PHYTOREMEDIATION POTENTIAL OF INDIGENOUS PLANTS AT NCHANGA MINE, CHINGOLA, ZAMBIA

by

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Author's Declaration

I, Lupupa Kachenga, do hereby declare that this dissertation represents my own work and that all the sources I have quoted have been duly acknowledged by means of complete references. I further declare that this dissertation has not previously been submitted for a Degree, Diploma, or any other qualification at this or any other University.

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Approval

This dissertation by LUPUPA KACHENGA is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Tropical Ecology and Biodiversity of the University of Zambia.

Examiners

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Dedication

I dedicate this dissertation to my dad and mum, Mr. Pearson Kachenga and Mrs. Grace Mulenga Kachenga. Your greatest gift to me is my education and I will forever be grateful for your support and continuous belief in me.

Copyright

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Abstract

Mining and smelting processes are among the key sources of soil contamination by heavy metals resulting in dramatic disturbances and loss of biodiversity. Phytoremediation is a cost effective technology that involves the efficient use of plants to eliminate or immobilize environmental contaminants. This, therefore requires identification of native plants that are able to accumulate heavy metals in their plant tissues at concentrations higher than that in the soil in which they are growing.

This research investigated the phytoremediation potential of indigenous plants growing at the tailings dams of Nchanga Mine in Chingola, Zambia. Tailings Dam Four (TD4) and a site 50m away from TD4 at Nchanga Mine were sampled. TD4 and Sampling Area Two were divided into four and two quadrats, respectively. Each quadrat was further divided into nine plots and three plots from each quadrat were randomly sampled. Composite soil samples were collected from the plots and a total of 175 individuals of plant species were collected and analysed. Atomic Absorption Spectrophotometry was used to determine the concentrations of copper (Cu) and zinc (Zn) in the soils and plant specimens.

The findings of the study showed that the concentrations of Cu and Zn in the soil ranged from 891.41 mg/kg to 15,617.47 mg/kg and 20.73 mg/kg to 96.85 mg/kg, respectively. *Arthraxon quartinianus* (A. Rich.) Nash.(a grass specie) had the highest concentration of Cu (1016.8 mg/kg) while *Cyperus rotundus* L. (a grass specie)had the lowest (29.35 mg/kg). *Arthraxon quartinianus* (A. Rich.) Nash.had the highest concentration of Zn (192.8 mg/kg) and *Crinum* L. (a herb)had the lowest (28.24 mg/kg). The Bioaccumulation Factors indicated that all the plant species studied are Cu excluders; and with the exception of C*rinum* L., all were Zn accumulators and *Arthraxon quartinianus* (A. Rich.) Nash.was ahyperaccumulator of Zn. Results showed that all the plant species studied had potential for phytoremediation and can therefore be used in management and possible decontamination of mine dumps and areas surrounding the mines. Further research is needed to cover other heavy metals such as Cobalt, Nickel and Cadmium.

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List of Abbreviations

AAS - Atomic Absorption Spectrophotometer

BAF - Bioaccumulation Factor

Cu - Copper

Fe - Iron

ICRCL- Interdepartmental Committee on the Redevelopment of Contaminated Land

Mn - Manganese

TD4 - Tailings Dam Four (4)

TF - Translocation Factor

Zn - Zinc

CHAPTER 1. INTRODUCTION

1.1 Background

The Mining industry is the backbone of Zambia's economy accounting for up to 80% of the country's foreign earnings (Ngoma, 2011). A large proportion of the world's minerals come from developing countries such as Brazil, China and Zambia. However, mining is one of the anthropogenic activities causing some of the most dramatic disturbances on biodiversity, water quality, and land use (Sheoran, et al, 2010). Increased mining activity increases the amount of degraded land. This includes bare stripped areas, loose soil piles, waste rock and overburden surfaces and subsided land areas. Surface mining or open pit mining causes up to 11 times more land destruction compared to underground mining; fertile land is transferred into wasteland or bog and local status of water and landscape deteriorates with some ecosystems being entirely destroyed (Ze-wei, et. al, 2000; Kumar, et. al, 2014).

Mining and smelting processes are among the key sources of heavy metal contamination of soil and water (Zhen-Guo et al., 2002). Although many metals are essential, all metals are toxic at a high concentration due to the oxidative stress they cause by formation of free radicals, as well as disrupting the function of pigments and enzymes (Malayeri et al., 2008). As a result, soil contaminated with heavy metals is rendered unsuitable for plant growth, thereby resulting in the loss of biodiversity (Ghosh and Singh, 2005).

In the recent past, there has been increasing concern for the environment and as such, post-mining reclamation of degraded land should be an integral feature of the mining spectrum (Sheoran et al., 2010). Reclamation is the process by which highly degraded land is returned to productivity and some measure of biotic function and productivity is restored (National Resources Conservation Service, 2006). There are a number of methods that have been used to cope with the soil pollution of degraded lands. Current techniques include excavation, chemical stabilization, soil washing or soil flushing (Mehes-Smithet al., 2013), but these methods are sophisticated techniques and are suitable for relatively small soil volumes at sites that require immediate action (Malayeri et al., 2008; Mehes-Smithet al., 2013). They are costly, time consuming and sometimes environmentally destructive rendering the soil unusable after treatment (Aboulrooset al., 2006; Lorestaniet al., 2011a).

In recent years, there are cost effective technologies that have been developed by scientists and engineers. These include the use of microorganisms or live plants to clean up the polluted areas and phytoremediation.

Phytoremediation is an emerging cost effective technology that has aesthetic advantages and long term applicability (Jadia and Fulekar, 2009; Susarlaet al., 2002). Phytoremediation is an integrated multidisciplinary approach and involves the efficient use of plants to eliminate, detoxify or immobilize environmental contaminants that are present in a growth matrix such as soil, water or sediments, through the natural, biological, chemical or physical processes of the plants (Jadia and Fulekar, 2009). The vegetation is capable of improving the nutrient conditions of the soil thereby setting the base for establishment of self-sustaining vegetation cover (Ssenku et al., 2014).

Plants that are able to take up heavy metals to a greater concentration than that in the soil in which they are growing are called hyperaccumulator plants (Baker and Brooks, 1989; McGrath and Zhao, 2003).Hyperaccumulators that grow in polluted areas can accumulate large concentrations of heavy metals in their shoots; consequently, the removal of metals from the soil can be enhanced considerably by the judicious selection of plant species (Nazir et al., 2011). Therefore, it is important to search for plants that spontaneously colonize these disturbed sites (Mehes-Smithet al., 2013). Research has shown that native plants growing naturally in hostile mining environments are potential phytoremediators and can be used to rehabilitate the disturbed sites (Prasad and Freitas, 2003; Sharma and Pandey, 2014). Identification of indigenous hyperaccumulator plants is therefore imperative for the successful implementation of phytoremediation for mine reclamation mainly because native plant species require less management and they are acclimatized to the native climatic conditions and seasonal cycle (Mwegoha, 2008; Lorestani et al., 2011b; Sarma, 2011).

Most Copper (Cu) and Zinc (Zn)hyperaccumulator plants that have been identified are specific to Europe, Asia, USA, and other African Countries like Congo DR and Zimbabwe. However, few species of Zambian native vegetation have been identified, namely,*Cheilanthes perlanata* (Pic. Serm.) Kornas, *Eragrostis racemosa* (Thunb.)Steud., *Bulbostylis pseudoperennis* Goetgh., *Aspilia ciliate*(Schumach.) Wild.,(Leteinturier et al., 2001), *Conyza cordata* Kuntze., *Persicaria puncata* (Elliot) Small.and*Persicaria capitata*(Buch. -Ham. Ex D. Don) H. Gross (Van der Ent et al.,2015).

Therefore, this research sought to identify native plant species growing at the Nchanga Mine that have potential for phytoremediation.

1.2 Statement of the problem

Zambia has a fairly rich biological diversity which is being impacted upon directly and indirectly by mining activities. Heavy metal soil contamination resulting from mining activities in various parts of the country is a problem that is not adequately addressed in spite of the danger this poses to the environment and human health (Ikenaka, 2010; Lindahl, 2014). Phytoremediation, an eco-friendly and cost effective method of rehabilitating and remediating impacts of mining requires identification and selection of locally available plant species that are established at the contaminated sites. However, there is little awareness and practice in Zambia about phytoremediation despite the tremendous amount of literature on its use for reclamation of mining areas elsewhere in the world. Though limited studies have been conducted in Solwezi, Kabwe and parts of the Copperbelt including Kitwe, Ndola and Mufulira, none has been conducted in Chingola despite the fact that the largest Open Pit Mine in Africa is in Chingola. Therefore, this research will attempt to fill this knowledge gap by identifying plants that have potential for phytoremediation, native to Zambia, particularly in Chingola at Nchanga mine.

1.3 Significance of the Study

Mining leads to loss of biodiversity of both flora and fauna (Lindahl, 2014). Phytoremediation is one method that can be employed to reclaim disturbed mine land. It does not destructively impact the soil structure and soil fertility as some conventional technologies do. It is an environmentally-friendly approach, cheap and low-tech alternative suitable for developing countries like Zambia. Therefore, research needs to be conducted to identify potential native phytoremediator plants for the purpose of ecological restoration and reclamation of land that is no longer used for mining.

This study will provide an inventory of the plants growing at Nchanga Mine and will contribute to science by filling part of the knowledge gap on native plants that have the potential for phytoremediation in Zambia, particularly in Chingola. The methods of this study can be used in combination with others for management, decontamination and possible rehabilitation of tailings storage facilities, polluted soils and consequent conversion of mine waste to safe resources using natural processes hence achieving sustainable rehabilitation of entire mines. This research undoubtedly has added more plants to the list of those already identified in other tropical countries and even here in Zambia.

1.4 Objectives of the Study

1.4.1 General Objective of the Study

The general objective of this study was to assess the phytoremediation potential of indigenous plants growing at the Nchanga Mine tailings dams in Chingola, Zambia.

1.4.2 Specific Objectives of the Study

To achieve this aim, the specific objectives were:

1. To determine the concentrations of Cu and Zn in the soil cover and tailings at Nchanga Mine;
2. To identify plant species growing at the Nchanga Mine;
3. To analyze the concentrations of Cu and Zn in the tissues of the plant species growing on the contaminated sites;
4. To identify plant species which have the potential for phytoremediation.

1.5 Hypotheses of the Study

This study tested the following hypotheses:

1. The native plants growing at Nchanga Mine do not have the potential for phytoremediation to clean up the heavy metals, Cu and Zn.
2. There is no relationship between the concentration of Cu and Zn in the soil and concentrations of the aforementioned heavy metals in the tissues of the plant species growing at the study site.

1.6 Research Questions

1. What are the concentrations of Cu and Zn in the soil cover and tailings at Nchanga Mine?
2. What types of plants grow in the vicinity of the mine?
3. What are the concentrations of Cu and Zn in the tissues of the plants growing at the Nchanga Mine?
4. Do any of the plant species growing at the study site accumulate Cu and Zn in their biomass?

CHAPTER 2. LITERATURE REVIEW

2.1 Mining and the Environment

Primary impacts of mineral exploitation are the removal of vegetation and disturbance of the ground both in the immediate vicinity of the principle mining activities and surrounding areas (Kumar et al., 2014). The excavations and dumping of the mine waste alter the hydrology, soil profile, topography and nutrient status of an area. Soil contamination may result from runoff from mine workings, tailings impoundments, pits and overburden, waste rock, mine development rock, ore and sub-ore piles and from acid rain (Rodricks, 1992). Deposition of wind-blown particulates from piles may also be a source of soil contamination. These factors lead to fragmentation of the biological habitats and consequent deleterious effects on the structure and floristic composition of plant communities and the local biodiversity as a whole (Meerts and Grommesch, 2001).

