

# Assessment of Seedling Establishment and Growth Performance of *Leucaena leucocephala* (Lam.) De Wit., *Senna siamea* (Lam.) and *Eucalyptus grandis* W. Hill ex Maid. in Amended and Untreated Pyrite and Copper Tailings

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## Abstract

Growth and survival performance of *Leucaena leucocephala* (Lam.) De Wit., *Senna siamea* Lam. and *Eucalyptus grandis* W. Hill ex Maid. in amended and untreated pyrite and copper tailings were evaluated under field conditions. The physico-chemical characteristics of the pyrite soil and tailings were determined. Growth in height, basal diameter and later dbh, relative growth rate due to height ( $RGR_h$ ) and basal diameter ( $RGR_d$ ) and survival were determined every after six months. A split block experimental design was used and the data collected were analyzed using a statistical package R, with an additional package lme4. Tailings and pyrite soils had extremely low pH, poor nutritional status, low organic matter content and elevated concentrations of available heavy metals as compared to the unpolluted soils and treated pyrite soil and copper tailings. Growth performance was extremely poor on the untreated pyrite soil and copper tailings for all the species but significantly enhanced by the application of compost and limestone. Treatment had a significant effect on all parameters at all sites. *Eucalyptus grandis* displayed a higher potential of phytomass accumulation than *Leucaena leucocephala* and *Senna siamea*. Even though *Leucaena leucocephala* grew fastest reaching reproductive maturity in 7 months after planting, relative

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growth rates of the three species were not significantly different at all sites. The three species can be used for phytostabilisation of the tailings at Kilembe tailings dam sites (KTDS) after treatment while at Low polluted pyrite trail site (LPPTS) and Highly polluted pyrite trail sites (HPPTS) *Senna siamea* is more suitable as *Eucalyptus grandis* and *Leucaena leucocephala* are susceptible to attacks by *Syncerus caffer* (Buffalos) and *Kobus kob thomasi* (Uganda Kob).

## Keywords

Seedling Establishment; Growth Performance; Pyrite; Phytostabilization; Tailings; QECA

## 1. Introduction

Copper mining activities in Kilembe that lasted for close to 30 years from 1956 to 1982 generated an enormous volume of cobaltiferous pyrite wastes to the tune of 1.13 million metric tonnes that were stockpiled near Kasese town, 11 km east of the mines [1]. Flotation tailings to the tune of 15 million metric tonnes were dumped in various areas in Kilembe valley in which the fast flowing River Nyamwamba is located [2]. In total there are four tailings dams in the area. The cobaltiferous pyrite wastes and the tailings dams have remained acidic and devoid of vegetation for the last 30 years. The acid mine drainage emanating from these wastes has over the years polluted the nearby ecosystems without any mitigation measures instituted leading to wide spread environmental pollution in Queen Elizabeth Conservation Area (QECA) [1] and old Kilembe Copper Mining Area. In QECA the acid mine drainage from the wastes scarred and damaged shallow rooted vegetation creating bare ground over a large area, now popularly referred to as the pyrite trail, originally covering a total area of about 150 hectares and a distance of about 11 km to Lake George [1].

Mitigation of the pollution most especially after closure of mining areas is still a global challenge most especially in the developing world due to the exorbitant costs involved when conventional techniques such as leaching of pollutant, vitrification, electro-kinetic treatment, excavation and off-site treatment are deployed [3]. Such methods are expensive and technically limited to small areas. Amongst the various strategies adopted for removal of toxic heavy metals from the contaminated sites, phytoremediation has emerged as an economical, eco-friendly and aesthetically acceptable technology in the recent years [4]-[10]. It is a technique that involves the use of plants and soil microbes for removal and cleaning of pollutants from the soil including heavy metals.

Among the plants used in phytoremediation, trees are preferred to the shallow rooted plants because of their ability to treat the contaminants at greater depths, as their roots have the potential to penetrate more deeply into the ground. However, their success largely depends on their establishment and growth performance on sites to be remediated. In this study, one naturalized leguminous tree species *Leucaena leucocephala* (Lam.) De Wit. (Family Fabaceae), one exotic leguminous tree species *Senna siamea* (Lam.) H. S. Irwin & Barneby (Family Fabaceae) and one non leguminous fast-growing tree species *Eucalyptus grandis* W. Hill ex Maid (Family Myrtaceae) were used in the field to assess their growth performance during the reclamation and redevelopment of polluted and degraded soils. Selection of the legumes has received justification from recent studies on plant communities of metal contaminated areas. Surveys of plant species surviving in long term heavy metal contaminated environments have revealed that legumes constitute a dominant portion of the populations in these communities [11], hence having potential for phytoremediation. *Eucalyptus grandis* is a tree species exhibiting great environmental plasticity, with the ability to grow in impoverished or marginal soils and to accumulate high quantities of heavy metals [12] while *Senna siamea* has been applied as a hyper-accumulator plant for bioremediation of fly ash dumps elsewhere [13].

The three selected tree species have characteristics that were hoped to enhance the phytoremediation of polluted and degraded soils. The tree species are locally known to be resistant to draught and termites, fast growing and produce vast amount of seeds. *Senna siamea* and *Leucaena leucocephala* are legumes capable of enhancing nitrogen fixation, hence improving soil fertility of the nutritionally impoverished tailings and pyrite soils. By growing very fast the new vegetation cover will minimize soil erosion and lixiviation of hazardous heavy metals to aquifers or river systems by controlling direct rainfall impacts on bare soils and promoting the retention of

water within the rhizosphere.

Plant growth requirements are key components that determine the growth and survival of introduced trees [14] in a phytoremediation process. However, mine spoil habitats are nutritionally impoverished; characterized by low nitrogen mineralization rates, low phosphate availability, low soil organic matter, poor soil structure, compacted sub-soil, poor drainage and low water holding capacity [15]. Like any other plant species, reduction of soil phytotoxicity is a precondition for growth of legumes on highly metal contaminated sites [11]. Limestone and compost have been reported to improve substrate fertility by increasing plant nutrients and organic matter content, and neutralizing acidity [16]. On the basis of being abundant and locally available, limestone and compost were selected for the study. Limestone was added to neutralize the acidity while compost was to improve upon the water-holding capacity and the impoverished nutritional status of the soils or tailings respectively. The main purpose of soil amendments was initially to facilitate the establishment of the test trees before growing on their own abilities beyond the treat soil layers.

Comparatively, scanty information is available on the response of many woody species commonly used in ecological restoration as compared to the grass species. Similarly, there is no information on the field performance of *Leucaena leucocephala*, *Senna siamea* and *Eucalyptus grandis* in the phytoremediation of tailings and mine wastes polluted soils that are characterised with heterogenous distribution of heavy metals as in this study area. The aim of this study was to assess the impact of the treatments on the survival and growth performance of the selected tree species. It was hypothesized that seedling establishment and survival and growth performance of the experimental tree species did not vary with site and treatment factor.

## 2. Materials and Methods

### 2.1. Study Area

The study area comprised of the pyrite trail (PT) in 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 (Figure 1).

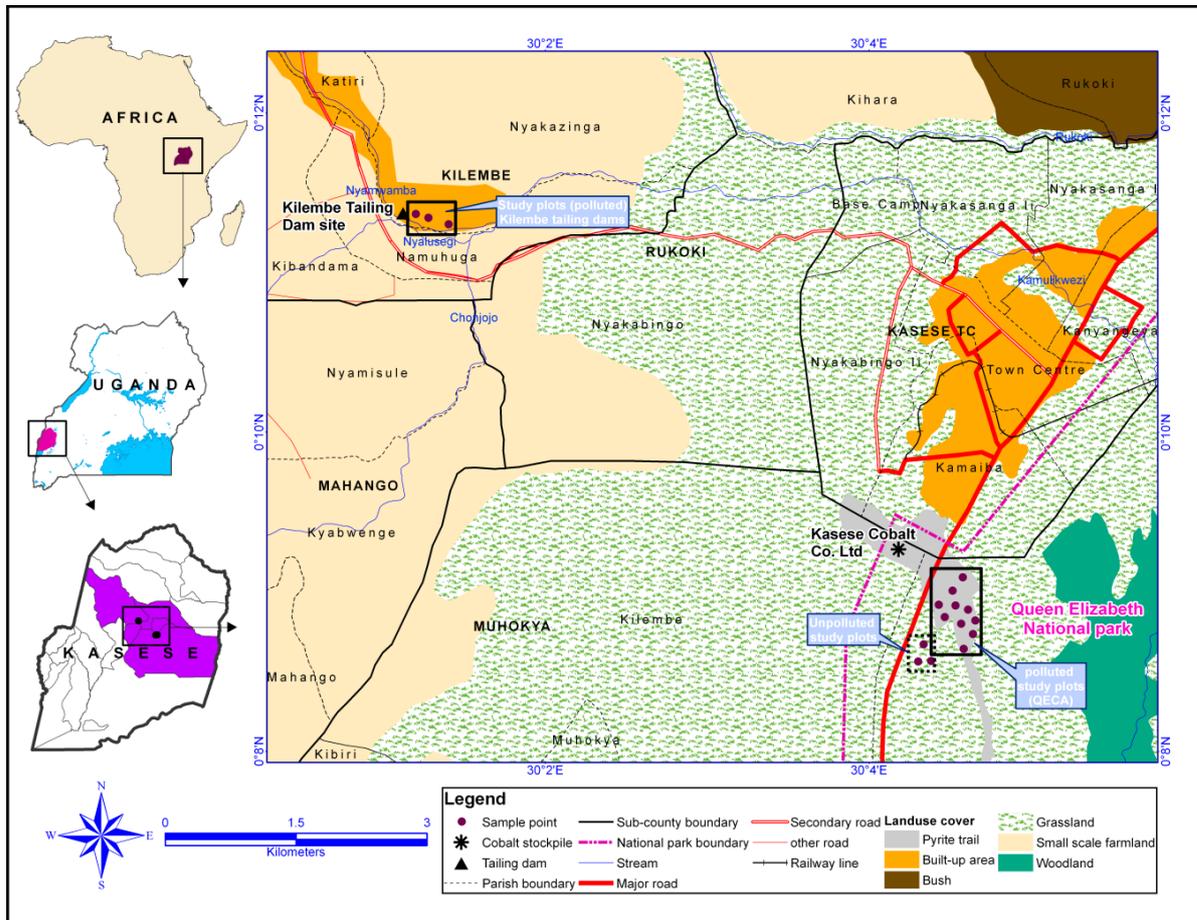
The study site experiences a tropical climate with rainfall which is bi-modally distributed with the wetter periods occurring from March to May and August to November. During the study period from May 2010 to February 2013, the temperatures for pyrite trail site showed minima ranging from 20.1° to 17.4°C and maxima ranging from 29.2° to 33.8°C. Records of temperatures for the Kilembe tailings dams were not available, but being located at higher altitude it was always cooler than the pyrite trail site which lies within the floor of the western arm of the great East African Rift Valley. The tailings dams are flattened at the top, characterised by longitudinal rows of depressions and elevations that were formed during the heaping process and gullies formed as result of water erosion. The flattened top is characterised 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 trees of *Acacia gerrardii* Benth and *Balanites aegyptiaca* (L.) Del. and islets of vegetation composed of *Capparis tormentosa* Lam., *Phytolacca dodecandra* L Hérit., *Fimbristylis ferruginea* (L.) Vahl, *Imperata cylindrica* (L.) P. Beauv., *Sporobolus pyramidalis* P. Beauv., *Typha latifolia* L. *Cynodon dactylon* (L.) Pers. covers most of the regenerated part of the pyrite trail. The surrounding vegetation consists largely of *Acacia* savannah woodland.

