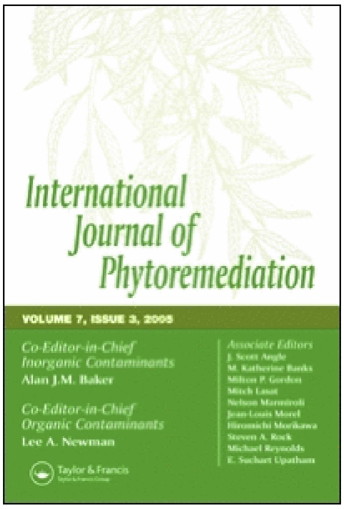

International Journal of Phytoremediation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713610150

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Accepted uncorrected manuscript posted online: 11 May 2010

First published on: 11 May 2010

To cite this Article Shutcha, Mylor Ngoy , Mubemba, Michel Mpundu , Faucon, Michel-Pierre , Luhembwe, Michel Ngongo , Visser, Marjolein , Colinet, Gilles and Meerts, Pierre(2010) 'Phytostabilisation of Copper-Contaminated Soil in Katanga: An Experiment with Three Native Grasses and Two Amendments', International Journal of Phytoremediation, 12: 6, 616 — 632, First published on: 11 May 2010 (iFirst)

To link to this Article: DOI: 10.1080/15226510903390411

URL: http://dx.doi.org/10.1080/15226510903390411

International Journal of Phytoremediation, 12:616–632, 2010

ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226510903390411

PHYTOSTABILISATION OF COPPER-CONTAMINATED SOIL IN KATANGA: AN EXPERIMENT WITH THREE NATIVE GRASSES AND TWO AMENDMENTS

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This study evaluates the feasibility of using the grass species* Rendlia altera*,* Monocymbium ceresiiforme*,* Cynodon dactylon*, and amendments (compost and lime) for the phytostabilisation of soils contaminated by Cu in the province of Katanga (Democratic Republic of Congo). Species were grown on control and Cu-contaminated plots (artificially contaminated with 2,500 mg kg^{-1} Cu) unamended (NA), amended with 4.5 kg compost m^{-2} or 0.2 kg lime m^{-2}.* R. altera *was also grown on contaminated plots amended with 22.5 kg compost m^{-2} or 1 kg lime m^{-2}. Plant survival, growth, and reproduction were monitored for two years. Cu-concentration in leaves of* R. altera *and* M. ceresiiforme *were analysed. pH and extractable Cu (0.01 M $CaCl_2$) in soil were analysed in April 2007 and 2008. Results showed that* R. altera *seems to be the best candidate because of its highest survival on NA, followed by* M. ceresiiforme*, while liming was necessary to ensure survival of* C. dactylon*. Lime increased plant reproduction and reduced Cu accumulation in leaves compared to compost. However, higher survival and number of spikes of* R. altera *obtained in experiment 2 with 22.5 kg compost m^{-2} suggest that lime x compost interactions should be investigated in further studies.

KEYWORDS Soil pollution, *Rendlia altera*, *Monocymbium ceresiiforme*, *Cynodon dactylon*, metallophytes, lime, compost, copper, phytostabilisation, Katanga

INTRODUCTION

In South-East of Katanga province (Democratic Republic of Congo) the "Katangan copperbelt" is rich in copper/cobalt ore bodies. Mining activities have existed there since pre-colonial times. As a result, over ten different types of contamination sources

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have been identified, including pre-colonial metallurgic sites, prospecting trenches, open quarries, heaps of overburden materials, transport of cupriferous rocks by railways and roads, removed of "sterile" rocks, alluvial deposits and atmospheric fallout from smelters (Leteinturier *et al.*, 1999).

In particular, ore smelting activities release large amount of SO_2 in the atmosphere as well as metalliferous dust. Heavy metals are then accumulated mainly in the surface horizons of soils in the surrounding area (Ayres *et al.*, 2002). Around non-ferrous ore smelting industries, high concentrations of heavy metals combined with low pH are responsible for the decline in biodiversity and appearance of bare soil exposed to erosion by rainfall and wind (Kozlov and Zverera, 2007). One well-documented example is the region of Sudbury (Canada) where a century of Cu-Ni smelting activities have resulted in 10,000 ha of bare soil and degradation of 36,000 ha of native forest (Winterhalder, 1996). Similar situations were also reported from the region of Harjavalta (Finland) where Cu-Ni smelting activities caused degradation of species diversity and coverage of Scots pine forest, inhibited nutrient cycling and impoverished soil decomposer community (Kiikkilä, 2003).

A similar situation exists in the vicinity of Lubumbashi. Emissions of SO_2 (which causes acid deposition) and metalliferous particles by the Cu-smelting industry for over 80 years has degraded *miombo* woodland (the dominant open forest of the Zambezi region) and caused its replacement by open grassland and bare soil in the area situated downwind of the copper smelter (Mbenza *et al.*, 1989; Malaisse, 1997). In such a strongly degraded landscape, large areas of bare soil are exposed to erosion by rainfall in the wet season and transport of particles by wind in the dry season (Munyemba *et al.*, 2008). This is a real concern to human health due to the high population density in the contaminated area. Recently, to assess human exposure to pollution in Katanga, Banza *et al.* (2009) measured concentrations of metals and metalloids in urine of people living close to mines or smelting plants. Concentrations of Co and other metals were higher in people living close to mines or smelting plants (including the site contaminated by the Cu-smelter industry of Lubumbashi), exceeding the baseline value of the Centers for Disease Control and Prevention (CDC).

Remediation of polluted soil in the outskirts of Lubumbashi should be a priority in view of the demographic expansion of the city. A variety of soil remediation methods exists, but most of them are expensive, technically complicated and cause additional adverse side effects on the environment (Cunningham and Ow, 1996). Therefore, a number of green technologies have been proposed. Phytostabilisation is the use of tolerant plants and amendments to contain degrade or reduce heavy metal mobility in soil (Berti and Cunningham, 2000; Salt *et al.*, 1995). The aim is to establish a permanent continuous vegetation cover to mitigate erosion, percolation and wind dispersion of contaminants. Plants suitable for phytostabilisation must develop dense root systems that stabilize soil thus preventing metal dispersion by water and/or wind erosion and be tolerant of other adverse soil conditions (Mendez and Maier, 2008; Cunningham and Ow, 1996). Amendments can be used to reduce metal bioavailability by increasing pH or acting as chelates thus facilitating plant establishment (Berti and Cunningham, 2000; Madejon *et al.*, 2006; Mench *et al.*, 2006; Kumpiene *et al.*, 2008).

