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Research article

Species richness and phytoremediation potential of mine wastelands-native trees across the Zambian Copperbelt Region

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ABSTRACT

Mining activities are among the key sources of soil metal contamination in the Zambian Copperbelt, resulting in drastic landscape transformation. Plant species growing naturally on mine wastelands represent an asset for remediation on the disturbed ecosystems in the region. However, little is known about the suitability of Zambian native tree and shrub species for phytoremediation. The current study was carried to determine tree species richness and abundance on seven mine wastelands across the Zambian Copperbelt and evaluate their phytoremediation potential. Field inventory and post-hoc ecological analyses allowed identification of 32 native tree species, belonging to 13 different families, of which Fabaceae (34%) and Combretaceae (19%) predominated. Most of the identified tree species were found to be Cu, Co, Cr, Ni and Mo excluders. Among them, *Rhus longipes* (Anacardiaceae), *Syzygium guineense* (Myrtaceae), *Senegalia polyacantha* (Fabaceae) and *Ficus craterostoma* (Moraceae) were revealed as the most dominant tree species across the studied tailing dams (TDs) making them ideal candidates for metal phytostabilization. And coincidentally, their richness was positively correlated with high soil Cu concentration, a sought-after trait for phytoremediation of heavily polluted environment. Intriguingly, most identified tree species proved not suited for phytostabilization of Mn, Zn, B and Ba. On the other hand, species such as *Annona senegalensis*, *Parinari curatellifolia*, *Dombeya rotundilifolia* actively translocated these metals to leaves (TF > 1), indicating their potential for phytoextraction of Cu, Co, Cr, Ni, and Mo notably. Species richness and abundance significantly varied across the seven studied TDs. This was however barely influenced by soil metal contents, suggesting additional drivers dictating tree species-environment relationship in the context of studied TDs. The findings of this study provide crucial information in prospect of tree-based ecological restoration of mine wastelands, having revealed a diversified floristic composition of wastelands-native trees in the region, and clarified their respective phytoremediation attributes.

1. Introduction

Mining is a major contributor to economic growth in many countries, especially the developing world [1]. However, it produces

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vast amounts of wastes that result in degraded landscapes. Thus, it is considered as one of the greatest drivers of land transformation, converting forests and other ecosystems into mine wastelands [2]. This is the case for the Central African Copperbelt, extending north-westward from Zambia into the Congolese Copperbelt in the Democratic Republic of Congo (DRC), where mining activities have left an ecological footprint through the generation of wastelands. For example, the Zambia Copperbelt is believed to have around 791 million tons of tailings, referred to as the materials left over after extraction of the valuable ore. This is estimated to occupy almost 9125 ha of land [3], which represents a loss of opportunity for the local population in terms of other land use such as agriculture, forestry, housing, ranching etc. [1]. Mine wastelands generally remain un-vegetated for an extended period [4,5] due to unfavourable conditions such as low pH, low nutrient and water retention, and elevated metal concentrations that they are associated with, which inhibits plant growth [6]. They are prone to all forms of erosion and are a major source of environmental pollution [7]. Social, economic and health effects of mine wastelands have been observed in many mining regions including in USA [8], South Africa [9], China [10], Ghana [2], Zambia [4,1], Democratic Republic of Congo [11] and Australia [12].

Several techniques have been developed to reduce environmental contamination from mine wastelands. Among them, is vegetation establishment on Tailings Storage Facilities (TSFs)/Tailings Dam (TD) via phytoremediation that provides a cost effective and environment friendly option of restoring the mining degraded landscape [13,14]. Phytoremediation has proved such effective that it is being used to initiate international environmentally-sound cooperations aimed at mitigating negative consequences of metals pollution [15]. This phytotechnology takes advantage of plant's metabolic ability to remediate contaminated sites through immobilization of the pollutants in the soil or by recovering them from soil and subsequently storing them into plant biomass, thereby improving the overall environmental quality of the contaminated ecosystem [16].

However, plant establishment on TSFs has been reported to be difficult due to the high metal concentrations affecting plant physiology and growth [17,18]. Species establishment and growth on TSFs stands as the critical step towards successful implementation of phytoremediation of mine wastelands. Studies on reports of well adapted plants on TSFs have been made in a number of regions globally i.e Japan [19], China [20–23], Tanzania [24], Mexico [25], Uganda [26], Zambia [3,5], Portugal [27] and South Africa [28]. In most cases, the focus has been limited on herbaceous and grass species. In recent years, there has been a surge of interest in tree species growing on metal-contaminated sites. However, the understanding of their phytoremediation potential still is fragmentary, as the attributes of plant for metal accumulation and relevant ecological data are seldom investigated together. Interest on indigenous trees occurring on mine wastelands is also on increase in Southern Africa. Recent studies [29] have documented several native tree species occurring on mining generated wastelands on the Zambian Copperbelt. Such plant species were observed to successfully colonize the mining generated wastelands; however, their phytoremediation potential remains ununderstood, though this is

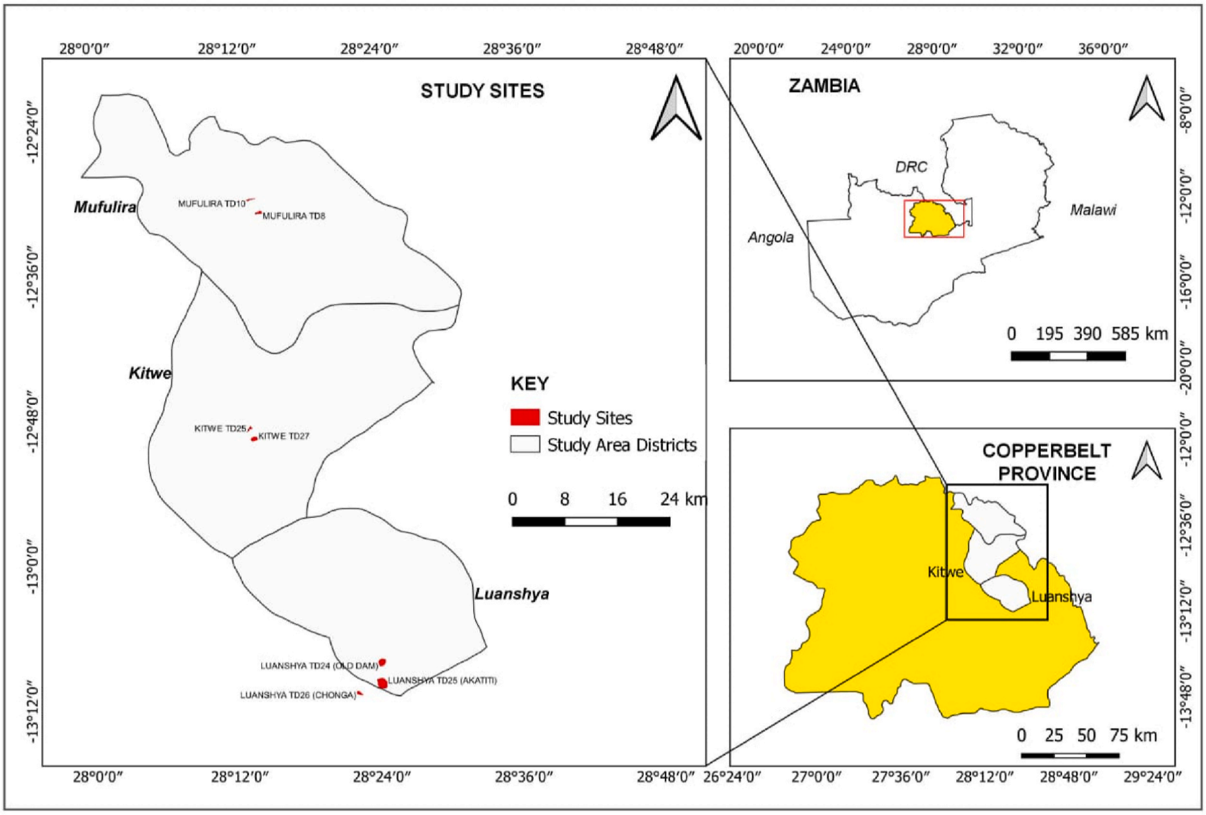

Fig. 1. Location of study sites within Copperbelt Province in Zambia.

crucial for informing precise decision making with regard to restoration of contaminated environments.

