Dynamics of plant species during phytostabilisation of copper mine tailings and pyrite soils, Western Uganda

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Abstract

Introduction: Destruction of vegetation resources emanating from deposition of mine wastes is a serious environmental problem. Conventional plant species restoration methodologies are costly and feasible only on a small scale. The current study was focussed on developing phytostabilisation protocols involving the application of limestone, compost, selected tree species and assessing the re-establishment of plants in polluted soils.

Methods: Early establishment of plant species under *Eucalyptus grandis*, *Senna siamea* and *Leucaena leucocephala* planted on mine tailings and pyrite soils amended with compost, limestone and limestone+compost was studied. Four plant inventories were conducted on the study plots and surrounding plant communities, involving enumeration of the plant species and estimation of their ground covers. Physico-chemical characteristics of the soils of the study plots were determined each time an inventory was conducted. Data were analysed using R statistical packages *vegan* and *lme4*.

Results: Mine tailings and pyrite soils had extremely low pH, poor nutritional status, low organic matter content and elevated concentrations of heavy metals as compared to the unpolluted soils. Before treatment, species richness, diversity and plant cover were extremely low with most of the ground being completely bare. Treatment of the soils significantly improved the physico-chemical characteristics starting a plant succession that increased the number of species from 18 to 215 different species, belonging to 131 genera and 34 families. Plots of the leguminous tree species *Senna siamea* and *Leucaena leucocephala* had significantly more species than the non-leguminous *Eucalyptus grandis*. Early changes in species composition of the restoration plots were minimal. Correspondence analysis (CA) revealed significant differences in species composition between the experimental plots and the plots at the unpolluted site.

Conclusion: Application of amendment material that significantly alters the physico-chemical characteristics of mine wastes is pre-requisite for their phytostabilisation. Leguminous tree species *Senna siamea* and *Leucaena leucocephala* have a higher potential for phytostabilisation of pyrite and copper tailings as their growth led to the establishment of understory plant communities with higher species diversity and cover.

Keywords: Compost, correspondence analysis, dynamics, limestone, phytostabilisation, pyrite, restoration, tailings

Introduction

Mining activities all over the world are known to generate enormous volumes of wastes that are dumped in various places that suffer from low water retention capacity [22], high electrical conductivity [13], extreme pH, low cation exchange

capacity, pollution by heavy metals and low organic matter and nutrient concentration [1,3,5]. These physico-chemical conditions persist in the environment for a long period of time impeding the establishment of plant communities on the wastes while inflicting damage to nearby plant communities

and causing serious environmental pollution. The polluted bare grounds consequently become susceptible to wind and severe water erosion, which may result in the contamination of nearby water bodies and soils with toxic metals [15]. Ecologically sound and cost effective means of controlling these negative consequences would in most cases involve reestablishment of plant communities that provide cover and help to phytostabilise the wastes.

Enormous volumes of both pyrite materials and copper tailings to the tune of 1.13 and 15 million metric tonnes were respectively generated from 1956 to 1982. These were respectively dumped in Western Uganda near Queen Elizabeth Conservation Area (QECA) to form a large cobaltiferous stockpile and in various places in Kilembe to constitute four tailings dams. The tailings dams and the cobaltiferous stockpile have remained devoid of vegetation since suspension of mining activities in 1982 leading to wide dispersal of pyrite materials laden with heavy metals into gardens and surrounding aquifers at Kilembe and into QECA to form the largely bare pyrite trail. These deleterious effects of copper mining activities on vegetation in Kilembe and QECA are still a major environmental concern in the area due to their persistence over years.

Mine wastes can cause significant environmental problems in the surrounding areas if they are not reclaimed [4], remaining exposed to different forms of erosion leading to heavy metal pollution. The heavy metal pollution in the environment often has a catastrophic impact on the structure and floristic composition of plant communities [36]. Even though there are different techniques that can be used for remediation, the establishment of a stable plant cover on the wastes is considered a suitable option to get long term reclamation [58]. The established vegetation may improve the nutrient conditions of the soil [12] and set the base for establishment of self-sustaining vegetation cover [11].

Ecological studies reveal that metalliferous mine wastes, such as tailings, can also be colonized by plants, starting with seeds or vegetative propagules that have entered the site, either from the surrounding vegetation or after a long-distance transport by wind or water [10]. However, plant succession in the metalliferous habitats is extremely slow and always leads to establishment of plant communities that are characterised by low species diversity for a prolonged period of time. Extremely slow natural colonisation has been going on in Kilembe, without any large areas being restored in the periphery of the tailings dams where fertile soils have been washed into the area by erosion and in the pyrite trail areas that receive fresh water. Coupled with being slow, plant succession within the pyrite trail has been rendered ineffective owing to regular resurgence of pollution when fresh seasonal inflow of heavily polluted storm water import polluted sediments into areas already recolonised by plants. Rather than the natural processes taking charge, this calls for human interventions in the revegetation of these areas. Such interventions have

also been recommended by [20], who suggested speeding up the regeneration process through mimicking the natural soil processes.

