A MULTIFARIOUS COMPARATIVE ECOTOXICOLOGICAL APPROACH ON A CATCHMENT SCALE FROM THREE MINE WASTELANDS FOR IMPROVED ENVIRONMENTAL MANAGEMENT AND RISK ASSESSMENT: INTEGRATING ABIOTIC, BIOTIC AND AGROECOSYSTEM APPROACHES

By

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DECLARATION

I hereby declare that the entirety of the work contained herein, represents my own work, I am the sole author of this work, and that this work has not in part or entirety been previously submitted to this or any other institution for obtaining any qualification.

I have equally read the current University ethics regulations and accede responsibility for any issues that may be raised from this study. I have endeavoured to identify all the potential risks linked with this research likely to emanate while conducting this research, obtained ethical clearance and recognize my obligations.

Signed by candidate

Signature:

Date: 14/02/2022

Abstract

Metal mobilization under acid or neutral rock drainage represents one of the major environmental impacts associated with mining of sulphidic minerals. To avert this, suitable handling of mine overburden material, waste rock, open pits and tailing storage facilities (TSF) is needed. This study addressed the risks associated with metal mobilization from the waste, including those resulting from the potential for ARD generation from the mine waste (tailings) following the recovery of copper from sulphidic ores, the impacts on the aquatic ecosystem, the agro-ecosystem and potential ecological restoration using phytomining technologies. The study is focused on the Kafue River basin on the Zambian Copperbelt and seeks to identify impacts and potential benefits through studying a grouping of TSFs and their impact in a single geographical region, allowing attributes of the facilities to be contrasted.

In this study, we have addressed the categorisation of ARD generation of Chibuluma TSF, TSF15A and TSF14 tailing samples and associated metal mobility using the standard static tests, UCT biokinetic test and column bioleach experiments. Owing to the potential for compromised water quality and exploratory studies alluding to this, an ecotoxicology study at the catchment scale was conducted seasonally for three years on water resources (Nselaki Stream, Fikondo Stream and Mululu Streams) and food crops in close proximity to the selected TSFs. The potential of phytomining technologies using native herbaceous plants to mitigate mobilization of metals from the copper mine wastelands was investigated.

Characterisation of the risk of ARD using data generated from the standard static and biokinetic tests was compared across the three samples. The biokinetic test supported the standard static test classification of non-acid forming, providing preliminary kinetic data on ARD generation. The three tailings have high neutralisation potential and are not acid forming over an initial period. Column bioleach tests allowed for differentiation of metals according to their leaching potential under conditions ranging from neutral through varying levels of acidity conditions, providing support evidence for potential ecological burdens. The results showed that low pH promoted significant release of Fe, Cu and Mn while release of metals Co, Ni, Zn and Pb remained considerably low. Low mobilization of metal species was observed under high pH, however, over time the sustained low mobilization of metal species is likely to cause significant ecological risks. The results better inform the risk posed by copper wastelands, through the combined use of a suit of tests (static, biokinetic and column leach

tests). Under high acidic conditions, Fe and Cu exhibited high ecological risk while the risk was moderate under non-acidic conditions. The ecological risk under acidic conditions for Ca, Al, Mg and Mn was observed to vary from low to moderate, while negligible ecological risk profiles were observed with elements of interest Pb, Co, Zn, and Ni. Our research further expanded the studies on monitoring abiotic and biotic ecosystem drivers in adjacent streams. Selected physiochemical indicators downstream were identified in relation to the influence of the mine wastelands. No significant difference in heavy metals was observed between the three streams at the significance level ($P > 0.05$), however, notable changes in chemical and physical signatures for selected elements was reported downstream of the selected TSFs. Multivariate analysis such as principal component analysis, indicated prevalent TSF interferences of Cu, Co, Mn, Zn, and Pb in water and sediment samples analysed.

The use of macroinvertebrates provided a useful approach to monitor the variation in the degree of impacts and characterise the ecological integrity of the streams, as well as evaluate the links with selected physiochemical contaminants. The various physiochemical markers used were useful in observing persistent impacts on macroinvertebrate taxa, which can be linked to severe anthropogenic impacts as well as timely warning indicators. Particularly, macroinvertebrate taxa tolerant to water pollution such as *Talitridae* and *Gnathobdellidae* were observed to be dominant species. The biotic monitoring results supported the abiotic test classification with regards to stream contamination. The use of macroinvertebrate community structures proved more useful to characterize the integrity of the ecosystem of the streams and determine the links with possible contaminants. Similarly, results from food crops irrigated using the selected streams reported significant elevation of metals Cu, Co, Mn, and Pb in the edible parts. The contamination load index (CLI) showed that the pollution index of Pb, at ≈43.8 in the vegetable samples, exceeded that of the other metals; equally, metal contamination was also determined in the edible vegetables for Cu, Co and Mn, but not consistently for Zn. One-way ANOVA at $p \leq 0.05$ and boxplot analysis suggests that heavy metal concentration in soils and crops did not vary significantly among the sites downstream of the TSF. In contrast, the soils in the upstream control sites showed much reduced metal content. These observations suggest that the TSFs may be the primary source of metal contamination in the selected streams.

The study presents phytomining as an improvement approach towards mitigating the impacts of metal mobilization and rehabilitation of wastelands. Further, it acknowledges the benefit of vegetation of TSFs. A rich diversity of indigenous herbaceous plant species was observed to thrive on the low-grade wastelands, with 622 indigenous herbaceous species from 21 families and 46 genera identified. Through analysis of the rhizosphere and above- and below-ground biomass of these plant species, the following plants reporting copper accumulation above 1000 ppm, terming them hyper-accumulators: *A. eucomus, B. alata, C. floribunda, C. ductylon, C. alternifolius, H. filipendula, E. scuber* and *V. glabra*. However, hyper-accumulation of Co, Zn and Mn was not observed despite accumulation to levels of 300, 200 and 1000 ppm respectively. Further, a number of the hyper-accumulators showed wide-spread acclimatisation to TSFs through their importance value index (IVI). Our findings suggest that phytomining using indigenous herbaceous plant species in Zambia has potential as a viable technology.

Overall, the approach of comparing catchments impacted by similar land use activities, was observed to be valuable and useful in current and future management of watersheds exposed to similar challenges. The study highlights useful monitoring methods, key risks requiring mitigation and highlights the need for interventions. The comparative catchment scale study is unique and rare which few studies have utilised to assess the likely impacts of mine wastelands, while also investigating potential remedial measures.

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AAS	Atomic Absorption Spectrometer
ABA	Acid Base Accounting
AGM	Above Ground Biomass
ANOVA	Analysis of Variance
ARD	Acid Rock Drainage
Al	Aluminium
As	Arsenic
Au	Gold
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BGM	Below Ground Biomass
Ca	Calcium
CACB	Central African Copperbelt
Cd	Cadmium
CeBER	Centre for Bioprocess Engineering Research
CEC	Cation Exchange Capacity
CF	Contamination Factor
CLI	Contamination Load Index
Co	Cobalt
Cu	Copper
D	Simpson's Index
DO	Dissolved Oxygen
DRC	Democratic Republic of Congo
EF	Enrichment Factor
FAO	Food Agriculture Organization
Fe	Iron
FS	Fikondo Stream
GDP	Gross Domestic Product
H'	Shannon Diversity Index
$HClO_4$	Perchloric Acid
HF	Hydrofluoric Acid
HNO_3	Nitric Acid
IRMA	Initiative for Responsible Mining Assurance
IVI	Importance Value Index
KRB	Kafue River Basin
LECO	Laboratory Equipment Corporation
MDS	Multidimensional Scaling
Mg	Magnesium
Mn	Manganese
MPA	Maximum Potential Acid Generation
MPC	Maximum Permissible Limit
MS	Mululu Stream
NAF	Non-Acid Forming
NAG	Net Acid Generation
NAPP	Net Acid Producing Potential
Ni	Nickel

NIPI	Nemerow Integrated Pollution Index
NS	Nselaki Stream
Pb	Lead
PCA	Principal Component Analysis
Pi	Pollution Index
PLI	Pollution Load Index
UCT	University of Cape Town
RDA	Redundancy Analysis
RDe	Relative Density
RDo	Relative Dominance
RF	Relative Frequency
RI	Ecological Risk Assessment
SASS	South African Scoring System
TDS	Total Dissolved Solids
TF	Translocation Factor
Ti	Titanium
TSF	Tailing Storage Facility
WHO	World Health Organization
XRD	X-Ray Diffraction
Zn	Zinc
Zr	Zirconium
ZRB	Zambezi River Basin

CHAPTER 1: INTRODUCTION

1.1. Background

Water security in Zambia is endangered by reduction of ecosystem services by impacted water resources from mining and related activities (Muma *et al*., 2020). Hence, sustainable water resource management is critical (Pan *et al*., 2019; Sdiri *et al*., 2018). Significant environmental impact of copper mining, persisting for many years, is a major source of surface and groundwater pollution in most mining regions (Knierzinger *et al*., 2021; Tyler Mehler *et al*., 2019; Valenzuela-Diaz *et al*., 2020). Notably, wastewater and solid waste from copper mines, including tailings storage facilities (TSF), are cited as the main cause of water pollution in mining regions in Brazil (Karaca *et al*., 2018), Chile (Aguilar *et al*., 2011), China (Liu *et al*., 2020) and Zambia (Mohapatra and Kirpalani, 2016). High concentrations of metals may enter the river ecosystem directly or indirectly, especially if the wastewater and solid waste are not treated effectively and key components recovered. Zhao *et al*. (2017) and Perlatti *et al*. (2021), in north-east China and north-east Brazil respectively, highlighted impacts of coal and copper mining, including acid rock drainage (ARD) and metal mobilization, on river chemistry and the diversity of intolerant macroinvertebrate taxa. Limited data are available to predict these impacts due to the variable and distinctive properties of mine waste. To enhance our understanding of these impacts, it is critical to explore long-term impacted sites to develop effective management strategies for mining areas.

The Kafue River catchment on the Copperbelt Province of Zambia faces similar risks with adverse impacts on the river traceable to copper mining activities. To determine the existing risks to the Kafue River catchment, critical lessons may be drawn from a multifarious comparative approach on a catchment scale from the tributaries (Nselaki Stream, Fikondo Stream, and Mululu Stream) of Kafue River. These three study areas share similar geographical, climatic, and geological conditions (Broughton, 2013; Shimaponda-Mataa *et al*., 2017); hence, this study presents a unique opportunity for comparative assessment of water resources impacted by mine wastelands with different histories in terms of their mineral content, age, integrity, vegetation etc. The study identifies the current state of the streams and highlights the potential of metal mobilisation from TSFs in close proximity to the streams and impact of stream irrigation of agroecosystems. The possibility of phytoextraction and phytomining for semi-passive metal recovery to reduce the negative impacts of copper TSFs on the ambient environment is evaluated. This study has potential to inform the management of water resources in Zambia and to develop management strategies of international riverine ecosystems passing through mining regions.

1.2. Rationale

Zambia's water resources include subsurface aquifers, rainfall, streams, rivers, wetlands, lakes, dams, evapotranspiration, and discharge from industries. Their management determines the quality and quantity of water available (Nachiyunde *et al*., 2013; Ntengwe, 2005). While Zambia exploits surface water (dams and rivers) mostly, groundwater is increasingly used (Chande and Mayo, 2019; Funder *et al*., 2010). Water resources across southern Africa are under pressure in the face of climate change, population growth, increasing industrialization and urbanization (Ebeke and Ntsama Etoundi, 2017; Levy *et al*., 2017).

Water resources provide a variety of ecological services that benefit end-users and the environment (Maze *et al*., 2016). The services provided by ecological infrastructure can be divided into four categories, detailed with examples (Böck *et al*., 2018; Schmidt *et al*., 2016) as follows:

- Provisioning services: provide water for consumptive use (domestic use, drinking and agriculture or industrial use), non-consumptive use (navigation, transport, and power generation), and aquatic organisms (medicines and food).
- Regulating services: maintain water quality (through water treatment and natural filtration), buffer flood flows, flood flow infrastructure, erosion control and disease.
- Supporting services: influence nutrient cycling, primary production, living space for animals and plants, and soil formation.
- Cultural services: provide nonmaterial benefits such as recreation, tourism, and spiritual benefits.