Species establishment is also influenced by environmental variables. Plants, like other living organisms, respond differently under different environmental conditions. Previous studies reported the effect of physicochemical properties of the tailings on plant establishment with less reference to metals [4,25,26]. Yet, understanding the influence of metals on plant species abundance on tailings dams is cardinal for the successful implementation of phytoremediation strategy as it provides a guide on soil amendments [25] and potential for manipulation of nutrients to enhance the survival of plants on degraded environments. To date, few studies have investigated the influence of metals on species richness and abundance on contaminated sites especially on copper mining generated wastelands, and available investigations have scarcely been multi-local to integrate the environmental variability and harness the intrinsic attributes of investigated plant species.

Hypothesizing the place of mine wastelands-native trees as a resource for phytoremediation technology, and considering the mineral-rich Zambian Copperbelt Region as an untapped asset in this regard, the present study was carried out in multiple metal-contaminated sites (i) to gain insights into the still understudied floristic composition of indigenous trees species occurring on the widespread mine wastelands in the region, (ii) and to evaluate the phytoremediation potential of these native tree species in prospect of sustainable ecological restoration of mine-generated wastelands in the region.

2. Materials and methods

2.1. Site description

The study was conducted in selected mining towns (Mufulira, Kitwe, Luanshya) of the Copperbelt Province, in Zambia. The province lies between latitude 13° 00' 0.00" S and longitude 28° 00' 0.00" E (Fig. 1), virtually encompassing the Zambian part of the Central African Copperbelt. It is the main mining region in Zambia, with mining history dating back to the 1920s [30].

The soils in the region are characterized by low base exchange capacity, resulting from the intense weathering processes [31]. They are best represented by ferralsol [32]. And like most ferralsol, the soils of the region are predominantly highly acidic, poor in humus and soil organic carbon, and generally rich in exchangeable aluminium (Al^{3+}) [31]. The Copperbelt province is one of the wetter regions of the country, falling under the third (III) agro-ecological zone, with an annual rainfall averaging 1000–1500 mm and temperature ranging from 7.8 °C to 23.7 °C [33]. The rainy season spans from November to March, followed by a cool dry season from April to August and a hot dry season from August to November [34,35].

Across the study area, seven decommissioned tailing dams (TDs) were selected for investigations i.e TD 25 and TD26 in Kitwe, TD24, TD25 and TD26 in Luanshya and TD 8 and TD10 in Mufulira (Fig. 1).

2.2. Sampling procedure

Field surveys were conducted on the selected TDs for collection of data regarding plant species as well as collection of soil samples. The transect method of sampling was adopted due to the pattern of vegetation distribution and establishment on the TDs. The starting point of the first line transect and sampling points were selected randomly. Thereafter, transects were established at every 200 m as recommended by Kambinga and Syampungani [5], and sampling points were established every 100 m along transects [24]. At each sampling point, a 20 m diameter circular plot was established for data observation, and the corresponding GPS coordinates were recorded from the centre of the plot.

2.3. Field data collection

In each sampling plot along transects, all tree species were taxonomically identified. And then the trees' diameter at breast height (DBH) was measured and recorded. For each tree species within a sampling plot, only individuals with larger DBH were retained as representative of the species, and used for the collection of root and leaf samples for later analyses of mineral composition. Hence, for each species within a sampling plot, leaf and root materials were collected to constitute composite samples of grabs from selected individuals. The collection of leaf samples was straightforward for tree with single stem. For trees with multiple stems by contrast, prior examination was required to identify the stem with the highest DBH value, which was considered for collection of leaf samples.

Similarly, soil samples were collected in different sampling plots following [4,24] with minor modifications. Briefly, for each sampling plot, three individual grabs of soil were collected from randomly selected locations within the plot, and then mixed to form a composite sample. Soils were collected in the upper layer (0–30 cm) known to be the habitat of biological factors influencing plant growth [36], and which is commonly used for soil sampling in disturbed environment including agricultural lands and mine-generated wastelands [37,38,39].

The different plant and soil samples were packed in a plastic bag, clearly labelled with site and species name, transect and plot number, and then taken to the laboratory for analyses.

2.4. Analyses of ecological data

Different ecological parameters i.e species richness and abundance, diameter at breast height (DBH) of trees, species importance value index (IVI), were investigated for each site. Species richness per site, which implies prior taxonomical identification, was determined by counting the number of species occurring on the site, while abundance was determined by counting the number of individuals of a species per plot and consecutively by site. Moreover, in addition to measurement of individuals' DBH, trees species were investigated for their relative density (Rd), relative frequency (RF) and relative dominance (RD). Ultimately, the species importance value index (IVI) was calculated to determine the overall species performance and dominance on the studied tailings dams (TDs) [40]. IVI was calculated as the sum of RF, Rd and RD divided by three [41,40] as follows:

$$IVI = RF + Rd + RD/3$$

Where

$$RF = \frac{\text{Frequency } \textit{of a species}}{\textit{Total frequencies of all species}} * 100 \quad (2)$$

$$Rd = \frac{\textit{Number of individuals of one species}}{\textit{Total number of all individuals counted}} * 100 \quad (3)$$

$$RD = \frac{\textit{Basal area per species}}{\textit{Total basal area}} * 100 \quad (4)$$

2.5. Chemical analyses of plant materials and soil samples

Prior to analyses, plant materials (root and leaf samples) were thoroughly washed with tap water and then rinsed with deionised water [42,43]. Moreover, to allow thorough desorption of metals, root samples were immersed in 20 mM of EDTA-Na_2 for 3 h and then extensively washed in running deionised water [44]. Both plant and soil samples were oven dried at 60 °C until constant dry weight [45,46]. Then dried materials were subjected to microwave assisted acid digestion following [7]. 3 g of either soil or plant samples (roots or leaves) were placed into respective 50 mL inert microwave vessels and digested (PerkinElmer Multiwave 3000) in 9 mL of concentrated nitric acid (65%, Suprapur) for 10–20 min. The samples were left to cool for 20–25 min, then filtered, and each extract was diluted with deionised water to a 30 mL mark. Finally, metal concentrations in different samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Precision and accuracy were achieved by measuring three blanks, and the final results were expressed in mg kg^{-1}.

2.6. Phytostabilization potential assessment

Insights into the phytostabilization potential of tree species on different tailings dams was gained by assessing their excluding abilities, expressed by the bioaccumulation factor (BAF) and translocation factor (TF). BAF and TF values were generated from data on metal concentrations in soil and in plant materials as follows [21]:

$$BAF = \frac{\text{Heavy metal concentraion in roots}}{\text{Heavy metal concentration in soil (tailings)}}$$

$$TF = \frac{\text{Heavy metal concentration in leaves}}{\text{Heavy metal concentration in roots}}$$

BAF <1 indicates a poor ability of a plant to accumulate metals from the soil to the roots, implying metal exclusion abilities, while values > 1 indicate high potential for metal bioaccumulation [47]. Likewise, TF > 1 means higher potential of metal upward movement within plant; and conversely, TF < 1 indicates restricted metals translocation towards aboveground parts [48,49,47]. On this basis, ideal species candidates for phytostabilization can be identified by lower BAF and TF values (<1).