2.2 Heavy Metals

Heavy metals are elements that occur naturally in the earth's crust and are characterized by high relative atomic mass and have metallic properties which include a conductivity, cation stability, malleability and ductility (Rodricks, 1992, Chibuike and Obiora, 2014). Heavy metals include elements such as Copper (Cu), Zinc (Zn), Cadmium (Cd), Cobalt (Co), Lead (Pb), Nickel (Ni), etc. Human activities can, however, cause increased concentration of heavy metals in the environment through geologic activities which include, but not limited to, mining, smelting of metals, vehicle emissions, industrial wastes, use of fertilizers and paints (Rodricks, 1992, Chibuike and Obiora, 2014; Harvey et al., 2015). Metals (or metalloids) that occur where they are unwanted, or in concentrations or forms that are injurious, may be considered as contaminants (Singh et al., 2011).

All living organisms (plants and animals) need different amounts of heavy metals for normal metabolic processes, growth and development. Nevertheless, all metals are toxic at high concentrations because of their non-biodegradability, persistence in soils and cumulative nature (Singh et al., 2011; Chibuike and Obiora, 2014). The effect of heavy metal toxicity varies according to the specific heavy metal involved. Exposure routes of heavy metals are via manual handling of heavy metal contaminated soil, air inhalation and diet (Ikenaka et al., 2010; Harvey et al., 2015). Heavy metals in the soil are taken up by plants along with water and ingestion of these plants exposes animals to heavy metals toxicity.

2.2.1 Effects of Selected Heavy Metals

High Concentrations of metals in the soil can lead to significant hazards to human, animal and ecosystem health, degradation of soil quality and reduced crop yield (Long et al., 2002).In humans, long term exposure to heavy metals can have detrimental effects on the central and peripheral nervous systems. In plants, exposure to heavy metals leads to oxidative stress, resulting in disturbance of cellular ionic homeostasis and cellular damage (Singh et al., 2011). The concentration of metals in soils ranges from 1mg/kg to as much as 100,000mg/kg (Long *et* al., 2002). According to the World Health Organization (WHO), the maximum permissible concentrations of Cu and Zn in soils are 20 mg/kg and 40 mg/kg respectively, and the maximum permissible values of Cu and Zn for plants are 10mg/kg and 50mg/kg, respectively (Ruqia et al., 2015).

2.2.1.1 Copper

Copper, normally in association with silver (as chalcosine) or iron (as chalcopyrite) is widely distributed in the earth's crust. It is also found in all living organisms. It is an essential trace element for normal plant growth. In animals, it is essential for normal metabolism and is a co-factor for many enzymes. Symptoms of Cu deficiency in humans include weight loss, bone disorders, graying of hair, microcytic hypochromic anaemia, hypopigmentation and demyelination of nerves, etc (Singh et al.,2011). However, there are diseases that are associated with increased levels of Cu: sickle cell anemia, diabetes mellitus, schizophrenia, Wilson's disease, etc. Copper is twice as toxic as Zn. Increased concentrations of copper in the soil results in decreased plant yields and death of livestock.

2.2.1.2 Zinc

Zinc is an abundant elementwith environmental sources whichinclude the use of Zn roofing materials, Zn mining and other industries. Zinc is an essential element in living organisms, making up an essential part of some enzymes in both plants and animals (Ghaderian and Ravandi, 2012). It is also needed for protein synthesis, DNA and RNA metabolism, etc. Deficiency of Zn however results in dwarfism, growth retardation, decreased appetite, skeletal abnormalities, skin lesions and poor healing of burns and wounds, whereas, high concentrations cause vomiting, dizziness, lethargy, etc. (Ghaderian and Ravandi, 2012).

2.2.2 Plant Tolerance

Metal tolerant plants have evolved mechanisms to minimize the effects of exposure to and accumulation of heavy metals. These are based on chelation and subcellular compartmentalization

(Singh et al., 2011). Two strategies are used by metal tolerant plants: exclusion and accumulation (Baker, 1981). Three types of plant-soil relationships based on these two strategies were proposed by Baker (1981); these are excluders, accumulators and indicators. Excluders restrict the transport of metals to the shoot and maintain relatively low concentrations of the metal in the shoot. Accumulators are able to translocate and accumulate high levels of metal in the above ground parts from both low and high soil metal concentration levels. Some accumulators may be hyperaccumulators, in that, they are able to accumulate very high concentrations of the metals. Indicators show an intermediate response to metal concentration in the soil and the concentration of the metal in the plant is somewhat a reflection of what is available in the soil.

2.3 Phytoremediation

Phytoremediation is the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates (Sarma, 2011). It is an integrated multidisciplinary approach to cleaning up soils that are contaminated (Jadia and Fulekar, 2009). It makes use of the unique and selective capabilities of plant root systems in addition to translocation, Bio-Accumulation and contaminant storage or degradation abilities of the whole plant body (Sharma and Pandey, 2014). Phytoremediation is categorized into two approaches: (i) Phytoextraction or phytoaccumulation and (ii) Phytostabilization (Malayeri et al., 2008).

Phytoextraction is the removal of heavy metals by aboveground parts of the plant achieved by planting metal accumulating plants on the contaminated soil which are later harvested to remove metals from the soil (Yoon et al., 2006; Usman and Mohamed, 2009; Lorestaniet al.,2011a; Singh et al., 2011; Usman et al., 2012). Phytostabilization is the reduction of the mobility of heavy metals using metal-tolerant plants (Yoon et al., 2006; Antosiewicz et al., 2008). The mobility and availability of heavy metals in the soil is generally low when soil pH is high (Nazir et al., 2011).

In order for phytoremediation to be feasible, the plants to be used need to be able to extract large concentrations of heavy metals into their roots; translocate them into the surface biomass and produce a large quantity of plant biomass (Bouwman et al., 2005; Jadia and Fulekar, 2009, Mehes-Smith *et* al., 2013).

2.3.1 Plants as Phytoremediators

Plant communities respond in different ways to soils that are contaminated by heavy metals (Malaisse*et* al., 1994). The response is dependent on the plants ability to accumulate and detoxify various heavy metals (Lorestaniet al.,2011b). Usman et al., (2012) showed that the extent of heavy

metal accumulation differed among the plant species, tissue types, as well as the types of heavy metals. Soil pH, clay content, organic matter content and the presence of other ions are factors that also influence the uptake of metals by plants (Kabata-Pendias and Pendias, 1992).

Plants that are natural metal hyperaccumulators accumulate high concentrations of heavy metals in their tissues and are generally tolerant to metal contaminants and other site conditions that limit plant growth (Padmavathiamma and Li, 2007). These plants can be used to clean-up contaminated soils provided their biomass and metal content are large enough to complete remediation within an acceptable period (Jadia and Fulekar, 2009). Native plants are often better in terms of survival, growth and reproduction under environmental stress (Nazir et al., 2011) unlike exotic plants.

The ability of a plant to accumulate metal from soils is expressed by the BAF (Usman et al., 2012).The Bio-Accumulation Factor (BAF) is used to calculate the phytoextraction efficiency (Meerts and Gronmesch, 2001; Yoon et al., 2006). Plants that have a BAF shoot value greater than one are classified as accumulators, whereas, plants that have BAF shoot values less than one are considered excluders (Baker, 1981; Usman et al., 2012).

2.3.2 Identification of Plants with Phytoremediation Potential

According to Freidland (in Sharma and Pandey, 2014), an ideal plant species for phytoremediation should have one of the following characteristics: (a) a low biomass with a very high metal accumulation capacity or (b) a high biomass with enhanced metal uptake potential.

A number of studies have been conducted to identify plants with potential for phytoremediation and well over 45 families of plants have been identified and reported from temperate to tropical regions as hyperaccumulators of heavy metals; these include Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae (Jadia and Fulekar, 2009; Sharma and Pandey, 2014).

The most important family identified with phytoremediation potential is Brassicaceae which contains a large number of species that are able to hyperaccumulate heavy metals, especially Zn and Cd and include (*Thlaspi caerulescens* (J. & C. Presl), *Thlaspi praecox*Wulfen, *Brassica juncea (*L.) Czern.,and *Brassica napus* L. (Sharma and Pandey, 2014).

In the identification of plants with potential for phytoremediation, emphasis is mainly placed on native plants, or plants indigenous to an area under study, primarily because of their resilience to climatic conditions and their tolerance to elevated concentrations of toxic metals (Prasad and Freitas,

2003). Introduction of invasive species for the purpose of phytoremediation can adversely affect the local biodiversity (Waoo, Khare and Ganguly, 2014).

2.3.3 Native Plants with Potential for Phytoremediation around the World

Many studies have been conducted in many parts of the world on plants that can hyperaccumulate heavy metals. Malayeri et al. (2008) identified accumulator plants that are effective for phytoremediation in a mine of Iron (Fe) and Cu, named Hame Kasi in Iran. Seventeen plant species and six soil samples were collected and content of heavy metals in both the soil and plant samples determined using Atomic Absorption Spectrophotometry. The BAF was also calculated for each of the seventeen species. Results showed that the concentration of the heavy metals exceeded natural limits. The plant species found to be hyperaccumulators were *Scariola orientalis* (Boiss.) Soják. 1962, *Cirsium comgestum* Fisch & C.A. Mey.ex DC., *Chenopodium botyrs* L.,and *Verbascum speciosum* Schrad.

In Pakistan, Nazir et al. (2011) evaluated the phytoremediation potential of 23 plant species and found that 15 of the species were Zn hyperaccumulators. These included *Alternanthera pungens* Kunth, *Brachiaria reptans* (L.) Gardner and Hubb, *Cenchrus pennisetiformis* Hochst. & Steud., *Cannabis sativa* L., *Chenopodium album* L.and *Eleusine indica* (L.) Gaertn.

Lorestani et al., (2011b) found *Euphorbia macroclada* Boiss. and *Verbascum speciosum*Schrad.to be Fe hyperaccumulator plants as well as being the most efficient in phytostabilization of Cu and Fe, while *Ziziphora clinopodiodes* Lam., *Cousinia* sp. 103_Salouk and *Chenopodium botyrs* L. were the most suitable for phytostabilization of Zn. This was in a study they conducted in the surrounding areas of Hame Kasi copper mine in Iran where they analyzed soil and plant samples.

A study by Usman et al. (2012) identified native plant species that have phytoremediative capacity at a Korean chromate copper arsenate contaminated site. Nineteen plant species were collected and soil samples from the same locations were analysed and the BAF calculated. Their results showed that *Iris ensata* Thunmb. was a hyperaccumulator of Chromium.

2.3.4 Native Plants with Potential for Phytoremediation in Africa

In Africa, few studies have been done pertaining to the identification of plants that have potential for phytoremediation. Studies were conducted in Katanga (Congo D. R.), Zambia and Zimbabwe (then Rhodesia) on the relationship between heavy metal content of soils and uptake of these metals by vegetation (Drew and Reily, 1972). Well over 600 metallophytes were described, some of which do

not occur in any other part of the world (Van der Ent and Erskine, 2015). These include the 'Zambian Copper flower', *Becium centralafricanum (B. homblei)* (De Wild.) Duvig. &Plancke., whose presence has been used extensively for mineral exploration in Katanga, Democratic Republic of Congo and the Zambian Copperbelt (Malaisse et al., 1999).However, these studies were limited to the ecology of areas contaminated with mining residues and the possible mechanisms of tolerance by these plants. With the increasing interest in phytoremediation, there has been further research carried out on plants that can be used for phytoremediation and a number of native plants have since been identified. *Bulbostylis cupricola* Goetgh. And *Pimpinella acutidentata* C. Norman were identified as copper hyperaccumulators by Malaisseet al. (1999). This research was done at Luiswishi in Upper Katanga region of the Democratic Republic of Congo. They studied the diversity of plant communities and leaf heavy metal content of the vegetation. Apart from the aforementioned copper hyperaccumulators found at the site, *Aeollanthus subacaulis* var. *Ericoides* (De Wild.) Ryding *and Senecio* cf.*Coronatus*) Thunb.) Harv., cobalt Hyperaccumulators, were noted. *Gnidia hockii* De Wild. was found to be a zinc Hyperaccumulator (Malaisseet al., 1999).

2.3.5 Native Plants with Potential for Phytoremediation in Zambia

In a study of copper vegetation at the Kansanshi Hill in Zambia, Leteinturier et al., (2001) found *Cheilanthes perlanata* (Pic.Serm.) Kornaś, *Eragrostis racemosa* (Thunb.) Steud., *Bulbostylis pseudoperennis* Goetgh., *Aspilia ciliate* (Schumach.) Wild. and *Glycine wightii* var. *Longicauda* (Schweinf.) Verdc. to be hyperaccumulators for copper.