Phytostabilisation of soil polluted by metal processing industries has been successfully applied using native tolerant and perennial grasses and various inorganic or organic amendments (Smith and Bradshaw, 1970, 1979; Ernst, 1996; O'dell and Classen, 2006). One of the best examples is the revegetation of areas around Sudbury mining and smelting region where the use of lime and tolerant grasses and legumes has permitted the revegetation of 3000 ha and colonization by native tree species (Winterhalder, 1996). In tropical

Africa, successful revegetation trials of mine spoil have been reported in Zimbabwe (Piha, 1995).

In Katanga, the native biodiversity represents a source of potentially useful species for phytostabilisation. Duvigneaud and Denaeyer-De Smet (1963) have described the original metallophyte flora of naturally Cu-enriched soil ("copper hills"). This flora is composed of ca. 600 species and different plant communities have been described. Leteinturier *et al.* (1999) suggested that some metallophyte species might present interesting characteristics for phytostabilisation. However, no experimental assessment of phytostabilisation has yet been performed in Katanga.

This paper reports one of the first phytostabilisation studies of metal-contaminated soil in tropical Africa. The aim was to assess the merits of three native cuprophytic grasses and two amendments for the phytostabilisation of Cu-contaminated soils in the vicinity of Lubumbashi. Our study consists of two experiments where the three grass species were cultivated on soil artificially contaminated with copper and amended with lime or compost. Our specific objectives were; (i) to assess survival, growth and reproduction of grasses on highly copper contaminated soil; (ii) to evaluate the effect of lime and compost amendments on the bioavailability of copper; and (iii) to assess Cu-mobility in soil and Cu-accumulation in the leaves of the three species under the different soil treatments.

MATERIALS AND METHODS

Experimental Plots

The experiments were performed in the experimental garden of the Agronomy Faculty of the University of Lubumbashi (11°27′ S, 27°28′ N and altitude of 1200 m; Temperature: 16–33°C; Annual precipitation: 1200 mm, rain season: November to April) in plots (1 × 12 m) contaminated with Cu at 2,500 mg kg^{-1} in form of copper sulphate ($CuSO_4 \cdot 5\ H_2O$). This very high concentration of Cu was chosen to completely eliminate non-tolerant species (as checked in a preliminary study) and represents a stringent tolerance test for the three species tested. For comparison, the total Cu-concentration (Extractable with HF + $HClO_4$ + HCl) in supposedly uncontaminated soils (*Miombo* forest and cultivated land) around Lubumbashi varies between 116 and 220 mg kg^{-1} (Andres, 2008).

The soil (density = 1.35) was contaminated in November 2005 to a depth of 15 cm with 2.0 kg $CuSO_4$ m^{-2} as follows: The upper 0–7.5 cm layer was removed and 1 kg $CuSO_4$ m^{-2} thoroughly mixed into the bulk soil. A second 1 kg $CuSO_4$ m^{-2} was applied to the soil at 7.5 cm depth and mixed thoroughly with the soil to a depth of 15 cm (from the original soil surface). Subsequently, the upper 0–7.5 cm layer was returned to each plot. This process aimed to obtain a reasonably homogeneous contamination in the 0–15 cm layer.

Two phytostabilisation experiments were established from November 2006 to April 2008 (experiment 1) and from February 2007 to April 2008 (experiment 2).

Species

Three grass species, *Rendlia altera* (Rendle) Chiov. (Synonyms: *R. cupricola* PA Duvign., *Microchloa altera* (Rendle) Stapf), *Cynodon dactylon* (L.) Pers. and *Monocymbium ceresiiforme* (Nees) Stapf. were tested. The three species were selected based on their frequent occurrence and abundance as colonists of Cu-contaminated soil in the area of Lubumbashi (personal observations).

R. altera is a caespitose perennial grass forming compact tufts. In South-East Katanga, this species is frequent on metal-polluted sites where it is one of the first colonisers and can form monospecific stands (Duvigneaud and Denaeyer-De Smet, 1963). It tolerates soil compacting and temporary waterlogging. It has been reported to accumulate 394 $\mu g\ g^{-1}$ Cu and 13 $\mu g\ g^{-1}$ Co in its leaves (Brooks *et al.*, 1987). In this study, vegetatively propagated material was used for planting. This material was collected in November 2006 from a population established at the experimental garden of the Faculty of Agronomy with plants originating from the population near the Lubumbashi Cu-smelter in 2005.

C. dactylon is a stoloniferous perennial grass. It is a cosmopolitan species often used for erosion control and frequently observed on cultivated soil as a weed. In Katanga, it occurs on a wide range of disturbed soils, including mines and other metal-contaminated soils, especially in urban areas (personal observations). It may evolve local Cu-tolerant ecotypes, which can be used for phytostabilisation (Shu *et al.*, 2002). Vegetative material was collected from a population established at the experimental garden with plants originating from a population adjacent to the Lubumbashi Cu-smelter.

M. ceresiiforme is a perennial grass widespread in open savannas and mountain grassland in tropical Africa. This species has been used as a bioindicator of acid soils (Hoare, 1990). In Katanga, it is a characteristic component of steppic savanna on natural metalliferous soil and a colonist of mine deposits. Vegetative material was collected from a population established at the experimental garden with plants originating from the population of the "Mine de l'Etoile" (Lubumbashi).