2.7. Statistical analysis

Statistical analyses were done using SPSS version 25.0. Significant differences of metal concentrations in roots and shoots were determined using a two-way ANOVA at 0.05 level of significance. A paired T-test was then used to determine the differences between metals in roots and shoots. Site specific metal concentration and data with replicates were presented as mean ± standard error. To determine the significant difference in species richness, abundance, and metal concentrations between the sites, a one-way Anova ($p < 0.05$) was conducted using SPSS version 25.0. All ordinations, including detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were performed using CANOCO version 5.1 [50]. DCA analysis was used to determine the gradient length [51] which is used to determine the suitable ordination method to apply to data [52]. Gradient lengths greater than 3 suggests the use of "unimodal ordination methods such as DCA and Canonical Correspondence Analysis (CCA) while gradient lengths less than 3 specify linear models" [53, 52] such as Principal Component Analysis (PCA) and Redundancy Analysis (RDA) [54,52]. In this study, the gradient length was 5.4, and therefore CCA was used to investigate relationship between environmental variables (metals) and species abundance on studied tailings dams. The statistical significance of tree species-environment correlations was tested by distribution free Monte Carlo permutation tests (499 permutations) [54,55]. Prior to the analysis, all variables were assessed for normality.

3. Results

3.1. Species richness and abundance

In total, 32 tree species from 13 families and 23 genera were recorded on Tailings Storage facilities (TSFs) across the three studied mining cities of the Zambian Copperbelt (Table 1). Species richness significantly ($p < 0.05$) varied across sites, with Luanshya TD24 and Mufulira TD8 showing higher richness, 20 and 17 different tree species respectively, whereas Kitwe TD26 had the lowest (10 species).

The tailing dams with the most species richness *i.e.* Luanshya TD24 (20 species) and Mufulira TD8 (17 species) were also the highest in species abundance (80 and 82 species respectively). Likewise, Kitwe TD26 presented the lowest number of individual trees (45).

Across studied sites, Fabaceae was the most represented among the 13 families inventoried, accounting for about one third of the total number of the species identified. Combretaceae was second highest family (with 6 species), followed by Anacardiaceae and Moraceae with 3 species each. Other families (Annonaceae, Chrysobalanaceae, Connaraceae, Ebenaceae, Malvaceae, Myrtaceae, Sapindaceae, Sterculiaceae, Phyllanthaceae) were represented by a single species (Table 1).

Remarkable variability was noted between tree species within each of the studied sites, with coefficient of variation varying from 155% (Kitwe TD25) to 243% (Luanshya TD26). Considerable variability of species IVI was also observed across tailings dams. For example, several tree species were present on certain tailings dams but absent on others. And in many cases, the same tree species presented contrasting IVI values across tailing dams. Of importance, was that the most dominant species were different from one tailings dam to another. *Peltophorum africanum* (Fabaceae) was identified as the most dominant species on Kitwe TD25, *Senegalia polyacantha* (Fabaceae) on Kitwe TD26, *Bauhinia thonningii* (Fabaceae) on Luanshya TD25, *Syzygium guineense* (Myrtaceae) on Luanshya TD26, *Ficus sycomorus* (Moraceae) on Mufulira TD10. Exceptionally however, the Anacardiacea *Rhus longipes* proved to be the most dominant tree species on 3 among the 7 investigated tailing dams *i.e.* Luanshya TD24, Mufulira TD8 and Mufulira TD10 (Table 2).

Based on the overall species performance as characterized by species IVI (Table 1), *Rhus longipes, Syzygium guineense, Senegalia polyacantha* and *Ficus craterostoma* notably, and to lesser extent, *Combretum molle*, *Bauhinia thonningii*, *Peltophorum africanum, Ficus sycomorus and Terminalis mollis* have been identified as the dominant native tree species on tailings dams across the three studied cities (Table 2).

3.2. Metal concentrations on Copperbelt tailings dams

Data of soil metal content show considerable variability from one metal to another, with copper (Cu) and manganese (Mn) being the most abundant metals in different TDs, and molybdenoum (Mo) the least represented (Fig. 2). For each given metal, the concentrations significantly varied ($p < 0.05$) between tailings dams, with Kitwe TD26 being singled out for the highest content of copper (Cu), cobalt (Co), manganese (Mn), and barium (Ba). Conversely, Mufulira TD8 and Mufulira TD10 exhibited the lowest content in nickel (Ni), boron (B), molybdenum (Mo), Ba and especially Co that was found in trace amounts (Fig. 2d).

It is intriguing to note that the three studied mining cities (Kitwe, Luanshya, Mufulira) could be discriminated based on the pattern of metal concentration in tailing dams. Hence, Mufulira TDs are the poorest in Ni, Co, Ba, B and Mo, while TDs in Luanshya are the most enriched in Zn and Ni (Fig. 2e f). Like in Mufulira, TDs in Luanshya also presents the lowest contents of Co and Ba, especially TDs 24

Table 1
Species richness and abundance on studied tailings dams (TD) in three mining cities of the Zambian Copperbelt (Kitwe, Luanshya, Mufulira).

Family			Research Sites						
			Kitwe		Luanshya			Mufulira	
	Overall number of genera	Overall number of species	TD25	TD26	TD24	TD25	TD26	TD8	TD10
			Number of species[a]						
Anacardiaceae	3	3	2(2)	1	2(2)	1	3(3)	2 (2)	1
Annonaceae	1	1	0[b]	0	0	1	0	1	1
Chrysobalanaceae	1	1	1	0	0	0	0	0	0
Combretaceae	2	6	2(2)	1	3(2)	4(2)	2(1)	4(2)	2(2)
Connaraceae	1	1	0	0	1	0	0	0	0
Ebenaceae	1	1	0	1	0	0	0	0	0
Fabaceae	8	11	8 (6)	4(4)	10 (8)	5(4)	4(4)	4(3)	4(4)
Malvaceae	1	1	0	0	0	0	0	1	1
Moraceae	1	3	0	2(1)	2(1)	1	1	2(1)	3(1)
Myrtaceae	1	1	1	1	1	1	1	1	1
Sapindaceae	1	1	1	0	0	1	1	0	0
Sterculiaceae	1	1	0	0	0	1	0	1	0
Phyllanthaceae	1	1	0	0	1	0	1	1	0
Total	23	32							
Species richness per site			15	10	20	15	13	17	13
Species abundance per site			66	45	80	65	66	82	54

[a] Number in parenthesis indicates the corresponding number of genera.
[b] "Zero" indicates absence of a family in a particular site.

Table 2
Species importance value index (IVI) per site on studied tailings dams (TD) in three mining cities of the Zambian Copperbelt (Kitwe, Luansha, Mufulira).