There are a number of changes in physico-chemical characteristics of soils that come along with the establishment of trees on a particular site that can effectively enhance plant species regeneration on acid mine waste soils. Trees produce sufficient biomass which can add more organic material than other plants to the soil both above and below ground [62]. Shrubs and trees can provide a more extensive canopy cover and establish a deeper root network for long-term erosion prevention [51], which is known to be one of the hindrances to plant species re-establishment. Shrubs and trees provide a high nutrient environment for undergrowth of plant species while reducing moisture stress and improving the physical characteristics of the soil in arid and semiarid regions [7,37,54]. Furthermore, trees can decrease metal mobility and toxicity by root growth. However, tree species differ in their capacity to modulate the soils to suitable conditions for reestablishment of other plant species. For example they are not equally effective soil generators [23]. Therefore selection of appropriate tree species is very critical to ensure establishment of self sustainable vegetation cover [59], hence a need to examine the locally growing trees for their suitability.

Reclamation programs on mine soils provide a rare opportunity to examine ecosystem development from scratch [45]. To our knowledge, no field reclamation trials involving the use of tree species have been conducted to examine their influence on dynamics of species during the redevelopment of ecosystems on barren pyrite and copper tailings. The aim of this study was to investigate the effect of applying limestone, compost and establishment of *Leucaena leucocephala* (Lam.) De Wit., *Senna siamea* (Lam.) H.S.Irwin & Barneby and *Eucalyptus grandis* W. Hill ex Maid., on pyrite and copper tailings on physico-chemical characteristics, colonisation and species enrichment during a phytoremediation process. The tree species selected for the study were locally growing in the area, known to be drought resistant and to grow very fast. *Leucaena leucocephala* is known to be suitable for effective and yet cheap inexpensive restoration of degraded mining sites through afforestation [34]. In addition, its leaf litter is essential resource for improving increases soil organic matter (SOM) and other essential nutrients including phosphorus that will keep on accumulating with time to create favorable environment for other important native plant species to colonize the site [33]. *Eucalyptus grandis* is known to exhibit great environmental plasticity with ability to grow in impoverished soils [2] while *Senna siamea* is tolerant to both limestone and moderately acid soils [28] and capable of growing on degraded infertile soils [30]. The significance of this study is to provide inexpensive and ecologically sound methodologies of stimulating the emergence of several plant species in the degraded areas leading to the establishment of bio-diverse and self-sustaining ecosystems. The ecosystem will

contribute to the stabilisation of degraded areas, control of further pollution, visual improvement and removal of threats to human beings and game animals. We hypothesized that soil improvements through amendment application and subsequent establishment and growth of the selected tree species would enhance plant species regeneration in the degraded areas.

Methodology

Description of study site

The study area comprised of the pyrite trail in Queen Elizabeth Conservation Area (QECA) located at the geographical coordinates of latitude 0° 8'53.03"N, longitude 30° 4'27.53"E and altitude of 949 meters above sea level and the four tailings dams in the vicinity of Kilembe Town area located at latitude of 0°11'16.12"N, longitude of 30°1'11.43"E and altitude of 1243 meters above sea level in Western Uganda (Figure 1). The area has a tropical climate with rainfall which is bi-modally distributed with the wetter periods occurring from March to May and August to November. The tailings dams were levelled at the top during the dumping process. The flattened top surface is covered by very fine polluted powdery soils that are easily transferred into nearby gardens and River Nyamwamba by eolian dispersal during the dry season. The pyrite trail is characterised by bare patches dotted with thickets of *Capparis tormentosa* Lam., trees of *Acacia gerrardii* Benth, *Acacia sieberiana* DC. and *Balanites aegyptiaca* (L.) Del. and islets of vegetation composed of *Phytolacca dodecandra* L Hérit, *Fimbristylis ferruginea* (L.) Vahl, *Imperata cylindrica* (L.) P.Beauv, *Sporobolus pyramidalis* P. Beauv., *Typha latifolia* L. and *Cynodon dactylon* that covers most of the regenerated part of the pyrite trail. The surrounding vegetation consists largely of *Acacia savannah* woodland.

Experimental design

The study area was categorised into four study sites coded as Kilembe tailing dams site (KTDS), low polluted pyrite trail site (LPPTS), highly polluted pyrite trail site (HPPTS) and unpolluted site (UPS). The categorisation was based on the results of the geochemical survey of the eight zones that were mapped out covering the entire study area. A split block experimental design was used with site as a blocking factor and with two treatment factors as amendment type categorised into un-amended (UA), limestone (LS), compost (Comp) and limestone+compost (LS+Comp) and the tree species grown. For purposes of comparison to gauge the regeneration success the UPS was included in the study. Establishment of plots, amendment applications in the sub-plots and planting of experimental tree species was done as per the description of [54].

Data collection

Four inventories of all plant species were conducted when most of the plant species would be identifiable, usually in the

month of June or December. Each sub-plot was thoroughly scanned for understory plant species. The common plant species in each sub-plot were identified in situ and recorded. The few species that could not be identified in the field were collected and later taken to the herbarium at Makerere University, Department of Biological Sciences, for proper identification. For each plant species recorded, its plant cover in the entire sub-plot was estimated by visual inspection [41] following cover classes of 1=0-5%, 2=5-25%, 3=25-50%, 4=50-75%, 5=75-95%, and 6=95-100% [16]. To ensure reliability of estimates, four people were involved and consensus was always sought for each species.