More recently, a study by van der Ent and Erskine (2015) on the potential of Zambian Cu-Co hyperaccumulator plants for phytoremediation of polluted soils in Ndola, Kitwe and Kansanshi showed a range of metallophytes and Cu- Co hyperaccumulator plants, which included Horseweed, *Conyza cordata* Kuntze. 1898, Dotted knotweed, *Persicaria puncata* (Elliot) Small. and *Persicaria capitata*.

However, no study has so far been conducted to identify hyperaccumulator plants in Chingola notwithstanding the fact that according to Lindahl (2014), it is among the towns ranked with the highest contamination of soils by heavy metals on the Copperbelt. This study will therefore seek to identify plants with potential for phytoremediation in Chingola, at Nchanga Mine.

CHAPTER 3. MATERIALSAND METHODS

3.1 Study Area

The study was conducted at Nchanga Mine in Chingola, a town in the Copperbelt Province of Zambia (Figure 1). It is situated at an elevation of 1340 meters above sea level on the Central African Plateau, 12° 30' S latitude, 27° 50' E Longitude and with a population of 210,073 according to the 2010 Census (Central Statistical Office, 2003,2011). Nchanga Mine which has the largest Open Pit Mine in Africa is operated by Konkola Copper Mines (KCM) Plc. The mine workings lie in an arc which is 11km long around the west and north of the town (Figure 2), covering nearly 30km^2, as such, the extent of the land degradation and soil contamination by heavy metals is of serious concern.

Fig1: Map Showing Chingola town on the map of Zambia (world maps online)

3.1.1 Climate

Chingola, like most towns in Zambia has three distinct seasons: the rainy season from November to April; a cool and dry season from May to August; and a hot and dry season from September to October. It has an average rainfall range between 1000 mm to 1200 mm per year and temperatures range from 10 degrees Celsius to 37 degrees Celsius depending on the time of the year (Konkola Copper Mine, 2014).

3.1.2 Vegetation and Wildlife

The principal vegetation type in the Copperbelt region is the Miombo woodland. Common trees found in the Miombo woodland include species of *Brachystegia*, *Isoberlinia*, and *Julbernadia*(Konkola Copper Mine, 2014). However, human activities such as mining have extensively disturbed the natural vegetation patterns.

3.2 Plant and Soil Sampling

3.2.1 Sampling Area Selection

A stratified random sampling approach was used. Two sampling areas were established; one at the Tailings Dam four (TD4) (Figures2 and 3.) as sampling Area one (01) and the other covering the immediate vicinity of the tailings impoundment (Figure 4) which served as the control site. The sampling area at TD4 was divided into four (4) quadrats: N1, W1, S1 and E1. Two (2) quadrats were established in the control site: N2 and W2. In each quadrat, nine (9) 10m×10m plots were established and three of them were randomly picked and sampled.

Figure2: Map showing location of TD4 (Google earth)

Figure3: Vegetation at TD4

Figure 4: Vegetation at Control site 50m away from TD4

3.2.2 Plant Collection

At least three specimen were collected for each plant species present from each plot. The plant specimens were tagged, put in a polythene bag and placed in a cooler box.

3.2.3 Plant Identification

The plant specimens were identified by use of taxonomic keys:(1) Flora Zambesiaca (Timberlake, 2012); (2) Flora of Zambia (Bingham et al., 2016); (3) Flora of Zimbabwe (Hyde et al., 2016); (4) Guide to Grasses of Southern Africa (Van Oudtshoorn, 2012); (5) Sasol First Field Guide to Grasses of Southern Africa (Smith, 2012); (6) Sasol First Field Guide to Wild Flowers of Southern Africa (Manning, 2008); and (7) Checklist of Zambian Vascular Plants (Phiri, 2005). Unidentified specimen were pressed and taken to the University of Zambia Herbarium for comparison with voucher specimen. (Figure 3).

Figure5: Plants in plant press ready for identification at the University of Zambia Herbarium

3.2.4 Soil Sampling

A soil probe was used to collect soil samples at depths of 15-20cm. Three soil samples were collected from each plot at different points. A composite sample from each plot was then prepared by mixing the three samples well. The composite samples were placed in labeled polythene bags and stored in airtight containers.

3.3 Plant Diversity Study

A line transect was laid in each quadrat, running north to south. A square 1m × 1m grid was used to record the plant species present and the number of individuals. The grid was placed along the transect every two meters and the species present in the grid were recorded.

3.4 Quantitative Analysis

The important quantitative analysis such as density, frequency, and abundance of the herbs and grass species were determined according to Curtis and McIntosh (1950).

3.4.1 Frequency

Frequency, sometimes called Frequency Index, is defined as the number of times a plant species occurs in a given number of quadrats of a particular size and is expressed as a percentage (Bonham, 1989). It refers to the degree of dispersion of a given individual species in an area. The frequency was calculated using the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Relative Frequency} = \frac{\text{Number of occurrences of a particular species}}{\text{Total number of occurrences of all species}} \times 100$$

3.4.2 Density

Density is essentially an expression of the numerical strength of a species determined by dividing the total number of individuals of each species in all quadrats by the total number of quadrats studied. The equation shown below was used:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Relative Density} = \frac{\text{Density of a particular species}}{\text{Sum of the densities of all species}} \times 100$$

3.4.3 Abundance

The number of individuals occurring per sampling unit was calculated using the equation:

$$\text{Abundance} = \frac{\text{Total number of individuals in all sampling units}}{\text{Total number of sampling units of occurrence}}$$

$$\text{Relative Abundance} = \frac{\text{Abundance of a particular species}}{\text{Sum of abundances of all species}} \times 100$$

Relative abundance refers to how common or rare a species is relative to other species in a specific location or community (Hubbell, 2001). The assigned abundance – categories as suggested by Dagar et al., (1991) are as follows:

>25	d	Dominant
15- 25	Va	Very abundant

10-15	a	Abundant
6- 10	f	Frequent
3 -6	o	Occasional
1- 3	r	Rare
<1	Vr	Very rare

3.4.4Similarity and Dissimilarity Indices

The similarity and dissimilarity indices were calculated as per Sorensen (1948) as shown below:

Index of similarity (S) = 2 C/ A+ B

Where, A= number of species in the community A

B= number of species in the community B

C= number or common species in both the communities

Index of dissimilarity = 1 - S

3.4.5 Species Richness

Species richness is a measure of the number of species per sample (Bonham, 1989). The more species present in a quadrat, the richer the quadrat. The Species richness was calculated using the method 'Margalef's index of richness' D_{mg} (Magurran, 1988):

$$D_{mg} = (S-1)/ \ln N$$

Where, S = Total number of species

N = Total number of individuals

3.4.6 Species Diversity

Richness and evenness are the components used to describe Species Diversity (Nkoa et al., 2015). Richness is the number of species present in an area and a3ccording to Booth et al. (2010), evenness indicates whether one or more species dominates an area, or whether the species are represented by approximately equal numbers.

The species diversity was computed using the Shannon- Wiener Index (H').It specifies both richness and evenness, and is moderately sensitive to sample size (Magurran, 1988). The equation is shown below:

$$H' = \Sigma [-pi (\ln pi)]$$

Where H = Shannon's diversity index;

pi = proportion of species *i* relative to the total number of species

3.4.7 Evenness

Evenness was calculated using the formula:

$$E = \frac{H'}{H_{maximum}}$$

Where, H'= is Shannon-Weiner Diversity index.

$$H_{maximum} = ln\ (S)$$

Where S is the number of species.

Evenness ranges from zero to one (0- 1) ranging from very limited evenness to very even distribution of species, respectively.

3.5 Chemical Analysis

Analysis of both the soil and plant samples was done at the University of Zambia, School of Mines Geochemical Laboratory in Lusaka.

3.5.1 Chemical Analysis of the Soil Samples

The soil samples were air dried in the laboratory at room temperature by spreading them out on transparent plastic for seven days. The samples were then passed through a 2mm sieve and ashed in porclain crucibles in a furnace at 450°C for three hours. Acids used in the extraction of the heavy metals were all Analytical Grade. One gram of each of the soil samples was placed in a 200mL conical flask, to which 0.2mL of sulfuric acid, 1mL nitric acid and 5mL of perchloric acid were added too. The mixture was then placed on a hotplate and heated to 180°C for 15 minutes. The mixture was allowed to cool and then filtered through Whatman No. 42 filter paper into 100mL volumetric flasks. Distilled water was added to the mark. The filtrate was then transferred to 100mL plastic bottles.

The concentrations of the Cu and Zn were determined using an Atomic Absorption Spectrophotometer (AAS), Analyst^{TM} 900 (Perkin Elmer Instrument, USA) with an acetylene flame. The concentration of the heavy metals were expressed in mg/kg dry weight.

3.5.2. Chemical Analysis of the Plant Samples

Plant samples were dusted with a light brush and then dried at room temperature in the laboratory for seven days. They were then placed in an electric steel oven for at least three hours at 110°C before being ground using a steel grinding mill. Three grams (3g) of each of the ground and dried samples were then ashed in a controllable muffle furnace at 450°C. The resulting ash was dissolved in 20ml of 1M analytical grade nitric acid and the solution was evaporated to near dryness. The samples were then filtered through ashless whatman filter paper into 100cm³ volumetric flasks. The residue on the filter paper was washed several times with distilled deionised water. The resulting filtrate was diluted to the mark using distilled deionised water. The concentrations of Cu and Zn were then determined using an Atomic Absorption Spectrophotometer, as in the soil samples above.

3.6 Analysis of Phytoremediation Potential

The phytoremediation potentialwas assessed by calculating the Bio-Accumulation Factor (BAF) of each individual plant.

BAF shoot = $(c_{shoot}) / (c_{soil})$

Where c_{shoot} is the metal concentration in the shoots and c_{soil} is the metal concentration in the soil (Ma et al., 2001; Usman et al., 2012).

Plants that have a BAF shoot value greater than one are accumulators, whereas, plants that have BAF shoot values less than one are considered excluders (Baker, 1981; Usman et al., 2012). Plants that are potential phytoremediators have BAF shoot value greater than ten (Ma et al., 2001).

3.7 Statistical Analysis

Correlation was used to determine if there was a relationship between the concentration of Cu and Zn in the soil, and the Cu and Zn concentrations in the plants. Correlation was also used to determine if there was a relationship between the soil Cu and Zn concentration and the plant species diversity.

CHAPTER 4. RESULTS

4.1 Types of Plants Growing at the Study Site

The vegetation at TD4 consisted mainly of grasses and herbs. There were a few shrubs and trees dotted on the perimeter to the North of the TD4. The control site had a somewhat denser vegetation than TD4, with more trees and shrubs in addition to the grasses and herbs.

The plants recorded in this study were the grasses and herbs. The grasses are perennial whereas the herbs are both perennial and annual. Out of the 16 plant species studied, nine (9) were herbaceous species, one (1) fern and six (6) grass species.

Fourteen (14) of the sampled plant species were identified and four (4) could not be identified. The plant species identified were *Nephrolepis* sp. Schott. (Figure 6), *Crinum* sp. L. (Figure 11), *Anthephora* sp. Schreb. (Figure 7), *Digitaria eriantha* Steud. (Figure 8), *Arthraxon quartinianus* (A. Rich.) Nash. (Figure 9), *Cyperus rotundus* L., *Chondrilla juncea* L., *Senecio*sp. L., *Amaranthus hybridus* L., *Vernonia* sp. Schreb. (Figure 10), *Cymbopogon densiflorus* (Steud.) Stapf., *Cornyza cordata* Kuntze., *Crassocephalum* sp. Moench. And *Kyllinga alba* Nees.

Figure 6 *Nephrolepis* sp. Schott.

Figure 7 *Anthephora* sp. Schreb.

Figure 8 *Digitaria eriantha* Steud.

Figure 9 *Arthraxon quartinianus* (A. Rich.) Nash.