### 2.2. 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 amendment type as a treatment factor categorised into untreated (UT), limestone (LS), compost (Comp) and limestone + compost (LS + Comp) and the tree species grown.

### 2.3. Establishment of Pilot Restoration Plots

A total of 12 restoration and 3 control plots measuring 15 m × 15 m were demarcated in different randomly

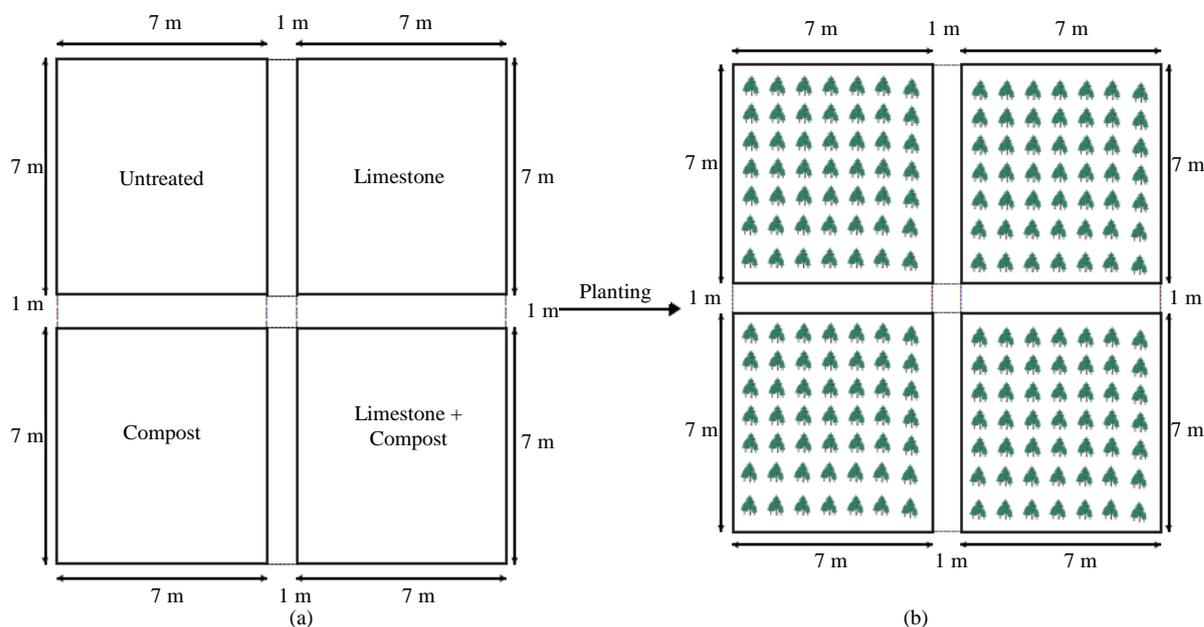


**Figure 1.** Location of the experimental sites at the tailings dams in Kilembe and the pyrite trail in QECA.

selected parts of the control and polluted area. Three plots were established on Kilembe tailings dams, three in the control and nine plots in the pyrite trail in QECA. Using hoes and the pick axes where necessary, the soils in the plots were dug up to the depth of 40 cm, and the large hard crumbs crashed to get the finest soil particles possible. Each plot was split into four sub-plots each measuring 7 m × 7 m, separated from each other by one meter (Figure 2(a)). In order to avoid anthropogenic and wild animal interference in the pyrite trail, the plots were fenced off by planting a live fence of *Euphorbia trirucalli* L. strengthened by reeds and *Eucalyptus* poles.

### 2.4. Treatment of Sub-Plots

Like any other plant species, reduction of soil phytotoxicity is a precondition for growth of legumes on highly metal contaminated sites [11]. Therefore, two abundant and locally available treatment materials limestone and compost were selected for the study. Limestone was added to neutralize the acidity while compost was for improvement of the water holding capacity and the impoverished nutritional status of the soils and tailings. The plots at UPS and one of the four sub-plots in each plot at KTDS, LPPTS and HPPTS were not subjected to any treatment that would result in significant change in physico-chemical characteristics except for the initial hoeing. Three treatment types were designed and randomly assigned to the remaining three sub-plots in each plot. One of the sub-plots in each plot at KTDS, LPPTS and HPPTS was treated with limestone at a rate of 2 tons per sub-plot. Another sub-plot was treated with compost at a rate of 1 ton per sub-plot. The remaining sub-plot was treated with a mixture of the two amendment materials prepared by thoroughly mixing 0.5 ton of wet compost with 1 ton of limestone. The treatment materials applied were then thoroughly mixed with the residual soil of the sub-plot by overturning them within the sub-plot four times. This was followed by regular watering to field capacity and allowing the treated pyrite soils and copper tailings to homogenize for a period of one month.



**Figure 2.** Design of the experimental plots: (a) Layout of the sub-plots; (b) Planting of the experimental trees.

## 2.5. Raising and Planting of Seedlings

Seeds of *Leucaena leucocephala*, *Senna siamea* and *Eucalyptus grandis* were secured, planted and raised at the nursery beds of the Afforestation and Soil Conservation Project of the Catholic Diocese of Kasese. Fifteen day old healthy seedlings of each species were selected and pure stands of each species planted (**Figure 2(b)**) in five different plots of which one was selected from the UPS, one from KTDS and the three from LPPTS & HPPTS.

## 2.6. Physico-Chemical Characterization of Copper Tailings and Pyrite Soil Samples

Each time growth parameters were measured in the field, rhizospheric copper tailings and pyrite soils were sampled. Their physico-chemical characterisation 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 [17] as described by [18] while total nitrogen was determined by the semi-micro Kjeldahl method [19]. 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 (3 g) 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 phosphorous in the extract was determined following Ammonium Molybdate-Ascorbic acid method [20] 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 (SHIMADZU AA-6800).

## 2.7. Determination of Survival Rate and Growth Performance of the Tree Species

The survival rate for each species under different treatments at the four sites was monitored throughout the study period. At regular time intervals of 6 months the number of trees surviving in each sub-plot was counted and recorded. The percentage survival of each species under the different treatments was calculated as the number of trees surviving by the end of each sampling period divided by the number of tree seedlings planted in a sub-plot (64 seedlings) multiplied by 100.

For growth performance, five trees were selected from each sub-plot and labelled. At regular intervals of six months, stem heights of the labelled trees were measured using common measuring tape [21] while basal di-

iameter was measured slightly above the root collar by using a vernier calliper [22]. Growth performance of the trees under each treatment was then evaluated as the mean relative growth rates in height ( $RGR_h$ ) and mean relative growth rate in diameter slightly above root collar ( $RGR_d$ ) using the formulae of classic growth analysis below [22] [23].

$$RGR_h = \frac{\log_e^{fh} - \log_e^{ih}}{t_2 - t_1} \quad (1)$$

where:  $RGR_h$  = height relative growth rate,  $\log_e$  = the natural logarithm,  $ih$  = mean height of the seedling at  $t_1$ ,  $fh$  = mean height of the seedling at  $t_2$  and  $t_2 - t_1$  = the period between two successive measurements (6 months).

$$RGR_d = \frac{\log_e^{fd} - \log_e^{id}}{t_1 - t_2} \quad (2)$$

where:  $RGR_d$  = diameter relative growth rate,  $\log_e$  = the natural logarithm,  $id$  = mean diameter of the seedling at  $t_1$ ,  $fd$  = mean diameter of the seedling at  $t_2$  and  $t_2 - t_1$  = the period between two successive measurements (6 months).

After 18 months of growth, diameter at breast height (dbh) was measured at 4.5ft (137 cm) above ground level using diameter tape (d-tape). Non quantifiable growth features of the trees were regularly observed and recorded.

## 2.8. Data Analysis

Data collected were analyzed using a statistical package R (version 2.13.2) developed by R development Core Team [24], with an additional package lme4 [25]. Prior to any statistical analysis, data distributions were checked for normality and homogeneity of variances. Data with strong deviations from the normal distribution or that were heteroscedastic were log-transformed and analyzed with parametric tests. Variability in means among parameters with data that were both normally distributed and homoscedastic were analyzed with analysis of variance (ANOVA) followed by a post-hoc test (Tukey Honest Significant Multiple Comparison) with means considered to be significantly different at  $p < 0.05$ . Correlation between growth and survival performance and physico-chemical characteristics were explored. A generalised linear mixed model (GLMM) was fitted to analyse variability of soil physico-chemical characteristics and the growth performance of the tree species. 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 strong deviations from normality or homoscedasticity, data were log-transformed before analysis.