Soil sample collection

Rhizospheric soils were collected at the same time the tree species were harvested for chemical analysis and a plant inventory was conducted. Sampling of the soil was undertaken using a 25 mm diameter stainless steel tube which was pushed to a depth of 30 cm and the core extruded from it with the aid of a stainless steel rod. The soil samples were picked in triplicates from each sub-plot whenever plant inventory was conducted. The samples were packed into labelled polythene bags, sealed and taken to the laboratory for physico-chemical characterisation.

Physico-chemical characterisation of soil samples

Physico-chemical characterisation of the soils samples was done at National Agricultural Research Laboratories (NARL) at Kawanda following standard procedures. Soil pH (soil: deionised water=1:2.5 w/v) was determined using a calibrated pH meter, organic matter content by Walkley-Black potassium dichromate wet oxidation [39] as described by [41] and total nitrogen was determined by the semi-micro Kjeldahl method [8]. Extraction of available phosphorous and heavy metals was done using Mehlich 3 extractant. In brief, the soil sample was dried in an oven at 45°C for 48 hours. The dried sample was pulverized to pass through a 2 mm sieve to remove any coarse particles. The sample was then sub-sampled to a very fine powder in a mortar. The dry sample (3g) was weighed into a 50 ml centrifuge vial and 30 ml of Melich 3 extractant was added. The mixture was then shaken at 200 rpm for 5 minutes and later left to stand for 10 minutes for settling before centrifuging at 2000 rpm for 5 minutes. The available phosphorus in the extract was determined following Ammonium Molybdate-Ascorbic acid method [31] using a UV/Visible spectrophotometer at 860 nm. The heavy metal concentrations representing largely available concentrations for plant uptake was determined with an atomic absorption spectrophotometer.

Data analysis

All statistical analyses were performed using R statistical package 2.13.2 [48] with the additional packages vegan [42] and lme4 [6]. Pearson correlation analysis was adopted to

Figure 1. Location of the experimental sites at the tailings dams in Kilembe and the pyrite trail in QECA.

establish the relationship between the established community characteristics and soil physico-chemical characteristics. A multivariate correspondence analysis (CA) was run to establish the variation in species composition that exists between the different plots at different sites and the species relationships with site factors. Mixed models of package lme4 were used to analyse variability of soil physico-chemical characteristics. For each parameter, the model was tested for normality and homogeneity of variance by the normal (Q-Q) plot and the plot of residuals against fitted values respectively. In case of deviations from normality or homoscedasticity, the statistical assumptions of the analysis could be fulfilled by using log-transformation.

Results and discussion

Physico-chemical characteristics of soils

Before treatments, the soils were characterised by extremely low pH values and organic matter content, poor nutritional status with respect to available phosphorous and total nitrogen, and high concentrations of available heavy metals. Elevated bioavailability of metals and low organic matter content, and extremely low pH values have also elsewhere been reported to be associated with soils of comparable nature [3,5,49]. All of the untreated copper tailings and pyrite soils were acidic with mean pH values ranging between 2.21 and 4.28 and significantly lower than the mean pH value of the unpolluted soils (Table 1). This range is characterized as extremely acid [57] and not suitable for plant growth due to

increased metal toxicities such as magnesium or manganese and reduced population of nitrogen fixing bacteria [49]. The occurrence of such low pH values most especially in pyrite soils from the LPPTS and HPPTS was possibly due to existence of unweathered pyrite materials, which usually contain a lot of pyritic sulphur in excess of their neutralizers (carbonates). Upon exposure to water and oxygen the sulphur species usually drop the soil pH to the range of 2.2-3.5 [49].

Amendments of the soils and planting of trees in this study significantly changed the physico-chemical characteristics of the soils to ranges that are suitable for the growth of plants at most of the sites. The mean pH rose to a range of 5.39 to 7.74 (Figure 2), which is characterised as strongly acidic to slightly alkaline [57]. The strongly acidic conditions were associated with soils amended with compost alone on which *Senna siamea* and *Leucaena leucocephala* were growing. The mean pH values of most of the amended sub-plots were well above the pH of 5.5 that was also reported by [38] and [43] to have favoured the germination of a number of plant species in the area under field conditions. Soil pH varied significantly across the tree species grown (linear mixed model, $\chi^2=12.69$, $df=2$, $p<0.01$) and amendments applied ($\chi^2=332$, $df=3$, $p<0.001$). The limestone and limestone+compost amended soils had significantly higher pH than the compost amended and unpolluted soils (Tukey's test, $p<0.05$). Variation across the sites was not significant ($\chi^2=3.94$, $df=2$, $p>0.05$).

The copper tailings and pyrite soils had significantly lower organic matter content, total nitrogen and available

Table 1. Mean ($\pm$ SEM, n=9) pH, organic matter content and Melich 3 extractable concentrations of phosphorous and heavy metals of unpolluted soils from UPS, copper tailings from KTDS and pyrite soils from LPPTS and HPPTS sites.