Other essential indirect or direct services provided by ecological infrastructure include erosion control, maintenance of biodiversity, nitrate and phosphate removal, sediment trapping, and removal of toxicants (Teixeira *et al*., 2019; Kotze *et al*., 2020). Effective ecological infrastructure provides significant benefits, thus, they need to be managed, maintained and restored (Loucks and van Beek, 2017). Improvement of water quality is one of the significant services provided for by water resources, from which, end users benefit. However, this is one area that is often impacted by various land use activities.

1.2.1. Land use in Zambia

Land use is a complex environmental and socio-economic issue requiring comprehensive understanding of interactions between the environment and anthropogenic activities (Handavu *et al*., 2019). Arguably, it is one of the most pervasive socio-economic forces influencing ecosystems. Land use change is integral to strategies aimed at effecting environmental changes and natural resource management, including water resources (Brown *et al*., 2013; Syampungani *et al*., 2014). These changes in land use are known to impact water quality, flow, and availability. Increased land disturbance may impact runoff and erosion, leading to a reduction in groundwater recharge and transformed hydrological patterns. This may increase introduction of sediments with their associated pollutants into receiving water resources, impacting ecosystem degradation and flow dynamics (Bond *et al*., 2019; Xu *et al*., 2018). Water flows are affected by the disturbed unnatural land, with potential degradation of the aquatic ecosystem and colonization by alien vegetation, with high water consumption than indigenous vegetation (Cessford and Burke, 2005). On the Zambian Copperbelt, the most significant land uses impacting water resources are mining operations, agriculture practices, industrial activities, and urbanization (Choongo *et al*., 2021; Mubanga and Kwarteng, 2020; Toyomaki *et al*., 2020). The above –mentioned human activities play a critical role in land use impact on adjacent water resources.

Metals and other dissolved salts such as sulphates, phosphate, and nitrates enter streams and rivers through discharge of treated and untreated liquid waste, leachate from disposal of solid wastes, and runoff, amongst others. Such pollution may be non-point source or point source. Non-point source pollution generally arises from hydrologic modification, land runoff, seepage, precipitation, drainage, or atmospheric deposition. And is reasonable for considerable metal mobilization, necessitating management of non- point source pollution. Point source pollution occurs when insufficiently treated discharge from mining, agriculture and other industries is released directly into a receiving waterbody, occurring through an identifiable, specific source.

1.2.2. Mining

Mining is an industry of strategic importance in Southern Africa. It is estimated that over half the world's diamonds, platinum and vanadium as well as 20% and 36% of cobalt and gold originate from this region (German *et al*., 2015). These minerals contribute greatly to employment creation and gross national product; many African countries depend on mineral exports for foreign exchange earnings (Ericsson and Löf, 2019). The largest and highest-grade sedimentary copper ore deposits are found on the Central African Copperbelt (CACB) with more than 200 Mt in reserves (Saintilan *et al*., 2018; Twite *et al*., 2019). Significant nickel, uranium, and zinc deposits are also contained in the CACB (Capistrant, 2012; Horn *et al*., 2021), and recent discoveries and project developments in the North Western Zambia demonstrate the continued perceptivity of the CACB (Nkuna *et al*., 2016).

The adverse environmental impacts of mining can radically alter the natural environment by stripping away the ground, and adding more chemicals and other toxic substances to surface and groundwater resources (Carvalho, 2017; Haddaway *et al*., 2019). The use of water during the processing of ore, discharge of mine effluent and seepage from tailings dams and waste rock impoundments are known to affect surface and groundwater resources (Burritt and Christ, 2018; Glotov *et al*., 2018). These may lead to contamination of water resources with pollutants including sulphates, metal(loid)s, salinity, acidity etc. (Burritt and Christ, 2018). Currently, a major environmental challenge associated with extraction of copper bearing sulphidic ores is acid rock drainage (ARD) and mobilization of metals (Acharya and Kharel, 2020; Dold, 2017). While the exposure of sulphidic minerals to air and water allows sulphide oxidation; the presence of naturally occurring sulphur and iron oxidising micro-organisms catalyses sulphide oxidation reactions, accelerating the rate of ARD formation (Shiers *et al*., 2016).

1.2.3. Mine Wastelands and Water Resources

Mine wastes, especially waste rock and mine tailing which are a mixture of processing fluid in tailing storage facilities (TSFs), are predominant sources of environmental liabilities responsible for degradation of aquatic ecosystems (Rakotonimaro *et al*., 2021). These remain after the recovery of economic minerals and metals (Kossoff *et al*., 2014; Simonsen *et al*., 2020). ARD and metal contamination is a major concern in regions with large, well established mining industries that specialise in coal, copper, and gold from sulphide ores; and other base metal industries rich in pyrite ore (Ferreira *et al*., 2021; Harrison *et al*., 2010). For instance, in the USA, it is estimated that nearly 180000 acres of freshwater reservoirs and 22000 km of streams are negatively impacted by ARD and metal contamination (Acharya and Kharel, 2020). ARD is recognised as a multifactor pollutant, and when accompanied with metal mobilization, can alter the aquatic ecosystem and water chemistry for centuries (Jeong *et al*., 2018; Karczewska *et al*., 2017; Vriens *et al*., 2019). Characteristics of ARD and metal mobilization vary widely depending on the conditions of the site such as amount of reactive waste, climate and nature (Cánovas *et al*., 2021; Nieva *et al*., 2018). Generally, ARD is associated with active and abandoned mines, and the consequent release of potentially toxic metals remains a major environmental concern around the world (Mohapatra and Kirpalani, 2016; Tayebi-Khorami *et al*., 2019; Wolkersdorfer *et al*., 2020). ARD with metal mobilization is a persistent contaminant of water resources, not seasonal as with surface runoff (Rambabu *et al*., 2020; Sun *et al*., 2020). A noticeable decrease in water quality of a catchment results (Akhavan and Golchin, 2021; Wang *et al.*, 2019).

ARD generation can result in contamination of agricultural land and crops irrigated by contaminated water resources (Fernández-Caliani *et al*., 2019; Madejón *et al*., 2021). Agriculture and mining practices are inextricably linked, taking place in the same area, and competing for available water resources (He *et al*., 2021; Musvoto and de Lange, 2019).

1.2.4. Urban Agriculture Practices

In developing countries, cities and towns are growing rapidly (Smart *et al*., 2015). Globally, the highest annual urban growth rate is in Sub-Saharan Africa, estimated at 4.1% and double the average global rate of 1.8% (World Health Organization and UN-Habitat, 2016). This rapid growth has resulted in challenges including water scarcity, food insecurity, poor and /or lack of shelter, and un-employment, particularly in poor areas. Economic vulnerability and periodic crises exacerbate these challenges (Cohen, 2006; Drechsel and Dongus, 2009; FAO, 2010).

Urban agriculture has gained increasing recognition in southern Africa towards mitigating food insecurity in rapidly growing cities (Smart *et al*., 2015). It is difficult although to assess the significance of urban agriculture in Zambia and across the global South given the differing contexts, policies, regions and economies (Frayne *et al.*, 2014); still, urban agriculture plays a relatively important role in food supply in most households. Crush *et al*. (2011) studied urban agriculture in 11 selected cities in southern Africa and reported a significant variation in in households growing food, from 3% in Windhoek (Namibia) and about 64% in Blantyre (Malawi). Urban agriculture is a critical adaptation strategy in response to population growth, food insecurities, loss for economic and employment opportunities which catalyse the search for alternative livelihoods (Dawley *et al*., 2010). In the case of the Zambian Copperbelt, mining operations are threatening the growth of urban agriculture especially those communities near mine operations. There is a need for more nuanced, critical, and place-specific research into the likely impact of mine wastelands on urban agriculture. In recent years, the Copperbelt province has experienced an economic decline, driving a large proportion of its population into urban agriculture as a means of supplementing household food supplies, income generation, and diversification of household economies (Mususa, 2012, 2010). Importantly, more in-depth case studies are needed to better understand the magnitude of the effects of mine wastelands on urban agriculture under different causal links.

1.3. Significance of Study

This study is focused on the impact of mine wastelands on aquatic systems in Zambia's Copperbelt using ecological assessment of the long-term risks. The study was designed to understand the potential for ARD generation, metal mobilization and its potential for impact on surrounding water resources and arable land through comparison of both active and historical mine wastelands, particularly tailings storage facilities (TSFs). It acknowledges and explores the impact of mine wastelands on water quality, aquatic community structures and food crops. Tributaries of the Kafue River catchment and associated copper TSFs are used as case studies of impact and potential for phytomining for remediation. Although studies focussed on potential for ARD generation and metal mobilization have been done elsewhere, they remain limited in Zambia. ARD characterization and prediction, and associated release of metal species, plays a significant role in planning, monitoring and management of mine waste.

Generally, the standard static tests, conventional kinetic tests and the recently developed biokinetic tests are often used to characterise and predict ARD generation (Hesketh *et al.*, 2010; Broadhurst and Harrison, 2015). Additionally, due to exclusive focus on the potential of acidity generation, there is a scarcity of knowledge on the deportment of metal species within neutral drainage (Plante *et al*., 2011, 2012). The copper wastelands in Zambia are a good example of a system with high neutralizing capacity and flowing neutral drainage (Sracek *et al*., 2012). Mobilization of metals related to ecological degradation, can occur under neutral conditions although aggravated by acid formation. Through this study, metal mobilization was explored through measurement of elemental concentrations in solutions resulting from the static and biokinetic ARD characterisation tests, and column bioleach tests, allowing for quantitative information on potential mobilization of selected metal species under disposal conditions. The integration of such analyses within common ARD characterisation protocols remains limited (Maest *et al*., 2005; Opitz *et al*., 2015; Parbhakar-Fox *et al*., 2013).

Furthermore, this study provides potential for improved monitoring of water quality associated with active and historic mining activities, informing potential interventions to reduce impact of mining activities on water resources and associated arable land areas. The importance of resource efficiency and rehabilitation are recognised; hence, through the study, potential to extract value from these low-grade resources (TSFs) and achieve natural rehabilitation through biomimicry is explored by studying the plant species thriving in metal rich areas and classifying these based on their functional traits (e.g., Bioconcentration factors, translocation factors etc). Using this knowledge, metal harvesting protocols are proposed as amendment and management protocols. Suitable plant species are selected based on their ability to exclude or accumulate metals on contaminated sites.

The desired outcome of the project was to provide innovations in the handling of regions surrounding the TSF to minimize negative impact, speed up rehabilitation and enhance resource recovery in the region.

1.4. Problem Statement

The impact of mining activities in the Kafue River catchment, Copperbelt Province has been a source of environmental concern, with a wide range of possible impacts anticipated to continue on receiving waters and aquatic ecosystems. Nselaki Stream, Fikondo Stream and Mululu Stream are at the center of these activities and hence ecological risk assessment of the impacts will need to be properly anticipated, monitored and suitable management actions set in place to ensure that impacts are kept to a minimum.

In order to achieve this, the importance of understanding the impact of copper mining wastelands (TSFs), is vital. Therefore, by comparatively studying the potential for ARD generation and metal mobilization of TSFs, useful information may be generated. This information may provide a sound and defensible scientific basis for the assessment of likely impacts, the evaluation of the significance of these impacts and the design of remedial measures related to water resources in close proximity to mine wastelands.

Few studies have had the opportunity to comparatively investigate three streams that are impacted by copper TSFs on different ends of the spectrum. Most studies on copper mining mainly focus retrospectively, but in this study, we were able to not only look retrospectively at the impacts of copper mining in the selected streams and arable land, but also understand the geochemical processes likely to influence behaviour patterns of copper TSFs. This was achieved through not only understanding the potential for ARD generation and metal mobility, but also putting it in context with the current water quality conditions in selected streams, type of aquatic community assemblages present and impacts on irrigated crops. The information from this study may therefore play a significant role in improving water resource management linked to copper mine wastelands and its associated risks

1.5. Aims and Objectives

The aim of this study was to collect, process and analyse multiple sets of data using a comparative catchment approach from Nselaki Stream, Fikondo Stream and Mululu Stream, as well as TSFs adjacent to these streams. The study seeks to identify impacts and potential benefits through studying a grouping of TSFs and their impact in a single geographical region, allowing attributes of the facilities to be contrasted. Additionally, the study exploited the potential of using native herbaceous plant species for TSF rehabilitation and metal recovery. Thus, this information may aid in predicting potential impacts of copper TSFs along with improved management and monitoring.