Figure 10 *Vernonia* sp. Schreb.

Figure 11 *Crinum* sp. L.

4.2 Plant Diversity Data

4.2.1 Species Recorded

Table 1 shows the Species recorded and how many individuals of each species. Sampling site N1 had the highest number of species recorded while W1 had the lowest number of species recorded (Figure 12).

Table 1: Number of individuals recorded for each species in each sampling site

S/N	Species	Quadrats					
		N1	N2	E1	S1	W1	W2
1	*Crinum* sp. L.	4	_	_	_	_	5
2	*Anthephora* sp. Schreb.	32	48	64	69	24	_
3	*Digitaria eriantha* Stued.	26	-	73	62	38	-
4	Sp 21	2	6	-	-	8	-
5	Sp 27	9	15	-	-	-	8
6	*Nephrolepis* sp. Schott.	8	-	3	-	-	18
7	*Senecio*sp.L.	4	12	-	-	-	-
8	*Arthraxon quartinianus* (A. Rich.) Nash	2	16	-	-	-	11
9	*Amaranthus hybridus* L.	6	13	8	7	-	-
10	*Cyperus rotundus* L.	-	8	3	-	-	-
11	*Vernonia* sp. Schreb.	-	9	-	13	6	-
12	*Cymbopogon densiflorus* (Steud.) Stapf	-	-	-	-	-	15
13	*Chondrilla juncea* L.	-	-	-	-	-	3
14	*Crassocephalum* Moench.	-	-	4	-	-	4
15	*Conyza cordata*Kuntze	-	-	-	79	-	-
16	*Kyllinga alba* Nees.	29	-	-	-	-	-

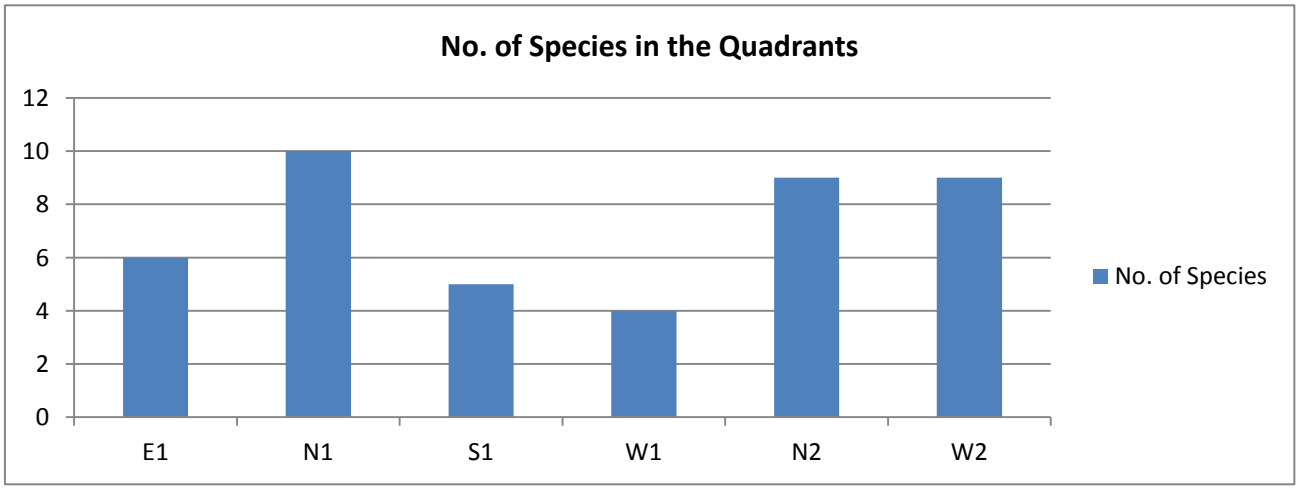

Figure 12: Number of species in the quadrats

4.2.2 Frequency, Abundance and Density

Anthephora Schreb. And *Digitaria eriantha* Steud. had the highest frequencies (83.33% each) and densities (39.5 and 40, respectively); and the lowest was recorded for *Kyllinga alba* Nees. (frequency 16.67% and density 4.83) (Table 2; Figure 13). *Anthephora* and *Digitaria eriantha* were more abundant (abundance = 47.4 and 48 respectively) and the least abundant was *Crassocephalum* Moench. (abundance = 4) and *Chondrilla juncea* L. (abundance = 3).

Table 2: Table showing the Relative frequency, density and abundance for the species studied.

Species	**No. Of Individuals**	**Frequency (%)**	**Relative Frequency**	**Density**	**Relative Density**	**Abundance**	**Relative abundance**
Crinum L.	9	33.33	1.1	1.5	1.1	4.5	1.5
Anthephora Schreb.	237	83.33	29.08	39.5	29.08	47.4	15.83
Digitaria eriantha Stued.	240	83.33	29.45	40	29.45	48	16.03
Sp 21	16	50	1.96	2.67	1.97	5.33	1.78
Sp 27	32	50	3.93	5.33	3.92	10.66	3.56
Nephrolepis Schott	29	50	0.25	4.83	3.56	9.67	3.23
Senecio L.	16	33.33	1.96	2.67	1.97	8	2.67
Arthraxon quartinianus (A. Rich.) Nash	29	50	0.25	4.83	3.56	9.67	3.23
Amaranthus L.	34	50	4.17	5.67	4.17	11.33	3.78
Cyperus rotundus L.	11	33.33	1.35	1.83	1.35	5.5	1.84
Vernonia Schreb.	28	50	3.44	4.67	3.43	9.33	3.12
Cymbopogon densiflorus (Steud.) Stapf	15	16.67	1.84	2.5	1.84	15	5.01
Chondrilla juncea L.	3	16.67	0.37	0.5	0.37	3	1
Crassocephalum Moench.	8	33.33	0.98	1.33	0.98	4	1.34
Conyza cordata Kuntze	79	16.67	9.69	13.17	9.7	79	26.39
Kyllinga alba Nees.	29	16.67	0.25	4.83	3.56	29	9.69

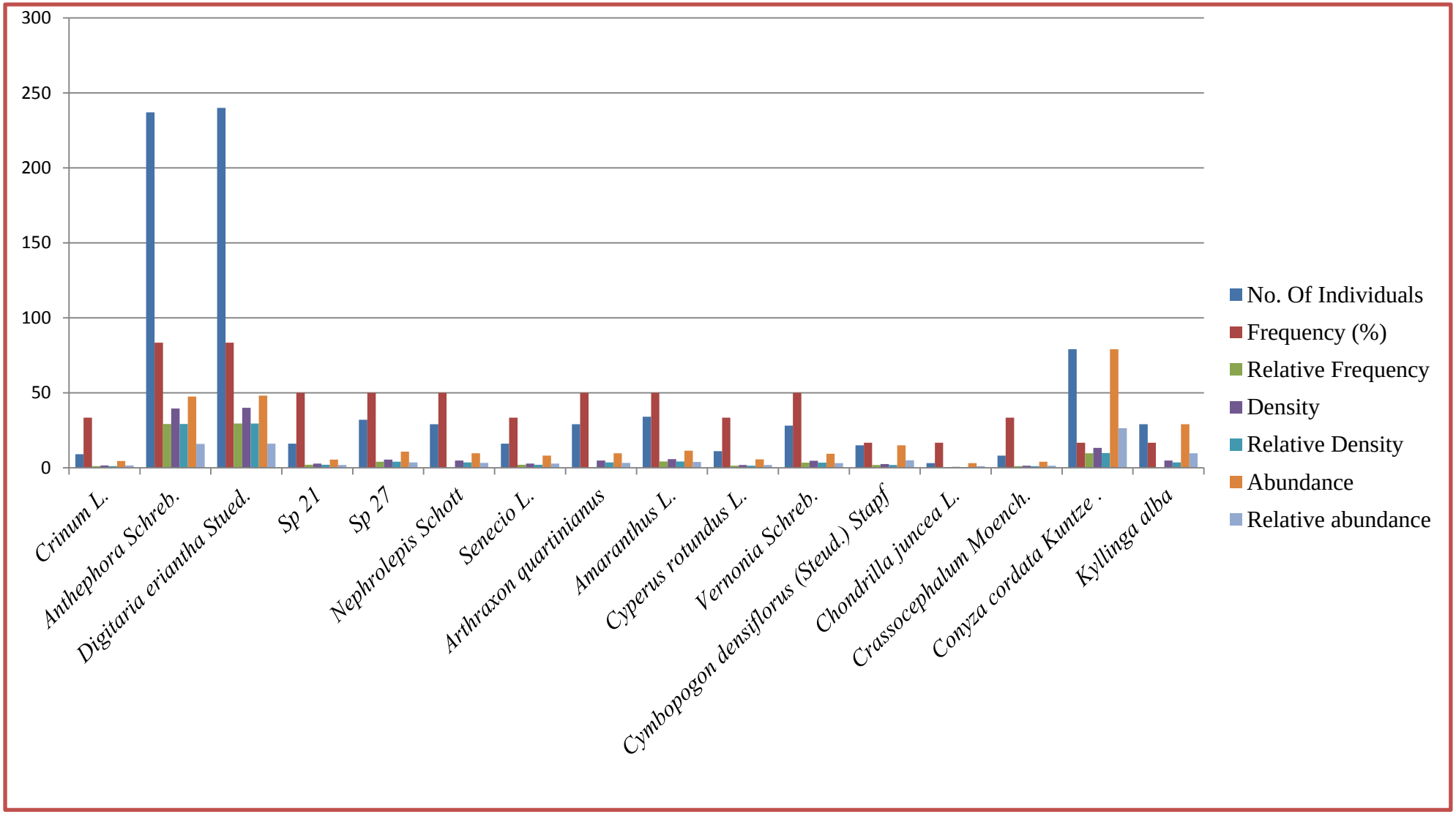

Fig. 13: Plant species' overall frequency, density and abundance

4.2.3 Species Richness

Of all the quadrats studied, N1 was the most species diverse (species richness = 35%)followed by W2 and N2 (species richness = 18% in both quadrats),while W1 (species richness = 8%) is the least diverse (Figure 14). Comparing between sampling areas, the immediate vicinity of TD4 is more diverse than TD4 though they both have relatively high species richness.