Parameter	Sites			
	UPS	KTDS	LPPTS	HPPTS
pH	6.29 $\pm$ 0.11c	4.28 $\pm$ 0.85b	3.41 $\pm$ 0.16b	2.21 $\pm$ 0.44a
Organic matter (%)	8.82 $\pm$ 1.84b	3.61 $\pm$ 0.94a	2.59 $\pm$ 0.84a	3.68 $\pm$ 1.08a
Total N (mg kg ⁻¹)	0.39 $\pm$ 0.06b	0.17 $\pm$ 0.03a	0.19 $\pm$ 0.08a	0.16 $\pm$ 0.07a
Phosphorous (mg kg ⁻¹)	91.49 $\pm$ 13.26b	6.18 $\pm$ 1.25a	7.30 $\pm$ 1.08a	4.48 $\pm$ 1.14a
Copper(mg kg ⁻¹)	8.14 $\pm$ 1.80a	15.11 $\pm$ 4.89a	24.30 $\pm$ 6.23ab	43.44 $\pm$ 13.39b
Cobalt (mg kg ⁻¹)	2.40 $\pm$ 0.47a	10.59 $\pm$ 3.21a	39.14 $\pm$ 9.68a	115.39 $\pm$ 12.06b
Nickel (mg kg ⁻¹)	1.25 $\pm$ 0.15a	6.43 $\pm$ 2.69a	4.38 $\pm$ 0.79a	18.39 $\pm$ 4.68b
Lead(mg kg ⁻¹)	trace	2.25 $\pm$ 0.31a	2.38 $\pm$ 0.25a	1.99 $\pm$ 0.37a

Means in each row followed by different letters are significantly different at $p < 0.05$, Tukey's HSD test.

phosphorous than unpolluted soils (Tukey's test, $p < 0.05$). The low organic matter content was attributed to the prolonged absence of plant growth, addition and decomposition of litter and low microbial activity. This partly accounts for the low available phosphorous and total nitrogen that are sometimes derived from mineralization of soil organic matter. Mean organic matter content varied significantly across sites ($\chi^2 = 28.02$, $df = 2$ and $p < 0.001$), tree species grown ($\chi^2 = 11.26$, $df = 2$, $p < 0.001$) and treatments applied ($\chi^2 = 45$, $df = 3$, $p < 0.001$). Total nitrogen content varied significantly across sites ($\chi^2 = 12.12$, $df = 2$, $p < 0.05$), tree species grown ($\chi^2 = 9.72$, $df = 2$, $p < 0.01$) and amendments applied ($\chi^2 = 63$, $df = 3$, $p < 0.001$). Available phosphorous content did not vary significantly across sites ($\chi^2 = 2.80$, $df = 2$, $p > 0.05$) and tree species grown ($\chi^2 = 1.40$, $df = 2$, $p > 0.05$) but significantly varied across amendments ($\chi^2 = 84.45$, $df = 3$, $p < 0.001$) with the content for compost amended soils being significantly higher than the content for any other soil

type (Tukey's, $p < 0.05$). Application of the amendment materials and growth of trees improved the organic matter content, most especially for compost and limestone+compost treated soils (Tukey's, $p < 0.05$) (Figure 2). The mean total nitrogen content increased upon treatment and growth of the tree species most especially for *Leucaena leucocephala* that is known to fix nitrogen symbiotically unlike *Senna siamea* and *Eucalyptus grandis*. Compost is the major source of nitrogen and available phosphorous in unfertile soils [20], and in this case could have been the source of the two nutrients through its mineralisation by microorganisms.

The mean available heavy metal concentrations for copper tailings and pyrite soils were generally higher than those of the unpolluted soils (Table 1). However it was only the concentrations of Cu, Co and Ni for the pyrite soils from HPPTS that were significantly higher (Tukey's, $p < 0.05$). Among the treated soils, the mean available concentrations of heavy metals varied significantly across sites, ($\chi^2 = 13.92$, $df = 2$ and $p < 0.001$) for Cu; ($\chi^2 = 31.41$, $df = 2$ and $p < 0.001$) for Co; ($\chi^2 = 18.99$, $df = 2$ and $p < 0.001$) for Ni but did not vary significantly for Pb ($\chi^2 = 0.11$, $df = 2$ and $p > 0.05$). Their variability among tree species grown was not significant for any of the heavy metals under study. Variation across treatments was significant for all heavy metals under study, (for Cu ($\chi^2 = 202.91$, $df = 2$, $p < 0.001$), for Co ($\chi^2 = 107.45$, $df = 3$, $p < 0.001$), for Ni ($\chi^2 = 49.04$, $df = 3$, $p < 0.001$) and for Pb ($\chi^2 = 6.04$, $df = 3$, $p > 0.05$). The results thus show that soil treatments effectively reduced rhizospheric available concentrations of heavy metals for all tree species (Figure 3). The reduction in available heavy metal concentrations could have been due to immobilization by organic matter applied and the adjustment of pH by limestone rendering most of the heavy metals unavailable. The dissolution of humic acid of organic matter applied at higher pH has been reported to be responsible for dissolution of Cu and Pb from soil [50]. Furthermore, the enrichment of the polluted soils with organic matter can also reduce the content of bioavailable heavy metals

as a result of complexation of free ions of heavy metals [52].