Family	Species	Kitwe		Luanshya			Mufulira	
		TD25	TD26	TD24	TD25	TD26	TD8	TD10
Anacardiaceae	*Lannea discolor*	9.0	0.0[a]	1.8	0.0	3.6	1.0	0.0
	Ozoroa insignis	0.0	0.0	0.0	0.0	1.6	0.0	0.0
	Rhus longipes	1.3	23.6	22.4	10.1	8.9	23.6	21.9
Annonaceae	*Annona senegalensis*	0.0	0.0	0.0	1.2	0.0	2.1	1.7
Chrysobalanaceae	*Parinari curatellifolia*	2.1	0.0	0.0	0.0	0.0	0.0	0.0
Combretaceae	*Combretum apiculatum*	0.0	0.0	0.0	1.4	0.0	0.0	0.0
	Combretum microphyllum	0.0	0.0	1.2	0.0	0.0	1.9	1.7
	Combretum molle	9.6	0.0	5.9	3.9	0.0	16.5	0.0
	Combretum zeyheri	0.0	0.0	0.0	1.2	0.0	6.7	0.0
	Terminalia mollis	0.0	2.2	0.0	5.0	5.5	7.6	0.0
	Terminalia stenostachya	1.5	0.0	0.0	0.0	14.2	0.0	1.7
Connaraceae	*Brysocarpus orientalis*	0.0	0.0	1.4	0.0	0.0	0.0	0.0
Ebenaceae	*Diospyros mespiliformis*	0.0	5.6	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Albizia adianthifolia*	0.0	0.0	6.5	0.0	0.0	0.0	0.0
	Albizia amara	4.0	0.0	0.0	3.1	0.0	3.4	0.0
	Albizia antunesiana	7.9	0.0	3.2	0.0	0.0	1.5	4.5
	Albizia versicolor	13.0	0.0	2.4	2.9	3.1	0.0	0.0
	Bauhinia petersiana	1.3	5.1	1.2	0.0	0.0	3.4	15.6
	Dichrostachys cinerea	2.1	0.0	1.3	1.2	0.0	0.0	2.4
	Peltophorum africanum	19.1	0.0	4.3	5.6	0.0	0.0	0.0
	Bauhinia thonningii	10.8	2.0	12.1	20.3	0.0	0.0	0.0
	Senegalia polyacantha	5.3	30.4	3.3	0.0	3.7	9.2	8.6
	Senna singueana	0.0	2.1	8.1	7.9	2.0	1.3	0.0
	Vachellia sieberiana	0.0	0.0	3.3	0.0	5.9	0.0	0.0
Malvaceae	*Azanza garckeana*	0.0	0.0	0.0	0.0	0.0	0.0	5.9
Moraceae	*Ficus capensis*	0.0	0.0	0.0	0.0	0.0	0.0	2.0
	Ficus craterostoma	0.0	7.1	9.3	18.4	8.4	5.8	8.4
	Ficus sycomorus	0.0	2.4	1.3	0.0	0.0	6.4	23.5
Myrtaceae	*Syzigium guineense*	8.3	19.6	5.8	14.1	40.4	3.0	2.0
Phyllanthaceae	*Phyllanthus guineensis*	0.0	0.0	1.6	0.0	1.5	3.1	0.0
Sapindaceae	*Dodonaea viscosa*	4.6	0.0	0.0	2.4	1.4	0.0	0.0
Sterculiaceae	*Dombeya rotundifolia*	0.0	0.0	0.0	1.3	0.0	1.5	0.0
	Min	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
	Max	**19.1**	**30.4**	**22.4**	**20.3**	**40.4**	**23.6**	**23.5**
	CV (%)	**155**	**235**	**157**	**173**	**243**	**171**	**198**

[a] *"Zero" indicates absence of a species in a particular site. CV stands for coefficient of variation.*

and 25 (Fig. 2d, g).

Overall, soil Mn and chromium (Cr) contents were comparable across different TDs in Kitwe, Luanshya and Mufulira, although Kitwe TD26 deviated from the general pattern. However, Cu contents were very variable, and no consistent comparison could be drawn across cities. The analysis of data across tailings dams reveals a similar pattern of distribution between cobalt and Ba (Fig. 2d, g), zinc (Zn) and Ni (Fig. 2e and f) and between B and Mo (Fig. 2h and i).

3.3. Metal influence on species richness and abundance on studied tailings dams

Kitwe TD26, the most metal enriched tailings dam across investigated sites (Fig. 2), presented the lowest values of tree species richness and abundance among studied tailings dams (Table 1). Similarly, Mufulira TD8, one of the least metal-enriched tailings dam was found to be higher in species richness and abundance, thus suggesting the existence of relationship, to some extent, between soil metal content and species richness and abundance on tailings dams.

Like its counterpart TD8, Mufulira TD10 also presented the lowest content of nickel (Ni), boron (B), molybdenum (Mo), barium (Ba), and cobalt (Co) (Fig. 2). By contrast however, its species richness and species abundance were significantly lower than TD8 (13 tree species <17; 54 individual trees <82; Table 1). A similar observation was made on Luanshya TD24 and TD25, which presented comparable profile of metal contents, yet higher tree species richness (20 > 15) and higher tree abundance (85 > 65) were recorded on Luanshya TD24, implying a relatively lesser influence of metal on tree species richness and abundance for these tailings dams.

Taken separately, it is apparent that the soil metal contents scarcely influenced tree species richness, as revealed by Monte Carlo permutation tests (Table 3). None of the detected metals affected species richness in Kitwe TD25, Kitwe TD26 and Luanshya TD24. Nonetheless, a significant influence was detected for Mn and chromium (Cr) in Luanshya TD25, Cr in Luanshya TD26, Mo in Mufulira TD8, and copper (Cu) and zinc (Zn) in Mufulira TD10.

Further, canonical correspondence analysis (CCA) was performed to get insights into the behaviour of tree species as influenced by metals on tailing dams where metals influence (Cu, Mn, Zn, Cr, Mo) was detected (Luanshya TD25 & 26, Mufulira TD8 & 10; Fig. 3). Both negative and positive influence of metals on tree species richness were recorded (Fig. 3a). *Rhus longipes, Annona senegalensis,*

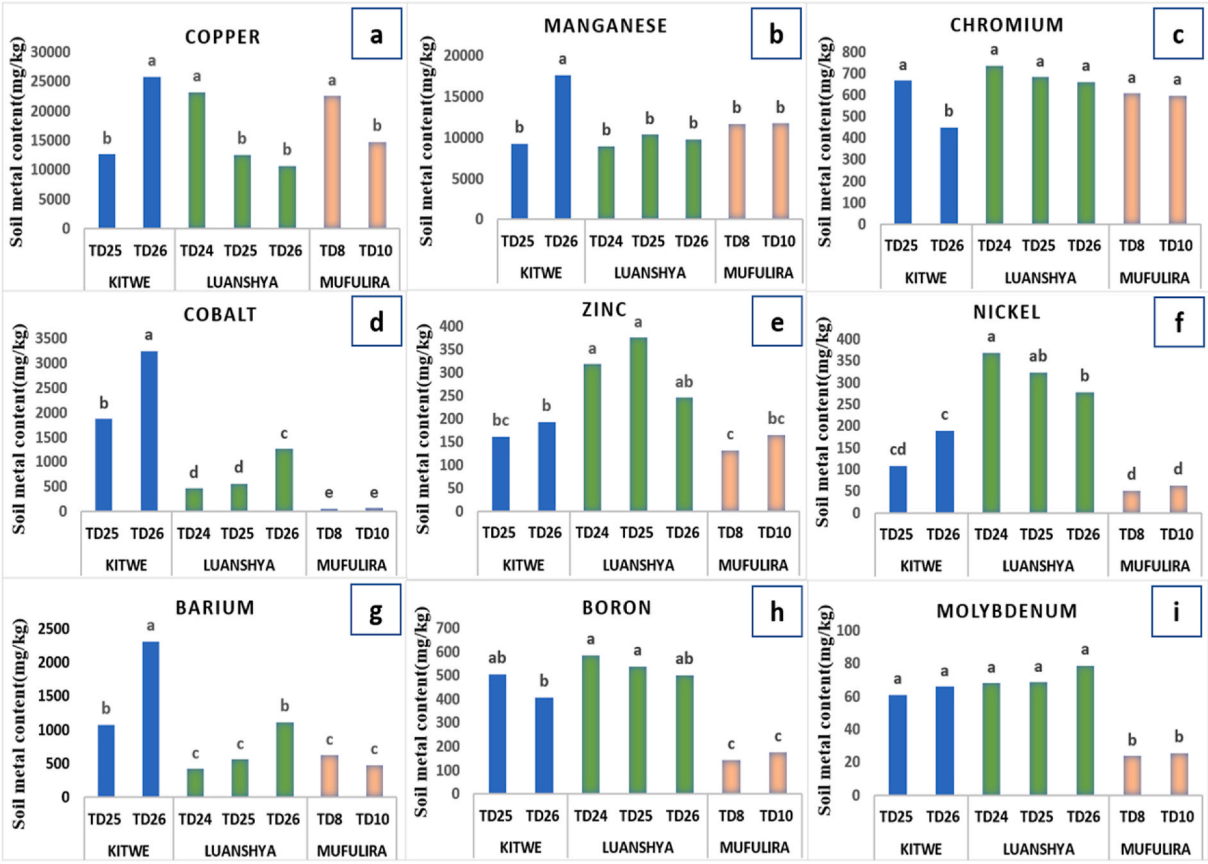

Fig. 2. Metal concentrations in soil from different tailing dams (TD) across the three studied mining cities along the Zambian Copperbelt (*i.e.* Kitwe; Luanshya; Mufulira).