Soil and Amendments

Soil of the experimental garden of the Agronomy Faculty was used as substrate for plants in this study. It is a ferralitic soil with clay—sandy texture. Before the establishment of the 2 experiments, soil samples were collected on different points of the experimental garden at a depth of 0–15 cm. All samples were mixed into a composite sample for soil description. Total carbon analysed after the method of Walkley and Black (Pauwels, 1992) was 1.9% while the pH (water) was 5.6. Total heavy metal contents (extracted with HF + HCl + $HClO_4$) were 168 mg kg^{-1} Cu, 22 mg kg^{-1} Co, 88 mg kg^{-1} Zn, 52 mg kg^{-1} Pb and 3 mg kg^{-1} Cd. The concentrations of heavy metal extractable with 1M ammonium acetate + EDTA (pH 4.65) were 34 mg kg-1 Cu, 3 mg kg^{-1} Co, 11 mg kg^{-1} Zn, 8.7 mg kg-1 Pb, 0.6 mg kg-1 Cd. These values are regarded as normal for uncontaminated soil in the area (for comparison on the polluted site targeted for phytostabilisation, the AA-EDTA (pH 4.65)-extractable Cu in the 0–20 cm depth varied between 840 and 2450 mg kg^{-1}). In the experimental garden, this soil is colonised by a species-rich weed flora (non Cu-tolerant).

Dolomitic lime ($CaCO_3.MgCO_3$) and organic matter were used as amendments for reducing Cu-mobility in soil. Lime was obtained from 'Carrière de chaux et calcaire' (Likasi, Democratic Republic of Congo). Organic matter was a homemade compost produced using weeds collected from non Cu-contaminated soil (total Cu in soil: 168 mg kg^{-1}) of the experimental garden of Agronomy Faculty after five months of composting. The compost has a pH (water) of 6.5 and content 40 g kg^{-1} of organic C and 0.33% of total N. Total heavy metals (extracted with HF + HCl + HClO4) were 32 mg kg^{-1} Cu, 3 mg kg^{-1} Co, 0.6 mg kg^{-1} Cd, 0.2 mg kg^{-1} Pb, 48 mg kg^{-1} Zn.

Experimental Design and Treatments

The two experiments were conducted using a randomised complete block design with six and five blocks for experiment 1 and 2, respectively. In both experiments, each treatment was represented once in a block. In experiment 1, the three species were separately cultivated on 1 m x 1 m plots. The treatments were as follows: uncontaminated (control), contaminated with 2,500 mg kg^{-1} Cu unamended (NA), Cu-contaminated amended with 4.5 kg compost/m^2 (Cu-compost1) and Cu-contaminated amended with 0.2 kg lime m^{-2} (Cu-lime1). In experiment 2 (*R. altera* only), the treatments were as follows: contaminated with 2,500 mg kg^{-1} Cu amended either with 22.5 kg compost m^{-2} (Cu-compost 2) or with 1 kg lime m^{-2} (Cu-lime2).

The plants (10 cm cuttings) were transplanted in November 2006 at the density of 16 individuals m^{-2}.

Measurements

Plants. For each species, survival, growth, and reproduction were monitored. Survival was checked in April 2007, and April 2008, for all species. Growth was estimated by the length of creeping shoots (*C. dactylon*) or tuft diameter (*R. altera* and *M. ceresiiforme*). Reproduction was assessed as the number of floral culms and the number of spikes (*R. altera* and *M. ceresiiforme*). Measurements were performed on all surviving plants.

In April 2008, fresh leaf samples were collected from individuals of *R. altera* and *M. ceresiiforme* for determination of Cu-content. Each plant sample was carefully washed in Alconox 1% (manufacturer catalogue ref. 1104 by Alconox Inc.) and rinsed in water as described by Faucon *et al.* (2007) and dried at 60°C for at least 48 h. Mineralisation was done by ashing 1–1.5 g samples in a muffle furnace at 550°C for 12 h. Ash was then dissolved with hydrochloric acid 30% p.a. MERCK Suprapur (Ref 100.318.) and filtered. Cu bound in silica (after filtering) was recovered by destroying silica with hydrofluoric acid 40% p.a. MERCK Suprapur (Ref. 100.335) after ashing the filter paper in muffle furnace at 550°C for 12 h. Copper was then determined by ICP-OES (Varian Vista MPX). Plant analyses were performed at the Laboratory of Plant Ecology and Biogeochemistry of the Université Libre de Bruxelles (Belgium).

Influence of Amendments on pH and Copper Mobility. Soil pH and Cu mobility were determined on soil samples collected in April 2007, and April 2008, for both experiments (0–15 cm). Before analyses, soil samples were dried and sieved with 2-mm mesh. Soil pH (H_2O) was measured using a glass electrode. Cu bioavailability was determined using 0.01 M $CaCl_2$. Five grams of soil were mixed with 50 ml of $CaCl_2$ 0.01 M and shaken for 72 h (Van Ranst *et al.*, 1999). Cu was then determined by ICP-OES. All soil analyses were performed at the Laboratory of Plant Ecology and Biogeochemistry of the Université Libre de Bruxelles.

Statistical Analyses. For each plot (treatments) mean and standard deviation of all surviving replicates were calculated. Statistical analyses were carried out separately for each species. Data from plants and soil were analysed by main-effect analysis of variance (ANOVA) without replication with block and treatments as main effects. When the effect of treatment was significant, post-hoc multiple comparison were performed with Fisher LSD test. All data were log-transformed before ANOVA. All statistical analyses were carried out using Statistica 7 (Statsoft, 2005).

Results

Plant Colonisation on Uncontaminated and Contaminated Soils. Uncontaminated plots were quickly and densely colonised by the species-rich weed flora from the experimental garden (*Acanthospermum hispidum* DC, *Ageratum conyzoïdes* L., *Bidens pilosa* L., *Cynodon dactylon* (L.) Pers., *Imperata cylindrica* (L.) Raeusch., *Setaria cfr pallide-fusca* (Schumach.) Stapf & C.E.Hubb., *Nicandra physaloides* (L.) Gaertn., *Pennisetum polystachyon* (L) Schult., *Rhynchelytrum roseum* (Nees) Stapf & C.E. Hubbard, *Tithonia diversifolia* (Hemls.) A. Gray, *Spilanthes cf. acmella* (L.) Murray, *Aspilia africana* (Pers.) C.D. Adams, *Crassocephalum houstonianum, Portulaca oleracea* L., *Cyperus vulgaris* Kunth, *Commelina diffusa* Burm. f., *Euphorbia hirta* L. In contrast, there was no plant colonisation on Cu-contaminated plots during two years.