The following objectives were established in order to achieve the aim of this study, and to:

1. Investigate the ecological risk posed by copper TSFs on selected adjacent water resources. This was achieved by assessing potential for ARD from the TSFs and mobilization metals related to ecological degradation.

2. Evaluate the impact of ARD generation and mobilization of metals on the aquatic ecosystem, through a multiple comparative approach of water quality in selected streams. To achieve this, the main abiotic water quality drivers in Nselaki Stream, Fikondo Stream, and Mululu Stream, were investigated.
3. Assess the impacts of surface water toxicity on the biota in selected streams, so as to understand the controlling factors that regulate a multitude of variables in an impacted environment. To attain this, the macroinvertebrate community structure changes in the selected streams were investigated using multiple lines of evidence.
4. Conduct a comparative study on the fate, transport and impact of metal mobilization taking place in selected streams on agroecosystems. To achieve this, metal concentration in soil and food crops irrigated by impacted streams were analysed.
5. Determine the potential of phytomining as a mitigation measure aimed at rehabilitation of TSFs and recovery of residual metals. Herbaceous plants with attributes such as high metal tolerance, high metal translocation rate from below-ground biomass to above-ground biomass, fast growth, and metal specificity were investigated.

1.6. Research Design

The study areas selected for this research were Nselaki Stream, Fikondo Stream and Mululu Stream within the Copperbelt Province of Zambia. These three catchments were comparable in terms of their geographical, climatic conditions, processing technology, land use patterns and similarity in the geology of mine wastelands anticipated to impact the streams (Broughton, 2013).

The study area was sampled in the wet and dry seasons over three consecutive years within Nselaki Stream, Fikondo Stream and Mululu Stream catchments. Sample collection was undertaken across different seasons to provide for seasonal variation and to cater for hydrological extremes. Samples were collected from the TSF, discharge point of TSFs, toe drain of the TSFs, upstream, downstream, arable land and crops irrigated by impacted streams, as well as noticeable major impoundment that are located within the study area. The sampling sites were selected to be representative of the different variations (such as impact and composition) within each catchment in order to obtain a reasonable inclusive representation.

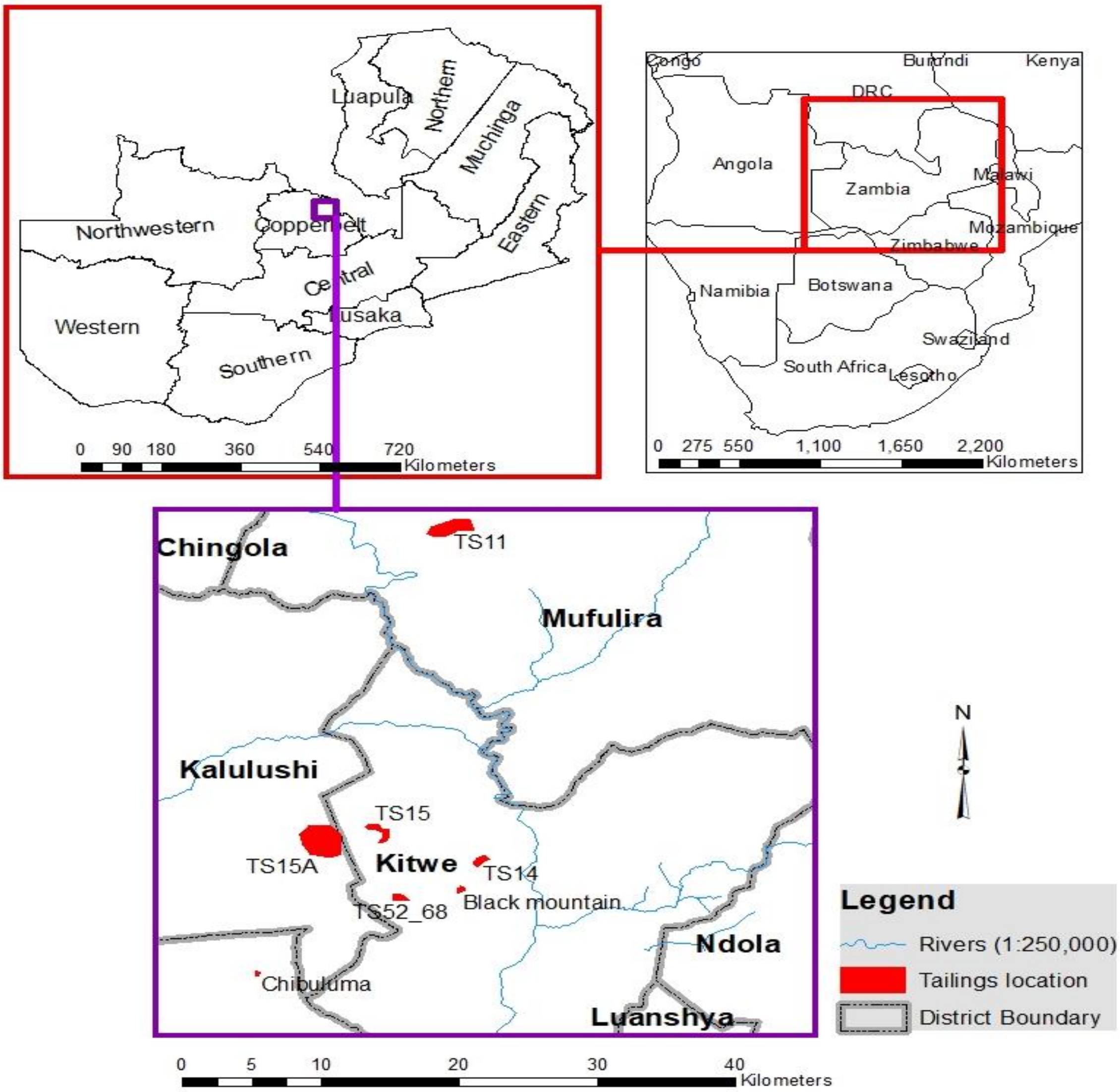

Figure 1-1: The location of the study areas selected for this case study

1.7. Thesis Structure

This thesis has been organised as follows.

- **Chapter 1:** The general introductory part describing the water resources and related problems are highlighted in this section. The need for improved monitoring of water resources to mitigate negative impact, speed up rehabilitation and enhance resource recovery is stated.
- **Chapter 2:** Deals with the impact of different land use practices with particular focus on copper mining. The study area is described in relation to the main anthropogenic activities that has potential to affect the water resources directly or indirectly in the region. It assesses the unique features of copper mine wastelands (TSFs), including ecological degradation of surface waters induced by ARD and associated metal

mobilization, and reviews the available literature regarding water quality, macroinvertebrates, and crop irrigation. Methods used to undertake ecological risk studies and some pitfalls are discussed.

- **Chapter 3:** Deals with ARD analysis and potential ecological risks associated with metal mobilization from the TSFs. The metal elements that are likely to have significant influence on the ambient environment are explained. Metal mobilization is simulated under varying column leach conditions, designed to mimic different tailing disposal conditions. The influence of pH on metal release over time is explained.
- **Chapter 4:** Deals with the use of physical and chemical water quality parameters to assess TSF induced changes in water resources. It compares the different physical and chemical signature of water between upstream and downstream sampling points. Additionally, differences in water quality between the streams is evaluated using the multivariate techniques such as principal component analysis (PCA), for the purposes of assessing the variations in TSF impacts between the water resources.
- **Chapter 5:** The influence of physical and chemical habitat change on macroinvertebrates are assessed in the streams. The different macroinvertebrate metrics and water quality variables are compared between the impacted sites and reference condition. This demonstrates how macroinvertebrates can be used to assess the influence of TSF impacts on stream water quality. The redundancy analysis (RDA) was incorporated to draw out and summarize variation by a set of descriptive variables (environmental variables) in a set of response variables (macroinvertebrate species). The sensitivity of different macroinvertebrate taxa is assessed, taxa that are able to distinguish disturbed sites from less impaired sites are identified.
- **Chapter 6:** Deals with food crops irrigated by TSF impacted streams. In this section, selected vegetable and soil samples are classified based on the metal contamination load. Selected elemental pollution assessment such as the contamination load index (CLI) and Nemerov integrated pollution index (NIPI) are discussed and applied. The spatial distribution of metal concentration in soils and food crops is compared across the sampling sites. The advantage of using this approach, it helps to validate the reported patterns from previous sections and is reliable because it has less variation.

- **Chapter 7:** Presents the use of phytomining technologies as a mitigation measure to mobilization of metals from TSFs, as well as metal recovery. In particular, the use of native herbaceous plant species is exploited based on the functional traits of plant species.
- **Chapter 8:** Deals with the general summary of the study. It compares the results and comments from different chapters for ecological risk purposes of mine wastelands. Based on the main findings from previous chapters, ecological risk issues are discussed and priorities for restoration of water resources are indicated.

Since the thesis has been structured as separate articles, duplication with regards to types of information such as methodologies, background etc., was unavoidable. For example, abiotic data from the same study sites were integrated across Chapters 4 to 6, thus selectively replicated.

1.8. References

Acharya, B.S., Kharel, G., 2020. Acid mine drainage from coal mining in the United States – An overview. Journal of Hydrology 588, 125061. https://doi.org/10.1016/j.jhydrol.2020.125061

Aguilar, R., Hormazábal, C., Gaete, H., Neaman, A., 2011. Spatial distribution of copper, organic matter and pH in agricultural soils affected by mining activities. Journal of Soil Science and Plant Nutrition 11, 125–145. https://doi.org/10.4067/S0718-95162011000300010

Akhavan, A., Golchin, A., 2021. Estimation of arsenic leaching from Zn–Pb mine tailings under environmental conditions. Journal of Cleaner Production 295, 126477. https://doi.org/10.1016/j.jclepro.2021.126477

Böck, K., Polt, R., Schülting, L., 2018. Ecosystem Services in River Landscapes, in: Schmutz, S., Sendzimir, J. (Eds.), Riverine Ecosystem Management: Science for Governing Towards a Sustainable Future, Aquatic Ecology Series. Springer International Publishing, Cham, pp. 413–433. https://doi.org/10.1007/978-3-319-73250-3_21

Bond, N.R., Burrows, R.M., Kennard, M.J., Bunn, S.E., 2019. Chapter 6 - Water Scarcity as a Driver of Multiple Stressor Effects, in: Sabater, S., Elosegi, A., Ludwig, R. (Eds.), Multiple Stressors in River Ecosystems. Elsevier, pp. 111–129. https://doi.org/10.1016/B978-0-12-811713-2.00006-6

Broadhurst, J.L., Harrison, S., 2015. A desulfurization flotation approach for the integrated management of sulfide wastes and acid rock drainage risks. 10th ICARDIMWA conference 2015

Broughton, D.W., 2013. Geology and ore deposits of the Central African Copperbelt (Text). Colorado School of Mines.

Brown, D.G., Verburg, P.H., Pontius, R.G., Lange, M.D., 2013. Opportunities to improve impact, integration, and evaluation of land change models. Current Opinion in Environmental Sustainability, Human Settlements and Industrial Systems 5, 452–457. https://doi.org/10.1016/j.cosust.2013.07.012

Burritt, R.L., Christ, K.L., 2018. Water risk in mining: Analysis of the Samarco dam failure. Journal of Cleaner Production 178, 196–205. https://doi.org/10.1016/j.jclepro.2018.01.042

Cánovas, C.R., Macías, F., Basallote, M.D., Olías, M., Nieto, J.M., Pérez-López, R., 2021. Metal(loid) release from sulfide-rich wastes to the environment: the case of the Iberian Pyrite Belt (SW Spain). Current Opinion in Environmental Science & Health 100240. https://doi.org/10.1016/j.coesh.2021.100240

Capistrant, P.L., 2012. Geology of the Enterprise hydrothermal nickel deposit, eastern Kabompo Dome, North-Western Province, Zambia (Thesis). Colorado School of Mines.