Table 3
Monte Carlo permutation tests results per site.

Metal	Study sites						
	KITWE		LUANSHA			MUFULIRA	
	TD25	TD26	TD24	TD25	TD26	TD8	TD10
Cu	0.644	0.608	0.768	0.130	0.064	0.482	**0.002**[a]
Mn	0.746	0.064	0.950	**0.018**[a]	0.074	0.850	0.628
Zn	0.518	0.369	0.862	0.538	1.000	0.662	**0.018**[a]
Cr	0.472	0.480	0.878	**0.048**[a]	**0.018**[a]	0.396	0.118
Co	0.862	0.306	0.916	0.958	0.936	0.680	0.088
Ni	0.758	0.460	0.854	0.246	0.266	0.974	0.600
Ba	0.686	0.548	0.918	0.930	0.952	0.302	0.906
B	0.525	0.408	0.850	0.086	0.198	0.458	0.102
Mo	0.208	0.872	0.662	0.236	0.422	**0.004**[a]	0.512

[a] The numbers in bold indicate statistically significant plant-environment interaction.

Terminalia mollis, Bauhinia petersiana, Senna singueana, Ficus craterostoma and to a lesser degree, *Combretum mole, Albizia amara, Albizia antunesiana, Dombeya rotundifolia* were found to be the most influenced tree species (Fig. 3b).

In general, the trend of metal influence on species richness was not consistent, as the richness of the same species could be negatively influenced by one metal but positively affected by another. In some cases, a given tree species was differently affected by the same metal across tailings dams. For example, *Rhus longipes, Terminalia mollis, Senna singueana, Syzygium guineensis,* were affected by Cr in opposite way in Luanshya TD25 and Luanshya TD26 (Fig. 3a and b). Interestingly however, *Lannea discolor, Albizia versicolor, Vachellia sieberiana and Ficus craterostoma* were positively influenced by Cr concentration in both Luanshya TD25 and TD26. Similarly, the richness of tree species including *Rhus longipes, Ficus craterostoma, Syzygium guineense, Phyllanthus guineesis* were positively affected by high concentration of Cu in Mufulira TD10, as were *Combretum microphyllum* and *Bauhimia petersiana* for Zn in Mufulira TD8, and

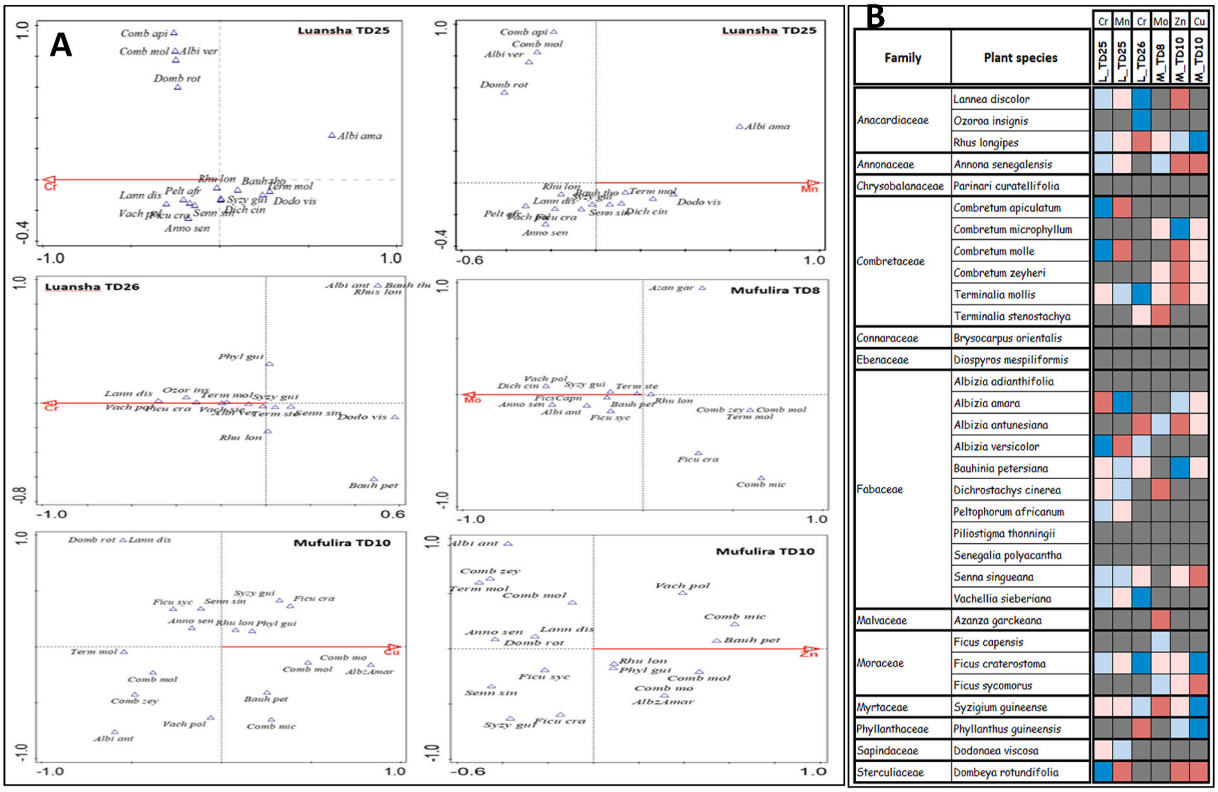

Fig. 3. Influence of metal concentrations on tree species richness and abundance on selected tailing dams (TD) in Zambian Copperbelt cities (i.e Luanshya (L); Mufulira (M)) as revealed by canonical correspondence analysis (a), and corresponding heatmap summarizing species behaviour under low and high soil metal concentration conditions (b). Colours are utilized to appreciate the nature of metal influence on tree species richness: "blue" for positive influence and "red" for negative influence. Metal influence at low concentrations is indicated by light colour (blue or red), while their influence at higher concentrations is marked by deep colour (blue or red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Albizia amara for Mn in Luanshya TD25.

3.4. Metal uptake and distribution in different tree species identified

Data on mineral accumulation in roots demonstrate a differential behaviour of metals. As a general pattern for tree species, copper (Cu), manganese (Mn), cobalt (Co), chromium (Cr) and nickel (Ni) were less absorbed in roots. Comparatively, a slightly higher uptake rate was noted for zinc (Zn), boron (B), molybdenum (Mo) and barium (Ba) (Fig. 3). The accumulation behaviour of minerals also somehow varied from one tailings dam to another. The bioaccumulation of metals including Cu, Mn, Zn, cobalt, Ni was restricted at relatively higher extent in Luanshya TD26 (L_TD26) as compared to other tailings dams (TDs). Likewise, Zn and cobalt bio-accumulation was noticeably restricted in Luanshya TD25, as were Ni in Luanshya TD24 or Cobalt and Ba in Kitwe TD25 (Fig. 3). Conversely, Mufulira TD8 and TD10 allowed a relatively higher accumulation of Zn, Ni, and Ba. Hence, it is apparent that the soil-to-roots transfer of metals was more limited in tailings dams located in Luanshya City (L_TD26, L_TD25, L_TD24) and Kitwe (K_TD25), whereas those found in Mufulira city favoured the flux of metals from soil to root tissues.