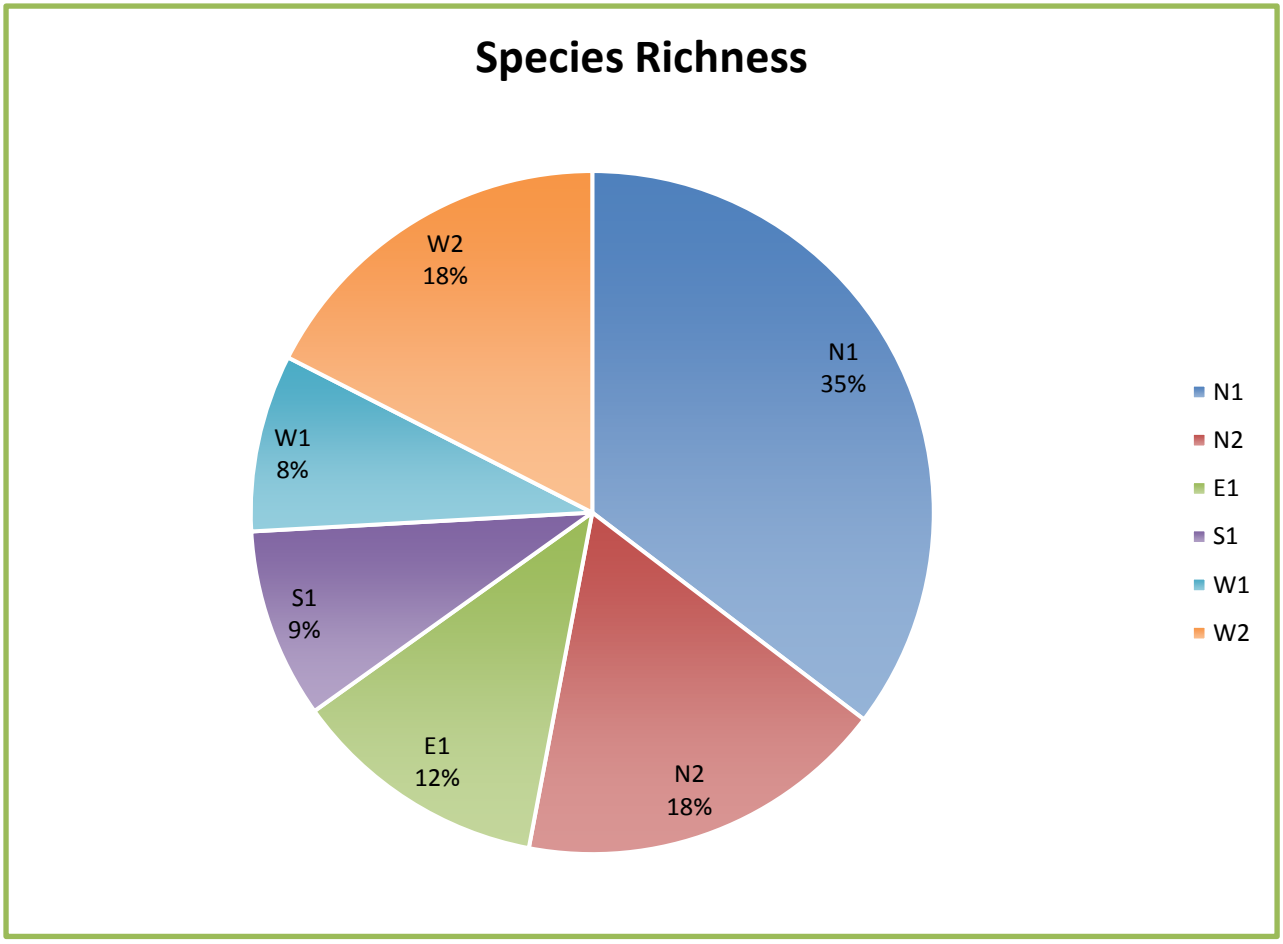

Figure 14: Species Richness of the quadrats

4.2.4 Species Diversity

Sampling site 50m away from TD4 had a higher diversity (H'= 2.31) than TD4 (H'= 1.84) (Table 4).

Table 4: Calculation of Species Diversity of TD4 and the immediate vicinity.

Species	**TD4**				**Immediate Vicinity**				**Overall**			
	ni	***Pi***	***lnpi***	***-pi(lnpi)***	**ni**	***pi***	***lnpi***	***-pi(lnpi)***	**ni**	***pi***	***Lnpi***	***-pi(lnpi)***
Crinum L.	4	0.01	-4.61	0.05	5	0.03	-3.51	0.11	9	0.01	-4.61	0.05
Anthephora Schreb.	189	0.32	-1.14	0.36	48	0.25	-1.37	0.34	237	0.29	-1.24	0.36
Digitaria eriantha Stued.	199	0.34	-1.08	0.37	0	0	0	0	240	0.29	-1.24	0.36
Sp 21	10	0.02	-3.91	0.08	6	0.03	-3.51	0.11	16	0.02	-3.91	0.08
Sp 27	9	0.02	-3.91	0.08	23	0.12	-2.12	0.25	32	0.04	-3.22	0.13
Nephrolepis Schott	11	0.02	-3.91	0.08	18	0.09	-2.41	0.22	29	0.04	-3.22	0.13
Senecio L.	4	0.01	-4.61	0.05	12	0.06	-2.81	0.17	16	0.02	-3.91	0.08
Arthraxon quartinianus (A. Rich.) Nash	2	0	0	0	27	0.14	-1.97	0.28	29	0.04	-3.22	0.13
Amaranthushybridus L.	21	0.04	-3.22	0.13	13	0.07	-2.66	0.19	34	0.04	-3.22	0.13
Cyperus rotundus L.	3	0.01	-4.61	0.05	8	0.04	-3.22	0.13	11	0.01	-4.61	0.05
Vernonia Schreb.	19	0.03	-3.51	0.11	9	0.05	-3	0.15	28	0.04	-3.22	0.13
Cymbopogon densiflorus (Steud.) Stapf.	0	0	0	0	15	0.08	-2.53	0.2	15	0.02	-3.91	0.08
Chondrilla juncea L.	0	0	0	0	3	0.02	-3.91	0.08	3	0	0	0
Crassocephalum Moench.	4	0.01	-4.61	0.05	4	0.02	-3.91	0.08	8	0.01	-4.61	0.05
Conyza cordata Kuntze	79	0.14	-1.97	0.28	0	0	0	0	79	0.1	-2.3	0.23
*Kyllinga alba*Nees.	29	0.05	-3	0.15	0	0	0	0	29	0.04	-3.22	0.13
Σ (sum of the columns	**583**	**1.02**		**H'=1.84**	**191**	**1**		**H'=2.31**	**815**	**1.01**		**H'=2.12**

4.2.5 Evenness

The immediate vicinity of TD4 had an evenness of 0.9 signifying that the species were highly even in the sampling area, whereas, TD4 had an evenness of 0.7 signifying a lower even distribution (Table 5).

Table 5: Evenness of TD4

Sampling Unit	**TD4**	**Immediate Vicinity**
S	14	13
H_{max}	2.64	2.56
H'	1.84	2.31
Evenness	0.7	0.9

4.2.6 Similarity Indices

N1 and N2, S1 and W1 were very similar in terms of species present. S1 and W2, W1 and W2 were not similar at all. Overall, the two main sampling areas, TD4 (sampling area one) and The Immediate Vicinity (sampling area two) were 81% similar (Table 6).

Table 6: Indices of Similarity and Dissimilarity of the sampling sites

Quadrats	Index of Similarity	Index of Dissimilarity
N1 and N2	0.67	0.33
N1 and E1	0.5	0.5
N1 and S1	0.4	0.6
N1 and W1	0.43	0.57
N1 and W2	0.47	0.53
N2 and E1	0.29	0.71
N2 and S1	0.31	0.69
N2 and W1	0.5	0.5
N2 and W2	0.27	0.73
E1 and S1	0.55	0.45
E1 and W1	0.4	0.6
E1 and W2	0.31	0.69
S1 and W1	0.67	0.33
S1 and W2	0	1
W1 and W2	0	1
TD4 and Immediate vicinity	0.81	0.19

4.3 Chemical Analysis of the Soil and Plant Specimens

4.3.1 Concentrations (mg/kg) of Cu and Zn in the soil

The concentrations of Cu and Zn in the soil are shown in Table 7. The sampling site S1 in TD4 recorded the highest Cu and Zn concentrations of 12,024.49 mg/kg and 67.99 mg/kg, respectively. N2 at the site 50m away from TD4 recorded the lowest Cu and Zn concentrations of 2,342.04 mg/kg and 24.22 mg/kg, respectively.

Table 7: Mean concentrations of Cu and Zn in the soil cover and Tailings at TD4 and the site 50m away from TD4.

Sampling Area		Cu mg/kg				Zn mg/kg			
		Mean	SE of Mean	Median	Range	Mean	SE of Mean	Median	Range
TD4	N1	5364.67	349.36	6042.54	1042.06- 6720.92	34.05	0.65	32.35	27.58- 37.94
	E1	4943.95	153.79	4737.41	3857.34- 6023.37	32.44	0.58	31.66	27.59- 36.34
	S1	12024.49	455.95	12397.77	8925.36- 15617.47	67.99	4.11	59.74	42.91- 96.85
	W1	5700.45	99.53	5857.67	5196.85- 6214.71	30.41	0.73	30.90	25.84- 33.46
Site 50m away from TD4	N2	2342.04	401.24	1813.80	891.41- 5112.44	24.22	0.86	23.59	20.73- 29.99
	W2	4381.25	350.12	3900.72	2403.52- 6251.75	28.85	0.34	29.69	26.99- 30.84

4.3.2 Concentrations of Cu and Zn in the plants growing at TD4

A total of 175 individuals of 16 plant species were collected and analyzed for heavy metals (Cu and Zn). Concentrations of Cu and Zn in the plants ranged from 29.35 mg/kg to 1,016.8 mg/kg, and 26.47 mg/kg to 192.4 mg/kg, respectively (Table 8 and 10). Tables 9 and 11 show the overall mean concentrations of Cu and Zn, respectively.

Table 8: Mean concentrations of Cu (mg/kg) in the plant species in the sampling sites

Species	N1		N2		E1		S1		W1		W2	
	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean
Crinum L.	58.91	0.69									50.2	
Anthephora Schreb.	132.92	9.31	43.17	10.31	65.78	5.92	139.63	4.03	360.7	11.62	29.35	0.49
Digitaria eriantha Stued.	135.61	8.13	144.94	3.04	64.77	2.33	1282.2	68.71	578.9	21.71	91.4	3.05
Sp 21	450.85								449	1.77		
Sp 27	118.86	5.22	112.47								213.56	34.25
Nephrolepis Schott	207.83	9.37			221.15	22.24					228.15	2.59
Senecio L.	204.78	7.44	213.4									
Arthraxon quartinianus (A. Rich.) Nash	109.02		1016.8	454.07							47.53	
Amaranthus hybridus L.	254.1	52.59	360.58	0.72	280.89	26.87	392.63					
Cyperus rotundus L.			20.99		46.97	1.78						
Vernonia Schreb.			167.08	51.26			588.46	13.14	434.4	184.38		
Cymbopogon densiflorus (Steud.) Stapf											53.78	
Chondrilla juncea L.											247.96	6.86
Crassocephalum Moench.					100.88	7.38					75.92	
Conyza cordata Kuntze							192.84	17.62				
Kyllinga alba Nees.	91.92	0.69										

Table 9: Overall Mean concentration of Cu (mg/kg) in the plant species

Species	TD4		Immediate Vicinity		Overall	
	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean
Crinum L.	58.91	0.69	50.2		56.73	2.23
Anthephora Schreb.	169.45	18.94	35.63	4.91	137.45	16.73
Digitaria eriantha Stued.	496.72	92.64	115.2	9.62	410.88	75.97
Sp 21	449.48	1.34			449.48	1.34
Sp 27	118.86	5.22	179.86	39.07	139.2	15.55
Nephrolepis Schott	214.49	10.57	228.15	2.59	222.07	5.12
Senecio L.	204.78	7.44	213.4		206.93	5.68
Arthraxon quartinianus (A. Rich.) Nash	641.39	338.82	774.48	402.25	641.39	757.62
Amaranthus hybridus L.	281.21	23.5	360.58	0.72	291.14	21.56
Cyperus rotundus L.	46.97	1.78	20.99		41.78	5.38
Vernonia Schreb.	542.23	53.86	167.08	51.26	479.71	61.6
Cymbopogon densiflorus (Steud.) Stapf			53.78		53.78	
Chondrilla juncea L.			247.96	6.86	247.96	6.86
Crassocephalum Moench.	100.88	7.38	75.92		96.72	7.32
Conyza cordata Kuntze	192.84	17.62			192.84	17.62
Kyllinga alba Nees	91.92	0.69			91.92	0.69

Table 10: Mean concentration of Zn (mg/kg) in the plant species in the sampling sites

Species	N1		N2		E1		S1		W1		W2	
	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean
Crinum L.	28.82	0.28									26.47	
Anthephora Schreb.	41.19	5.18	38.15	9.29	76.76	1.57	83.81	3.13	55.47	1.22	29.08	1.3
Digitaria eriantha Stued.	26.16	1.19	29	0.69	31.52	0.54	174.32	16.13	69.77	20.64	28.59	0.38
Sp 21	64.68								61.82	0.77		
Sp 27	34.64	1.42	31.06								105.7	20.58
Nephrolepis Schott	53.63	4.45			46.14	22.15					55.83	1.49
Senecio L.	108.6	5.63	87.34									
Arthraxon quartinianus (A. Rich.) Nash	72.71		192.4	61.65							38.84	
Amaranthus hybridus L.	48.24	4.7	61.26	1.08	54.98	4.55	65.22					
Cyperus rotundus L.			27.21		38.7	0.64						
Vernonia Schreb.			69.95	3.66			31.9	0.66	53.75	2.9		
Cymbopogon densiflorus (Steud.) Stapf											44.9	
Chondrilla juncea L.									59.04	2.26		
Crassocephalum Moench.					57.66	1.97					52.74	
Conyza cordata Kuntze							91.51	3.43				
Kyllinga alba Nees.	33.06	0.88										

