense*. Growth stimulation by elevated copper concentrations has rarely been reported in plants. For instance, in the copper moss *Scopelophila cataractae* (Bryophyte) optimal growth is conditioned by a surplus of copper in the medium (Shaw 1994). Growth stimulation by elevated copper concentrations in growth medium was also observed in the copper mines populations of *Elsholtzia splendens* (Jiang et al. 2008) and *E. haichowensis* (Lou et al. 2004). On the contrary, copper tolerant and non-tolerant genotypes of *Mimulus guttatus* were similarly affected by decreasing copper supply, which is against the cost of living in the presence of copper excess. Mechanisms underlying a higher requirement of copper in cuprophytes need further study (Harper et al. 1997, 1998). In Zn hyperaccumulators, the higher Zn requirement is proposed to be related to efficient sequestration mechanisms and deregulated Zn deficiency response (reviewed in Verbruggen et al. 2009).

The concentrations of Cu tolerated by *H. katangense* in the present study are remarkably high, as compared to *N. plumbaginifolia*. Similarly high Cu tolerance was found in *Elsholtzia haichowensis, E. splendens* from China (Lou et al. 2004; Weng et al. 2005; Xia and Shen 2007; Jiang et al. 2008) and *Silene cobalticola* from DR Congo (Baker et al. 2000).

The copper concentrations measured in this study in the shoot of *H. katangense*, whether in situ or in hydroponics at physiological concentrations, were always below the conventional hyperaccumulation threshold (1,000 mg kg^{-1} DW). Those data are in marked contrast with previously reported values for *H. katangense* (Brooks et al. 1980). Reports of copper concentrations above 1,000 mg kg^{-1} DW in a number of species from the copper belt in Congo (Malaisse et al. 1979, 1994, 1999; Reeves and Baker 2000; Reeves 2006) are in fact exclusively based on specimens collected in the field, but these plants failed to display the phenomenon in the laboratory (Morrison et al.

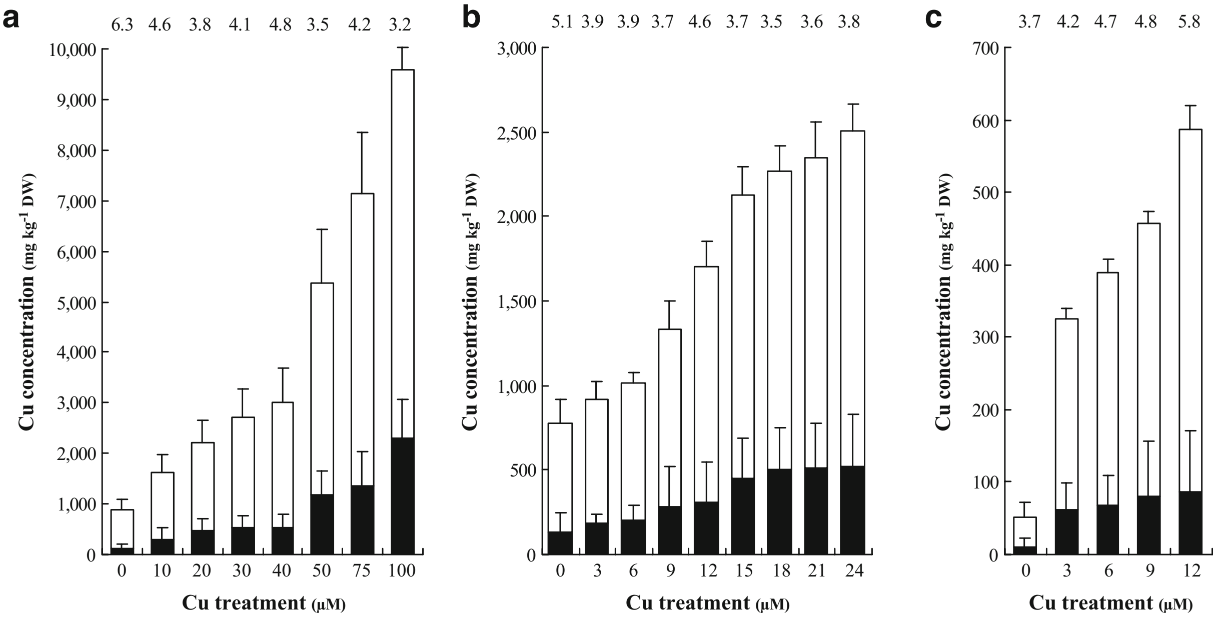

Fig. 6 Cu concentrations of *Haumaniastrum katangense* and *Nicotiana plumbaginifolia* plants grown in hydroponics Conditions are described in Fig. 2 for *Haumaniastrum katangense* **a** & **b** and *Nicotiana plumbaginifolia* **c** white bars refer to root Cu concentration, black bars refer to shoot Cu concentration; n=10. The root:shoot Cu concentrations ratios are added on the top of each bar

1979; Macnair 2003). A possible explanation for this discrepancy is a failure to wash contaminated dust off the leaf surface (Faucon et al. 2007).