Effect of Amendments on pH and Bioavailable Cu. Analysis of variance revealed a significant effect of treatments on soil pH and extractable Cu while there was no block effect (Table 1). The largest pH (Table 2) increase was found after liming application in both experiments (0.2 kg m^{-2}: from 4.7 ± 0.1 to 5.2 ± 0.2; 1 kg m^{-2}: from 4.7 ± 0.1 to 6.6 ± 0.2 in April 2008), while compost amendment did not significantly influence pH compared with unamended plots.

In plots amended with 1 kg lime m^{-2}; pH increased steadily up to 7.4 ± 0.3 in April 2007, thereafter decreased to 6.6 ± 0.2 in April 2008.

In experiment 1, the lowest concentration of 0.01 M $CaCl_2$-extractable Cu in soil (Table 1) was found in the control uncontaminated plots (2.1 ± 0.8 mg kg^{-1}) compared to all treatments on contaminated plots ($P < 0.001$). In April 2007 0.01 M $CaCl_2$-Cu concentrations were 2.5 to 3 times lower with 0.2 kg lime m^{-2} (129 ± 88 mg kg^{-1}) compared Cu-contaminated unamended plots (376 ± 85 mg kg^{-1}) and Cu-contaminated

Table 1 Analysis of variance of pH and Cu concentration in soil

	df	F	F
		April 2007	April 2008
Experiment 1			
pH			
Bloc	5	0.74 NS	2.80 NS
Treatments	3	9.46***	36.00***
Error	15		
Cu			
Bloc	5	0.33 NS	1.09 NS
Treatments	3	126.20***	951.26***
Error	15		
Experiment 2			
pH			
Bloc	4	0.94 NS	0.26 NS
Treatments	1	357.60***	136.08***
Error	4		
Cu			
Bloc	4	3.84 NS	0.64 NS
Treatments	1	719.28***	5722.31***
Error	4		

NS = No significant difference; * = Significant difference ($P < 0.05$); ** = Highly significant difference ($P < 0.01$); and *** = Very highly significant difference ($P < 0.001$).

Table 2 pH and extractable Cu in different soil treatments at two dates (6 and 18 months after amendment). Means ± SD. Means followed by the same letter are not different after LSD test (0.05)

	pH		Cu $CaCl_2$ 0.01 M (mg kg^{-1})	
	2007	2008	2007	2008
a. Experiment 1				
Control	5.3 ± 0.1 a	5.0 ± 0.1 A	2.1 ± 0.9 c	2.1 ± 0.8 C
Cu Unamended	4.7 ± 0.1 b	4.6 ± 0.1 B	376 ± 85 a	399 ± 66 A
Cu Compost 1 (4.5 kg m^{-2})	4.6 ± 0.2 b	4.6 ± 0.1 B	329 ± 55 a	351 ± 55 A
Cu Lime 1 (0.2 kg m^{-2})	5.2 ± 0.2 a	5.2 ± 0.2 A	129 ± 88 b	96 ± 13 B
b. Experiment 2				
Cu Compost 2 (22.5 kg m^{-2})	4.8 ± 0.1 f	4.8 ± 0.2 F	160 ± 87 e	250 ± 20 E
Cu Lime 2 (1 kg m^{-2})	7.4 ± 0.3 e	6.6 ± 0.2 E	0.8 ± 0.3 f	0.2 ± 0.04 F

compost-amended plots (329 ± 55 mg kg^{-1}). No significant difference was found between Cu concentration in unamended and compost-amended plots. In experiment 2 there was a significant difference in 0.01 M $CaCl_2$-extractable Cu between lime and compost treatments ($P < 0.001$). Extractable Cu was strongly reduced on plots amended with 1 kg lime m^{-2} (0.8 mg kg^{-1}). In contrast, the effect of compost (22.5 kg m^{-2}) was more limited (160 ± 87 mg kg^{-1} Cu). A similar pattern was observed in April 2008.

From April 2007 to April 2008, concentration of 0.01 M $CaCl_2$-extractable Cu increased in plots amended with compost in both experiments and in unamended plots (Table 2). The largest increase was observed in experiment 2 for the compost treatment (22.5 kg m^{-2}) with $CaCl_2$-extractable Cu increasing from 160 ± 87 to 250 ± 20 mg kg^{-1}. In contrast, in both experiments the opposite situation was observed for the Cu-contaminated plots amended with lime where the concentration of $CaCl_2$-extractable Cu in soil decreased in Cu-contaminated plots amended with lime applied at 0.2 kg m^{-2} and 1.0 kg m^{-2} with values of 129 ± 88 and 96 ± 13 and 0.8 ± 0.3 and 0.2 ± 0.04, respectively.

Plant Survival. For *R. altera*, in experiment 1 survival at both sampling dates (Figure 1) was significantly lower ($P < 0.05$) in the control plots (<20% survival rate) as compared to contaminated plots (>50% survival rate). No significant difference was observed among the three Cu-contaminated treatments i.e. amendments did not affect survival. However, in experiment 2, survival did not vary from April 2007 to April 2008 in all treatments (Figure 2). At both dates survival was higher on plots amended with 22.5 compost kg m^{-2} (81%) compared to plots amended with 1 kg lime m^{-2} (54%) ($P < 0.05$).

For *M. ceresiiforme,* in April 2007 survival was similar on control plots and plots amended with compost (>45%) (Figure 1). There was a significant difference between control and contaminated plots unamended or amended with lime ($P < 0.05$). In April 2008, survival had not changed except for a steady decrease on contaminated plots amended with compost (23%).

In April 2007, highest survival ($P < 0.001$) of *C. dactylon* (Figure 1) was observed in the control (100%) and the Cu-contaminated 0.2 kg m^{-2} lime treatment (99%) as compared to the other two contaminated treatments (<20%). In April 2008, survival was still 100% in the control. In contrast, no plant survived in contaminated plots either unamended or amended with compost. In the plots amended with lime, 36.5% of the plants survived.