Carvalho, F.P., 2017. Mining industry and sustainable development: time for change. Food and Energy Security 6, 61–77. https://doi.org/10.1002/fes3.109

Cessford, F., and Burke, J. (2005). National State of the Environment Project. Inland Water. Background Research Paper Produced for the South Africa Environment Outlook Report: Inland Water. Department of Environmental Affairs and Tourism, Pretoria, South Africa

Chande, M.M., Mayo, A.W., 2019. Assessment of groundwater vulnerability and water quality of Ngwerere sub-catchment urban aquifers in Lusaka, Zambia. Physics and Chemistry of the Earth, Parts A/B/C, 18th WaterNet/WARFSA/GWPSA Symposium on Integrated Water Resources Development and Management: Innovative Technological Advances for Water Security in Eastern and Southern Africa - Part B 112, 113–124. https://doi.org/10.1016/j.pce.2019.03.004

Choongo, P., Eijdenberg, E.L., Lungu, J., Chabala, M., Taylor, T.K., Masurel, E., 2021. Chapter 10 - The Influence of Environmental Pollution and Drought on the Satisfaction with Life of Entrepreneurs in Zambia's Mining Sector, in: Chaiechi, T. (Ed.), Economic Effects of Natural Disasters. Academic Press, pp. 147–160. https://doi.org/10.1016/B978-0-12-817465-4.00010-8

Cohen, B., 2006. Urbanization in developing countries: Current trends, future projections, and key challenges for sustainability. Technology in Society, Sustainable Cities 28, 63–80. https://doi.org/10.1016/j.techsoc.2005.10.005

Crush, J., Hovorka, A., Tevera, D., 2011. Food security in Southern African cities: The place of urban agriculture. Progress in Development Studies 11, 285–305. https://doi.org/10.1177/146499341001100402

Dawley, S., Pike, A., Tomaney, J., 2010. Towards the Resilient Region? Local Economy 25, 650–667. https://doi.org/10.1080/02690942.2010.533424

Dold, B., 2017. Acid rock drainage prediction: A critical review. Journal of Geochemical Exploration 172, 120–132. https://doi.org/10.1016/j.gexplo.2016.09.014

Drechsel, P., Dongus, S., 2009. Dynamics and sustainability of urban agriculture: examples from sub-Saharan Africa. Sustainability Science 5, 69. https://doi.org/10.1007/s11625-009-0097-x

Ebeke, C.H., Ntsama Etoundi, S.M., 2017. The Effects of Natural Resources on Urbanization, Concentration, and Living Standards in Africa. World Development 96, 408–417. https://doi.org/10.1016/j.worlddev.2017.03.026

Ericsson, M., Löf, O., 2019. Mining's contribution to national economies between 1996 and 2016. Mineral Economics 32, 223–250. https://doi.org/10.1007/s13563-019-00191-6

FAO (Ed.), 2010. Addressing food insecurity in protracted crises, The state of food insecurity in the world. FAO, Rome.

Fernández-Caliani, J.C., Giráldez, M.I., Barba-Brioso, C., 2019. Oral bioaccessibility and human health risk assessment of trace elements in agricultural soils impacted by acid mine drainage. Chemosphere 237, 124441. https://doi.org/10.1016/j.chemosphere.2019.124441

Ferreira, R.A., Pereira, M.F., Magalhães, J.P., Maurício, A.M., Caçador, I., Martins-Dias, S., 2021. Assessing local acid mine drainage impacts on natural regeneration-revegetation of São Domingos mine (Portugal) using a mineralogical, biochemical and textural approach. Science of The Total Environment 755, 142825. https://doi.org/10.1016/j.scitotenv.2020.142825

Frayne, B., McCordic, C., Shilomboleni, H., 2014. Growing Out of Poverty: Does Urban Agriculture Contribute to Household Food Security in Southern African Cities? Urban Forum 25, 177–189. https://doi.org/10.1007/s12132-014-9219-3

Funder, M., Mweemba, C., Nyambe, I., van Koppen, B., Ravnborg, H.M., 2010. Understanding local water conflict and cooperation: The case of Namwala District, Zambia. Physics and Chemistry of the Earth, Parts A/B/C, 10th WaterNet/WARFSA/GWP-SA Symposium: IWRM - Environmental Sustainability, Climate Change and Livelihoods 35, 758–764. https://doi.org/10.1016/j.pce.2010.07.022

German, L., Schoneveld, G.C., Wertz-Kanounnikoff, S., Gumbo, D.J., 2015. Chinese trade and investment and its impacts on forests: A scoping study in the miombo woodlands [Chinese] (Working Paper). https://doi.org/10.17528/cifor/005543

Glotov, V.E., Chlachula, J., Glotova, L.P., Little, E., 2018. Causes and environmental impact of the gold-tailings dam failure at Karamken, the Russian Far East. Engineering Geology 245, 236–247. https://doi.org/10.1016/j.enggeo.2018.08.012

Haddaway, N.R., Cooke, S.J., Lesser, P., Macura, B., Nilsson, A.E., Taylor, J.J., Raito, K., 2019. Evidence of the impacts of metal mining and the effectiveness of mining mitigation measures on social–ecological systems in Arctic and boreal regions: a systematic map protocol. Environmental Evidence 8, 9. https://doi.org/10.1186/s13750-019-0152-8

Hampwaye, G., Rogerson, C.M., 2010. Economic Restructuring in the Zambian Copperbelt: Local Responses in Ndola. Urban Forum 21, 387–403. https://doi.org/10.1007/s12132-010-9096-3

Handavu, F., Chirwa, P.W.C., Syampungani, S., 2019. Socio-economic factors influencing land-use and land-cover changes in the miombo woodlands of the Copperbelt province in Zambia. Forest Policy and Economics 100, 75–94. https://doi.org/10.1016/j.forpol.2018.10.010

Harrison, S., Broadhurst, J.L., Opitz, A., Fundikwa, B., Stander, H.-M., Mostert, L., Juarez, Amaral Filho, J., Kotsiopoulos, A., 2019. An Industrial Ecology Approach to Sulphide-containing Mineral Wastes to Minimise ARD Formation Part 2: Design for Disposal and Extraction of Products of Value. Water Research Commission Report: TT822/1/20

Harrison, S.T., Broadhurst, J.L., Van Hille, R., Oyekola, O., Bryan, C., Hesketh, A., Opitz, A., 2010. A systematic approach to sulphidic waste rock and tailings management to minimise acid rock drainage formation. Water Research Commission Report: 1831/1/10

He, B., Wang, W., Geng, R., Ding, Z., Luo, D., Qiu, J., Zheng, G., Fan, Q., 2021. Exploring the fate of heavy metals from mining and smelting activities in soil-crop system in Baiyin, NW China. Ecotoxicology and Environmental Safety 207, 111234. https://doi.org/10.1016/j.ecoenv.2020.111234

Hesketh, A.H., Broadhurst, J.L., Bryan, C.G., van Hille, R.P., Harrison, S.T.L., 2010. Biokinetic test for the characterisation of AMD generation potential of sulfide mineral wastes. Hydrometallurgy, 18th International Biohydrometallurgy Symposium, IBS2009, Bariloche-Argentina, 13-17 September 2009 104, 459–464. https://doi.org/10.1016/j.hydromet.2010.01.015

Horn, S., Gunn, A.G., Petavratzi, E., Shaw, R.A., Eilu, P., Törmänen, T., Bjerkgård, T., Sandstad, J.S., Jonsson, E., Kountourelis, S., Wall, F., 2021. Cobalt resources in Europe and the potential for new discoveries. Ore Geology Reviews 130, 103915. https://doi.org/10.1016/j.oregeorev.2020.103915

Jeong, S.W., Wu, Y.-H., Cho, Y.C., Ji, S.W., 2018. Flow behavior and mobility of contaminated waste rock materials in the abandoned Imgi mine in Korea. Geomorphology 301, 79–91. https://doi.org/10.1016/j.geomorph.2017.10.021

Kampunzu, A., Cailteux, J., Kamona, F., Intiomale, M.M., Melcher, F., 2009. Sediment-hosted Zn–Pb–Cu deposits in the Central African Copperbelt. Ore Geology Reviews - ORE GEOL REV 35, 263–297. https://doi.org/10.1016/j.oregeorev.2009.02.003

Karaca, O., Cameselle, C., Reddy, K.R., 2018. Mine tailing disposal sites: contamination problems, remedial options and phytocaps for sustainable remediation. Reviews in Environmental Science and Biotechnology 17, 205–228. https://doi.org/10.1007/s11157-017-9453-y

Karczewska, A., Kaszubkiewicz, J., Kabała, C., Jezierski, P., Spiak, Z., Szopka, K., 2017. Chapter 6 - Tailings Impoundments of Polish Copper Mining Industry—Environmental Effects, Risk

Assessment and Reclamation, in: Bech, J., Bini, C., Pashkevich, M.A. (Eds.), Assessment, Restoration and Reclamation of Mining Influenced Soils. Academic Press, pp. 149–202. https://doi.org/10.1016/B978-0-12-809588-1.00006-2

Kirchhoff, C.J.; B., 2008. Sustainable Water Management in the Zambezi River Basin. Journal of the International Institute 15

Knierzinger, W., Huang, J.-J.S., Strasser, M., Knorr, K.-H., Drescher-Schneider, R., Wagreich, M., 2021. Late Holocene periods of copper mining in the Eisenerz Alps (Austria) deduced from calcareous lake deposits. Anthropocene 33, 100273. https://doi.org/10.1016/j.ancene.2020.100273

Kotze, D., Macfarlane, D., Edwards, R., Madikizela, B., 2020. WET-EcoServices Version 2: A revised ecosystem services assessment technique, and its application to selected wetland and riparian areas. Water SA 46. https://doi.org/10.17159/wsa/2020.v46.i4.9084

Kossoff, D., Dubbin, W.E., Alfredsson, M., Edwards, S.J., Macklin, M.G., Hudson-Edwards, K.A., 2014. Mine tailings dams: Characteristics, failure, environmental impacts, and remediation. Applied Geochemistry 51, 229–245. https://doi.org/10.1016/j.apgeochem.2014.09.010

Levy, J., Hay, C., Chandipo, R., Jordan, K., Nyambe, I., Mutiti, S., 2017. Drinking Water Provision and Quality in Low-Income Peri-Urban Communities of Lusaka, Zambia. Annals of Global Health, Poster Abstracts from the 8th Annual CUGH Conference: Healthy People, Healthy Ecosystems: Implementation, Leadership & Sustainability in Global Health 83, 92. https://doi.org/10.1016/j.aogh.2017.03.205

Liu, J., Wu, J., Feng, W., Li, X., 2020. Ecological Risk Assessment of Heavy Metals in Water Bodies around Typical Copper Mines in China. International Journal of Environmental Research and Public Health 17, 4315. https://doi.org/10.3390/ijerph17124315

Lottermoser, B.G., Ashley, P.M., 2005. Tailings dam seepage at the rehabilitated Mary Kathleen uranium mine, Australia. Journal of Geochemical Exploration 85, 119–137. https://doi.org/10.1016/j.gexplo.2005.01.001

Loucks, D.P., van Beek, E., 2017. Water Resources Planning and Management: An Overview, in: Loucks, D.P., van Beek, E. (Eds.), Water Resource Systems Planning and Management: An Introduction to Methods, Models, and Applications. Springer International Publishing, pp. 1–49. https://doi.org/10.1007/978-3-319-44234-1_1

Madejón, P., Caro-Moreno, D., Navarro-Fernández, C.M., Rossini-Oliva, S., Marañón, T., 2021. Rehabilitation of waste rock piles: Impact of acid drainage on potential toxicity by trace elements in plants and soil. Journal of Environmental Management 280, 111848. https://doi.org/10.1016/j.jenvman.2020.111848

Maest, A.S., Kuipers, J.R., Travers, C.L., and Atkins, D.A., 2005. Predicting Water Quality at Hardrock Mines: Methods and Models, Uncertainties, and State-of-the-Art

Maze, K., Barnett, M., Botts, E.A., Stephens, A., Freedman, M., Guenther, L., 2016. Making the case for biodiversity in South Africa: Re-framing biodiversity communications. Bothalia - African Biodiversity & Conservation 46, 1–8. https://doi.org/10.4102/abc.v46i1.2039