Plant community structure and cover before experimentation

In the initial plant inventories at the three polluted sites, a total of 18 different species were recorded from 18 genera and 7 families (Table 2). Total number of plant species recorded for each site was highest at KTDS with 9 species, followed by LPPTS and least at HPPTS with 8 and 6 species respectively. The estimated plant cover was (0-5%) for all the species but overall plant cover ranged from the highest recorded at KTDS (26%) followed by LPPTS and least at HPPTS with 18% and 13% respectively. Diversity of species represented by Shannon-Wiener index followed the same pattern. The low plant cover, species diversity and richness could have been mainly due to extremely acidic conditions of pH of 2.96 to 4.36 that was characteristic of background soils and far below the pH range of 6.0-7.0 that is ideal for growth of many plant species [21]. At such low pH values, the higher available concentrations of the heavy metals coupled with low levels of nitrogen and available phosphorous could have also contributed to the poor germination, seedling establishment and growth of many plant species in the area. This is supported by the finding of [26] that heavy metal toxicity in addition to phosphorous and nitrogen deficiencies are major factors limiting revegetation of contaminated soils.

Plant species belonging to the family Poaceae were more frequently found at the three polluted sites than species from other families. The total number of different species from Poaceae at the three sites was 9 while Asteraceae had 2 and the rest of the families had one species each. *Digitaria abyssinica* (A. Rich.) Stapf and *Dryopteris* sp were the only species distributed at all three sites. The dominance of grasses could be attributed to the tolerance advantage grasses have over dicots, of being insensitive to ethylene produced in

such stressful environments [24] that exacerbate damage to plants [29], ultimately leading to disappearance of some plant species from the site.

Amidst the harsh environmental condition, the monocotyledonous species *Cynodon dactylon*, *Digitaria abyssinica*, *Fimbristylis ferruginea*, *Pycreus macrostachyos*, *Sporobolus pyramidalis*, *Paspalum scrobiculatum*, and the dicotyledonous *Vernonia amygdalina* and *Pluchea ovalis* were growing on tailings and pyrite soils. Their occurrence on heavily polluted soils is indicative of their potential for phytoremediation of the site, since the plant species used for phytoremediation of a particular site should be those that have earlier colonised it for a long time and are completely adapted to the polluted environments [15]. Featuring prominently were the ferns *Pallaea calomelanos* and *Dryopteris* sp. At some plots they were exclusively occurring on the untreated sub-plots. Their survival is partly linked to their ability to form symbiotic association with *Anabaena* which is a nitrogen fixing microorganism that draw nitrogen from the atmosphere and enrich their rhizosphere [21].

Understory plant community changes during restoration

The change in the physico-chemical characteristics of the soils after application of limestone, compost and growth of tree species sparked off a plant succession process that led to the rise in species diversity, richness and plant cover. A total number of 215 different understory plant species belonging to 131 genera and 34 families were recorded during the entire study period in which four plant inventories were conducted. Most of the plant species found in the study site belongs to family Asteraceae, Poaceae, Commelinaceae, Euphorbiaceae and Fabaceae. The species which were common at all sites included *Paspalum scrobiculatum* L., *Imperata cylindrica* (L.) Pal., *Cynodon dactylon* (L.) Pers., *Digitaria abyssinica* (A. Rich.) Stapf, *Panicum maximum* Jacq., *Conyza sumatrensis* (Retz.) E.H. Walker, *Pluchea ovalis* (Pers.) DC., *Vernonia amygdalina* Del., *Commelina africana* L. and *Commelina benghalensis* L. *Fimbristylis ferruginea* Vahl. and *Pycreus macrostachyos* (Lam.) J. Rayn were exclusively found at HPPTS and LPPTS while *Setaria sphacelata* (Schumach.) Moss., *Oxalis corniculata* L. and *Dyschoriste radicans* Nees at KTDS. Some species like *Lycopersicon esculentum* Mill, *Amaranthus hybridus* L. subsp. *cruentus* (L.) Thell. and *Citrullus lanatus* (Thunb) Matsum. & Nakai var. *lanatus*, were introduced into the area with compost. However, there were no species associated with limestone treatment. *Pellaea calomelanos* (Sw.) Link and *Dryopteris* sp were more associated with the untreated soils.

The number of species on each sub-plot was smaller at the time of the first inventory, increasing in the second and the third inventories and declining/increasing slightly or remaining constant in the last inventory (Table 3). The Shannon-weaver index followed a similar trend. Species richness is one of the various criteria for evaluating restoration success [25,47]. Therefore the continuous rise in species richness with time on

Table 2. Species composition, diversity (H) and plant cover before restoration plot establishment recorded in three 7x7 m quadrants in each site.