Effect of Amendments on Plants Growth and Reproduction. For *R. altera*, tuft diameter did not vary among treatments in both experiments (Tables 4 and 5). For

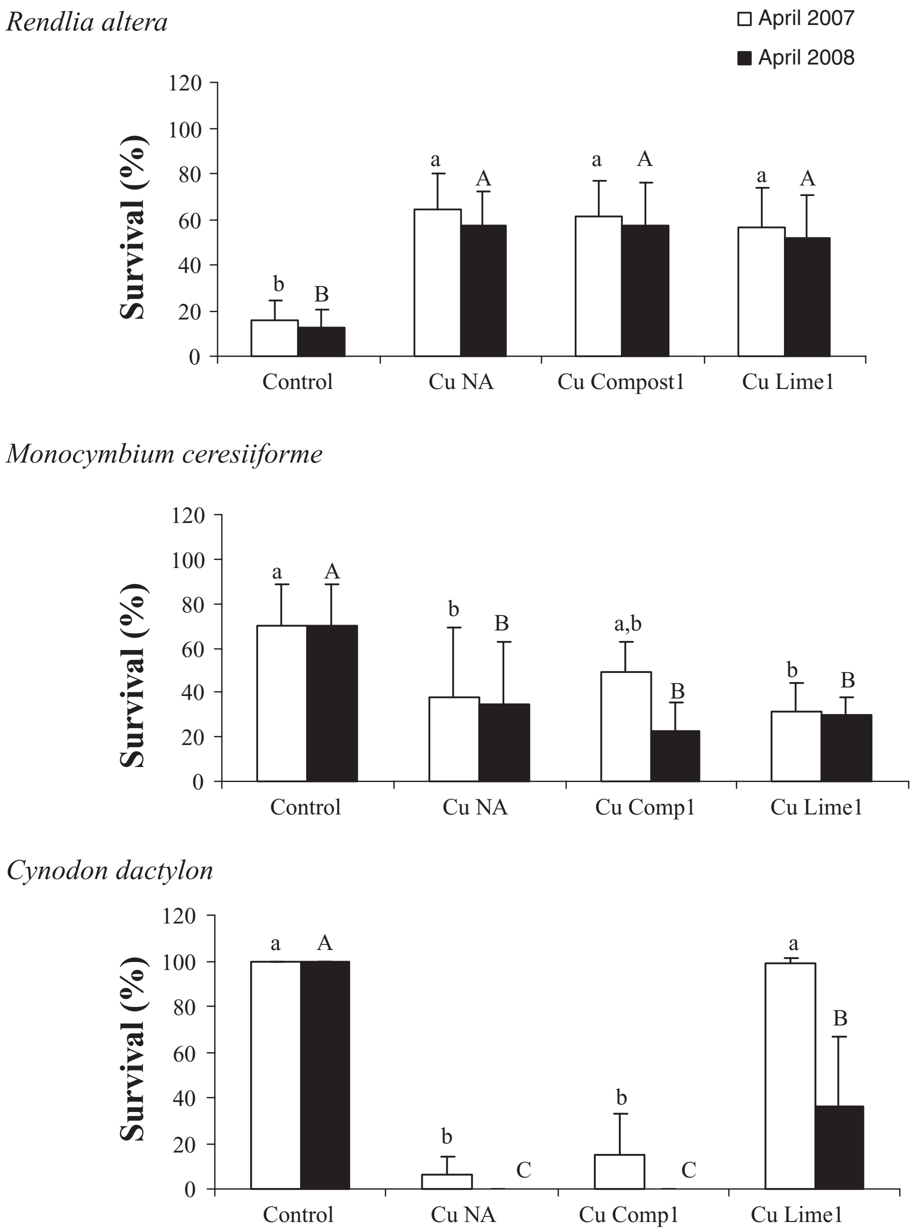

Figure 1 Survival of *R. altera*, *M. ceresiiforme* and *C. dactylon* 6 and 18 months after transplanting in the Experiment 1. Means ± SD. Control (uncontaminated, unamended), Cu NA (contaminated, unamended), Cu Comp1 (contaminated, 4.5 compost Kg m^{-2}), Cu Lime (contaminated, 0.2 lime Kg m^{-2}). Different letters represent significant difference (0.05) after LSD test.

M. ceresiiforme, growth and reproduction responded strongly to the different amendments. ANOVA (Table 3) showed a significant variation for tuft diameter with the highest diameter ($P < 0.001$) in 0.2 kg lime/m^2 treatment compared to unamended and compost treatments. There was no significant difference between unamended and compost treatments.

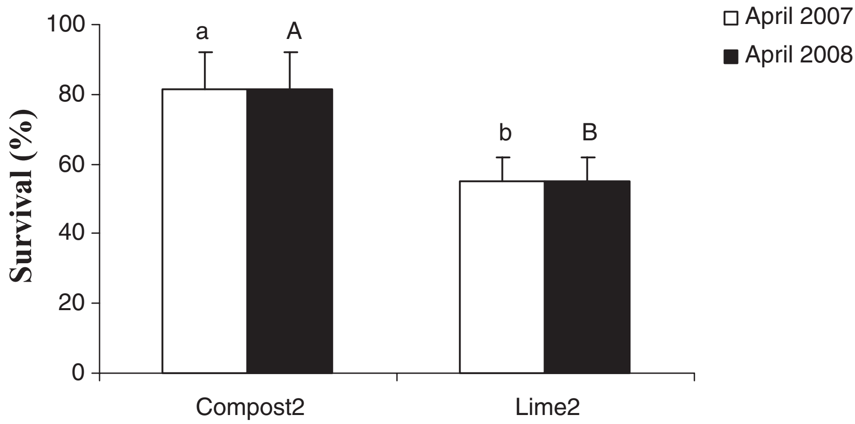

Figure 2 Survival of *R. altera* 15 months after transplanting in the Experiment 2. Means ± SD. Comp2 = 22.5 Kg compost m^{-2}; and Lime2 = 1 Kg lime m^{-2}. Different letters represent significant difference (0.05) after ANOVA.