Mohapatra, D.P., Kirpalani, D.M., 2016. Process effluents and mine tailings: sources, effects and management and role of nanotechnology. Nanotechnology for Environmental Engineering 2, 1. https://doi.org/10.1007/s41204-016-0011-6

Moncur, M.C., Ptacek, C.J., Blowes, D.W., Jambor, J.L., 2005. Release, transport and attenuation of metals from an old tailings impoundment. Applied Geochemistry 20, 639–659. https://doi.org/10.1016/j.apgeochem.2004.09.019

Moreno Abril, S.I., Dalmolin, C., Costa, P.G., Bianchini, A., 2018. Expression of genes related to metal metabolism in the freshwater fish Hyphessobrycon luetkenii living in a historically contaminated area associated with copper mining. Environmental Toxicology and Pharmacology 60, 146–156. https://doi.org/10.1016/j.etap.2018.04.019

Mubanga, R.O., Kwarteng, K., 2020. A comparative evaluation of the environmental impact assessment legislation of South Africa and Zambia. Environmental Impact Assessment Review 83, 106401. https://doi.org/10.1016/j.eiar.2020.106401

Muma, D., Besa, B., Manchisi, J., Banda, W., 2020. Effects of mining operations on air and water quality in Mufulira district of Zambia: A case study of Kankoyo Township. Journal of the Southern African Institute of Mining and Metallurgy 120, 287–298. https://doi.org/10.17159/2411-9717/952/2020

Mususa, P., 2012. Mining, welfare and urbanisation: the wavering urban character of Zambia's Copperbelt. Journal of Contemporary African Studies 30, 571–587. https://doi.org/10.1080/02589001.2012.724873

Mususa, P., 2010. 'Getting by': life on the Copperbelt after the privatisation of the Zambia Consolidated Copper Mines. Social Dynamics-a Journal of The Centre for African Studies University of Cape Town - SOC DYNAMICS 36, 380–394. https://doi.org/10.1080/02533951003790546

Musvoto, C., de Lange, W.J., 2019. A framework for selecting crops for irrigation using mining contaminated water: An example from the Olifants basin of South Africa. Journal of Environmental Management 231, 49–58. https://doi.org/10.1016/j.jenvman.2018.09.058

Mwitwa, J., German, L., Muimba-Kankolongo, A., Puntodewo, A., 2012. Governance and sustainability challenges in landscapes shaped by mining: Mining-forestry linkages and impacts in the Copper Belt of Zambia and the DR Congo. Forest Policy and Economics 25, 19–30. https://doi.org/10.1016/j.forpol.2012.08.001

Nachiyunde, K., Ikeda, H., Okuda, T., Nishijima, W., 2013. Assessment of Dissolved Heavy Metal Pollution in Five Provinces of Zambia. Journal of Environmental Protection 4, 80–85. https://doi.org/10.4236/jep.2013.41B015

Nieva, N.E., Borgnino, L., García, M.G., 2018. Long term metal release and acid generation in abandoned mine wastes containing metal-sulphides. Environmental Pollution 242, 264–276. https://doi.org/10.1016/j.envpol.2018.06.067

Nkuna, V.L., Naidoo, T., Amos, S.R., 2016. Ivanhoe Mines' giant Kamoa copper discovery in the DRC - a metallurgical perspective. Journal of the Southern African Institute of Mining and Metallurgy 116, 547–552. https://doi.org/10.17159/2411-9717/2016/v116n6a9

Ntengwe, F.W., 2005. An overview of industrial wastewater treatment and analysis as means of preventing pollution of surface and underground water bodies—the case of Nkana Mine in Zambia. Physics and Chemistry of the Earth, Parts A/B/C, Integrated Water Resources Management (IWRM) and the Millennium Development Goals: Managing Water for Peace and Prosperity 30, 726–734. https://doi.org/10.1016/j.pce.2005.08.014

Opitz, A.K.B., Broadhurst, J.L., Harrison, S.T.L., 2015. Assessing Environmental Risks Associated with Ultrafine Coal Wastes Using Laboratory-Scale Tests [WWW Document]. Advanced Materials Research. https://doi.org/10.4028/www.scientific.net/AMR.1130.635

Organization, W.H., UN-Habitat, 2016. Global report on urban health: equitable healthier cities for sustainable development. World Health Organization.

Pan, Y.-R., Wang, X., Ren, Z.J., Hu, C., Liu, J., Butler, D., 2019. Characterization of implementation limits and identification of optimization strategies for sustainable water resource recovery through life cycle impact analysis. Environment International 133, 105266. https://doi.org/10.1016/j.envint.2019.105266

Parbhakar-Fox, A., Lottermoser, B., Bradshaw, D., 2013. Evaluating waste rock mineralogy and microtexture during kinetic testing for improved acid rock drainage prediction. Minerals Engineering, Process Mineralogy 52, 111–124. https://doi.org/10.1016/j.mineng.2013.04.022

Perlatti, F., Ferreira, T.O., Romero, R.E., Costa, M.C.G., Otero, X.L., 2015. Copper accumulation and changes in soil physical–chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: Implications for restoration of mine sites. Ecological Engineering 82, 103–111. https://doi.org/10.1016/j.ecoleng.2015.04.085

Perlatti, F., Martins, E.P., de Oliveira, D.P., Ruiz, F., Asensio, V., Rezende, C.F., Otero, X.L., Ferreira, T.O., 2021. Copper release from waste rocks in an abandoned mine (NE, Brazil) and its impacts on ecosystem environmental quality. Chemosphere 262, 127843. https://doi.org/10.1016/j.chemosphere.2020.127843

Plante, B., Benzaazoua, M., Bussière, B., 2011. Kinetic Testing and Sorption Studies by Modified Weathering Cells to Characterize the Potential to Generate Contaminated Neutral Drainage. Mine Water Environment 30, 22–37. https://doi.org/10.1007/s10230-010-0131-3

Plante, B., Bussière, B., Benzaazoua, M., 2012. Static tests response on 5 Canadian hard rock mine tailings with low net acid-generating potentials. Journal of Geochemical Exploration 114, 57–69. https://doi.org/10.1016/j.gexplo.2011.12.003

Rakotonimaro, T.V., Guittonny, M., Neculita, C.M., 2021. Compaction of peat cover over desulfurized gold mine tailings changes: Arsenic speciation and mobility. Applied Geochemistry 104923. https://doi.org/10.1016/j.apgeochem.2021.104923

Rambabu, K., Banat, F., Pham, Q.M., Ho, S.-H., Ren, N.-Q., Show, P.L., 2020. Biological remediation of acid mine drainage: Review of past trends and current outlook. Environmental Science and Ecotechnology 2, 100024. https://doi.org/10.1016/j.ese.2020.100024

Saintilan, N.J., Selby, D., Creaser, R.A., Dewaele, S., 2018. Sulphide Re-Os geochronology links orogenesis, salt and Cu-Co ores in the Central African Copperbelt. Scientific Reports 8. https://doi.org/10.1038/s41598-018-33399-7

Schmidt, K., Sachse, R., Walz, A., 2016. Current role of social benefits in ecosystem service assessments. Landscape and Urban Planning 149, 49–64. https://doi.org/10.1016/j.landurbplan.2016.01.005

Sdiri, A., Pinho, J., Ratanatamskul, C., 2018. Water resource management for sustainable development. Arabian Journal of Geosciences 11, 124. https://doi.org/10.1007/s12517-018-3411-z

Shiers, D.W., Collinson, D.M., Watling, H.R., 2016. Life in heaps: a review of microbial responses to variable acidity in sulfide mineral bioleaching heaps for metal extraction. Research in Microbiology, Special issue on Biohydrometallurgy: recent advances in microbiology and molecular biology 167, 576–586. https://doi.org/10.1016/j.resmic.2016.05.007

Shimaponda-Mataa, N.M., Tembo-Mwase, E., Gebreslasie, M., Achia, T.N.O., Mukaratirwa, S., 2017. Modelling the influence of temperature and rainfall on malaria incidence in four endemic provinces of Zambia using semiparametric Poisson regression. Acta Tropical 166, 81–91. https://doi.org/10.1016/j.actatropica.2016.11.007

Simonsen, A.M.T., Solismaa, S., Hansen, H.K., Jensen, P.E., 2020. Evaluation of mine tailings' potential as supplementary cementitious materials based on chemical, mineralogical and physical characteristics. Waste Management 102, 710–721. https://doi.org/10.1016/j.wasman.2019.11.037

Smart, J., Nel, E., Binns, T., 2015. Economic crisis and food security in Africa: Exploring the significance of urban agriculture in Zambia's Copperbelt province. Geoforum 65, 37–45. https://doi.org/10.1016/j.geoforum.2015.07.009

Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Sun, J., Strosnider, W.H.J., Nairn, R.W., LaBar, J.A., 2020. Water quality impacts of in-stream mine tailings on a headwater tributary of the Rio Pilcomayo, Potosí, Bolivia. Applied Geochemistry 113, 104464. https://doi.org/10.1016/j.apgeochem.2019.104464

Syampungani, S., Clendenning, J., Gumbo, D., Nasi, R., Moombe, K., Chirwa, P., Ribeiro, N., Grundy, I., Matakala, N., Martius, C., Kaliwile, M., Kabwe, G., Petrokofsky, G., 2014. The impact of land use and cover change on above and below-ground carbon stocks of the miombo woodlands

since the 1950s: a systematic review protocol. Environmental Evidence 3, 25. https://doi.org/10.1186/2047-2382-3-25
Tayebi-Khorami, M., Edraki, M., Corder, G., Golev, A., 2019. Re-Thinking Mining Waste through an Integrative Approach Led by Circular Economy Aspirations. Minerals 9, 286. https://doi.org/10.3390/min9050286
Teixeira, H., Lillebø, A.I., Culhane, F., Robinson, L., Trauner, D., Borgwardt, F., Kuemmerlen, M., Barbosa, A., McDonald, H., Funk, A., O'Higgins, T., Van der Wal, J.T., Piet, G., Hein, T., Arévalo-Torres, J., Iglesias-Campos, A., Barbière, J., Nogueira, A.J.A., 2019. Linking biodiversity to ecosystem services supply: Patterns across aquatic ecosystems. Science of The Total Environment 657, 517–534. https://doi.org/10.1016/j.scitotenv.2018.11.440
Torremans, K., Gauquie, J., Boyce, A.J., Barrie, C.D., Dewaele, S., Sikazwe, O., Muchez, Ph., 2013. Remobilisation features and structural control on ore grade distribution at the Konkola stratiform Cu–Co ore deposit, Zambia. Journal of African Earth Sciences 79, 10–23. https://doi.org/10.1016/j.jafrearsci.2012.10.005
Toyomaki, H., Yabe, J., Nakayama, S.M.M., Yohannes, Y.B., Muzandu, K., Liazambi, A., Ikenaka, Y., Kuritani, T., Nakagawa, M., Ishizuka, M., 2020. Factors associated with lead (Pb) exposure on dogs around a Pb mining area, Kabwe, Zambia. Chemosphere 247, 125884. https://doi.org/10.1016/j.chemosphere.2020.125884
Twite, F., Broughton, D., Nex, P., Kinnaird, J., Gilchrist, G., Edwards, D., 2019. Lithostratigraphic and structural controls on sulphide mineralisation at the Kamoa copper deposit, Democratic Republic of Congo. Journal of African Earth Sciences 151, 212–224. https://doi.org/10.1016/j.jafrearsci.2018.12.016
Tyler Mehler, W., Gagliardi, B., Keough, M.J., Pettigrove, V., 2019. Evaluating freshwater mining sediment toxicity in Tasmania: Achieving strong multiple lines of evidence. Science of The Total Environment 651, 1243–1252. https://doi.org/10.1016/j.scitotenv.2018.09.245
Valenzuela-Diaz, M.J., Navarrete-Calvo, A., Caraballo, M.A., McPhee, J., Garcia, A., Correa-Burrows, J.P., Navarro-Valdivia, L., 2020. Hydrogeochemical and environmental water quality standards in the overlap between high mountainous natural protected areas and copper mining activities (Mapocho river upper basin, Santiago, Chile). Journal of Hydrology 588, 125063. https://doi.org/10.1016/j.jhydrol.2020.125063
Vriens, B., Peterson, H., Laurenzi, L., Smith, L., Aranda, C., Mayer, K.U., Beckie, R.D., 2019. Long-term monitoring of waste-rock weathering at the Antamina mine, Peru. Chemosphere 215, 858–869. https://doi.org/10.1016/j.chemosphere.2018.10.105
Wang, P., Sun, Z., Hu, Y., Cheng, H., 2019. Leaching of heavy metals from abandoned mine tailings brought by precipitation and the associated environmental impact. Science of The Total Environment 695, 133893. https://doi.org/10.1016/j.scitotenv.2019.133893
Wolkersdorfer, C., Nordstrom, D.K., Beckie, R.D., Cicerone, D.S., Elliot, T., Edraki, M., Valente, T., França, S.C.A., Kumar, P., Lucero, R.A.O., Soler i Gil, A., 2020. Guidance for the Integrated Use of Hydrological, Geochemical, and Isotopic Tools in Mining Operations. Mine Water Environment 39, 204–228. https://doi.org/10.1007/s10230-020-00666-x
Xu, Y., Cai, Y., Sun, T., Yang, Z., Hao, Y., 2018. Coupled hydrodynamic and ecological simulation for prognosticating land reclamation impacts in river estuaries. Estuarine, Coastal and Shelf Science 202, 290–301. https://doi.org/10.1016/j.ecss.2017.12.009
Zhao, Q., Guo, F., Zhang, Y., Ma, S., Jia, X., Meng, W., 2017. How sulfate-rich mine drainage affected aquatic ecosystem degradation in northeastern China, and potential ecological risk. Science of The Total Environment 609, 1093–1102. https://doi.org/10.1016/j.scitotenv.2017.07.276