## 3. Results

### 3.1. Physico-Chemical Characteristics of Soils

The physico-chemical characteristics of the untreated and treated tailings and pyrite soils are presented in **Table 1**. All the untreated copper tailings and pyrite soils had extremely low pH ranging between  $2.96 \pm 0.35 - 4.36 \pm 0.89$ , poor nutritional status with respect to total nitrogen and available phosphorous, low organic matter content and elevated concentrations of available heavy metals as compared to the unpolluted soils. The pH of the treated soils varied within the range of acidic to slightly alkaline with mean values ranging between  $4.33 \pm 0.78 - 7.70 \pm 0.44$  while that of unpolluted soils ranged between  $5.88 \pm 0.49 - 6.25 \pm 0.61$ . Application of the amendment materials improved the organic matter content most especially for compost and limestone+compost treated soils, available phosphorous and total nitrogen content. Total nitrogen content was generally higher with compost treatment for all the tree species. All the treatments effectively reduced rhizospheric available concentrations of heavy metals for all tree species at all sites.

### 3.2. Height and Diameter of the Seedlings of the Experimental Tree Species

The genotypic characteristics of the seedlings with respect to height and diameter varied significantly (ANOVA,  $p < 0.05$ ). Regarding diameter, *Leucaena leucocephala* had highest diameter of  $0.380 \pm 0.020$  cm, followed by *Eucalyptus grandis* with mean diameter of  $0.256 \pm 0.013$  cm and lowest in *Senna siamea* at  $0.232 \pm 0.019$  cm

**Table 1.** Mean ( $\pm$ SEM, n = 4), pH, organic matter content and concentrations of total nitrogen and available phosphorous and heavy metals in untreated, treated and unpolluted soils. The abbreviation OM, TN and AP denotes organic matter, total nitrogen and available phosphorous respectively.

Site	Tree species	Treatment	Melich 3 extractable concentrations of heavy metals (mg·kg <sup>-1</sup> )							
			pH	OM (%)	TN (mg·kg <sup>-1</sup> )	AP (mg·kg <sup>-1</sup> )	Cu	Co	Ni	Pb
KTDS	<i>E. grandis</i>	UT	3.73 $\pm$ 0.25	3.11 $\pm$ 1.08	0.14 $\pm$ 0.06	2.46 $\pm$ 0.40	14.32 $\pm$ 2.38	14.98 $\pm$ 2.89	3.90 $\pm$ 0.54	2.04 $\pm$ 0.12
		LS	6.88 $\pm$ 0.91	2.94 $\pm$ 0.50	0.13 $\pm$ 0.04	17.31 $\pm$ 5.43	7.86 $\pm$ 1.52	4.68 $\pm$ 1.31	0.90 $\pm$ 0.35	1.58 $\pm$ 0.25
		Comp.	5.67 $\pm$ 0.89	4.30 $\pm$ 0.67	0.23 $\pm$ 0.07	70.27 $\pm$ 18.43	8.76 $\pm$ 1.00	5.87 $\pm$ 1.16	1.54 $\pm$ 0.33	0.89 $\pm$ 0.10
		LS + Comp	6.61 $\pm$ 0.83	3.92 $\pm$ 1.33	0.19 $\pm$ 0.06	42.71 $\pm$ 9.04	9.04 $\pm$ 1.85	3.40 $\pm$ 0.82	1.25 $\pm$ 0.47	0.53 $\pm$ 0.15
	<i>Senna siamea</i>	UT	4.75 $\pm$ 0.82	3.97 $\pm$ 1.05	0.20 $\pm$ 0.09	10.74 $\pm$ 3.63	27.75 $\pm$ 3.31	15.22 $\pm$ 1.44	4.00 $\pm$ 0.47	2.53 $\pm$ 0.79
		LS	7.43 $\pm$ 0.28	4.87 $\pm$ 1.06	0.25 $\pm$ 0.07	24.92 $\pm$ 6.41	9.91 $\pm$ 2.02	2.90 $\pm$ 0.29	1.17 $\pm$ 0.63	0.40 $\pm$ 0.09
		Comp.	6.40 $\pm$ 0.72	6.27 $\pm$ 1.62	0.34 $\pm$ 0.11	89.67 $\pm$ 17.02	9.12 $\pm$ 0.99	6.30 $\pm$ 0.73	1.27 $\pm$ 0.51	0.16 $\pm$ 0.01
		LS + Comp	7.16 $\pm$ 0.78	5.99 $\pm$ 0.62	0.31 $\pm$ 0.07	76.69 $\pm$ 10.27	9.11 $\pm$ 1.33	4.56 $\pm$ 1.17	2.29 $\pm$ 0.43	0.39 $\pm$ 0.16
	<i>L. leucocephala</i>	UT	4.51 $\pm$ 0.39	3.14 $\pm$ 0.72	0.15 $\pm$ 0.01	1.79 $\pm$ 0.16	13.61 $\pm$ 4.60	15.65 $\pm$ 4.18	4.17 $\pm$ 0.52	1.09 $\pm$ 0.34
		LS	7.83 $\pm$ 0.37	4.51 $\pm$ 1.09	0.20 $\pm$ 0.06	25.49 $\pm$ 11.72	6.22 $\pm$ 0.82	3.47 $\pm$ 1.04	2.64 $\pm$ 0.81	0.58 $\pm$ 0.12
		Comp.	6.70 $\pm$ 1.17	5.84 $\pm$ 0.89	0.24 $\pm$ 0.07	204.00 $\pm$ 73.10	7.12 $\pm$ 1.96	1.47 $\pm$ 0.19	2.77 $\pm$ 0.95	0.63 $\pm$ 0.11
		LS + Comp	7.56 $\pm$ 0.47	5.22 $\pm$ 0.97	0.22 $\pm$ 0.06	104.06 $\pm$ 32.17	9.26 $\pm$ 2.87	3.56 $\pm$ 0.055	2.26 $\pm$ 0.24	0.79 $\pm$ 0.19
LPPTS	<i>E. grandis</i>	UT	2.96 $\pm$ 0.35	3.76 $\pm$ 0.84	0.14 $\pm$ 0.04	21.65 $\pm$ 5.98	15.02 $\pm$ 1.98	93.29 $\pm$ 17.48	10.09 $\pm$ 1.16	1.62 $\pm$ 0.32
		LS	6.95 $\pm$ 0.76	3.22 $\pm$ 0.47	0.16 $\pm$ 0.03	30.18 $\pm$ 6.67	5.66 $\pm$ 1.27	44.70 $\pm$ 8.23	4.29 $\pm$ 1.56	0.54 $\pm$ 0.10
		Comp.	4.33 $\pm$ 0.78	9.16 $\pm$ 1.19	0.33 $\pm$ 0.11	125.61 $\pm$ 27.16	6.43 $\pm$ 1.40	36.78 $\pm$ 3.12	5.57 $\pm$ 1.47	0.38 $\pm$ 0.12
		LS + Comp	6.75 $\pm$ 0.68	5.25 $\pm$ 0.73	0.22 $\pm$ 0.06	63.97 $\pm$ 19.41	7.10 $\pm$ 1.57	35.67 $\pm$ 5.59	4.93 $\pm$ 1.15	0.37 $\pm$ 0.09
	<i>Senna siamea</i>	UT	2.99 $\pm$ 0.56	5.15 $\pm$ 1.33	0.25 $\pm$ 0.08	26.28 $\pm$ 5.07	12.43 $\pm$ 1.35	88.48 $\pm$ 14.27	15.88 $\pm$ 2.33	1.62 $\pm$ 0.24
		LS	6.92 $\pm$ 0.71	5.16 $\pm$ 1.79	0.26 $\pm$ 0.06	37.86 $\pm$ 12.43	6.95 $\pm$ 1.70	28.82 $\pm$ 5.75	3.69 $\pm$ 0.62	0.43 $\pm$ 0.10
		Comp.	4.53 $\pm$ 0.36	9.18 $\pm$ 1.50	0.37 $\pm$ 0.14	139.38 $\pm$ 31.21	5.12 $\pm$ 1.93	53.10 $\pm$ 8.33	6.30 $\pm$ 1.65	0.69 $\pm$ 0.02
		LS + Comp	6.50 $\pm$ 0.65	6.26 $\pm$ 1.77	0.27 $\pm$ 0.07	64.63 $\pm$ 17.54	7.68 $\pm$ 1.29	33.42 $\pm$ 2.48	2.64 $\pm$ 0.57	0.71 $\pm$ 0.21
	<i>L. leucocephala</i>	UT	4.36 $\pm$ 0.89	1.36 $\pm$ 0.18	0.09 $\pm$ 0.02	5.02 $\pm$ 0.49	16.18 $\pm$ 3.53	49.70 $\pm$ 11.6	13.22 $\pm$ 3.49	1.75 $\pm$ 0.21
		LS	7.58 $\pm$ 0.37	1.56 $\pm$ 0.30	0.09 $\pm$ 0.03	18.26 $\pm$ 3.54	7.20 $\pm$ 1.38	10.65 $\pm$ 1.11	6.29 $\pm$ 0.96	0.51 $\pm$ 0.10
		Comp.	6.94 $\pm$ 1.13	4.31 $\pm$ 0.59	0.25 $\pm$ 0.07	157.76 $\pm$ 25.57	4.83 $\pm$ 0.98	9.21 $\pm$ 0.81	4.96 $\pm$ 1.61	0.42 $\pm$ 0.06
		LS + Comp	7.57 $\pm$ 0.39	1.84 $\pm$ 0.15	0.21 $\pm$ 0.06	78.19 $\pm$ 10.34	6.22 $\pm$ 1.00	5.92 $\pm$ 0.66	6.40 $\pm$ 2.00	0.51 $\pm$ 0.13
HPPTS	<i>E. grandis</i>	UT	3.26 $\pm$ 1.00	1.80 $\pm$ 0.19	0.11 $\pm$ 0.05	9.31 $\pm$ 2.92	37.65 $\pm$ 8.89	125.11 $\pm$ 25.01	29.20 $\pm$ 9.04	2.46 $\pm$ 1.09
		LS	7.02 $\pm$ 0.33	1.89 $\pm$ 0.25	0.13 $\pm$ 0.04	38.04 $\pm$ 3.50	7.17 $\pm$ 1.01	36.78 $\pm$ 4.96	3.91 $\pm$ 1.86	1.13 $\pm$ 0.27
		Comp.	6.83 $\pm$ 0.67	4.36 $\pm$ 0.80	0.17 $\pm$ 0.06	280.17 $\pm$ 51.66	5.83 $\pm$ 1.34	18.63 $\pm$ 1.39	7.82 $\pm$ 2.74	0.48 $\pm$ 0.13
		LS + Comp	7.02 $\pm$ 0.55	3.77 $\pm$ 0.13	0.17 $\pm$ 0.05	172.75 $\pm$ 28.56	7.92 $\pm$ 1.69	21.81 $\pm$ 0.53	4.69 $\pm$ 1.62	0.75 $\pm$ 0.23
	<i>Senna siamea</i>	UT	3.39 $\pm$ 1.22	1.50 $\pm$ 0.15	0.09 $\pm$ 0.01	12.29 $\pm$ 3.03	40.58 $\pm$ 7.58	107.89 $\pm$ 19.76	9.78 $\pm$ 3.08	1.26 $\pm$ 0.14
		LS	7.37 $\pm$ 0.56	1.59 $\pm$ 0.18	0.21 $\pm$ 0.06	13.95 $\pm$ 3.25	14.76 $\pm$ 4.04	15.09 $\pm$ 4.96	2.80 $\pm$ 0.70	0.76 $\pm$ 0.17
		Comp.	4.60 $\pm$ 1.21	4.01 $\pm$ 0.36	0.15 $\pm$ 0.04	127.88 $\pm$ 14.58	18.75 $\pm$ 1.80	31.62 $\pm$ 5.95	3.22 $\pm$ 0.96	0.81 $\pm$ 0.11
		LS + Comp	7.30 $\pm$ 0.72	1.63 $\pm$ 0.14	0.13 $\pm$ 0.04	41.16 $\pm$ 8.42	11.84 $\pm$ 2.26	29.32 $\pm$ 8.77	3.66 $\pm$ 0.93	0.73 $\pm$ 0.16
	<i>L. leucocephala</i>	UT	4.50 $\pm$ 0.81	1.57 $\pm$ 0.17	0.11 $\pm$ 0.02	20.13 $\pm$ 3.27	36.14 $\pm$ 6.92	72.19 $\pm$ 12.62	10.42 $\pm$ 0.66	1.36 $\pm$ 0.14
		LS	7.70 $\pm$ 0.44	2.21 $\pm$ 0.31	0.13 $\pm$ 0.01	36.11 $\pm$ 5.17	6.94 $\pm$ 1.98	11.69 $\pm$ 2.46	2.53 $\pm$ 0.74	1.09 $\pm$ 0.27
		Comp.	6.64 $\pm$ 0.81	4.42 $\pm$ 0.80	0.17 $\pm$ 0.03	125.56 $\pm$ 22.46	7.06 $\pm$ 1.06	11.19 $\pm$ 3.56	3.72 $\pm$ 1.01	0.63 $\pm$ 0.15
		LS + Comp	7.55 $\pm$ 0.64	2.24 $\pm$ 0.37	0.14 $\pm$ 0.03	80.82 $\pm$ 16.97	10.35 $\pm$ 2.66	12.92 $\pm$ 2.79	2.58 $\pm$ 0.68	0.72 $\pm$ 0.19
Unpolluted	<i>E. grandis</i>		6.24 $\pm$ 0.61	9.83 $\pm$ 1.93	0.39 $\pm$ 0.08	40.92 $\pm$ 4.45	4.25 $\pm$ 0.77	4.50 $\pm$ 0.96	2.571 $\pm$ 0.06	0.28 $\pm$ 0.03
	<i>Senna siamea</i>		6.24 $\pm$ 0.56	9.04 $\pm$ 1.12	0.37 $\pm$ 0.04	42.70 $\pm$ 4.47	4.09 $\pm$ 0.42	4.65 $\pm$ 0.14	2.96 $\pm$ 0.01	0.15 $\pm$ 0.04
	<i>L. leucocephala</i>		5.88 $\pm$ 0.49	9.84 $\pm$ 1.92	0.39 $\pm$ 0.09	50.69 $\pm$ 6.75	5.54 $\pm$ 0.66	5.04 $\pm$ 0.11	3.00 $\pm$ 0.19	0.18 $\pm$ 0.01