The number of floral culms per individual of *M. ceresiiforme* (Table 4) was significantly different ($P < 0.001$) among treatments with the highest values observed for 0.2 kg m^{-2} lime Cu-contaminated amended treatment (22.8 ± 9.9). For *R. altera*, in Experiment 1 the number of floral culms and number of spikes per individual (Table 4) were significantly higher ($P < 0.01$) in the 0.2 kg m^{-2} lime amended Cu-contaminated treatments as compared with the Cu-contaminated unamended and 4.5 kg m^{-2} compost treatments with values of 37.5 ± 8.0 and 211 ± 61.0, 23.0 ± 10.0 and 125.0 ± 54.0 and 28.9 ± 8.9 and 134 ± 54.0, respectively. In Experiment 2, only the number of spikes per individual was found to vary significantly ($P < 0.001$) between treatments with the highest value associated with the application of 22.5 kg compost m^{-2}. No significant difference was observed in the number of floral culms between the lime 1.0 kg m^{-2} and 22.5 kg compost m^{-2} treatments (Table 5).

For *C. dactylon* as no plant survived on contaminated plots unamended and compost-amended, no ANOVA was applied. Mean length of plants on plots amended with 0.2 kg lime/m^2 was 42.5 ± 22.3 cm with limited soil cover.

Effect of Amendments on Cu Accumulation in Leaves. For *R. altera*, Cu accumulation in leaves varied significantly among treatments. In experiment 1, Cu concentration was highest ($P < 0.001$) in leaves on contaminated plots unamended or amended with compost (66.2 ± 26.4 and 76.3 ± 22.1 mg kg^{-1} Cu), followed by plots amended with lime (35.6 ± 12.6 mg kg^{-1}) and the control (<20 mg kg^{-1}) (Figure 3). In experiment 2, Cu-accumulation was lower ($P < 0.05$) on plots amended with 1 kg lime m^{-2} (25.2 ± 3.4 mg kg^{-1}) than plots amended with 22.5 kg compost m^{-2} (38.4 ± 11.3 mg kg^{-1}) (Figure 4).

The same pattern holds true for *M. ceresiiforme*, except that the lime treatment reduced Cu to the same level as in the uncontaminated treatments (<60 mg kg^{-1}). In all treatments, *M. ceresiiforme* accumulated higher Cu concentrations in its leaves compared to *R. altera*.

Discussion

Effect of Amendments on Soil. Contamination of soil with $CuSO_4$ has acidified soil to pH values where Cu is highly bioavailable for plants (Reddy *et al.*, 1995). This can

Table 3 Analysis of variance of tuft diameter, number of floral culms, number of spikes and Cu concentration in leaves of *R. altera* and *M. ceresiiforme*

	df	F *R. altera*	F *M. ceresiiforme*
Experiment 1			
Tuft diameter			
Bloc	5	0.56 NS	2.92 NS
Treatments	2	0.04 NS	38.32***
Error	10		
Number of floral culm			
Bloc	5	1.39 NS	1.39
Treatments	2	8.25**	19.90***
Error	10		
Number of spikes			
Bloc	5	1.19 NS	
Treatments	2	9.29**	
Error	10		
Experiment 2			
Number of floral culms			
Bloc	4	1.16 NS	
Treatments	1	2.01 NS	
Error	4		
Number of spikes			
Bloc	4	4.72 NS	
Treatments	1	86.38***	
Error	4		
Cu concentration in leaves			
Experiment 1			
Bloc	5	1.469 NS	0.4 NS
Treatments	2	35.962***	33.97***
Error	10		
Experiment 2			
Bloc	4	1.192 NS	
Treatments	1	8.343*	
Error	4		

NS = No significant difference; * = Significant difference ($P < 0.05$); ** = Highly significant difference ($P < 0.01$); and *** = Very highly significant difference ($P < 0.001$).

explain why the species-rich weed flora of the experimental garden failed to establish on plots contaminated with 2,500 mg kg^{-1} added Cu. This demonstrates that 2,500 mg kg^{-1} of added Cu represents a stringent tolerance test for plants studied for their potential use in phytostabilisation of contaminated soil in Katanga.

Only lime amendment increased soil pH while no variation was observed with compost. Increasing soil pH by amendment with lime is common in agriculture and it is explained by the action of calcium (Kumpiene *et al.*, 2008). However, an increase in soil pH can be transient as observed in experiment 2 where the highest decrease in soil pH from April 2007 to April 2008 was observed on Cu-contaminated plots amended with 1 kg of lime m^{-2} compared to other treatments (Table 2). Derome and Saarsalmi (1999) also reported this situation for a site at Harjavalta (Finland) which they explained by the fact that the initial Ca derived from the treatments may have displaced protons and aluminium from the exchange sites resulting in acidification.

Table 4 Growth and reproduction in experiment 1 in April 2008 (18 months after transplanting)

	Unamended	Compost1 (4.5 Kg m^{-2})	Lime1 (0.2 Kg m^{-2})
Rendlia altera			
Tuft diameter (mm)	53.5 ± 13.8 a	53.3 ± 5.3 a	51.9 ± 9.3 a
Number of floral culm/ind.	23.0 ± 10.3 b	28.9 ± 8.9 b	37.5 ± 8.0 a
Number of spike/ind.	125.3 ± 54.0 b	134 ± 54 b	211 ± 61 a
Monocymbium ceresiiforme			
Tuft diameter (mm)	27 ± 14 b	40 ± 10 b	67 ± 2.0 a
Number of floral culm/ind.	3.3 ± 1.7 b	9.4 ± 5.6 b	22.8 ± 9.9 a
Cynodon dactylon	(−)*	(−)*	42.5 ± 22.3

Means ± SD of plant diameter, number of floral culms/individual and number of spikes/individual. Different letters represent significant difference (0.05) after LSD test.
*(−) = No survivor.