CHAPTER 2: LITERATURE REVIEW

2.1. Zambian Water Resources

Zambia is endowed with abundant water resources, with available surface water sourced from the Congo and Zambezi River Basin (Petersen-Perlman, 2016). Three quarters of the country is covered by the Zambezi River Basin (ZRB), which comprises three sub-basins – Luangwa, Kafue, and Kabompo. The Kafue River Basin (KRB) lying wholly within Zambia, is the largest sub-catchment of ZRB (Kalumba and Nyirenda, 2017). KRB also has the largest economic activity covering 20% of the land in Zambia and is home to nearly half of the country's population. The KRB traverses Copperbelt, Central, Lusaka, and Southern Provinces with major mining, agriculture and industrial activities concentrated in these provinces (Kambole, 2003). It is the heart of the economic and developmental base for Zambia, and a major water supply to more than 40% of urban and rural population in Zambia, especially those living in the KRB (Schelle and Pittock, 2006). The Kafue River catchment constitutes a unique environment with international acclaim, as it provides habitats for extensive biological diversity and endemism (Ellenbroek, 1987; von der Heyden and New, 2004). In recent times, hydrochemical and toxicological studies have highlighted the adverse effect of mining interlinked operations on the ecology and chemistry of Kafue River catchment, and the threat on downstream resource end-users (Chileshe *et al.*, 2020; Kapungwe, 2013; Muma *et al.*, 2020; Ntengwe and Maseka, 2006). The aforementioned issues are closely related to pollution of water resources and complicated to mitigate. Their prevention is required to ensure suitable and sufficient water for both humans and ecosystem demands, since fresh water quality and availability and well-functioning ecosystems are essential for human wellbeing as well as for economic and social development (Apostolaki *et al.*, 2020; Kumar *et al.*, 2021).

2.2. Kafue River Catchment

2.2.1. Background

The Copperbelt Province is home to one of the biggest copper deposits in Africa (Sracek *et al.*, 2012). Mining operations on the Copperbelt occur primarily within the Kafue River catchment zone (Figure 2-1), as such, most of the pollutants originate in the upper reaches of the Kafue River (Sracek, 2015). The Copperbelt cities are the main economic centers in the upper Kafue River catchment and are located in the concentrated area of mining and industrial activities

(Muma *et al.*, 2020). As a result of these intense activities, the upper Kafue River has experienced various stresses and impacts for many years, and these impacts are still occurring today (Kalumba and Nyirenda, 2017; Kapungwe, 2013; M'kandawire *et al.*, 2017; Norrgren *et al.*, 2000;). Notably, tributaries of Kafue River such as Lubengele Stream, Mushishima Stream, Changa River, Mufulira River, Musakashi Stream, Mwambashi Stream, Mindolo Stream, Uchi Stream, Kitwe Stream, Mululu Stream, Fikondo Stream, and Wanshimba Stream, impacted by mining related activities, equally contribute to contamination of Kafue River (Sracek *et al.*, 2012).

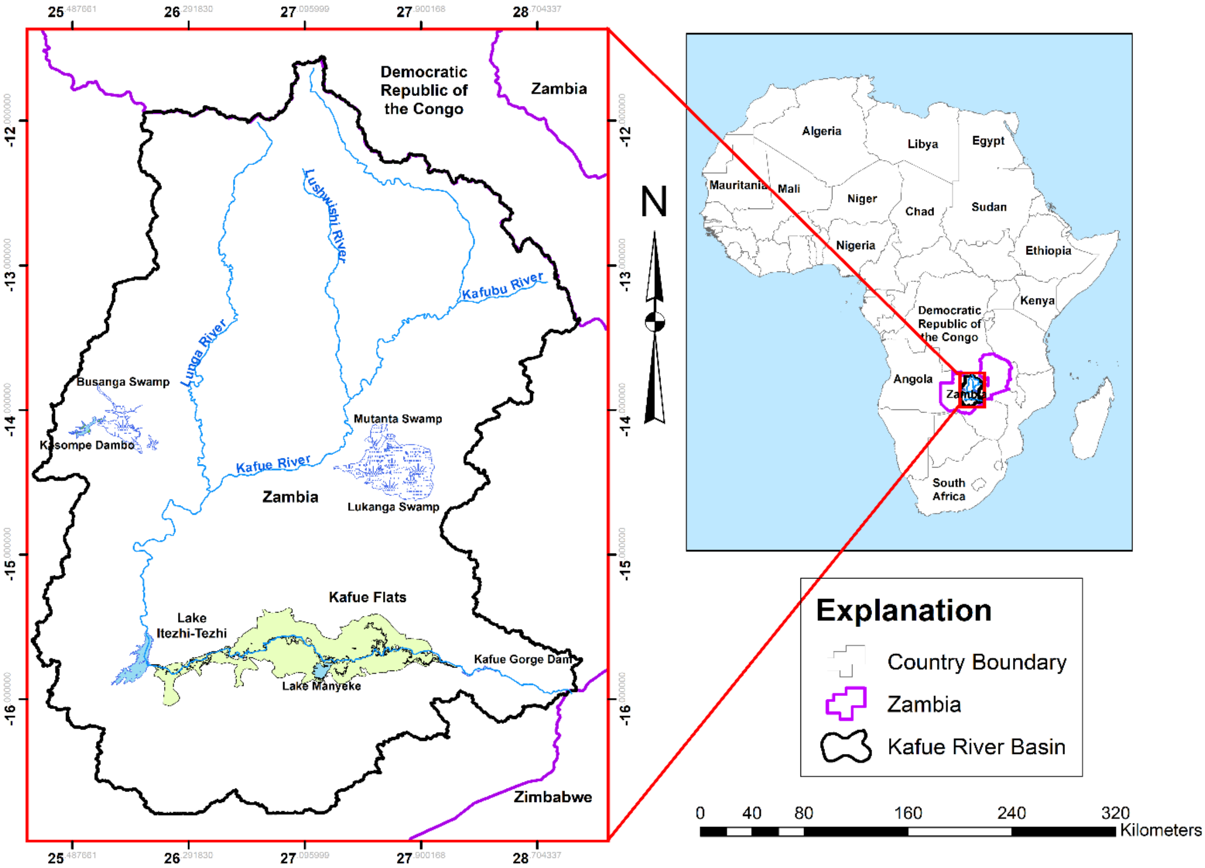

Figure 2-1: The Kafue River catchment on the Copperbelt Province of Zambia

With the expansion of the mining activities especially small-scale mines and other ancillary sectors in the Copperbelt region, urbanisation and demand for water is equally likely to increase (Banda and Chanda, 2021; Hilson, 2020). For this reason, water protection and conservation are necessary to address shortfalls in water supply experienced in upper Kafue River catchment owing to water pollution. One of the major pollution problems associated with copper mining is ARD discharge into water resources (Karaca *et al.*, 2018; Simate and Ndlovu, 2014), metal mobilization (Sracek *et al.*, 2012), complex effluents from mining and emerging industries (Oberholster *et al*., 2010, 2008), untreated industrial wastewater as well as habitat destruction and runoff (Edokpayi *et al*., 2017; Ntshane and Gambiza, 2016; Sonter *et al.*, 2018). All the above-mentioned environmental issues can negatively affect water quantity and quality.

In the course of extracting copper and cobalt reserves (mining), large volumes of waste such as waste rock and tailing material is generated, which may result in an increased rate of ARD production and mobilization of metals (Guo *et al*., 2013; Jiang *et al*., 2021). However, when sulfidic mineralization with exploitable Co and Cu is ingrained in carbonate rich sandstone, and shale and argillite; neutral drainage with relatively low concentration of dissolved metals is likely to occur (Langman *et al*., 2019). In contrast, where neutralization capacity in rocks is low, pH values in water resources are low and concentration of dissolved metals are high (Hudson-Edwards *et al*., 1999). The high sulphide oxidation rates in places with poor neutralization capacity could be the difference, owing to oxidation by ferric iron and absence of ferric oxyhydroxide coatings on the primary sulphides (Sracek *et al*., 2012). Additionally, neutralization by carbonates may result in a sequence of reactions, where most metals co-precipitate or precipitate as hydroxides directly in mine tailings (Sracek *et al*., 2010). A typical example of a high neutralization capacity system is the Zambian Copperbelt. Typically, neutralization occurs within the mining wastes in the Copperbelt owing to the high carbonate content of gangue rocks (Sracek *et al*., 2010, 2012). Transport in suspension often plays a significant role in high neutralization capacity systems, dissolved form may be less significant than contaminant transport in suspended load (Yuan *et al*., 2021). Authigenic suspended particles constituting Mn- and Fe-oxyhydroxides may transport most metals (Kimball et al., 2002, 1995). Formation of secondary Mn and Fe particles, enriched in Cu and Co in the Kafue River catchment have been reported by Pettersson and Ingri (2001). Suspended particles settle during low discharge periods; however, they can be re-suspended during high discharge in rainy periods. Efflorescent salts that are precipitated in dry periods may dissolve at the beginning of rainy periods with a resulting acid pulse and high metal concentration (Sracek *et al*., 2010).

2.2.2. Climate and Topographical Characteristics

The Zambian climate is typified by three recognizable seasons: (i) warm and wet season from November till April; (ii) dry and cool season which follows the rainy season (April) till August; and (iii) dry and hot season beginning in August and ending in November prior to a new rainy season (Libanda *et al*., 2020). Notably, cumuliform and cumulonimbus clouds are common during the months November to April, and are often punctuated by thunderstorms, heavy rainfall, and sunshine. These elements may motivate the formation of ARD, metal mobility, and runaway resurgence of mine tailings (Fosso-Kankeu *et al*., 2017; Lyu *et al*., 2019). Water resources located within the mine catchment areas are susceptible to impact of mine wastelands during this period. The precipitation patterns in the catchment are shown in Figure 2-2. On average, precipitation is above 1200 mm in the Kafue River catchment (Hachigonta *et al*., 2008).

The topography of the Kafue River catchment varies, ranging from 1083 m above sea level in Luanshya and Masaiti districts to over 1700 m in Chingola district (Figure 2-2). It is best described as a plateau with vast land in the area (Libanda *et al*., 2020; Libanda and Ngonga, 2018).