(Figure 3). Seedling diameter was significantly higher in *Leucaena leucocephala* than in the other two species (Tukey’s test,  $p < 0.05$ ), but there were no significant differences between those of *Eucalyptus grandis* and *Senna siamea* (Tukey’s test,  $p > 0.05$ ). *Eucalyptus grandis* seedling height was the highest at  $24.88 \pm 1.370$  cm, followed by that of *Leucaena leucocephala* at  $11.20 \pm 0.255$  cm and lowest in *Senna siamea* at  $6.90 \pm 0.210$  cm. The mean seedling height of each species significantly differed from each other (Tukey’s test,  $p < 0.05$ ).

### 3.3. Final Diameter and Height Attained by the Trees after 18 Months of Growth

Assessment of species growth performance was based on diameter and height attained at a particular time and site and their respective relative growth. For *Eucalyptus grandis* the diameters ranged between  $2.88 \pm 0.85$  cm for the untreated sub-plot at the HPPTS to  $10.26 \pm 1.72$  cm for the limestone + compost sub-plot at the LPPTS (Table 2). *Eucalyptus grandis* trees grown in the compost, limestone + compost sub-plots at all sites and limestone subplot at LPPTS had relatively higher diameters in the range of  $4.18 \pm 0.29$  cm to  $6.98 \pm 0.83$  cm as compared to those grown in the limestone treated sub-plots at KTDS and HPPTS with diameter of  $4.18 \pm 0.29$  cm and  $6.98 \pm 0.83$  cm respectively. With the exceptional case of trees grown in the limestone treated sub-plots at KTDS, all the trees grown in the amended sub-plots at all sites performed better than the trees grown in the

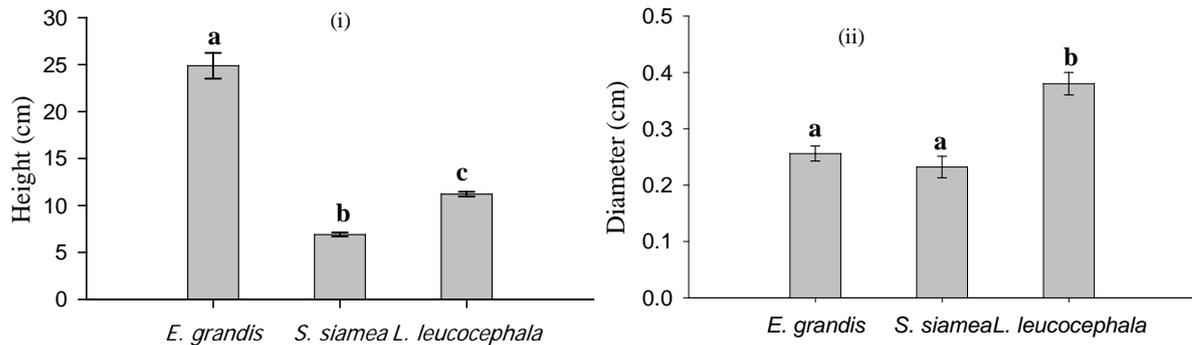


Figure 3. (i) Mean height and (ii) basal diameter of the seedlings (n = 10). Bars followed by different letter in each case are significantly different (Tukey,  $p < 0.05$ ). Error bars denote SEM.

Table 2. Mean diameters and heights attained by the tree species under different treatments at the different sites after 18 months of growth.

Site	Treatment	<i>Eucalyptus grandis</i>		<i>Senna siamea</i>		<i>Leucaena glauca</i>	
		Diameter	Height	Diameter	Height	Diameter	Height
KTDS	Untreated	$3.68 \pm 0.28c$	$159.80 \pm 48.3c$	$3.72 \pm 0.13c$	$146.60 \pm 28.05c$	$2.62 \pm 0.35d$	$141.20 \pm 26.84e$
	Limestone	$4.18 \pm 0.29c$	$180.00 \pm 35.64c$	$5.60 \pm 0.62c$	$334.80 \pm 26.78c$	$5.78 \pm 0.51ce$	$454.20 \pm 33.03d$
	Compost	$9.60 \pm 0.99d$	$722.80 \pm 66.63ad$	$8.16 \pm 0.54d$	$503.00 \pm 47.64ad$	$6.10 \pm 0.90ce$	$344.60 \pm 19.38bc$
	LS+Comp.	$9.62 \pm 0.61d$	$753.80 \pm 36.16ad$	$7.98 \pm 0.79d$	$543.00 \pm 21.82ad$	$6.48 \pm 0.49e$	$384.40 \pm 35.28bd$
LPPTS	Untreated	$3.93 \pm 1.05c$	$234.00 \pm 60.11c$	**	**	$2.38 \pm 0.61d$	$81.20 \pm 8.35f$
	Limestone	$9.20 \pm 0.47ad$	$773.20 \pm 16.50d$	$5.32 \pm 0.70ad$	$202.80 \pm 23.15d$	$6.16 \pm 0.75ce$	$321.00 \pm 27.78ab$
	Compost	$8.27 \pm 0.25bd$	*	$6.78 \pm 0.91bd$	$293.60 \pm 16.59be$	$4.88 \pm 0.86c$	$271.40 \pm 39.94a$
	LS+Comp.	$10.26 \pm 1.72d$	$847.20 \pm 128.89d$	$6.32 \pm 0.53d$	$293.00 \pm 27.26d$	$6.54 \pm 1.29e$	$385.60 \pm 26.02bd$
HPPTS	Untreated	$2.88 \pm 0.85c$	$157.75 \pm 50.86c$	$3.05 \pm 0.80c$	$101.50 \pm 20.47c$	$4.60 \pm 0.56ad$	$188.33 \pm 14.84a$
	Limestone	$6.98 \pm 0.83b$	$467.40 \pm 87.31b$	$3.39 \pm 0.74b$	$109.20 \pm 20.39b$	$4.88 \pm 0.75abc$	$298.20 \pm 22.02ab$
	Compost	$8.60 \pm 1.10ad$	$714.20 \pm 11.34ad$	$4.50 \pm 0.73ad$	$145.00 \pm 26.94ad$	$5.13 \pm 0.49bc$	$270.00 \pm 09.16ac$
	LS+Comp.	$7.72 \pm 0.82ab$	$577.60 \pm 38.36ab$	$5.23 \pm 0.97ab$	$225.25 \pm 32.02ab$	***	***
Unpolluted.	$6.32 \pm 0.34b$	$501.80 \pm 22.12b$	$6.24 \pm 0.27b$	$471.80 \pm 24.95b$	$5.16 \pm 0.64ce$	$310.00 \pm 10.00ab$	