In general, the identified tree species in the present study effectively restricted metal transfer from soil into roots, as they showed a bioaccumulation factor (BF) < 1 in all studied tailings dams (Fig. 3). Such restriction was particularly strong in *Albizia amara* (for cobalt, Cr and Ni) and *Ficus craterostoma* (for Ni), but relatively lower in *Albizia adianthifolia* (for Zn and Mo notably). Contrast to the general trend, *Diospyros mespiliformis* actively accumulated Ba in roots up to BF equivalent to 115% ($\log2 = 6.9$) in Kitwe TD26 (Fig. 3).

The translocation factor (TF) was determined to appreciate the flux of metals from roots to leaves. Like the soil-to-roots transfer, the pattern of mineral transfer from roots to leaves varied among metals, with Cu, Co, Cr, Ni and Mo translocation being strongly restricted in all tree species (TF < 1), except for *Annona senegalensis*, *Parinari curatellifolia*, *Dombeya rotundilifolia* that demonstrated a higher ability of translocation (TF > 1). Another group of metals such as Mn, Zn, B and Ba presented an opposite trend and were actively translocated to leaves (TF > 1) in all tree species, excepts *Diospyros mespiliformis, Albizia amara, Albizia antunesiana Dodonnaea viscosa and Dombeya rutundifolia* that effectively restricted Ba translocation (TF < 1), and *Parinari curatelifolia* that was effective in restricting Mn translocation (Fig. 5).

Differences were also noticed regarding the pattern of roots-to-shoots metal translocation between tailings dams. A greater restriction was observed in Luanshya TD24 for Cu, cobalt, Cr, Ni and Mo. The bioaccumulation of cobalt was also remarkably limited in Luanshya TD25, as was that of Mn in Kitwe TD25. By contrast, a greater root-to-shoot transfer was recorded in Mufulira TD8 notably for Cu and Cobalt. Hence, in similar fashion to BF (Fig. 3), a dichotomy in TF is apparent between Luanshya and Kitwe city on one side (lower translocation) and Mufulira city on the other side (higher translocation; Fig. 4).

4. Discussion

4.1. Species richness and abundance of trees accross studied tailings dams

In the Zambia Copperbelt Region, the environmental impacts of metals results from both historical and ongoing mining operations. And in most cases, the areas around tailings dams in the region are prone to air pollution, hence the neighbouring population is exposed to high concentrations of PM10 with potential to provoke lower respiratory symptoms and reduced lung functions [1]. These toxic tailings dams are also known to be blown into the nearest agricultural land, thereby endangering plant growth, while also posing threat to human and animal health through contamination in the food chain, as food metal contents are generally found above the permissible threshold for safety [56]. Moreover, these tailings dams generally flow in surficial water bodies, posing eminent water pollution in aquatic ecosystem and undesirable effects on irrigation [1].

Restoration of vegetation cover on tailings dams in the Zambian Copperbelt is expected to control the metal pollution associated to these mine wastelands and remove the threats to human health in the region. The selection of appropriate plant species is therefore crucial to fulfil this objective, for which the occurrence of native tree species constitutes an asset that is largely untapped in the region. The present study has identified a total of 32 tree species naturally occurring on tailing dams across three major mining cities of the Zambian Copperbelt (Kitwe, Mufulira and Luanshya). As much as 13 different families were represented. Such a variety of species from various families shows the possibility of vegetation diversification that is essential for ecological restoration of disturbed ecosystems. The various families possess different ability that aid in vegetation restoration on the wastelands [57]. A number of reports have demonstrated a favourable impact of vegetation diversification for microbes colonization of the rhizosphere, known to improve plant growth via stimulation of growth regulators and mobilization of essential nutrients [58] whilst sequestering metal contaminants [59].

The overall species richness identified in this study clearly demonstrates a remarkable ability of indigenous tree species of the region to colonize mining generated wastelands despite the high levels of metals. This corroborates previous observation by Kambingá and Syampungani [5] who noted the good growth performance of most native species on a tailings dam in Kitwe. Similar results were also reported for gold mine tailings in Zimbabwe [60]. Nonetheless, the species richness recorded across tailings dams in this study remains relatively low when compared to the floristic composition in the neighbouring local forests (55 species) [3]. This may be an indication that some local native species are not adapted to the tailings dam environment. A few studies have also reported lower

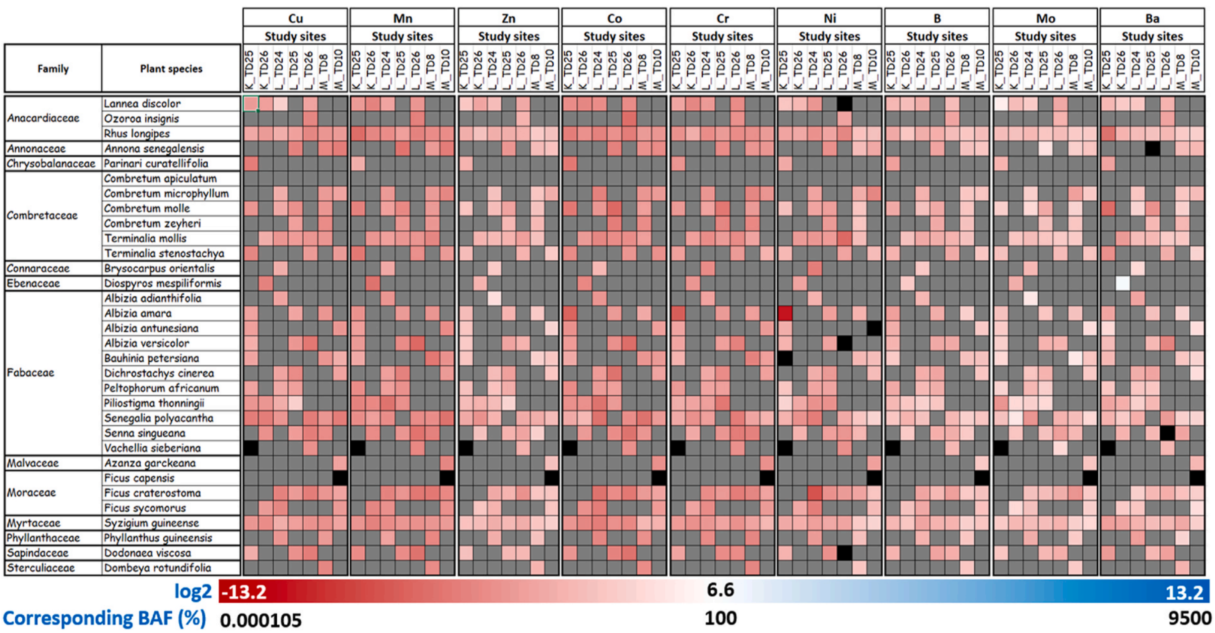

Fig. 4. Pattern of metals bioaccumulation factor (BAF) in different tree species occurring on tailing dams (TD) across the three studied mining cities along the Zambian Copperbelt (i.e. Kitwe: "K_"; Luansha: "L_"; Mufulira: "M_"). BAFs (log transformed) are quantified by colour: "red" and "blue" reflecting the ability of plant for metals exclusion or accumulation respectively, with "white" representing the point of demarcation. "Grey" indicates absence of plant species on the given site, while "black" indicates no detected values for a species that is present at the site (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