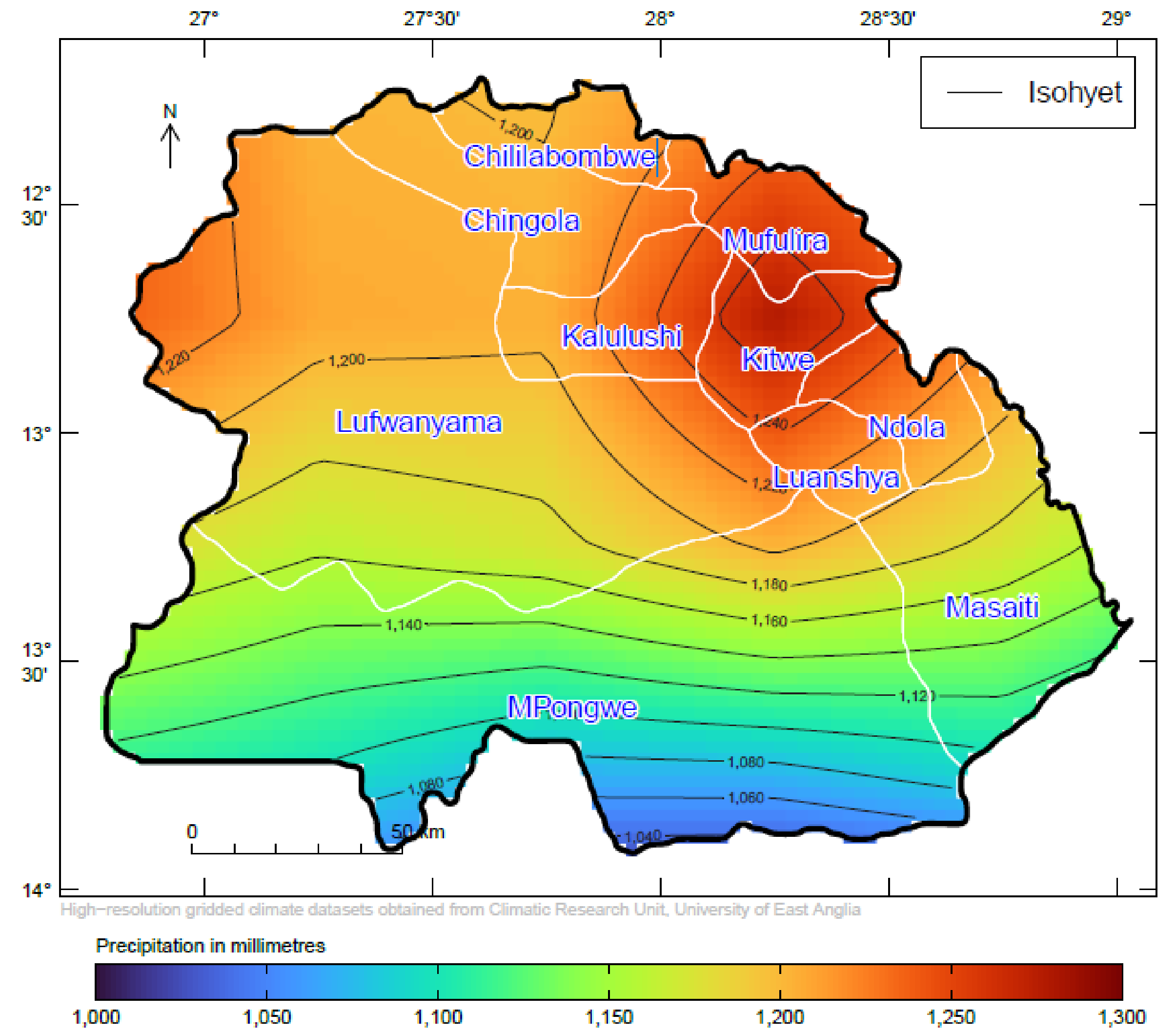

Figure 2-2: The mean annual rainfall of the Kafue River catchment on the Copperbelt Province in Zambia

2.2.3. Geology of the Kafue River Catchment (Zambian Copperbelt)

Kafue River catchment overlies a portion of Chambishi, Luanshya-Baluba, Nchanga-Chingola, Konkola-Musoshi, Mufulira and Nkana-Mindola mining areas (Saintilan *et al*., 2018; Sillitoe *et al*., 2011). The main host rocks in the area are Neoproterozoic sedimentary and Mesoproterozoic metamorphic basement rocks, which are associated with shales, sandstones, conglomerates and mudstones (Bull *et al*., 2011; Wilderode *et al*., 2014). The copper deposits in the catchment occur within the upper and lower Roan Subgroup (Figure 2-3). Economic copper reserves rich in Cu-Co are mostly found in the lower Roan Subgroup, as a result, most mining related activities are located in this region (Broughton, 2013). The upper Roan subgroup consists mainly of platformal mixed carbonates, quartzite, shale and clastic rocks.

The two recognised mineralization assemblages are Cu-Co sulphides and pyrites, with Cu-Co sulphide carrollite widespread in the region (Davey *et al.*, 2020; Rainaud *et al.*, 2005). The overlying formations of the upper and lower Roan is a variety of lithologies with sandstones, shales and dolomites (Broughton, 2013).

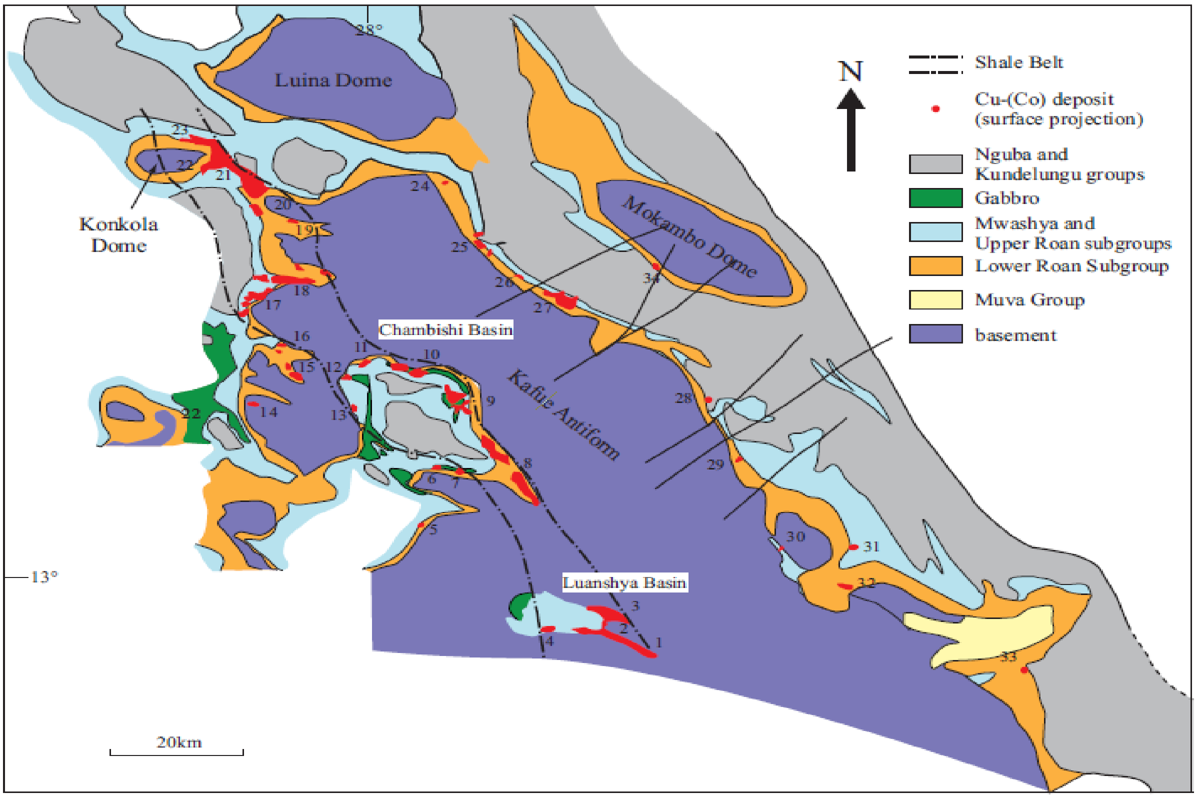

Figure 2-3: Map showing the geological representation of ore deposits in the Kafue River catchment on the Zambian Copperbelt (Broughton, 2013). The numbers designate the major Cu-Co deposits in the region

2.2.4. Copper and Cobalt Mining

Mining is an extremely important economic activity in Zambia, it contributes about 10% of the country's GDP and over 70% of export (Kolala and Dokowe, 2021; Mulenga, 2017; Ngubo, 2016). Study by Kolala and Umar (2019) has shown linkages between decline in copper production and prices, has significant impact on economic growth and rise in unemployment level in Zambia. Similarly, Mukosa *et al.* (2020) reported, as at 2019, the rate of unemployment in Zambia had reached 18.4% owing to a reduction in copper production, resulting in youth unemployment rising at the rate of 18.2% (with 18.7% female and 18% male). The strategic importance of copper mining to Zambia(ns) cannot be over emphasized. Other than copper and cobalt, Zambia is enriched with minerals such as zinc, uranium, nickel, gold, lead, emeralds, quartz and other precious minerals (Muchez *et al*., 2010; Sikamo *et al*., 2016). Mining mostly occurs on the Copperbelt and North-western Province, nearly 80% of copper production in Zambia is contributed by Kansanshi, Konkola, Mopani and Lumwana mines (Mwaanga *et al*., 2019).

Copper mining in the Kafue River catchment started around 1920s (Sikamo *et al*., 2016), since then, large volumes of mine waste have been generated, and more is being formed (Sikaundi, 2013; Festin *et al*., 2019). Mining is one of the major contributors of pollutants in the Kafue River catchment and has a diversity of ecological consequences (Mbewe *et al*., 2016; Nalishuwa, 2015; Yabe *et al*., 2010). For instance, Sracek *et al*., (2012) reported that metal contaminants in Kafue River contributed more than 95% of the contaminant load. A study by M'kandawire *et al.* (2017) showed that most of the streams within the Kafue River catchment had a high risk for metal contamination. The pollution load index (PLI) and contamination factor (CF) were used to assess the ecological risks, where PLI > 1 designated polluted site, CF < 1 stood for low pollution, 1 ≤ CF ≤ 3 represented moderate pollution, 3 ≤ CF < 6 meant considerable pollution, and CF ≥ designated high pollution (Loska *et al*., 1997; Suresh *et al.*, 2011). The study, summarised in Table 2-1 and 2-2, reported high pollution load index and contamination factor of metals Cu, Co, Mn, and As in aquatic environment around copper mining areas. The ecological risk assessment (RI) showed that Cu, As and Ag were the major pollutants. Similarly, studies by Liu et al. (2020) on seven mines in China, have shown significant metal pollution in aquatic environment around copper mine areas. The sediment ecological risk analysis indicated that Cu, Cd and Pb were the main pollutants (Table 2-3).

Mine wastelands have been observed to be potential sources of metal contamination (Worlanyo and Jiangfeng, 2021). Over the last century, ore-mining has resulted in the generation of enormous amounts of tailings globally (Chen et al., 2018). Nearly all the generated tailings are piled up in tailing ponds or open pit. Exposure of sulfide minerals in the tailings water and atmospheric oxygen, can result in ARD formation and mobilization of metals (Fan et al., 2016; Sağlam and Akçay, 2016). Mobilization of metals and their transport and fate are a topic of concern.