Values are means  $\pm$  SEM (n = 5). Means with different letters within the columns for a particular species and site indicate significant difference between values; Tukey’s test, ( $P < 0.05$ ). \*Missing values due to destruction of the sub-plot by buffalos; \*\*Missing values due to survival; \*\*\*Missing value due to attack by Uganda Kob.

unpolluted site with respect to diameter. Height for *Eucalyptus grandis* ranged from  $157.75 \pm 50.86$  cm for trees grown in the untreated sub-plot at HPPTS to  $847.20 \pm 128.89$  cm for the trees grown in limestone + compost sub-plot at LPPTS. Apart from the trees grown on the limestone treated sub-plot at KTDS and HPPTS, all the trees in the treated plots were significantly taller than those grown in the unpolluted site (Tukey’s test,  $p < 0.05$ ).

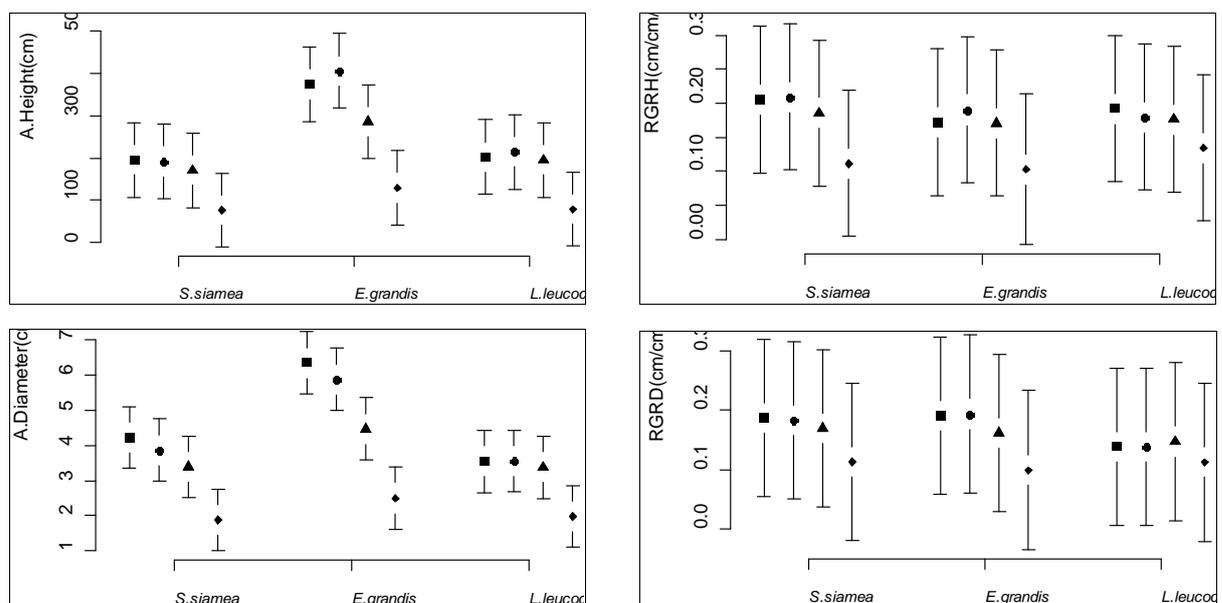
For *Senna siamea* diameter range was in the order of  $3.05 \pm 0.80$  in the untreated sub-plots at HPPTS to  $8.16 \pm 0.54$  cm in the compost treated sub-plots at KTDS. *Senna siamea* trees grown in the compost, limestone + compost at KTDS and LPPTS had significantly wider diameters than the rest of the trees in untreated and treated sub-plots and at the unpolluted site (Tukey’s test,  $p < 0.05$ ). Height was in the range of  $101.50 \pm 20.70$  cm to  $543.00 \pm 21.82$  cm in the limestone + compost sub-plot at KTDS. It is only trees grown in the compost and limestone +compost sub-plots at KTDS that were significantly taller than those grown at the unpolluted site (Tukey’s test,  $p < 0.05$ ).

In the case of *Leucaena leucocephala*, diameter was in the range of  $2.38 \pm 0.61$  cm in the untreated sub-plots at LPPTS to  $6.54 \pm 1.29$  cm at the same site. It is only trees grown on compost and limestone + compost sub-plots at KTDS and LPPTS that had significantly higher diameters than trees grown at the unpolluted site (Tukey’s test,  $p < 0.05$ ). Heights ranged from  $81.20 \pm 8.35$  cm for trees grown on the untreated sub-plot at LPPTS site to  $385.60 \pm 26.02$  cm for trees grown on limestone + compost sub-plot at the same site. Among the trees grown on the treated sub-plots it was only trees grown on compost and limestone + compost; and limestone and limestone + compost sub-plots at LPPTS that were significantly taller than those grown at the unpolluted site (Tukey’s test,  $p < 0.05$ ).

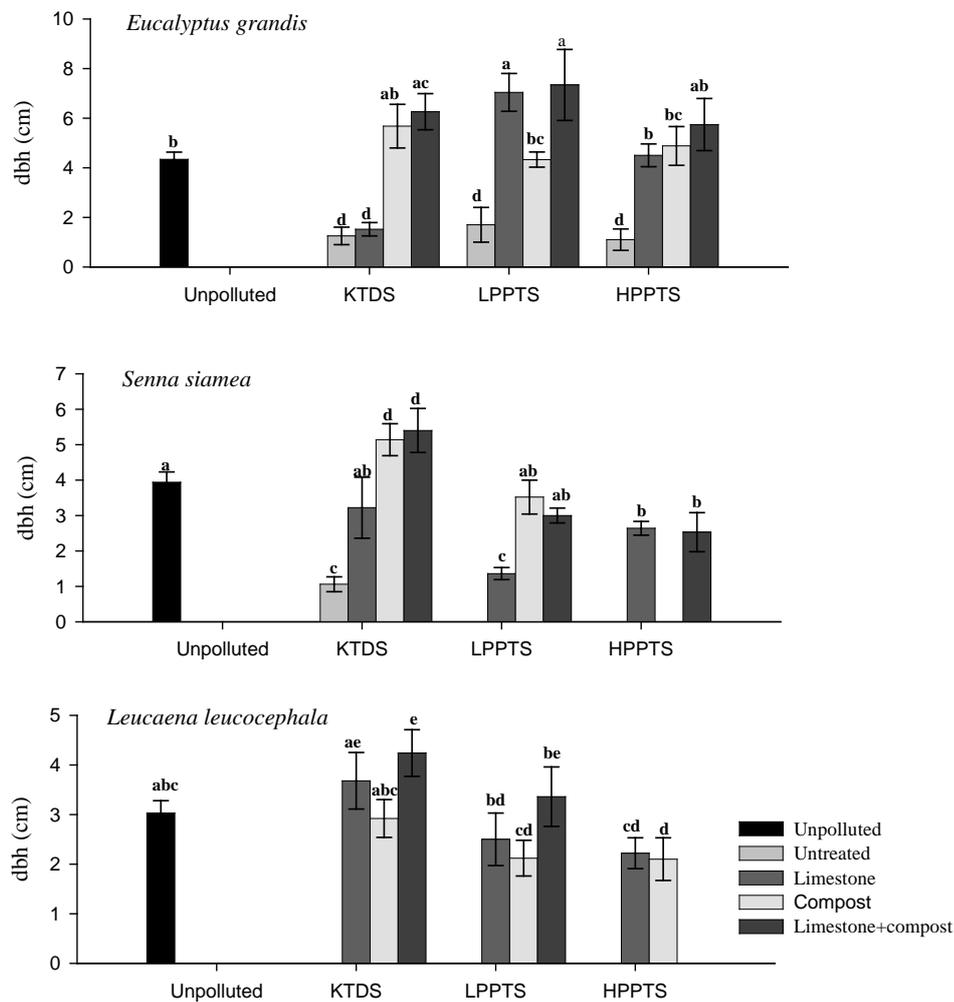
Means for species:treatment effect extracted from the model for the entire growth period show *Eucalyptus grandis* with the highest average height and diameter that considerably varied among the treatment (Figure 4). Mean average height attained by *Eucalyptus grandis* for the different treatments was in the order of limestone + compost > compost > limestone > untreated while for average diameter was in the order of compost > limestone + compost > limestone > untreated. Mean average height and diameter attained for *Senna siamea* and *Leucaena leucocephala* were within the same range with minimal variations across treatments.