Table 11: Overall Mean concentration of Zn (mg/kg) in the plant species

Species	TD4		Immediate Vicinity		Overall	
	Mean	SE of Mean	Mean	SE of Mean	Mean	SE of Mean
Crinum L.	28.82	0.28	26.47		28.24	0.62
Anthephora Schreb.	64.56	3.32	33.2	4.26	57.06	3.36
Digitaria eriantha Stued.	74.39	12.38	28.77	0.35	64.13	10.04
Sp 21	62.54	0.9			62.54	0.9
Sp 27	34.64	1.42	80.81	27.57	50.03	11.11
Nephrolepis Schott.	49.89	9.47	55.83	1.49	53.19	4.08
Senecio L.	108.6	5.63	87.34		103.3	6.65
Arthraxon quartinianus (A. Rich.) Nash	72.71		154	58.09	137.8	47.84
Amaranthus hybridus L.	53.79	3.33	61.26	1.08	54.72	2.98
Cyperus rotundus L.	38.7	0.64	27.21		36.4	2.35
Vernonia Schreb.	38.46	3.45	69.95	3.66	43.71	4.56
Cymbopogon densiflorus (Steud.) Stapf.			44.9		44.9	
Chondrilla juncea L.			59.04	2.26	59.04	2.26
Crassocephalum Moench.	57.66	1.97	52.74		56.84	1.8
Conyza cordata Kuntze.	91.51	3.43			91.51	3.43
Kyllinga alba Nees.	33.06	0.88			33.06	0.88

4.4 Analysis of Phytoremediation Potential

All plants studied had BAF Cu values of less than 1 (Table 12). With the exception of *Crinum* L., the plant species had BAF Zn values greater than 1 (Table 13). *Arthraxon quartinianus*(A. Rich.) Nash. had the highest BAF Zn value of 10.77

Table 12: Categorization of plant species based on their BAF Cu values

	Species	**BAF (TD4)**	**BAF (Immediate Vicinity)**
Excluders	*Crinum* L.		0.01
	Anthephora Schreb.	0.04	0.02
	Digitaria eriantha Steud.	0.1	0.04
	Sp 21	0.77	-
	Sp 27	0.04	0.35
	Nephrolepis Schott.	0.03	0.05
	Senecio L.		0.24
	Arthraxon quartinianus (A. Rich.) Nash.	-	0.5
	Amaranthus hybridus L.		0.07
	Cyperus rotundus L.	0.01	0.02
	Vernonia Schreb.	0.1	0.15
	Cymbopogon densiflorus (Steud.) Stapf.	-	0.01
	Chondrilla juncea L.		0.35
	Crassocephalum Moench.	-	0.01
	Conyza cordata Kuntze.	0.03	-
	Kyllinga alba Nees.	0.09	-

Table 13: Categorization of plants based on their BAF Zn values

	Species	**BAF (TD4)**	**BAF (immediate vicinity)**
Excluders	*Crinum* L.	-	0.86
Accumulators	*Anthephora* Schreb.	1.73	1.26
	Digitaria eriantha Steud.	1.89	1.07
	Sp 21	2.3	-
	Sp 27	1.28	3.92
	Nephrolepis Schott.	1.68	1.93
	Senecio L.	-	4.12
	Amaranthus hybridus L.	-	2.05
	Cyperus rotundus L.	1.22	1.31
	Vernonia Schreb.	1.91	3.18
	Cymbopogon densiflorus (Steud.) Stapf.	-	1.66
	Chondrilla juncea L.	-	1.95
	Crassocephalum Moench.	-	1.95
	Conyza cordata Kuntze.	1.56	-
	Kyllinga alba Nees.	1.26	-
Hyperaccumulator	*Arthraxon quartinianus* (A. Rich.) Nash	-	10.77

4.5 Statistical Analysis

4.5.1 Correlation between Soil Metal Concentration and Plant Metal Concentration

Tables 14 and 15 show the correlation coefficient between soil Cu and Zn concentration and the concentration of Zn and Cu in the plant species. The correlation between Cu concentration in the soil and Cu concentration in the plant species was 0.376, whereas, the correlation between concentration of Zn in the soil and concentration of Zn in the plant species was 0.359.The correlation coefficient was weak, but positive. This shows that there was a positive relationship between the concentration of Cu and Zn in the soil and Cu and Zn concentrations in the plants.

Table 14: Correlation between Cu concentration in the soil and Cu concentration in the plant species

		Soil Cu Concentration	Concentration of Copper in the plants
Soil Cu concentration	Pearson Correlation	1	0.376**
	Sig. (2-tailed)		0.000
	N	175	175
Concentration of Copper in the plants	Pearson Correlation	0.376**	1
	Sig. (2-tailed)	0.000	
	N	175	175

**. Correlation is significant at the 0.01 level (2-tailed).

Table 15: Correlation between concentration of Zn in the soil and concentration of Zn in the plant species

		Soil Zn concentration	Concentration of Zinc in the Plants
Soil Zn concentration	Pearson Correlation	1	0.359**
	Sig. (2-tailed)		0.000
	N	175	175
Concentration of Zinc in the Plants	Pearson Correlation	0.359**	1
	Sig. (2-tailed)	0.000	
	N	175	175

**. Correlation is significant at the 0.01 level (2-tailed).

4.5.2 Correlation between Shannon- Weiner diversity index and soil metal concentration

A negative, but significant correlation was found between the concentration of Cu and Zn in the soil, and the Shannon-Weiner diversity index (-0.716 for Cu and -0.648 for Zn) (Table 16 and Table 17). This means that an increase in Cu and Zn concentration in the soil resulted in a decrease in plant species diversity. The curve estimates for the above mentioned parameters also depict this negative relationship (Figure15 and Figure 16).

Table 16: Correlation between plant species diversity and Cu concentration in the soil

		Plant Species diversity	Soil_Cu_Concentration
Plant Species diversity	Pearson Correlation	1	-0.716
	Sig. (2-tailed)		-0.110
	N	6	6
Soil_Cu_Concentration	Pearson Correlation	-0.716	1
	Sig. (2-tailed)	-0.110	
	N	6	6

Table 17: Correlation between plant species diversity and Zn concentration in the soil

		Plant Species diversity	Soil_Zn_concentration
Plant Species diversity	Pearson Correlation	1	-0.648
	Sig. (2-tailed)		-0.164
	N	6	6
Soil_Zn_concentration	Pearson Correlation	-0.648	1
	Sig. (2-tailed)	-0.164	
	N	6	6

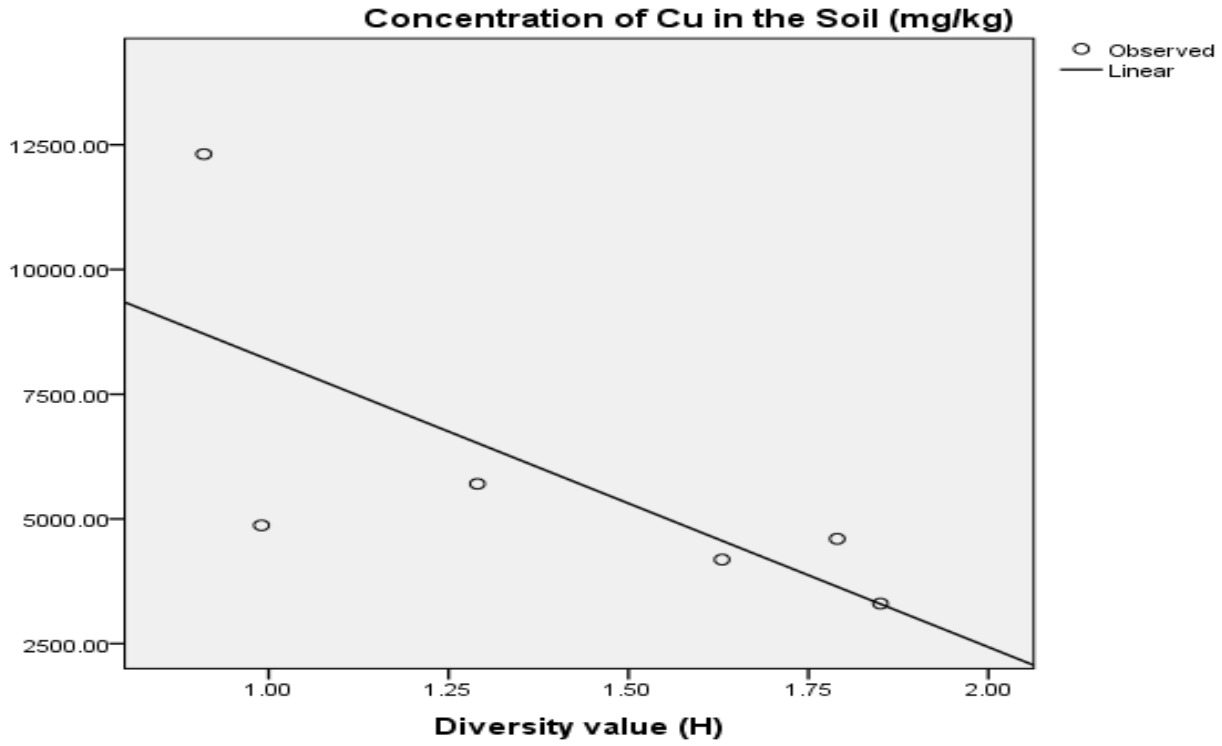

Fig 15: Curve estimate for concentration of Zn in the soil and plant species diversity

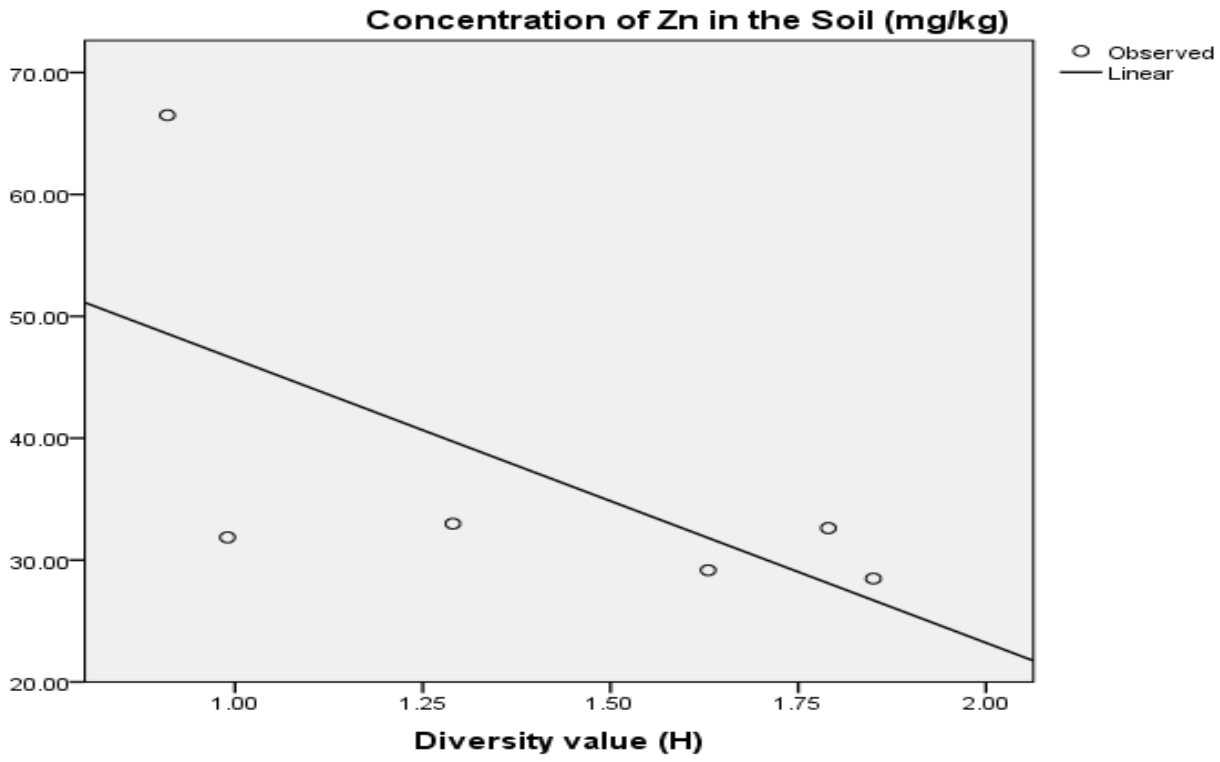

Fig 16: Curve estimate for concentration of Cu in the soil and Plant species diversity

CHAPTER 5.DISCUSSION

5.1 Concentrations of Cu and Zn in the Soil Cover and Tailings at Nchanga Mine

The study site was characterized by high concentrations of Cu and Zn as was expected, considering the fact that the study area was located in the mine environment, and at an old tailings dump to be precise. The concentrations in the soil ranged from 2,342.04 mg/kg to 12,024.49 mg/kg and 24.22 mg/kg to 67.99 mg/kg for Cu and Zn respectively (Table 7). The quadrats in TD4 had some of the highest values, compared to quadrats in the control site, 50m away from TD4. The results showed that the soil had very high concentrations of Cu, much higher than the threshold value of 20 mg/kg according to WHO (Ruqia et al., 2015) and 130 mg/kg, according to ICRCL (1987). The concentrations of Zn were higher than the permissible values of 40 mg/kg according to WHO (Ruqia et al., 2015), but below the threshold value of 300 mg/kg according to ICRCL (1987).