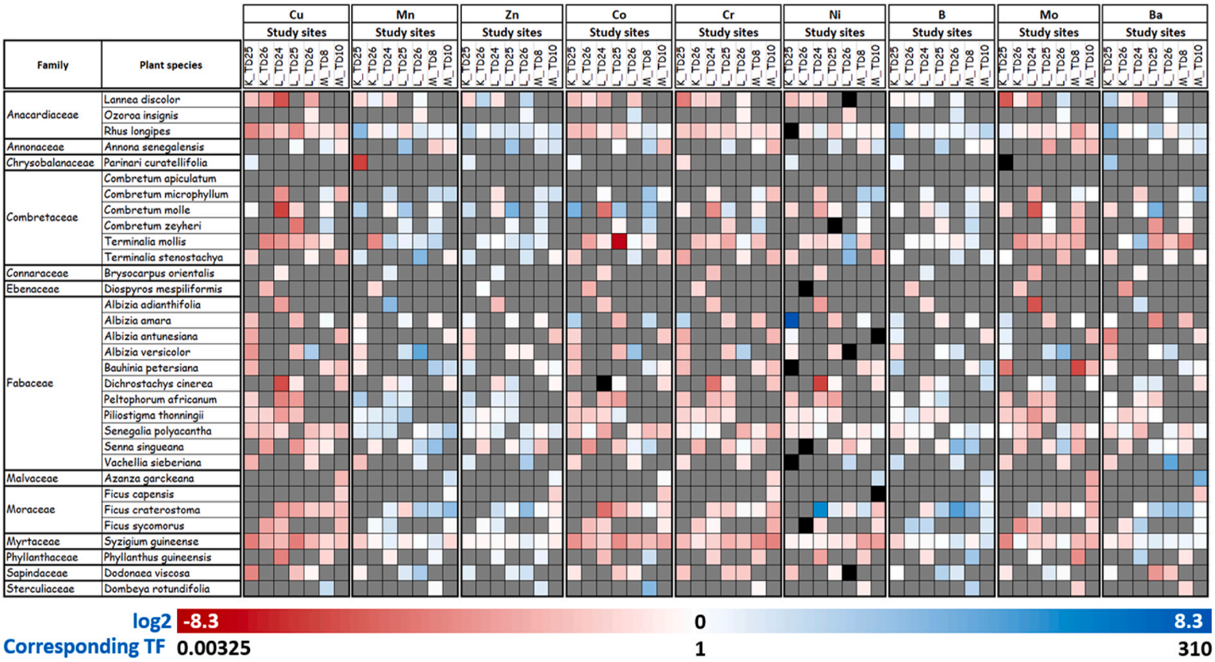

Fig. 5. Pattern of metals translocation factor (TF) in different tree species occurring on tailing dams (TD) across the three studied mining cities along the Zambian Copperbelt (i.e. Kitwe: "K_"; Luansha: "L_"; Mufulira: "M_"). TFs (log transformed) are quantified by colour: "red" and "blue" reflecting the ability of plant for metals exclusion or accumulation respectively, with "white" representing the point of demarcation. "Grey" indicates absence of plant species on the given site, while "black" indicates no detected values for a species that is present at the site (For interpretation of the references to colour in this figure legend. The reader is referred to the Web version of this article). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

species richness in metal contaminated sites than the uncontaminated. For example, [61] reported eight (8) species in slimes and 21 species in a natural woodland in Zimbabwe. Similarly, [62] reported 22 plant species on contaminated sites compared to 40 species in uncontaminated areas in China.

Logically, the lower species richness in contaminated sites compared to the uncontaminated ones gives an indication of the restrictive effect of metal contamination on plant development and survival. Tailings dams are characterized by high metal contents and left-over chemicals, low nutrient and water holding capacity, high salinity or acidity and poor physical structure [63, 3, 64] making it difficult for most plant species to thrive. Elevated metal concentrations were observed on studied tailings dams in the current study (Fig. 2), with most metal (except zinc and chromium) exceeding the screening benchmark limit (Appendix 1), especially Cu concentration, which was over 100 times higher than the screening benchmark [65]. The same trend was observed for manganese (Mn), cobalt (Co), boron (B), and molybdenum (Mo); although relatively low differences were observed between nickel (Ni) and barium (Ba) concentrations that were less than 2 times higher relative to the CCMERC guideline (Appendix 1). Coincidentally in this study, the tailings dam most enriched in copper, manganese, cobalt, barium (Kitwe TD26, Fig. 2) also presented the least species richness (Table 1), showing a possible relationship between species richness and abundance and the environmental variables such as soil metal concentration.

Species abundance shows how well a species functions in an ecosystem in relation to other species; and normally, species with high abundance are well adapted and can reproduce in an area. [66] suggested that the analysis of common and uncommon species in an area could be revealed by species abundance, which is key for conservation and management purposes. In this study, species richness and abundance also varied across studied tailings dams. Interestingly, the tailing dams with the highest species richness i.e. Luanshya TD24 (20 species) and Mufulira TD8 (17 species), were also the highest in species abundance (Table 1). For example, Kitwe TD26 (the most enriched in soil metal contents) exhibited the lowest species abundance, substantiating the view of possible metal influence on tree species richness and abundance on tailing dams in the Zambian Copperbelt.

Among identified families, Fabaceae (11 species) and Combretaceae (6 species), followed by Anacardiaceae (3 species) and Moraceae (species) were the most represented (Table 2). The preponderant role of Fabaceae in disturbed ecosystems is widely reported, with a few studies demonstrating their potential for colonization of mine generated wastelands [20,21]. The innate tolerance to metal stress is commonly put forward to account for such easy adaptability of Fabaceae to harsh environment [26]. Most importantly, Fabaceae are considered as ideal candidates in prospect of ecological restoration. In Mediterranean areas for example, the combined use of local grasses ecotypes and Fabaceae species resulted in marked increase in biomass production for the overall system, and a positive impact on plant succession [67]. Unlike the fabaceae family, Combretaceae, Anacardiacea and Moraceae have been scarcely investigated for their potential to colonize metal contaminated sites. While their presence is proved in forest ecosystems neighbouring tailings dams [3], factors explaining their higher frequency on studied tailings wastelands remain unknown to date. The potential causes could be in relation to (i) a better adaptability to harsh conditions favoured by their genetic background, (ii) a positive ecological interaction in the biophysical context of studied tailings dams, or (iii) a better mode of dispersal from neighbouring uncontaminated sites towards studied tailings dams. Further investigations are warranted to validate the above assumptions and gain insights on the actual factors and mechanisms underlying the observed differential species richness across studied tailing dams. The future investigations shall clarify the environmental factors and/or the biological and ecological drivers making some tree species unique to certain mine tailings dams, while other tree species are absent at certain mine tailings dams.

At species level, species importance value index (IVI), a measure attesting the good growth performance of a species [68], was used to evaluate how dominant a species is in each tailings dams. On this basis, *Rhus longipes* (Anacardiaceae)*, Syzygium guineense* (Myrtaceae)*, Senegalia polyacantha* (Fabaceae) and *Ficus craterostoma* (Moracease) have been identified as the most dominant native tree species on tailings dams, (Table 2). Such dominance, combined with their occurrence in almost all studied tailing dams indicates their good adaptability for growth and reproduction in harsh environment. Therefore, they represent good candidates for phytoremediation of mine wastelands of the region. Other species such as *Combretum molle*, *Bauhinia thonningii*, *Peltophorum africanum, Ficus sycomorus and Terminalis mollis* also had high IVI value, indicating their dominance and good growth performance, and may be considered for phytoremediation of tailings dams in the Zambian Copperbelt.