### 3.4. dbh of the Three Tree Species after 18 Months of Growth

Results of dbh after 18 months of growth are presented in Figure 5. *Eucalyptus grandis* dbh ranged between  $1.10 \pm 0.43$  cm for trees grown on the untreated soil to  $7.34 \pm 1.43$  cm for trees grown on limestone + compost treated pyrite soils at the LPPTS. The latter trees and those grown on limestone treated soils at LPPTS had



**Figure 4.** Means and respective 95% confidence intervals extracted from the model for Species:Treatment effect on average height, average diameter,  $RGR_h$  and  $RGR_d$ . The symbols ■, ●, ▲, and ◆ represent Compost, limestone + compost, limestone, and untreated respectively.



**Figure 5.** Mean dbh (n = 5) of each tree species attained after 18 months of growth at different sites and treatment. Bars followed by different letters for a particular species are significantly different (Tukey's, p < 0.05). Some bars are missing owing to failure of trees for a particular treatment to reach dbh measurable height.

significantly higher dbh than those grown on the unpolluted soils. For *Senna siamea* dbh was in the range of  $1.07 \pm 0.208$  cm for the untreated tailings to  $5.40 \pm 0.628$  cm in the limestone + compost tailings both at KTDS. Trees grown on the limestone + compost treated soils had significantly higher dbh than those grown at the unpolluted site (Tukey's test, p < 0.05). *Senna siamea* trees grown on untreated pyrite soils at LPPTS and HPPTS, and compost treated pyrite at HPPTS and *Leucaena leucocephala* trees grown on untreated soils and soils treated with limestone + compost at HPPTS had not reached the height of 4.5 ft above the ground at which dbh measurements were taken and were therefore taken to be 0 cm. *Leucaena leucocephala* trees grown on limestone and limestone + compost treated soils at KTDS had significantly higher diameter than those grown on the unpolluted soils.

### 3.5. Relative Growth Rate Performance of the Tree Species

Results of relative growth due to diameter  $RGR_d$  and height  $RGR_h$  are presented in Table 3. Relative growth rate was higher for all the species in the first month of growth but later declined to the lowest rates in the last six months of growth. A similar trend was observed for the trees grown on unpolluted soils. Site did not have significant effect on both  $RGR_d$  ( $\chi^2 = 0.19$ , df = 2, p > 0.05) and  $RGR_h$  ( $\chi^2 = 6.77$ , df = 2, p > 0.05) Table 4. Similarly both  $RGR_h$  and  $RGR_d$  did not vary significantly across tree species ( $\chi^2 = 0.07$ , df = 2, p > 0.05) and ( $\chi^2 = 0.14$ ,

**Table 3.** Variation of  $RGD_d$  and  $RGD_h$  ( $\text{cmcm}^{-1}\text{month}^{-1}$ ) of the tree species with time under different treatments at the different sites.

Site	Species	Treatment	6 Map		12 Map		18 Map	
			$RGD_d$	$RGD_h$	$RGD_d$	$RGD_h$	$RGD_d$	$RGD_h$
KTDS	<i>E. grandis</i>	Untreated	0.200 ± 0.021a	0.122 ± 0.179a	0.172 ± 0.016a	0.148 ± 0.036a	0.072 ± 0.017a	0.036 ± 0.011ab
		Limestone	0.254 ± 0.045b	0.192 ± 0.044b	0.130 ± 0.034ab	0.110 ± 0.059a	0.082 ± 0.041a	0.029 ± 0.016a
		Compost	0.438 ± 0.026c	0.374 ± 0.036c	0.120 ± 0.031b	0.132 ± 0.016a	0.048 ± 0.010a	0.056 ± 0.017b
		LS + Comp	0.420 ± 0.014c	0.380 ± 0.029c	0.126 ± 0.011b	0.124 ± 0.019a	0.057 ± 0.025a	0.065 ± 0.028ab
	<i>Sena siamea</i>	Untreated	0.314 ± 0.050a	0.408 ± 0.013a	0.122 ± 0.035a	0.062 ± 0.018a	0.030 ± 0.016a	0.035 ± 0.019a
		Limestone	0.332 ± 0.060a	0.456 ± 0.029b	0.160 ± 0.024a	0.136 ± 0.026b	0.043 ± 0.020a	0.055 ± 0.013ab
		Compost	0.386 ± 0.051a	0.510 ± 0.019c	0.146 ± 0.035a	0.152 ± 0.013b	0.062 ± 0.021a	0.054 ± 0.013ab
		LS + Comp	0.362 ± 0.028a	0.498 ± 0.016a	0.166 ± 0.009a	0.166 ± 0.011b	0.065 ± 0.021a	0.065 ± 0.012b
	<i>L. leucocephala</i>	Untreated	0.198 ± 0.043a	0.372 ± 0.028a	0.096 ± 0.029a	0.014 ± 0.005a	0.028 ± 0.019a	0.033 ± 0.018a
		Limestone	0.306 ± 0.025b	0.468 ± 0.022b	0.102 ± 0.016a	0.058 ± 0.013bc	0.044 ± 0.007a	0.084 ± 0.015b
		Compost	0.262 ± 0.031b	0.450 ± 0.012b	0.120 ± 0.030a	0.048 ± 0.019b	0.080 ± 0.017b	0.073 ± 0.024b
		LS + Comp	0.278 ± 0.015b	0.440 ± 0.007b	0.114 ± 0.018a	0.084 ± 0.017c	0.082 ± 0.023b	0.068 ± 0.018b
LPPTS	<i>E. grandis</i>	Untreated	0.134 ± 0.077a	0.098 ± 0.019a	0.160 ± 0.079a	0.093 ± 0.064a	0.085 ± 0.032a	0.105 ± 0.050a
		Limestone	0.265 ± 0.061b	0.239 ± 0.044b	0.159 ± 0.040a	0.102 ± 0.043ab	0.054 ± 0.010ab	0.073 ± 0.021a
		Compost	0.386 ± 0.040c	0.319 ± 0.070c	0.149 ± 0.042a	0.163 ± 0.052b	0.029 ± 0.008a	-
		L + Comp	0.391 ± 0.039c	0.377 ± 0.042c	0.111 ± 0.027a	0.070 ± 0.029a	0.073 ± 0.015b	0.085 ± 0.027a
	<i>Senna siamea</i>	Untreated	0.104 ± 0.055a	0.160 ± 0.031a	-	-	-	-
		Limestone	0.302 ± 0.024b	0.306 ± 0.017b	0.138 ± 0.019ab	0.120 ± 0.028a	0.082 ± 0.012b	0.139 ± 0.017a
		Compost	0.346 ± 0.031b	0.392 ± 0.029c	0.170 ± 0.007a	0.184 ± 0.017b	0.046 ± 0.018a	0.053 ± 0.016b
		LS + Comp	0.370 ± 0.049b	0.400 ± 0.020c	0.118 ± 0.044b	0.148 ± 0.025ab	0.063 ± 0.013a	0.077 ± 0.011b
	<i>L. leucocephala</i>	Untreated	0.188 ± 0.047a	0.282 ± 0.024a	0.074 ± 0.036a	0.020 ± 0.012a	0.041 ± 0.013a	0.024 ± 0.007a
		Limestone	0.210 ± 0.044a	0.382 ± 0.013b	0.170 ± 0.035b	0.092 ± 0.008b	0.085 ± 0.020a	0.081 ± 0.004b
		Compost	0.222 ± 0.045ab	0.386 ± 0.025b	0.170 ± 0.037b	0.102 ± 0.065b	0.029 ± 0.010a	0.041 ± 0.019a
		LS + Comp	0.306 ± 0.054b	0.450 ± 0.024ab	0.130 ± 0.023ab	0.096 ± 0.011b	0.038 ± 0.011a	0.043 ± 0.019a
HPPTS	<i>E. grandis</i>	Untreated	0.184 ± 0.077a	0.098 ± 0.011a	0.160 ± 0.079a	0.094 ± 0.014a	0.057 ± 0.039a	0.094 ± 0.008a
		Limestone	0.265 ± 0.061b	0.239 ± 0.044b	0.159 ± 0.040a	0.102 ± 0.043ab	0.079 ± 0.038a	0.098 ± 0.014a
		Compost	0.386 ± 0.040c	0.319 ± 0.070c	0.149 ± 0.042a	0.163 ± 0.012b	0.049 ± 0.023a	0.056 ± 0.023b
		LS + Comp	0.391 ± 0.039c	0.377 ± 0.041	0.111 ± 0.027a	0.070 ± 0.001a	0.040 ± 0.022a	0.046 ± 0.019b
	<i>Senna siamea</i>	Untreated	0.150 ± 0.023a	0.152 ± 0.026a	0.236 ± 0.018a	0.242 ± 0.016a	0.064 ± 0.022a	0.042 ± 0.027a
		Limestone	0.264 ± 0.056b	0.268 ± 0.027b	0.144 ± 0.049b	0.164 ± 0.038b	0.065 ± 0.032a	0.029 ± 0.011a
		Compost	0.240 ± 0.014ab	0.285 ± 0.007b	0.225 ± 0.091ab	0.175 ± 0.078ab	0.047 ± 0.029a	0.043 ± 0.007a
		LS + Comp	0.283 ± 0.005b	0.313 ± 0.005b	0.160 ± 0.000ab	0.180 ± 0.036ab	0.048 ± 0.013a	0.035 ± 0.002a
	<i>L. leucocephala</i>	Untreated	0.135 ± 0.049a	0.275 ± 0.064ab	0.195 ± 0.049a	0.105 ± 0.064a	0.045 ± 0.011a	0.035 ± 0.026a
		Limestone	0.200 ± 0.058ab	0.220 ± 0.072b	0.167 ± 0.048a	0.200 ± 0.069a	0.043 ± 0.029a	0.036 ± 0.023a
		Compost	0.232 ± 0.026b	0.384 ± 0.018a	0.150 ± 0.021a	0.145 ± 0.019a	0.028 ± 0.003a	0.095 ± 0.021b
		LS + Comp	0.171 ± 0.025a	0.233 ± 0.048b	0.214 ± 0.023a	0.176 ± 0.046a	-	-
Unpolluted	<i>E. grandis</i>		0.390 ± 0.008	0.352 ± 0.007	0.108 ± 0.005	-	0.037 ± 0.014	-
	<i>Senna siamea</i>		0.390 ± 0.016	0.458 ± 0.012	0.116 ± 0.007	0.130 ± 0.078	0.045 ± 0.009	0.119 ± 0.024
	<i>L. leucocephala</i>		0.146 ± 0.027	0.160 ± 0.0147	0.153 ± 0.020	0.397 ± 0.003	0.107 ± 0.060	0.071 ± 0.005

Values are means ± SEM (n = 5). Means with different letters within the columns for a particular species and site indicate significant difference between values; Tukey's test, ( $p < 0.05$ ).