In fact, the pattern of accumulation of copper in shoots and roots in response to increasing concentrations in the growth medium is similar for *N. plumbaginifolia* and *H. katangense*. However, much higher Cu concentrations were observed in *H. katangense* than in *N. plumbaginifolia*, especially at low external concentrations of copper. In both species, Cu mainly accumulated in roots. This strategy is typical of metal excluders. It has been repeatedly documented in several ecotypes or species adapted to high Cu soil (e.g. *Silene vulgaris* and *Elsholtzia splendens* (Schat et al. 1993; Song et al. 2004; Weng et al. 2005)). However, it should be noted that typical root/shoot Cu concentration ratios of these two latter Cu excluders are above 10, while on average those in *H. katangense* were below 5.

Table 1 Copper accumulation in *Haumaniastrum katangense* (mg kg^{-1}DW)

	Plants in hydroponics		In soil	In site samples	
	Roots	Shoots	Shoots	Leaves	Stem
NEC	1,402	305	49	67	28
EC_{50}	1,839	511	124	-	-
EC_{100}	7,273	2,302	200	-	-

No copper sequestration mechanism has been shown up to now and its cellular localization in cuprophytes like *H. katangense* needs to be studied. Tolerance is thought to be related to the capacity to efflux copper excess out of the cytoplasm. The HMA5 ATPase seems to be a key factor in Cu tolerance of *A. thaliana* where it contributes to root detoxification of Cu excess (Andrés-Colás et al. 2006; Kobayashi et al. 2008). The role of HMA5 homologues remains to be investigated in Cu hypertolerant plants.

Conclusion

Our data offer the first experimental evidence for extremely high copper tolerance in the cuprophyte *Haumaniastrum katangense*. Several components of fitness showed apparent stimulation by elevated levels of copper. Our results are consistent with the hypothesis that *H. katangense* is extremely susceptible to pathogenic fungi, a possible by-product of adaptation to soil with relaxed pathogen pressure. However, Cu also enhanced performance in sterile medium, suggesting genuinely high copper requirement. The mechanisms of this high copper requirement deserve further study. Finally our results are consistent with the ecological range of *H. katangense* in the field, i.e. a marked preference for Cu contaminated soil.

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References

Andrés-Colás N, Sanceno V, Rodrıguez-Navarro S, Mayo S, Thiele DJ, Ecker JR, Puig S, Penarrubia L (2006) The Arabidopsis heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. Plant J 45:225–236

Baize D, Tercé M (2002) Les éléments traces métalliques dans les sols: approches fonctionnelles et spatiales, INRA editions, Paris

Baker AJM (1981) Accumulators and excluders – strategies in the response of plant to heavy metal. J Plant Nutr 3:643–654

Baker AJ, Brooks RR, Pease AJ, Malaisse F (1983) Studies on copper and cobalt tolerance in the three closely related taxa within the genus *Silene* L. (Caryophyllaceae) from Zaïre. Plant Soil 73:377–385. doi:10.1007/BF02184314

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Bañuelos G, Terry N (eds) Phytoremediation of contaminated soil and water. CRC Press LLC, Boca Raton, pp 85–108

Brooks RR, Reeves RD, Morrison RS, Malaisse F (1980) Hyperaccumulation of copper and cobalt – a review. Bull Soc Roy Bot Belg 113:166–172

Brooks RR, Gregorie J, Madi L, Malaisse F (1982) Phytogéochimie des gisements cupro-cobaltifères de l'anticlinal de Kasonta (Shaba-Zaïre). Geo-Eco-Trop 6:219–228

Brooks RR, Malaisse F (1985) The heavy metal-tolerant Flora of Southcentral Africa – a multidisciplinary approach. Balkema, Rotterdam, p 199

Duvigneaud P, Denaeyer-De Smet S (1963) Cuivre et végétation au Katanga. Bull Soc Roy Bot Belg 96:92–231

Faucon MP, Shutcha M, Meerts P (2007) Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil. Plant Soil 301:29–36. doi:10.1007/s11104-9405-3

Faucon MP, Colinet G, Mahy G, Luhembwe NM, Verbruggen N, Meerts P (2009) Soil influence on Cu and Co uptake and plant size in the cuprophytes *Crepidorhopalon perennis* and C. *tenuis* (*Scrophulariaceae*) in SC Africa. Plant Soil 317:201–212. doi:10.1007/s11104-008-9801-3