4.2. Phytoremediation potential of identified tree species

Phytoremediation in terms of phytostabilisation has been proposed as a suitable technique to control the environmental risks of metal-enriched tailing dams. Selection of candidate species suitable for phytostabilization is a key aspect of phytostabilization [25]. In addition to the production of large biomass, plant species with phytostabilization potential exclude metals [69] and have high ability to adapt to contaminated sites [25]. In this study, the total metal contents (Cu, Mn, Co, Cr, Zn, Ni, Ba, B, Mo) were measured in tailings, in plant shoots and plant roots, and the biocaccumulation factor (BF) and translocation Factor (TF) were estimated to evaluate plant suitability for phytostabiliation. All the inventoried tree species presented a BF < 1 in all studied tailings dams except for *Diospyros mespiliformis* that effectively accumulated Ba in roots (BF $= 1.15$) in Kitwe TD26 (Fig. 3). Moreover, the 9 identified dominant tree species (*Rhus longipes, Syzygium guineense, Senegalia polyacantha, Ficus craterostoma, Combretum molle*, *Bauhinia thonningii*, *Peltophorum africanum, Ficus sycomorus*) effectively restricted the root-to-shoot translocation of Cu, Co, Cr, Ni and Mo, thereby satisfying all the requirements for phytostabilization species (BF and TF < 1). However, these species, were unable to restrict Mn, Zn, B and Ba translocation from root towards shoots (BF < 1, TF > 1; Figs. 3 and 4) making them unsuited for phytostabilization of these metals [22]. Alternatively, they present the potential to be used for phytoextraction. Such differential accumulation abilities towards various metals entails the need for careful selection of these tree species while considering remediation of multi-metal-contaminated sites. To date, there is little reports on metals accumulation in these 9 dominant species, except for *Senegalia polyacantha,* which was reported to be Cu accumulator in a metal-enriched land in Zimbabwe [70]. Therefore, further investigations are still needed on the identified dominant tree species, notably their comparative phytoremediation potential in the same experimental setup, which will provide further insights for proper phytomanagment of metal-polluted ecosystems in the Zambian Copperbelt region using these tree species.

Although showing lower IVI values, *Annona senegalensis*, *Parinari curatellifolia* and *Dombeya rotundilifolia* could be singled out of the 32 identified native tree species for their particular ability to accumulate Cu and Co in shoot tissues (Fig. 4). And exceptionally, P*arinari curatellifolia* appears to possess good potential for Ni accumulation and can be considered for use in Ni phytomining.

4.3. Variability of species richness across studied tailings dams as influenced by soil metal concentration

Although an association between species richness and metal contents on tailings dams emerged, results of canonical correspondence analysis attested a relatively lesser influence of metals, restricted on Luanshya TD25 (for Mn and Cr), Luanshya TD26 (for Cr), Mufulira TD8 (for Mo) and Mufulira TD10 (for Cu and Zn; Fig. 3). Such weak association plausibly implies that environmental factors other than soil metal concentrations might have had greater influence on species richness and abundance on studied tailings dams. Factors such as metal speciation which drives metals mobility in soil and their availability for uptake by plant can be considered to tentatively explain the observed discrepancies between i.e Mufulira TD8 and Mufulira TD10 or between Luanshya TD24 and Luanshya TD25, which presented comparable soil metals status (Fig. 2) but significantly differed in tree species richness and abundance (Table 1). The interaction between metals and plants may depend on metal solubility and reactivity with inorganic and organic molecules of such plant species [71]. Likewise, metal mobility may be influenced by the soil pH and organic matter [72]. Variations in these variables may explain the difference in the responses of tree species to the same metal in different sites as observed in this study. Additional investigations on soil chemistry on studied tailings dams are needed to provide a better understanding of common and possibly specific environmental factors dictating tree species richness and abundance across studied tailings dams in the Zambian Copperbelt region.

Normally, high metal concentrations negatively impact species richness, plant performance and the overall abundance [63], which was also observed for several cases in this study. Of importance however, the dominant species *Rhus longipes, Syzygium guineense* and *Ficus craterostoma* were positively affected by high concentration of Cu in Mufulira TD10, implying their remarkable ability for tolerance of the stress associated with excess soil Cu concentration. The average soil Cu concentration recorded in this study (17425.59 mg kg^{-1}) is among the highest reported on tailings dams, which may be attributed to the grade of copper ore in the region [73] and the extraction technology used. With such high concentration of Cu, it is evident that tree species growing on Copperbelt tailings dams are highly tolerant to Cu; and the fact that *Rhus longipes, Syzygium guineens* and *Ficus craterostoma* are positively affected by Cu clearly underlies their place as viable option for vegetation restoration programs of Cu tailings in the region. We cannot rule out the possibility that the three species (*Rhus longipes, Syzygium guineens* and *Ficus craterostoma*) beneficiate from a favourable genetic makeup endowing them with capability to thrive under Cu-enriched environments. In the same way, although showing relatively lower IVI values, tree species including *Lannea discolor, Albizia versicolor, Vachellia sieberiana and Ficus craterostoma* were positively influenced by Cr (Fig. 3) and may be assumed as potential candidates for phytomanagement of Cr-polluted environment.

5. Conclusion

The present study was carried out to gain insights into the floristic composition of native tree species growing on Zambian Copperbelt tailings dams and to determine their metal accumulation and exclusion strategies for possible application in phytoremediation. Obtained results revealed a rather diversified floristic composition of native trees on studied tailings (32 species, from 13 different families and 22 genera), demonstrating the existence of great opportunity to enhance ecological restoration of mine wastelands in the region through vegetation diversification, by targeting species combination for optimal management of varied metal-contaminated ecosystems across the region. Eight species from different families including *Rhus longipes* (Anacardiaceae), *Syzygium guineense* (Myrtaceae), *Senegalia polyacantha* (Fabaceae), *Ficus craterostoma* (Moraceae), and at lesser extent *Combretum mole* (Combretaceae), *Bauhinia thonningii* (Fabaceae), *Peltophorum africanum* (Fabaceae) *and Ficus sycomorus* have been identified to have appreciable Importance Value Index and metal exclusion ability, making them suited for phytostabilization of Cu, Co, Cr, Ni and Mo notably. Among them, particular emphasis has been placed on *R. Longipes, S. guineense and F. craterostoma,* which in addition to being ubiquitous and dominant, were positively correlated to high Cu concentration, indicating their remarkable ability for tolerance to Cu stress that is largely expected in tailings dams of the Zambian Copperbelt region. These results strengthen the possibility for restoration of mine wastelands in the Zambian Copperbelt through phytostabilization, using the identified species. Obtained results further revealed the possibility of using other tree species such *Annona senegalensis*, *Parinari curatellifolia*, *Dombeya rotundilifolia* for phytoextraction of metals including Cu, Co, Cr, Ni and Mo.

While investigations on environmental variables suggested scarce influence of metals on observed ecological data, the differential behaviour of plant species across studied sites led to hypothesize existence of additional environmental and ecological factors, and possibly biological factors driving tree species richness and abundance on mine wastelands in the Zambian Copperbelt region, which warrants further investigations.

Overall, the present study has provided sought-after information to contribute to precise decision making for a tree-based ecological restoration of mine wastelands towards efficient mitigation of environmental impacts arising from tailings dams in the Zambian Copperbelt region. Further, by revealing the dominant tree species occurring on the mine wastelands, this study provides a solid basis to advance research relating to phytoremediation of metal contaminated environment in the region, with future research focus expected to shift towards understanding the eco-physiological basis underlying the successful establishment of identified dominant tree species on mine wastelands, which was not explored in the present study.

Author contribution statement

NALUKUI MATAKALA, Master's: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

PAXIE WC. CHIRWA, PhD; STEPHEN SYAMPUNGANI, PhD: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

THEODORE MULEMBO MWAMBA, PhD: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13585.

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