**Table 4.** Survival performance of the tree species under different treatments with time at the different study sites.

Site	Treatment	<i>Eucalyptus grandis</i>			<i>Senna siamea</i>			<i>Leucaena leucocephala</i>		
		Time	Time	Time	Time	Time	Time	Time	Time	
		6 Map	12 Map	18 Map	6 Map	12 Map	18 Map	6 Map	12 Map	18 Map
KTDS	Untreated	100.00	59.38	43.75	96.88	76.56	43.75	90.63	65.63	39.06
	Limestone	98.44	75.00	71.88	89.06	71.88	67.19	97.69	92.19	90.63
	Compost	100.00	79.69	78.13	100.00	95.31	93.75	95.31	92.19	92.19
	LS + comp.	98.44	84.38	76.56	98.44	92.19	92.19	92.19	78.13	78.13
LPPTS	Untreated	37.50	12.50	3.57	87.50	51.56	0.00	46.94	2.04	2.04
	Limestone	92.86	33.30	33.30	100.00	91.84	85.71	77.55	51.02	48.98
	Compost	71.43	67.86	66.07	100.00	69.39	67.35	77.55	44.90	44.90
	LS + comp.	87.76	87.76	81.63	93.88	67.35	67.35	91.84	85.71	83.67
HPPTS	Untreated	51.56	7.81	1.56	63.27	44.90	14.29	12.24	4.08	2.04
	Limestone	84.38	62.50	59.38	89.29	44.90	44.90	28.57	18.37	18.37
	Compost	95.92	81.63	73.47	45.24	11.90	7.14	53.03	36.73	32.65
	LS + comp.	95.92	71.43	69.39	66.67	35.71	35.71	18.37	10.20	10.20
	Unpolluted	81.97	N/A	69.30	93.47	93.47	90.95	51.76	51.76	56.25

N/A—No record taken after destruction by Buffalos. Map denotes month after planting.

df = 2,  $p > 0.05$ ) respectively, but significantly varied across treatments ( $\chi^2 = 18.75$ , df = 3,  $p < 0.001$ ) and ( $\chi^2 = 26.37$ , df = 3,  $p < 0.001$ ). Relative growth for all the tree species grown on untreated soils remained lower than that of the trees grown on the unpolluted and treated copper tailings and pyrite soils.

### 3.6. Survival Performance of the Tree Species

Survival performance for all species was remarkably poor on untreated soils at all sites ranging between 1.56% at HPPTS to 43.75 at KTDS for *Eucalyptus grandis*; 0.00% at LPPTS to 14.29% at HPPTS for *Senna siamea* and 2.04% at LPPTS and HPPTS to 39.06% at KTDS for *Leucaena leucocephala* (Table 4). Survival of the three tree species on treated soils was generally high ranging between 33.30% on limestone treated soils at LPPTS to 78.13% on compost treated soils at KTDS for *Eucalyptus grandis*; from 7.14% on compost treated soils at HPPTS to 93.75% on compost treated soils at KTDS and 10.20% on compost treated soils at HPPTS to 92.19% on compost treated soils at KTDS. At HPPTS the survival performance of *Senna siamea* on treated soils was lower than that for the trees of the same species grown on untreated soils at both KTDS and HPPTS. Survival performance of the tree species was significantly affected by site and treatment ( $\chi^2 = 6.76$ , df = 2,  $p < 0.05$ ) and ( $\chi^2 = 26.37$ , df = 3,  $p < 0.001$ ) respectively, but did not vary significantly across the tree species ( $\chi^2 = 0.14$ , df = 2,  $p > 0.05$ ).

### 3.7. Site, Treatment and Time Effects on the Growth Performance and Survival Performance of the Tree Species

Results of the model test showed that variation in average height, average diameter,  $RGR_h$ ,  $RGR_d$  and survival were significant due to time and treatment (Table 5) ( $p < 0.05$ ). Variation in average height and survival performance of the tree species due to site was significant ( $p < 0.05$  and  $p < 0.001$  respectively) but on the other hand not statistically significant with respect to average diameter,  $RGR_h$  and  $RGR_d$  ( $p > 0.05$ ). Apart from  $RGR_h$

**Table 5.** Significance tests of Time, Site, Species and Treatment and their interactions on Average height, Average diameter,  $RGR_h$ ,  $RGR_d$  and Survival performance by the model.

Factors	Av. height	Av. diameter	$RGR_h$	$RGR_d$	Survival
Time	***	***	***	***	***
Site	*	NS	NS	NS	***
Tree species	***	***	NS	NS	***
Treatment	***	***	***	***	**
Site:Species	NS	NS	NS	NS	***
Site:Treatment	NS	NS	NS	NS	NS
Species:Treatment	NS	NS	NS	NS	NS
Species:Time	NS	NS	NS	*	NS

Level of significance: \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  and NS (not significant),  $p > 0.05$ .

and  $RGR_d$  all the other parameters varied significantly amongst the three tree species ( $p < 0.05$ ). Interactive effects of Species:Treatment and Site:Treatment had no significant effect on all parameters while Site:Species and Species:Time had significant effects on only survival and  $RGR_d$  respectively ( $p < 0.05$ ).

### 3.8. Relationships between Growth and Survival Performance and Soil Physico-Chemical Characteristics

Computation of correlation coefficients showed that pH, organic matter, available phosphorous and total nitrogen were positively correlated with all the growth performance parameter and survival performance as shown in **Table 6**. pH had a significant positive correlation with height,  $RGR_h$ ,  $RGR_d$  and survival ( $p < 0.05$ ) but with basal diameter the correlation was not significant ( $p > 0.05$ ). Soil organic matter content was positively and significantly correlated with basal diameter, height and survival ( $p < 0.05$ ) but had an insignificant positive correlation with relative growth rate ( $p > 0.05$ ).

Phosphorous had significant positive correlation with only  $RGR_h$  and survival while total nitrogen correlation was not only significant with basal diameter. Heavy metals had negative correlations with some parameters. Copper had significant negative correlation with basal diameter and height ( $p < 0.05$ ) but on the contrary had significant positive correlation with relative growth and survival performance ( $p < 0.05$ ). Cobalt had a negative correlation with survival that was not significant and insignificant positive correlation with  $RGR_h$ , height, and basal diameter. It was only positively and significantly correlated with  $RGR_d$ . Nickel had significant positive correlation with relative growth rate and insignificant negative correlation with all the other parameters. Lead was positively and significantly correlated with relative growth rate ( $p < 0.01$ ) but positively and not significantly correlated with survival. Its correlation with height and basal diameter was negative.

### 3.9. Observable Growth Features

Field observations were made on growth features of the tree species under different treatments. *Eucalyptus grandis* grown on untreated soils had developed chlorotic purple leaves. Such leaves were not observed in *Eucalyptus grandis* growing on unpolluted soils but occasionally occurred in some pockets of treated soils. Foliage density was observed to be very high in the early stages of growth for the trees grown on treated soils most especially for compost and limestone + compost treated soils but relatively lower for those grown on unpolluted soils. The trees maintained their foliage both in dry and wet seasons. *Senna siamea* grown on untreated soils also developed chlorotic yellow leaves which were totally not observed on the trees grown on unpolluted soils but occasionally occurred on trees grown on treated pyrite and tailings. Foliage density was remarkably higher for trees grown on treated tailings at KTDS and unpolluted soils, lower for plants grown on treated pyrite soils and very low on untreated soils. Symptoms of chlorosis were not observed in *Leucaena leucocephala* but there were fluctuations in foliage density with more leaves lost during the dry season. Rooting pattern varied with time and site.

**Table 6.** Correlation coefficients (r) between soil physico-chemical characteristics and growth and survival performance parameters.

Parameter	pH	OM	P	N	Cu	Co	Ni	Pb
Basal diameter	0.06 <sup>NS</sup>	0.23*	0.16 <sup>NS</sup>	0.07 <sup>NS</sup>	-0.32**	0.06 <sup>NS</sup>	-0.09 <sup>NS</sup>	-0.41**
Height	0.22*	0.21*	0.11 <sup>NS</sup>	0.19*	-0.18*	0.05 <sup>NS</sup>	-0.10 <sup>NS</sup>	-0.18 <sup>NS</sup>
$RGR_d$	0.27**	0.04 <sup>NS</sup>	0.12 <sup>NS</sup>	0.33**	0.45**	0.20*	0.37**	0.35**
$RGR_h$	0.29**	0.11 <sup>NS</sup>	0.20*	0.34**	0.44**	0.14 <sup>NS</sup>	0.29*	0.35**
Survival	0.30**	0.54**	0.46**	0.38**	0.37**	-0.17 <sup>NS</sup>	-0.003 <sup>NS</sup>	0.13 <sup>NS</sup>

<sup>NS</sup> = not significant, \*level of significance:  $p < 0.05$ , \*\*level of significance:  $p < 0.01$ .