Lime reduced Cu mobility more effectively than compost. Only the application of 22.5 kg compost m^{-2} reduced $CaCl_2$-extractable Cu by more than 50% as compared to the Cu-contaminated unamended plots. The observed reduction in Cu-mobility following lime addition can be explained by an increase in soil pH (Chaignon *et al*., 2002; 2003; Madejon *et al*., 2006), with Cu precipitating as Cu carbonate at high pH which is not bioavailable for plants (Kumpiene *et al*., 2008; Derome and Saarsani, 1997). For the compost amendment, reduction of Cu mobility without pH increase can be explained by the well-known affinity of Cu for organic matter and chelation by humic compounds, which are able to decrease Cu mobility even at low pH values (Narwal and Singh, 1998; Walker *et al*., 2004).

The increase in $CaCl_2$-extractable Cu-concentration in plots amended with compost in both Experiments 1 and 2 in April 2008 (as compared with April 2007) may in large part be due to the decomposition of the applied compost and loss of soil organic matter (Hso and Lo, 2001). Very high mineralisation rate of organic matter in tropical regions could be a serious constraint for reducing Cu-mobility in contaminated soil in Katanga. However, as the nature of organic matter may influence the rate of decomposition and the pH-variation (Zhou and Wong, 2001) we suggest that the choice of organic matter must retain much attention in future studies.

Our results point to lime as the best amendment to reduce Cu bioavailability in our soils. Moreover, lime is relatively cheap and easy to obtain commercially in SE Katanga. However, considering the positive effects of the heavy application rate of compost in experiment 2, the effect of a mixture of lime and compost must be investigated in future studies.

Plant Survival, Growth, and Reproduction. *Rendlia altera* was by far the most Cu tolerant species and seems to be a very good candidate for phytostabilisation of

Table 5 Growth and reproduction of *Rendlia altera* in experiment 2 in April 2008 (18 months after transplanting)

	Cu compost2 (22.5 Kg m^{-2})	Cu lime2 (1 Kg m^{-2})
Number of floral culms/ind.	13.4 ± 1.8 a	11.7 ± 3.8 a
Number of spikes/ind.	77.8 ± 23.5 a	45.1 ± 15.4 b

Means ± SD of plant diameter, number of floral culms/individual and number of spikes/individual. Different letters represent significant difference (0.05) after LSD test.

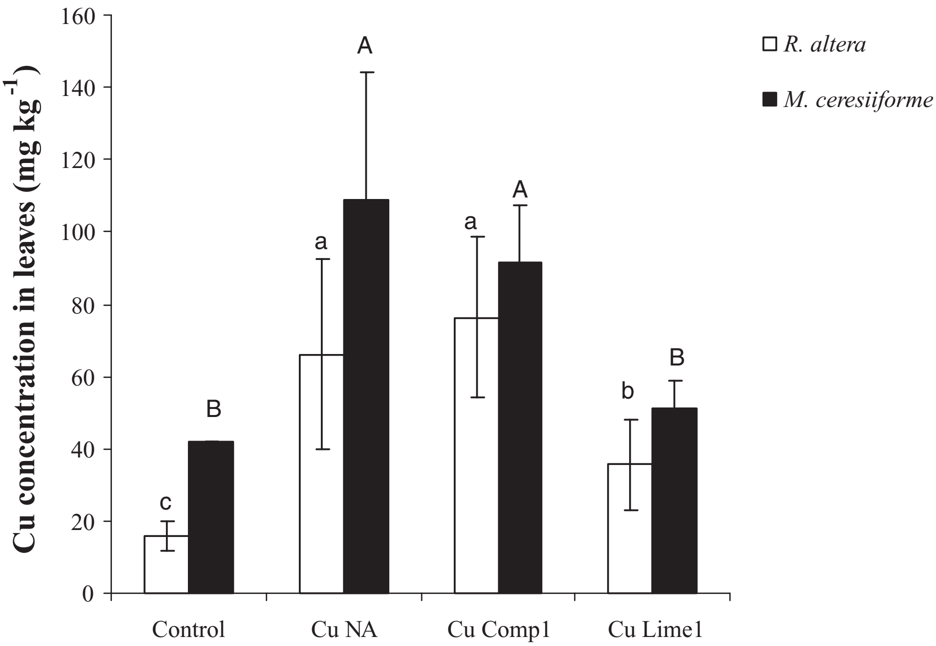

Figure 3 Copper accumulation in leaves of *Rendlia altera* and *Monocymbium ceresiiforme* in experiment 1. Means ± SD. Control = uncontaminated and unamended; Cu NA = contaminated, unamended; Cu Comp1 = contaminated + 4.5 kg compost m^{-2}; and Cu Lime2 = contaminated + 0.2 kg lime m^{-2}. Different letters represent significant difference (0.05) after LSD test.

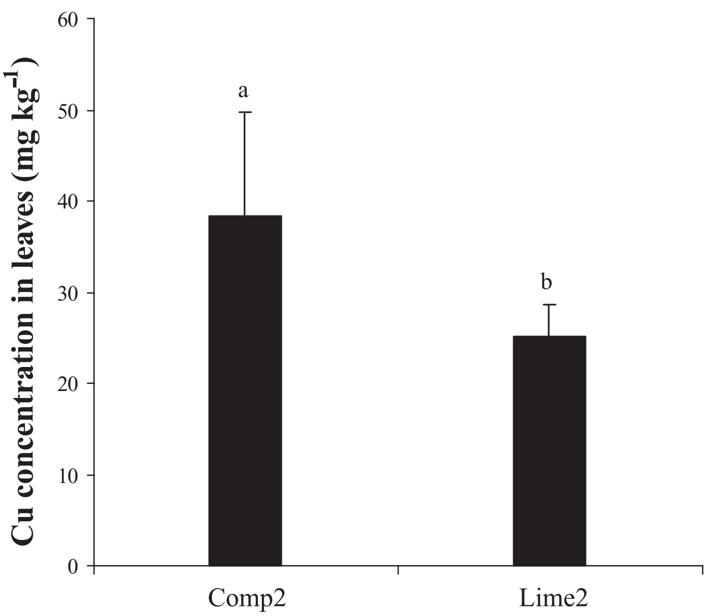

Figure 4 Copper accumulation in leaves of *Rendlia altera* in experiment 2. Means ± SD. Comp2 = 22.5 kg compost m^{-2}; and Lime2 = 1 kg lime m^{-2}. Different letters represent significant difference (0.05) after LSD test.

highly Cu-contaminated soils in Katanga. *R. altera* presented the highest survival in unamended treatments compared to the other species. Amendments did not increase survival rates possibly indicating high intrinsic tolerance of Cu. This also suggests that phytostabilisation with *R. altera* may require lower amounts of amendments compared to other two species.