Site	Species	Family	Cover class	H	Plant cover (%)
KTDS	<i>Abutilon mauritianum</i> (Jacq.) Medic.	Malvaceae	1	2.197	26.47
	<i>Commelina benghalensis</i> L.	Commelinaceae	1	--	--
	<i>Cymbopogon nardus</i> (L.) Rendel	Poaceae	1	--	--
	<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	1	--	--
	<i>Digitaria abyssinica</i> (Hochst. Ex A. Rich.) Stapf	Poaceae	1	--	--
	<i>Dryopteris</i> sp	Adiantaceae	1	--	--
	<i>Hyparrhenia filipendula</i> (Hochst.) Stapf	Poaceae	1	--	--
	<i>Paspalum scrobiculatum</i> (A. Rich.) Stapf	Poaceae	1	--	--
	<i>Setaria sphacelata</i> (Schumach.) Moss	Poaceae	1	--	--
	Bare	--	5	--	--
LPPTS	<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	1	1.946	17.97
	<i>Digitaria abyssinica</i> (A. Rich.) Stapf.	Poaceae	1	--	--
	<i>Dryopteris</i> sp	Adiantaceae	1	--	--
	<i>Fimbristylis ferruginea</i> Vahl.	Cyperaceae	1	--	--
	<i>Paspalum scrobiculatum</i> (A. Rich.) Stapf.	Poaceae	1	--	--
	<i>Pluchea ovalis</i> (Pers.) DC.	Asteraceae	1	--	--
	<i>Pycnus macrostachyos</i> (Lam.) J. Rayn	Cyperaceae	1	--	--
	<i>Sporobolus pyramidalis</i> (Lam.) Hitch.	Poaceae	1	--	--
	Bare	--	6	--	--
HPPTS	<i>Digitaria abyssinica</i> (A. Rich.) Stapf.	Poaceae	1	1.604	12.82
	<i>Dryopteris</i> sp	Adiantaceae	1	--	--
	<i>Imperata cylindrica</i> (L.) Pal.	Poaceae	1	--	--
	<i>Pallaea calomelanos</i> (Sw.) Link.	Pteridaceae	1	--	--
	<i>Pluchea ovalis</i> (Pers.) DC.	Asteraceae	1	--	--
	<i>Vernonia amygdalina</i> Del.	Asteraceae	1	--	--
	Bare	--	6	--	--

most of the treated sub-plots was reflective of a progressive succession and restoration process.

The number of understory plant species recorded varied significantly among the tree species grown ($\chi^2=11.96$, $df=2$, $p=0.03$), with plots on which leguminous tree species were growing having more species than plots on which *Eucalyptus grandis* was growing (Tukey's test, $p<0.05$). However, the number species growing on plots of leguminous tree species *Senna siamea* and *Leucaena leucocephala* did not vary significantly (Tukey's test, $p>0.05$). The number of species recorded for the unpolluted plots remained high throughout the study.

Nitrogen has been reported to be a major limiting nutrient on mine spoils to plant species establishment and maintenance of healthy growth and persistence of vegetation [53,60]. Thus, the higher species diversity and richness of understory plant species on plots of leguminous tree species than on plots of the non-leguminous *Eucalyptus grandis* could have been due to the legume's usual dramatic effect on soil fertility through

the production of readily decomposable nutrient rich litter and turnover of fine roots and nodules [49]. Mineralization of such nitrogen rich organic litter from these legumes could have allowed substantial transfer of nitrogen to companion species and subsequent cycling, thus enabling the development of a self-sustaining ecosystem [61].

Since the sites were devoid of diverse plant species for a long period of time, recruitment of more colonisers from distant unpolluted areas was critical. This was remarkably reflected by the understory plant species communities that sprung up on the treated sub-plots at KTDS, most especially on *Leucaena leucocephala* and *Senna siamea* plots which were dissimilar to the nearby plant community. The communities that sprung up were composed of herbaceous plant species that were prominently growing in gardens on slopes of the nearby hills. The inflow of fresh water and soils from the hills over years could have enriched the soils with plant propagules from which the new plant species emerged. Even though plant propagules immigration from the surrounding plant

Table 3. Variation in number of families, species richness and diversity with time of each sub-plot and unpolluted plots.