The tap root was still positively geotropic for all the species at KTDS and treatment but at LPPTS and HPPTS the tap roots had turned out to be negatively geotropic. Secondary roots were thin in untreated soils and thick in treated soils growing superficially. Nodulation was species specific occurring only on roots of *Leucaena leucocephala* and absent on roots of *Senna siamea* and as expected on roots of *Eucalyptus grandis*. Nodulation was only observed on young roots of *Leucaena leucocephala* grown on unpolluted soils and treated pyrite and tailings but not in the untreated pyrite and copper tailings. Despite insecticidal properties of *Leucaena leucocephala*, it was prone to attack by small insects that fed on the tips.

#### 4. Discussions

Growth performance of plant species is a consequence of multitude of factors comprising of among others climatic factors, soil (substrate) physico-chemical characteristics and genetic potential. Seedling height and diameter for the three species differed significantly despite being raised under the same environmental settings. The three species belong to different genera and families in case of *Eucalyptus grandis* and most likely possess different genetic potentials for expression of the characters. Growth and establishment of the three tree species on untreated soils was extremely poor. Even *Eucalyptus grandis* with proven environmental plasticity, its ability to grow in impoverished or marginal soils and ability to accumulate high quantities of heavy metals [12], could not cope up especially at LPPTS and HPPTS.

Generally, the failure of the three species to grow and establish themselves could partly be due to extremely acidic pH range of 2.96 to 4.36 that was characteristic of the untreated soils and far below the pH range of 6.0 - 7.0 that is ideal for growth of many plant species [26]. At such pH ranges growth is also adversely affected due to increased metal toxicities such as magnesium or manganese and reduced population of nitrogen fixing bacteria [27]. According to [28], at a pH of 2.4 there is a high mobility of elements such as Zn, Cu, Fe and Mn which become phytotoxic and adversely affect growth. In the current study higher availability of Cu, Co, Ni and Pb were observed (Table 2), in untreated soils as compared to unpolluted and treated soils. The availability of Cu, Ni and Pb could have had an adverse effect as depicted by correlation results with basal diameter and height (Table 5). The reduction in nitrogen fixing bacteria might be responsible for the absence of nodulation in *Leucaena leucocephala* growing on untreated soils. Poor growth and hindrance of nodulation of *Leucaena leucocephala* has also been reported in soils with low soil pH lower than 5.5 [29] while best growth occurs in soils with pH from 6.0 to 7.5 [30] which were characteristic of limestone and limetone + compost treated soils.

In this study relative growth rate was adopted for inter-specific comparison of the tree species due to their significant differences in average height and diameter among the seedlings [31]. Both  $RGR_h$  and  $RGR_d$  did not vary significantly across species ( $p > 0.05$ ) and site but varied significantly with treatment and time ( $p < 0.05$ ). This implies that the treatments were effective regardless of species and site. The significant variation with time may be ascribed to the usual pattern of growth followed with time, entailing slow growth rates in the initial stages, faster growth in the exponential phase and very slow growth in stationary phase.

Average height and diameter were considered as proxies for phytomass accumulation. The average heights and diameter measured were reflective of the species phytomass accumulation potential. Phytomass production by the tree species in a given period of time and its ability to accumulate heavy metals are very crucial for the success of phytoremediation. For the three species average height and diameter were substantially low for the trees grown on untreated soils (Figure 5), but significantly improved for the trees grown on treated soils for all

the species. The inability of the trees to grow and accumulate phytomass on the untreated pyrite and tailings demonstrated their low potential to remediate them through either phytoextraction or phytostabilisation and the need for application of amendment materials.

Nitrogen and phosphorous are the elements that most commonly limit tree growth [32]. Plants grown on acid soils commonly undergo phosphorous deficiency [33]. Their low concentrations as compared to unpolluted and treated soils could have also significantly retarded growth of the trees in untreated soils. It has been reported that phosphorous deficient plants show an enhanced exudation of carboxylic acids, such as citric and malic acid [34]. Carboxylate exudation could play a role in the mobilization of heavy metals in the rhizosphere and enhance their uptake to levels that are phytotoxic and thus prohibitive to growth.

However, as per the model results, the treatments were effective regardless of the species and site at which the species was grown. Average height and diameter for *Eucalyptus grandis* were substantially higher than those of the two leguminous species which had comparably similar diameter and heights for the three treatment regimes. The superiority *Eucalyptus grandis* demonstrated over *Senna siamea* and *Leucaena leucocephala* may not necessarily be linked with the species adaptability to the harsh soil condition but rather to the species' genetic potential since the roots had not penetrated to the deeper levels that were extremely harsh. The higher growth rate of the species on treated soils is attributed to reduction of the availability of the heavy metals through change of pH of soil to slightly alkaline in most of the soils and binding of heavy metals by the relatively high organic matter. The organic matter has frequently been reported to have a dominant role in controlling the behaviour of copper in the soil because it possesses important binding site for the element in compost and amended soils [35]. Nitrogen and phosphorous that most commonly limit tree growth [32], were relatively more abundant in treated soils.

Survival of the plant is one of the basic parameters to study the growth performance of the plants under a given set of environmental conditions [36]. Survival performance was generally poor on untreated soils for all the species most especially at LPPTS and HPPTS. Survival of the tree was under the influence of both climatic, physicochemical characteristics of soils and destructions by wild animals. Climatic conditions at KTDS could have favoured the survival of the trees more as compared to the other sites. Based on previous records of rainfall totals KTDS receives mean annual rainfall of 1370 mm while LPPTS and HPPTS receive 890 mm [37]. During the study period, temperatures at LPPTS and HPPTS ranged between 17.4°C to 33.8°C but KTDS being located at higher altitude it was always cooler than the pyrite trail site. With such climatic conditions at KTDS as compared to LPPTS and HPPTS, the evapotranspiration rates could have been low leading to higher water retention and enhancement of survival of the trees.

Contrary to the expectations, survival performance and growth of *Senna siamea* was very poor on compost treated soils at HPPTS. Treatment with compost adjusted the mean soil pH to 4.12 which is below the pH of 5.5 - 7.5 at which it grows best [38], thus high mortality of the seedlings. Similarly, survival of *Eucalyptus grandis* on compost treated soils at LPPTS and on unpolluted soils was remarkably low. This was due to frequent attacks to this species by *Syncerus caffer* (Buffalos) that defoliated and uprooted the trees. Similarly *Leucaena leucocephala* survival was lower at LPPTS and HPPTS partly due to frequent browsing by *Kobus kob thomasi* (Uganda kob), despite the physical barriers that were put in place to restrict their access to the plant. This points to the unsuitability of this species for phytoremediation in protected area as it may in turn serve as link through which heavy metals may be channelled into food chains of wild life. Survival performance was positively and strongly correlated with pH, organic matter, available phosphorous, total nitrogen as expected and strangely with available copper. The strange relationship with copper could have been due the decline in survival performance with time as the rhizospheric available copper did, due to phytoextraction by the trees.

Trees grown on untreated pyrite and tailings were characterised with low foliage density and chlorotic leaves in contrast with those grown on unpolluted and treated pyrite and tailings which had high foliage density and green leaves. Higher availability of the heavy metals in untreated pyrite and tailings as compared to those treated with limestone and compost could have lead to high uptake of heavy metals leading to chlorosis, as a result of decreased rate of chlorophyll biosynthesis and content [39]. Chlorosis may also be attributed to the deficiency of nutrients such as nitrogen, magnesium that usually characterise tailings and pyrite soils.

Roots are necessary for the growth and development of a plant and their modifications have an effect on other plant parts [40]. The roots formed by the trees growing on untreated soils were observed to be thin as compared to those formed by trees growing on treated tailings and pyrite soils and unpolluted soils, leading to poor growth. The relatively higher concentration of available heavy metals could have inhibited root elongation through interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis as sug-

gested by [41]-[44]. Upon absorption, compartmentalization and accumulation of heavy metals occur in the vacuoles of root cells thus limiting heavy metal transportation to shoots [45], consequently culminating into root cell metabolism disorders and depressed root growth [46]. *Senna siamea* lacked nodules and this in conformity with earlier reports in which it has been reported as a non-nodulating woody legume [47]. Failure of nodulation of *Leucaena leucocephala* in untreated soils may be attributed to low pH of the untreated tailings and pyrite soils. In acid soils malformation of roots of *Leucaena leucocephala*, poor growth of the entire plant, poor survival of *Rhizobium* and impairment of its nodulation have been reported [29]. The curving of the roots could be associated with the uneven vertical distribution of heavy metals and other physico-chemical characteristics, with the deeper layers of the soil profiles possessing levels that are prohibitive to growth. This explanation is in line with observation of [16] that plant roots grow selectively into the soil, taking advantage of the high degree of heterogeneity in the distribution of heavy metals, by avoiding the most contaminated part of the mine tailings. Thus the change in direction of growth following concentration gradients may result into such deformity in roots.

## 5. Conclusion

Pyrite and copper tailings were extremely acidic with relatively higher concentrations of available heavy metals, low organic matter content and deficient in nutrients. Proper establishment and growth of *Eucalyptus grandis*, *Senna siamea* and *Leucaena leucocephala* on untreated pyrite and copper tailings is unattainable. Thus, application of amendments purposely to boost the establishment of the species during a phytoremediation programme is recommended. The three species have great potential for phytostabilisation of pyrite and copper tailings. However, *Senna siamea* would be preferred to *Eucalyptus grandis* and *Leucaena leucocephala* in QECA as their use is limited by the potential attacks from *Syncerus caffer* and *Kobus kob thomasi* respectively.

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## Abbreviations

Dbh = Diameter at breast height  
 RGR<sub>h</sub> = Relative growth rate due to height  
 RGR<sub>d</sub> = Relative growth rate due to height  
 KTDS = Kilembe Tailings Dam site  
 LPPTS = Low polluted pyrite trail site  
 HPPTS = Highly polluted pyrite trail site  
 UPS = Unpolluted site  
 QECA = Queen Elizabeth Conservation Area  
 NARL = National Agricultural Research Laboratories  
 GLMM = Generalised Linear mixed models