Survival of *C. dactylon* and *M. ceresiiforme* in uncontaminated plots were higher than *R. altera*. This result needs further investigation since it may indicate elevated Cu requirements in *R. altera*. This would explain why *R. altera* is restricted to Cu-rich soil in Katanga (Duvigneaud, 1958; Duvigneaud and Denaeyer De-Smet, 1963). Another hypothesis is that *R. altera* has high susceptibility to diseases and soil pathogens. This hypothesis has been put forward to explain the fact that certain species of Katangan flora are more abundant on contaminated soils than on normal soils, particularly *Haumaniastrum katangense* (Malaisse and Brooks, 1982; Brooks and Malaisse, 1990; Paton and Brooks, 1996). However, *R. altera* is also present on normal soils in other regions of southern Africa (Pope, 1999; Renvoize, 1974) implicating that Katangan populations may represent a distinct ecotype.

Liming has increased survival of *C. dactylon,* probably due to decreased Cu-bioavailability (Brun *et al*., 2003; Sebastiani *et al*., 2004). Survival of *M. ceresiiforme* on Cu-contaminated plots was not influenced by amendments. Survival of *R. altera* was higher with 22.5 kg compost m^{-2} as compared to 1 kg lime m^{-2}, possibly due to excessively high pH in the latter treatment in year 1 (pH = 7.4). This suggests that the timing of pH stabilisation in soil must be investigated before transplanting.

On Cu-contaminated plots amended with lime *C. dactylon* has not formed a compact mat as compared to control, which may seriously impair soil stabilisation. It appeared that soil cover of *M. ceresiiforme* was better (larger tuft diameter) with lime amendment as compared to the other Cu-contaminated treatments. In contrast, vegetative growth of *R. altera* did not respond to amendments possibly due to constitutive Cu tolerance in this species.

0.2 kg lime m^{-2} or 22.5 kg compost m^{-2} amendments have increased number of floral culms and number of spikes of *R. altera* and *M. ceresiiforme* as compared to unamended plots, suggesting improved seed production. This may be due to decreased copper bioavailability and better macronutrient uptake (Brun *et al*., 2003). Improved seed production is desirable since it may facilitate establishment of dense compact mats on highly Cu-contaminated soil and favour long-term stabilisation of plant cover.

Cu Uptake by Plants. Cu-concentration was lower in leaves of *R. altera* and *M. ceresiiforme* from plots amended with lime, in line with the lower Cu bioavailability in soil (Brun *et al*., 1998). Low Cu-concentrations in the leaves of both species are encouraging because of reducing risk of food chain contamination. However, it is not known whether these grasses are actually grazed.

Cu concentrations in leaves of *R. altera* in this study are much lower compared to values reported in previous studies (220–394 mg kg^{-1}) (Brooks *et al*., 1987). Such high values may have been due to surface contamination by dust as shown by Faucon *et al*. (2007).

Implication for Phytostabilisation. Our results indicate the possibility of using local grass species for phytostabilisation of metal contaminated soils in Katanga. In this study, *Rendlia altera* seems to be the best candidate because of its remarkably high survival and good growth in all treatments. Using lime, *M. ceresiiforme* is another possible candidate. Lime also improved shoot length of *C. dactylon*, but stolons failed to root and plant cover remained poor. Lime (0.2 kg m^{-2}) had a larger positive effect on growth and reproductive parameters than compost (4.5 kg m^{-2}). It also reduced copper accumulation in leaves of *R. altera* and *M. ceresiiforme*. These results are in line with those obtained with liming application for revegetation of barren soils at Sudbury Cu-Ni smelting area (Winterhalder, 1996) where liming has reduced metal bioavailability and increased plant survival, growth and reproduction.

However, the higher survival and number of spikes of *R. altera* obtained with 22.5 kg compost m^{-2} supports the need to combine the positive effect of lime on reduction of Cu bioavailability and translocation to shoots and the positive effect of organic matter on macronutrients availability (Mench *et al*., 2006; Ye *et al*., 2000).

CONCLUSION

This paper reports one of the first experimental phytostabilisation studies of metal contaminated soil in tropical Africa. The results are encouraging, as they indicate the feasibility to use local tolerant grass species, in combination with lime and compost amendments for phytostabilisation of Cu-contaminated soil in Katanga. In particular, *Rendlia altera*, a local cuprophyte in Katanga stands out as a most promising species deserving further investigation. However, this result must be taken with caution, as our experimental soil was contaminated with Cu only while several different metals (Cu, Zn, Pb, ...) occur at elevated levels in the soils contaminated by atmospheric fall out from the Cu-smelter near Lubumbashi. In situ field experiments are urgently needed to test if *Rendlia altera* and *Monocymbium ceresiiforme* can thrive in such conditions. Future studies should also try combinations of compost and lime, and mixtures of several species. Screening of other local metallophytes is also desirable, especially nitrogen fixing species.

ACKNOWLEDGMENTS

Authors, Mylor Ngoy Shutcha and Michel Mpundu Mubemba, contributed equally to this work. This study was carried out in the framework of the project REMEDLU, a collaboration between the Université de Lubumbashi, the Université Libre de Bruxelles and the Faculté des Sciences Agronomiques de Gembloux and funded by the Coopération Universitaire au Développement (CUD, Belgium). We thank all the partners of this project. We also thank Wolf Gruber for his invaluable help for plant and soil analyses, Ckeface Kamengwa Kissi and Jean-Marc Kaumbu for their assistance. Financial support to two of us (Mylor Ngoy Shutcha and Michel Mpundu Mubemba) by the NGO Biodiversité au Katanga (BAK) is also gratefully acknowledged.

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