Soils from S1 had the highest concentration of Cu (12, 024.49 mg/kg), followed by W1 (5,700.45 mg/kg) and N1 (5,364.67 mg/kg). With regards to Zn, S1 had the highest concentration (67.99 mg/kg) followed by N1 (34.05 mg/kg) and E1 (32.44 mg/kg). The difference in the concentrations of Cu and Zn in the quadrats in TD4 can be attributed to the direction of flow of the tailings discharged into the tailings dump. The flow is from north to south, with the most tailings settling on the southern part of the tailings dump. The dump is in such a way that it slants to the south. The high concentrations in the immediate vicinity of the tailings dump, 50m away had Zn and Cu concentrations which were ranging from 2,343.04 mg/kg to 4,381.25 mg/kg, which however, lower than TD4, are still higher than recommended values. This may be due to waste rock, leachate from mine tailings and deposition of wind-blown particulates from piles (Rodricks, 1992).

5.2 Plant Species Growing at the Study Area

Despite the presence of very toxic concentrations of heavy metals such as Zn and Cu, it is very unlikely to find the tailings dumps or the areas surrounding them devoid of vegetation (Aggangan, et al., 2015). This is because there are plants that are able to tolerate the highly toxic concentrations of heavy metals. Most plants collected were annual or perennial herbs and grasses. Some are endemic to Zambia or sub-Saharan Africa, but they are not exclusively endemic to metalliferous areas. Some of the plant species collected have been noted in other parts of the country which do not have metalliferous soils (Phiri, 2005). Since these plants can grow naturally on both metal enriched soils and on non metalliferous soils, they are categorized as pseudometallophytes (Ghaderian and Ravandi, 2012).

A total of 16 plant species were studied, of which, 14 were identified to genus level. Five were from the family Asteraceae, four from family Poaceae, two from Cyperaceae, and one each from Amaryllidaceae, Lomariopsidaceae and Amaranthaceae (Appendix 1). In terms of growth habit, nine were herbs, one fern and six grasses.

The highest number of species was recorded in N1 of TD4, followed by N2 and W2 from the control site. Overall, the site 50m away from TD4 (the control site) had higher species richness than TD4. Species with the highest densities and frequencies were *Anthephora* sp. Schreb. and *Digitaria eriantha* Steud. These two plant species were present in nearly all sampling sites. *Kyllinga alba* Nees., *Conyza cordata* Kuntze., *Cymbopogon densiflorus* (Steud.) Stapf. *and Chondrilla juncea* L. were each present in only single sampling sites.

Similarity indices showed that N1 and N2 were very similar with respect to species present. S1 and W1 were also quite similar. However, S1 and W2 as well as W1 and W2, were not similar to each other at all. All in all, TD4 and the area 50m away from it were about 81% similar. The Shannon-Weiner diversity index (H) was found to be 2.31 for the control site and 1.84 for TD4. This indicated that there was a richer diversity in the control site compared to TD4.

5.3 Relationship between Concentrations of Cu and Zn in the Soil and Species Diversity

The study found a negative correlation between soil Zn and Cu concentrations and the Shannon-Weiner diversity (H), implying that as the soil Cu and Zn concentrations increased, the species diversity decreased. This highlights the effects of heavy metal pollution in soil on the biodiversity. These findings are corroborated by Vangrosveld et al (1996) in Chibuike and Obiora (2014) who reported that the diversity of higher plant species was very low in areas which were polluted with Zn and Cu. Similarly, Bagatto and Shorthouse (1999) noted that an increase in Cu concentration in the soil resulted in a decrease in floral diversity. As such, heavy metal concentration in the soil can predict the species diversity in polluted areas (Koptsik et al., 2003).The effect of heavy metal toxicity, however, varies according to the specific metal involved, but overall impact on the species diversity is negative.

5.4 Concentrations of Cu and Zn in the Tissues of Plant Species Growing at the Study Site

According to WHO (Ruqia et al., 2015), the maximum acceptable values of Cu and Zn in plants is 10 mg/kg and 50 mg/kg, respectively. The Zn content of plants in this study was ranging from 26.47 mg/kg to 174.32 mg/kg, with *Digitaria eriantha* Steud., *Conyza cordata* Kuntze. and *Arthraxon quartinianus* (A. Rich.) Nash. having higher values than other species. Plant species with high values of Cu concentration recorded included *Arthraxon quartinianus* (A. Rich.) Nash. (1,016.8 mg/kg), *Digitaria eriantha* Steud.(1,282.2 mg/kg) and *Vernonia*sp. Schreb. (588 mg/kg). Cu content in plants was lowest in *Cyperus rotundus* L. (20.99 mg/kg) and *Crinum* L. sp.(50.2 mg/kg). The concentration of Zn in the plants was mostly above the recommended values according to WHO, but below the threshold value of 300 mg/kg according to ICRCL (1987).

5.5 Relationship between Cu and Zn Concentrations in the Soil and in the Plants

Statistical analysis using correlation showed that there was a weak positive relationship between the concentrations of Cu and Zn in the soil and the concentration of Cu and Zn in the plants. This weak positive correlation indicates that the concentration of the heavy metals in the plants is weakly correlated to the concentration of the heavy metals in the soil. It is thus expected that when the concentration of the heavy metals in the soil is high, the concentration in the plants may be correspondingly high. This was illustrated by the high concentrations recorded in plants that were growing in the study sites having high Cu and Zn concentrations. S1 had the highest Cu and Zn concentrations recorded, 12,024.49 mg/kg and 67.99 mg/kg, respectively. *Digitaria eriantha* Steud. had its highest Cu concentration recorded in S1, 1,282.2 mg/kg, compared to values of 578.9 mg/kg in W1, 135.61 mg/kg in N1, 144.94 mg/kg in N2, 91.4 mg/kg in W2 and 64.77 mg/kg in E1. *Vernonia* sp. Schreb. and *Conyza cordata* Kuntze. also had their highest Cu concentrations recorded in S1 (588.46 mg/kg and 192.84 mg/kg, respectively) compared to other quadrats.

This, however, is not always the case, as in some soils, high Cu levels have been shown to be associated with insoluble copper compounds which have low bioavailability of copper to plants (Badilla-Ohlbaum et al., 2001). In addition, plants differ considerably in their ability to assimilate the heavy metals rendering the relationship between soil metal content and metal content in plants unpredictable (Robert, 1979).

5.6 Phytoremediation Potential of the Plants Studied

Nearly all values of Cu and Zn concentrations recorded in the plants studied were higher than the WHO recommended values. Nonetheless, plants have developed mechanisms that allow them to thrive in toxic environments. And it is these plants that have a high probability of being potential phytoremediators. Excluders only tolerate metals in the substrate by restricting the uptake of the metals into the roots (Baker, 1981). Accumulators on the other hand present specialized mechanisms that allow them to accumulate or even hyperaccumulate metals in their shoots (Jadia and Fulekar, 2009).

Hyperaccumulation of Zn is exceptionally rare due to the readiness with which it can be precipitated as the insoluble sulfate in the rhizosphere, thus minimizing the probable uptake and transport to the shoots of the plants (Ghaderian and Ravandi, 2012). Fromvarious studies conducted so far, 13 taxa have been identified as Zn hyperaccumulators (Ghaderian and Ravandi, 2012). The Bio-Accumulation factors calculated showed that *Crinum*sp. (BAF= 0.86)is a Zn excluder, *Arthraxon quartinianus* (A. Rich.) Nash. (BAF= 10.77)is a Zn hyperaccumulator and the remaining species (BAF ranging from 1.07- 4.12) are all Zn accumulators.

A number of Cu hyperaccumulators have been identified all over the world which include*Becium centralafricanum (B. homblei)* (De Wild.) Duvig. and Plancke., *Bulbostylis cupricola* Goetgh., *Pimpinella acutidentata* C. Norman, *Cheilanthes perlanata* (Pic. Serm.) Kornas., *Eragrostis racemosa* (Thunb.) Steud., *Bulbostylis pseudoperennis* Goetgh., *Aspilia ciliate* (Schumach.) Wild. and *Glycine wightii* var. *Longicaud* (Schweinf.) Verdc., *Conyza cordata* Kuntze, *Persicaria puncata* (Elliot) Small. and *Persicaria capitata* Buch. -Ham. Ex D. Don) H. Gross (Malaisse et al., 1999; Malasisse, et al., 1999; Leteinturier et al., 2001; Van der Ent et al., 2015). However, in this study, no Cu hyperaccumulator was identified. All the plant species were found to be Cu excluders (BAF ranging from 0.01 to 0.77).

It is possible that part of the measured Cu and Zn in the plant samples may have been from sample contamination which could not be removed completely during sample washing. Faucon et al. in Ghaderian and Ravandi (2012) highlighted the fact that improperly washed specimen tended to have relatively high concentrations of heavy metals.

The high number of Zn and Cu excluders found in this study attests to findings of other researchers that the majority of metal tolerant plants colonizing mineral wastes are excluders (Baker, 1981; Ghaderian and Ravandi, 2012).

CONCLUSION AND RECOMMENDATIONS

Only *Crinum* L. sp.was found to be a Zn excluder; the rest were Zn accumulators, with *Arthraxon quartinianus* (A. Rich.) Nashfound to be a hyperaccumulator of Zn. With regards to Cu, all the 16 plant species were found to be Cu excluders. The plant species identified in this study, thus, have potential for phytoremediation as excluders, accumulators and hyperaccumulators. They represent potential for remediation of soils heavily polluted by heavy metals.

The plants identified in this study could be used in the mine areas in Zambia and elsewhere to contain the heavy metals (Cu and Zn) in the soil and to help decontaminate the mine dumps. In addition, further research needs to be done to identify indigenous plants with potential for phytoremediation of other heavy metals such as Cobalt, Nickel, Lead and Cadmium.