Harper FA, Smith SE, Macnair MR (1997) Can an increased copper requirement in copper-tolerant *Mimulus guttatus* explain the cost of tolerance? I. Vegetative growth. New Phytol 136:455–467

Harper FA, Smith SE, Macnair MR (1998) Can an increased copper requirement in copper-tolerant *Mimulus guttatus* explain the cost of tolerance? II. Reproductive phase. New Phytol 140:637–654

Hermans C, Verbruggen N (2005) Physiological characterization of Mg deficiency in *Arabidopsis thaliana*. J Exp Bot 56:2153–2161. doi:10.1093/jxb/eric215

Jiang LY, Yang XE, Chen JM (2008) Copper tolerance and accumulation of *Elsholtzia splendens* Nakai in a pot environment. J Plant Nutr 31:1382–1392

Kobayashi Y, Kuroda K, Kimura K, Southron-Francis JL, Furuzawa A, Kimura K, Iuchi S, Kobayashi M, Taylor GJ, Koyama H (2008) Amino acid polymorphisms in strictly conserved domains of a P-Type ATPase HMA5 are involved in the mechanism of copper tolerance variation in *Arabidopsis*. Plant Physiol 148:969–980. doi:10.1104/pp.108.119933

Leteinturier B, Malaisse F (2000) The copper flora of Katanga: a phytogeochimical analysis. Geo-Eco-Trop. 23:31–48

Lou LQ, Shen ZG, Li XD (2004) The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper-enriched soils. Environ Exp Bot 51:111–120. doi:10.1016/j.envexpbot.2003.08.002

Macnair MR (2003) The hyperaccumulation of metals by plants. Adv Bot Res 40:63–105

Malaisse F, Grégoire J, Morrison RS, Brooks RR, Reeves RD (1979) Copper and cobalt in vegetation of Fungurume, Shaba Province, Zaïre. Oikos 33:472–478

Malaisse F, Brooks RR (1982) Colonisation of modified metalliferous environments in Zaire by the copper flower *Haumaniastrum katangense*. Plant Soil 64:289–293

Malaisse F, Brooks RR, Baker AJM (1994) Diversity of vegetation communities in relation to soil heavy metal content at the Shinkolobwe copper/cobalt/uranium mineralization, Upper Shaba, Zaïre. Bull Soc Roy Bot Belg 127:3–16

Malaisse F, Baker AJM, Ruelle S (1999) Diversity of plant communities and leaf heavy content at Luiswishi copper/cobalt mineralization, Katanga. Dem. Rep. Congo. Biotechnol Agron Soc Environ 3:104–114

Morrison RS, Brooks RR, Reeves RD, Malaisse F (1979) copper and cobalt uptake by metallophytes from Zaïre. Plant Soil 53:535–539

Morrison RS, Brooks RR, Reeves RD, Malaisse F, Horowitz P, Aronson M, Merriam GR (1981) The diverse chemical forms of heavy metals in tissue extracts of some metallophytes from Shaba province, Zaïre. Phytochemistry 20:455–458. doi:10.1016/S0031-9422(00)84165-5

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:437–497

Paton A, Brooks RR (1996) A re-evaluation of *Haumaniastrum* species as geobotanical indicators of copper and cobalt. J Geochem Expl 56:37–45

Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, Ye H (2006) Copper cofactor delivery in plant cells. Plant Biol 9:256–263

Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals. Wiley, New York, pp 193–221

Reeves RD (2006) Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N (eds) Phytoremediation of metal-contaminated soils. NATO Science Series: IV: earth and environmental sciences. Vol 68 pp 1–25. Spinger, New York

Schat H, Kuiper E, Ten bookum WM, Vooijs R (1993) A general model for the genetic control of copper tolerance in *Silene vulgaris*: evidence from crosses between plants from different tolerant populations. Heredity 70:142–147

Shaw AJ (1994) Adaptation to metals in widespread and endemic plants. Environ Health Persect 102(Suppl 12):105–108

Song J, Zhao F, Luo YM, McGrath SP, Zhang H (2004) Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. Environ Pollut 128:307–315

Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperacumulation in plants. New Phytol 181:759–776

Weng G, Wu L, Wang Z, Luo Y, Christie P (2005) Copper uptake by four *Elsholtzia* ecotypes supplied with varying levels of copper in solution culture. Environ Int 31:880–884. doi:10.1016/j.envint.2005.05.032

Xia Y, Shen Z (2007) Comparative studies of copper tolerance and uptake by three plant species of the genus *Elsholtzia*. Bull Environ Contam Toxicol 79:53–57. doi:10.1007/s00128-007-9222-7

Ye ZH, Baker AJM, Wong MH, Willis AJ (2003) Copper tolerance, uptake and accumulation by *Phragmites australis*. Chemosphere 50:795–800