Site	Tree Spp	Treatment	Number of families				Species richness (S)				Species diversity (H)			
			Time (Month(s))				Time (Month(s))				Time (Month(s))			
			1*	6	12	18	1*	6	12	18	1*	6	12	18
KTDS	<i>E. grandis</i>	UT	1	11	2	3	1	13	5	5	0.000	2.564	0.807	0.807
		Limestone	1	4	6	3	2	7	10	6	0.693	1.556	0.973	0.589
		Compost	3	11	7	7	4	22	17	15	0.990	2.590	1.438	1.572
		LC	5	5	7	7	6	16	20	19	1.792	3.091	3.001	1.971
	<i>Senna siamea</i>	UT	2	6	8	9	4	12	20	17	1.386	2.187	2.611	2.150
		Limestone	3	9	9	12	6	17	29	28	0.989	2.443	2.045	2.211
		Compost	6	9	11	9	8	24	27	31	2.079	2.987	2.734	2.894
		LC	4	9	15	16	4	13	40	37	1.386	1.791	3.214	2.754
	<i>L. leucocephala</i>	UT	5	4	8	9	6	6	19	19	1.792	1.418	2.611	2.283
		Limestone	10	7	9	12	17	16	17	22	2.549	2.379	2.251	2.499
		Compost	3	6	9	9	8	4	22	18	1.723	0.738	2.474	2.251
		LC	2	9	9	9	5	16	21	19	2.379	1.230	2.402	1.971
LPPTS	<i>E. grandis</i>	UT	0	1	0	0	0	2	0	0	0.000	0.693	0.000	0.000
		Limestone	1	7	4	5	1	9	11	9	0.000	2.197	1.823	1.615
		Compost	1	2	9	2	1	3	18	4	0.000	1.002	2.220	1.040
		LC	0	4	5	4	0	11	7	5	0.000	2.398	1.284	1.040
	<i>Senna siamea</i>	UT	2	2	4	3	0	2	8	6	0.000	0.693	2.079	1.791
		Limestone	3	10	13	8	4	17	25	21	1.097	2.485	2.619	2.422
		Compost	4	6	9	7	0	12	20	16	0.000	2.088	2.342	2.185
		LC	5	8	8	6	0	14	16	14	0.000	2.186	2.039	1.953
	<i>L. leucocephala</i>	UT	2	3	5	4	5	6	9	7	1.213	1.792	1.857	1.556
		Limestone	1	3	5	5	1	9	5	8	0.000	1.823	1.499	1.149
		Compost	2	4	8	8	3	13	19	15	1.099	2.182	2.241	2.105
		LC	5	5	6	8	5	11	16	17	1.418	2.005	2.087	2.242
HPPTS	<i>E. grandis</i>	UT	0	0	0	0	0	0	0	0	0.000	0.000	0.000	0.000
		Limestone	0	7	3	4	0	12	5	5	0.000	2.485	1.791	1.609
		Compost	0	1	6	3	0	2	9	4	0.000	0.693	2.197	1.386
		LC	0	2	3	1	0	3	3	1	0.000	1.097	1.099	0.000
	<i>Senna siamea</i>	UT	0	3	3	1	0	4	5	1	0.000	1.386	1.386	1.386
		Limestone	2	4	4	7	2	7	12	13	0.693	1.616	1.490	1.490
		Compost	1	6	5	7	1	8	12	8	0.000	1.683	1.723	1.723
		LC	2	6	5	7	2	11	14	13	0.693	2.005	1.776	1.776
	<i>L. leucocephala</i>	UT	0	4	4	3	0	5	5	5	0.000	1.609	1.265	1.205
		Limestone	0	3	3	4	0	7	4	5	0.000	1.783	1.297	1.297
		Compost	0	11	6	6	0	19	8	7	0.000	2.611	1.394	1.394
		LC	0	5	3	5	0	7	5	7	0.000	1.697	0.945	0.945
Unpolluted	<i>L. leucocephala</i>	--	7	10	12	11	8	15	26	20	1.393	2.344	3.000	2.890
	<i>Senna siamea</i>	--	6	13	13	9	11	21	22	19	2.054	2.995	2.940	2.708
	<i>E. grandis</i>	--	6	6	11	9	7	12	19	17	1.556	2.006	2.670	2.446

*Conducted at the end of the month for homogenisation of soil sample.

communities could have occurred also at HPPTS and LPPTS, there could have been loss of viability due to the harsher physico-chemical properties of storm water in which they are carried within the pyrite trail and soils where they are deposited. Thus, even though the existence of patches of natural vegetation has been pointed out as an important environmental factor determining in part, availability of colonisers and the high richness values in the initial stages of succession [35], the remarkable species turnover in the initial stages of the study, most especially at KTDS, was attributed to seed immigration [44] from distant plant communities. There were also minimal introduction of edible plant species from unsterilized compost that was applied. The contribution of climatic factors to seedling emergence and establishment has been pointed out by [32]. KTDS was characterised by relatively lower temperatures and higher rainfall totals that substantially contributed to establishment of numerous plant species.

The goal for rehabilitation of mined land is often to restore the pre-disturbed land use or ecosystem [18]. Similarly, establishment of plant communities whose species composition matches those of the unpolluted site through dispersal of seeds from the surrounding undisturbed plant communities was hoped for. However, the CA analysis showed a wide variation between the plant communities established on plots of each tree species within the pyrite trail (LPPTS and HPPTS) and the tailings dams (KTDS) with those in the unpolluted area (Figure 4a). This was mainly due to the remarkable differences that still existed between the remediated soils and those at the undisturbed site. Failure to restore the original community on mine sites has been attributed by other authors to radical changes to almost every component of the landscape as well as persistent non-natural features [8,16,27,40,56]. The radical changes to the landscape and non-natural feature were still characteristic of the area under study. The plant communities established under the leguminous *Senna siamea* and *Leucaena leucocephala* were similar, having low loadings along the CA1 axis (Figure 4b). Those established under *Eucalyptus grandis* had high loadings on CA1. Ordination of species with time as constraining variable showed no variation in species composition with time (data not shown).