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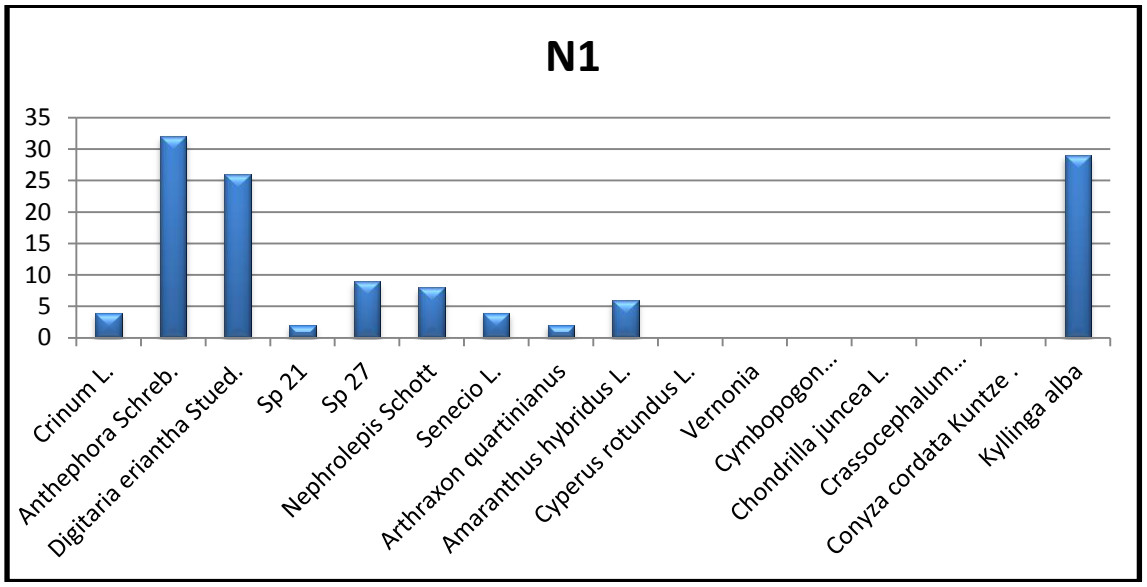
APPENDICES

Appendix 1: List of Plant Species Studied

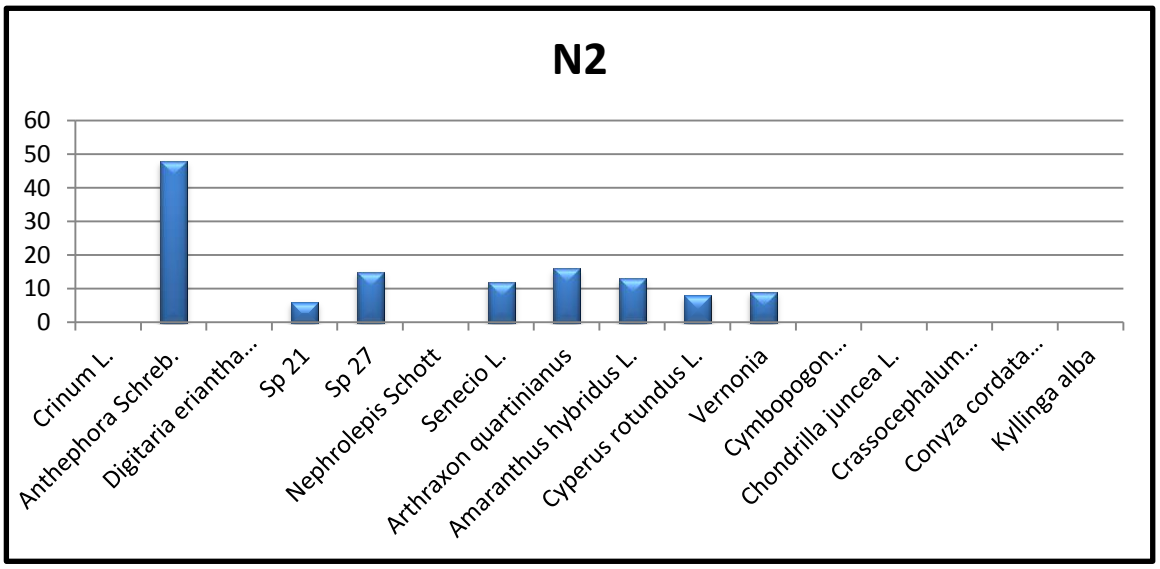
Plant species	**Family**	**Habit**	**Life Cycle**
Crinum L.	Amaryllidaceae	Herb	Perennial
Anthephora Schreb.	Poaceae	Grass	Perennial
Digitaria eriantha Stued.	Poaceae	Grass	Perennial
Sp 21	n.a.	Herb	n.a.
Sp 27	n.a.	Herb	n.a.
Nephrolepis Schott	Lomariopsidaceae	Fern	Perennial
Senecio L.	Asteraceae	Herb	Perennial
Arthraxon quartinianus (A. Rich.) Nash	Poaceae	Grass	Perennial
Amaranthus hybridus L.	Amaranthaceae	Herb	Annual
Cyperus rotundus L.	Cyperaceae	Grass	Perennial
Vernonia Schreb.	Asteraceae	Herb	Perennial
Cymbopogon densiflorus (Steud.) Stapf	Poaceae	Grass	Perennial
Chondrilla juncea L.	Asteraceae	Herb	Perennial
Crassocephalum Moench.	Asteraceae	Herb	Annual
*Conyza cordata*Kuntze	Asteraceae	Herb	Perennial
Kyllinga alba	Cyperaceae	Grass	Perennial

Appendix 2: Total individual distribution of species in each quadrat

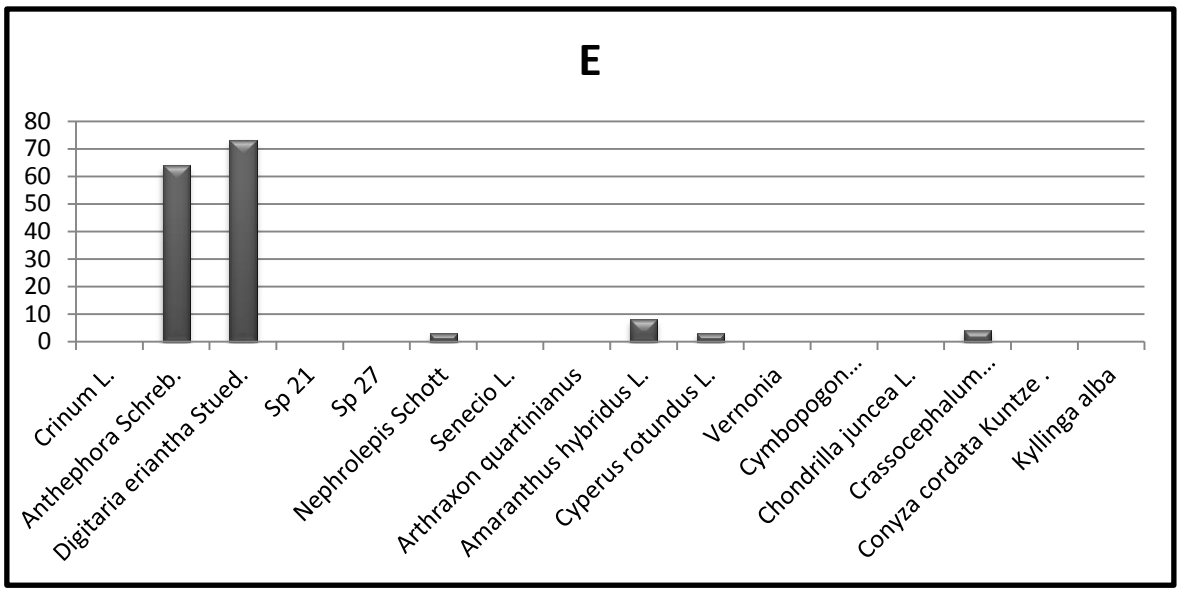
Appendix 2a: Number of individuals of respective species in N1



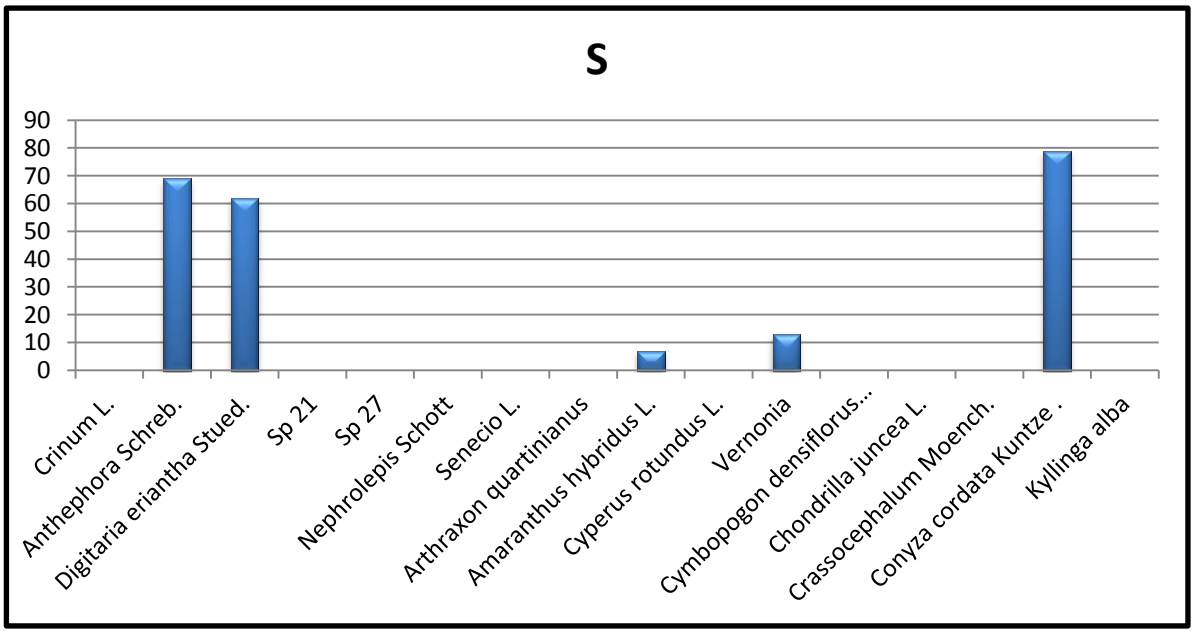
Appendix 2b: Number of individuals of respective species in N2



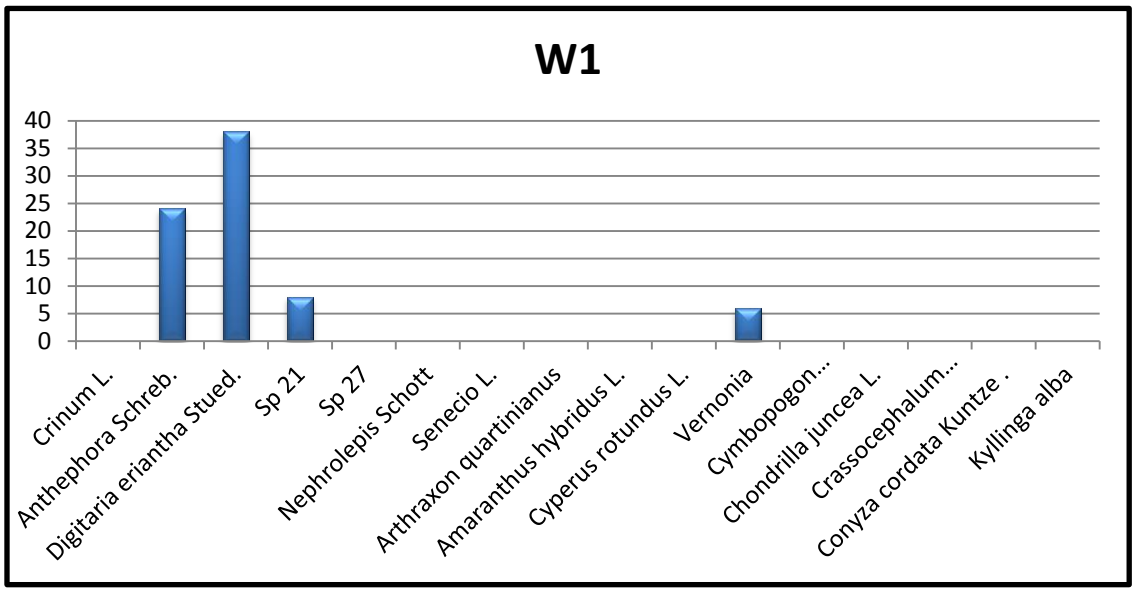
Appendix 2c: Number of individuals of respective species in E



Appendix 2d: Number of individuals of respective species in S



Appendix 2e: Number of individuals of respective species in W1



Appendix 2f: Number of individuals of respective species in W2