Percentage plant cover changes and distribution of plant species

Results of understory plant cover after 18 months are shown in Figure 5. On *Eucalyptus grandis* plots, the untreated sub-plots at LPPTS and HPPTS were bare while the sub-plot at KTDS had a plant cover of 37% which was higher than that of limestone treated sub-plot (11%), compost treated sub-plot (10%) and limestone+compost treated (2.5%) at HPPTS in that order. The highest plant cover of 98% was recorded on limestone treated sub-plot at KTDS. The plant cover for the rest of the sub-plots at the same site was in the order of compost treated

Figure 5. Percentage understory plant cover of the different sub-plots at different sites, 18 month after treatment of copper tailings at KTDS and pyrite soils at LPPTS and HPPTS.

sub-plot > limestone+compost treated sub-plot with 90% and 89% cover respectively while at LPPTS it was in the order of compost/limestone+compost > limestone treated sub-plot with 62% and 57%. The cover on the unpolluted plot was less than that of the treated sub-plots at KTDS; compost and limestone+compost treated sub-plots at LPPTS.

On *Senna siamea* plots the plant cover was low for the untreated sub-plots at HPPTS (9%), LPPTS (13%) and the compost treated sub-plot at HPPTS (34%). For the rest of the plots the cover was high ranging from 71% on the untreated sub-plot at KTDS to 98% for the lime+compost treated sub-plot at the same site. With *Leucaena leucocephala* plots, plant cover was lower for the untreated sub-plots at LPPTS (34%) and HPPTS (51%). For the rest of the sub-plots, plant cover was high ranging from 72% on the untreated sub-plot to 98% on the compost treated sub-plot at KTDS.

For both *Leucaena leucocephala* and *Senna siamea* plant cover on the unpolluted plots was lower than that of the sub-plots that received an amendment treatment. This may be attributed to the nature of the canopy formed by the experimental tree species and the type of species that had established. *Senna siamea* and *Leucaena leucocephala* formed dense canopies that strongly limited light penetration to the ground floor, leading to gradual disappearance or the dormancy of the grass species which are known to be shade intolerant. However, with the dense canopies and limited

light penetration on *Senna siamea* and *Leucaena leucocephala* plots at KTDS plant cover by understory species remained high. The species that established on these plots were mainly shade tolerant herbaceous species that thrived under limited light supply. The untreated sub-plots without canopies were dominated by grasses due to poor growth of the trees. The canopies also controlled the distribution of the species on the sub-plots with grasses distributed at the periphery and the shade tolerant herbs at the centre. The high plant cover on the plots for each tree species demonstrated their potential for phytostabilisation of the pyrite and copper tailings through enhanced establishment and growth of other species.

Relationship between the soil physico-chemical characteristics and plant community structure and cover

A correlation analysis revealed significant and positive correlations between pH, organic matter, total nitrogen and strangely with copper (Table 4). Correlations between available phosphorous with species richness and diversity were also surprising but not significant ($p > 0.05$). There was a negative significant

Table 4. Correlation coefficients (r) (n=136) between soil physico-chemical characteristics and understory plant community characteristics.

Parameter	pH	OM	P	N	Cu	Co	Ni	Pb
Species richness	0.26**	0.27***	-0.02 ^{NS}	0.27***	0.25**	-0.37***	-0.26**	0.07 ^{NS}
Species diversity	0.24**	0.24**	-0.01 ^{NS}	0.22**	0.23*	-0.35***	-0.21*	0.16*
No. Of families	0.25**	0.28***	0.03 ^{NS}	0.29***	0.24**	-0.34***	-0.26***	0.06 ^{NS}
Plant cover	0.40***	0.22**	0.04 ^{NS}	0.24**	0.22**	-0.39***	-0.25**	0.15 ^{NS}

NS=not significant, *level of significance: $p < 0.05$, **level of significance: $p < 0.01$ and ***level of significance: $p < 0.001$.

correlation between cobalt and nickel with all the four parameters ($p < 0.05$).

Conclusions

The physico-chemical characteristics of the pyrite soils and copper tailings were exceptionally harsh limiting the re-establishment of numerous plant species in areas where they were found. Solitary treatment of copper tailings and pyrite soils by the growth of tree species did not improve their physico-chemical characteristics to pave way for re-establishment of other plant species. However amelioration of their physico-chemical characteristics through the application of compost and limestone led to the germination of emigrated viable propagules and re-establishment of plant species. Grasses and ferns were more common on untreated copper tailings and pyrite soils than herbaceous plant due to their superior resistance to their harsh physico-chemical characteristics. In the early stages, plant species dynamics was minimal amongst the re-established plant communities. The plant communities established on treated pyrite soils and copper tailings were influenced by the growth of tree species

and greatly differed from those in the unpolluted sites.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	JES	MN	IB	LK	HOO
Research concept and design	✓	✓	✓	--	✓
Collection and/or assembly of data	✓	✓	✓	--	✓
Data analysis and interpretation	✓	--	✓	✓	✓
Writing the article	✓	--	--	--	--
Critical revision of the article	✓	✓	✓	✓	✓
Final approval of article	✓	✓	✓	✓	✓
Statistical analysis	✓	--	✓	✓	--

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