Table 2-1: PLI and CF of elements in streams within the Kafue River catchment

Seasons	Sampling Site	Contamination factor (CF)													PLI
		Pb	Al	As	Cu	Fe	Hg	Ni	Co	Zn	Mn	Cd	Se	Cr	
Warm/Rainy	Chililabombwe	2,6	1,6	1,6	145	2,2	0,9	1,3	28	3,5	12	0,7	7,4	1,1	3,6
	Chingola-Kanyemo	2,4	0,8	1,3	146	1,8	0,5	1,2	27	2,8	3,8	0,5	8,4	1	2,8
	Chingola-Hippo Pool	2,3	0,6	4	68	1,5	0,4	1	15	2,6	3	0,4	1,3	0,7	2
	Kafue Flats	1	2,2	1	0,4	2,1	0,5	1,7	0,8	1,4	0,6	ND	1,8	1,7	1,1
	Kafue Town	2,3	0,7	1,3	0,5	0,9	0,5	0,6	0,4	2,1	0,4	0,5	0,7	0,6	0,7
Dry/Cold	Chililabombwe	2,2	1,3	1,2	72	1,8	0,8	1,4	13	2,2	5,9	0,2	2,8	1,1	2,3
	Chingola-Kanyemo	2,4	0,8	1,2	129	1,8	0,8	1,1	18	1,5	5,5	0	1,7	0,9	1,7
	Chingola-Hippo Pool	1,7	0,2	3,2	50	1,2	0,6	0,5	7,1	1,1	2,7	0	ND	0,3	0,9
	Kafue Flats	1,4	2,8	1,1	0,4	2,7	0,5	1,7	0,7	1	1,3	ND	ND	2	1,2
	Kafue Town	0,8	1,3	0,8	0,2	1,6	0,5	1,3	0,4	0,4	0,8	0,5	ND	1,4	0,7
Dry/Hot	Chililabombwe	2,4	0,6	2	302	1,4	1,1	0,8	27	2,8	12,3	0,1	ND	0,6	2,3
	Chingola-Kanyemo	2,9	0,5	1,5	261	1,6	0,9	0,5	29	2,5	9,3	0,1	ND	0,5	2
	Chingola-Hippo Pool	3,6	0,6	8,8	156	2	1	0,7	19	3,1	9,2	0,2	ND	0,8	2,7
	Kafue Flats	1,2	1,7	1,6	1	2,2	0,8	1,1	1,1	1,5	2,9	0	ND	1,3	0,9
	Kafue Town	0,8	1,6	1,5	0,6	2,7	0,8	3,2	0,9	0,9	2,9	0	ND	1,8	0,8

Table 2-2: RI and Er metal of elements at sites in the Kafue River catchment

Seasons	Sampling Site	Potential ecological risk factors (Er $_{metal}$)								RI
		As	Cr	Ag	Cu	Pb	Ni	Cd	Zn	
Warm/Rainy	Chililabombwe	16	2	34	726	13	6	21	4	822
	Chingola-Kanyemo	13	2	21	732	12	6	16	3	806
	Chingola-Hippo Pool	40	1	15	338	11	5	11	3	425
	Kafue Flats	10	3	18	2	5	8	0	1	49
	Kafue Town	13	1	18	2	12	3	15	2	66
Dry/Cold	Chililabombwe	12	2	32	359	11	7	6	2	431
	Chingola-Kanyemo	12	2	33	643	12	6	0	1	709
	Chingola-Hippo Pool	32	1	24	252	8	2	0	1	320
	Kafue Flats	11	4	22	2	7	9	0	1	55
	Kafue Town	8	3	20	1	4	6	16	0	59
Dry/Hot	Chililabombwe	20	1	43	1510	12	4	2	3	1595
	Chingola-Kanyemo	15	1	38	1307	14	2	1	3	1381
	Chingola-Hippo Pool	88	2	42	778	18	4	6	3	939
	Kafue Flats	16	3	33	5	6	5	0	2	70
	Kafue Town	15	4	30	3	4	16	0	1	72

Table 2-3: Ecological risk index of sediments in rivers close proximity to copper mine wastelands in China

Region	Cd	Cu	Zn	Hg	Pb	Cr	NI	RI
Tongling	8430	292	12.9		29.2	2		8766
Dabaoshan	621	192	8.05	37.5	129	1.75		989
Dahongshan	85.7	33.1	0.46		2.61	0.73	6.67	129
Daye	4620	269	3.64	173	11.1	1.84	15.2	5093
Jinchuan	8233	1816	14.4		524	20.01	1563	2171
Baiyin	10847	186	49.7		1001	1.83	3.32	12088
Dexing	42.9	161	1.3	35.3	9.79	4.64		255

2.2.5. Other Land-Use Practices in the Kafue River Catchment

Aside from mining, agricultural practices, afforestation, and industrial activities, amongst others take place in the catchment and are shown in Figure 2-4 (Chibuye and Buitendag, 2020;

Hampwaye and Rogerson, 2010). Agriculture characterised by maize and vegetable production is one of the major users of freshwater resources in the catchment (Akayombokwa *et al*., 2015). Small scale and commercial farmers use dry-land cultivation and irrigation due to continual availability of stream water throughout the year. Irrigated farming contributes significantly to the agricultural output in the Kafue River catchment (Kapungwe, 2012). Agriculture can equally affect water quality, flow and availability (Watts *et al*., 2015; Zia *et al.*, 2013), through increased runoff introducing contaminants in receiving water resources, resulting in degradation of the ecosystem and changes in water flow dynamics.

Figure 2-4 summarises the most significant land uses in the Kafue River catchment on the Zambian Copperbelt. Mining and agriculture are the major land use activities. These have potential to contaminate groundwater and surface water through either point source or non-point source release mechanisms (Duda and Nawar, 1996; Khatri and Tyagi, 2015; Motevalli *et al*., 2019). Point source pollution is the direct release of pollutants from discrete carriages such as pipes, into the ambient environment under regulated conditions, while non-point source pollution is not identifiable with specific sources. An example is runoff from gardens and mining wastelands.

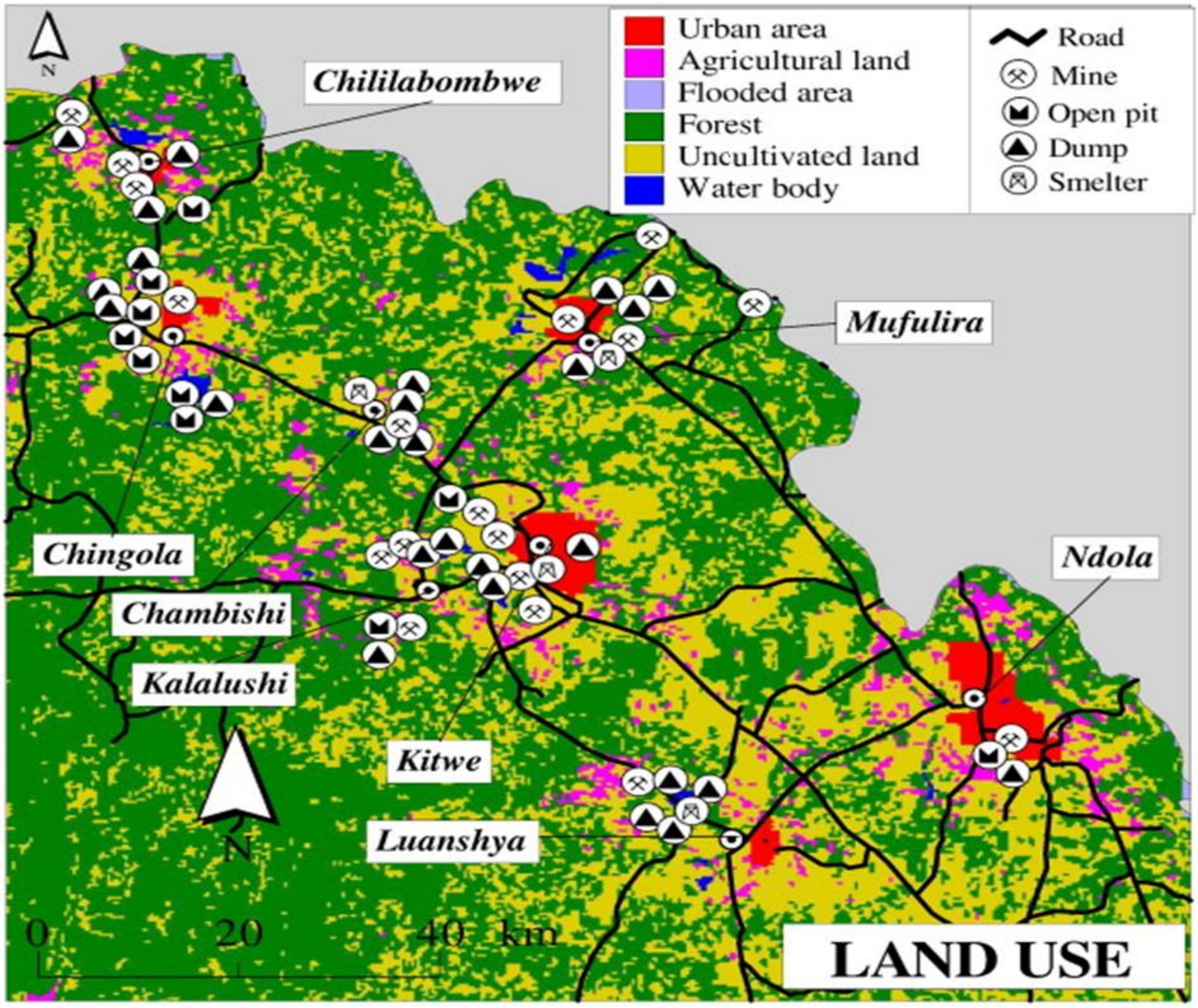

Figure 2-4: A synopsis of the major land-use practices within Kafue River catchment on the Copperbelt Province (Albanese et al., 2014)

2.2.6. Overall

For more than 80 years, copper mining, agriculture, urbanization and industrialization have placed substantial pressures on the Kafue River catchment (Ntengwe and Maseka, 2006; Podolský *et al.*, 2015; Sikamo *et al*., 2016). Ironically, this development has provided researchers with a unique opportunity to study this region and gain insights into the long-term effects of copper mining Zambian water resources. This is useful since copper production in the Kafue River catchment is projected to reach 1.5 million MT/yr by 2030 (GBR, 2014). This growth is likely to exacerbate and prolong the already deteriorated nature of Kafue River catchment through, inter alia, increased metal contamination. This does provide a significant opportunity to investigate the interaction of mine wastelands, ARD generation and mobilization of metals and its interaction with aquatic ecosystems and agroecosystems. The study may provide researchers with the ability to identify distinct factors that regulate a multitude of variables such that monitoring, and management efforts may be tailored more optimally to minimise contamination of water resources in the region.

2.3. Monitoring Ecological Integrity and Impact of Mine Wastelands (TSFs)

Ecological integrity encompasses ecosystem characteristics like resistance, recovery, and self-organisation abilities (Manolaki *et al*., 2021; Müller *et al.*, 2000). It can be defined as the ability of an ecological system to support and maintain a community of organisms and habitats with structure and composition, diversity, and functional organisation (Parrish et al., 2003). Monitoring ecological integrity provides for the assessment of integrated effects of activities occurring in a catchment (Mattson and Angermeier, 2007). Effective monitoring and reporting on ecological integrity is a vital component in natural resource management (Wurtzebach and Schultz, 2016). This requires a consistent monitoring program and the development of benchmarks and thresholds. For instance, in USA, scholars have noted that a well-designed monitoring program can help to detect threats to resources, trends in resource conditions and adaptation of effective mitigation measures (Deluca et al., 2010; Hanberry et al., 2015; Larson et al., 2013). Study of mine waste composition, potentiality for ARD generation and associated mobilization of metals, biotic and abiotic monitoring, provides useful indirect indicators of the integrity of aquatic ecosystems (Aazami *et al*., 2015). Understanding the characteristics of the source of contaminants and mobilisation relative to the aquatic ecosystem is a useful tool in monitoring and setting up remedial actions for the polluted aquatic ecosystems.

2.3.1. Approaches to Characterising ARD Potential

ARD is produced by oxidative dissolution of minerals rich in sulphide such as arsenopyrite, chalcopyrite, pyrite, marcasite, sphalerite, pyrrhotite and galena (Beauchemin *et al*., 2010; Hesketh *et al*., 2010). Sulphide rich tailings pose serious ecological risks when oxidized on exposure to air and water (Betrie *et al*., 2015; Fallon *et al*., 2017; Fan *et al*., 2016; Shu *et al*., 2018). The oxidation of sulphide tailings can generate acid mine drainage that may cause sustained dissolution of heavy metals from the tailing reservoir (Khoeurn *et al*., 2019; Kotsiopoulos and Harrison, 2018; Shu *et al*., 2018) into the surrounding environment.

2.3.1.1. *Review of Approaches to Characterising ARD Potential*

The application of mineralogical characterization to mine waste has potential to improve risk assessment, provide appropriate mine planning guidance and optimize remediation design for mine wastelands (Jamieson *et al*., 2015). Mineral characterization of the tailings, especially carbonate and sulphide phases is important in predicting ARD and metal(loid) leaching potential (Beauchemin *et al*., 2010). In order to facilitate effective management of ARD and metal deportment, accurate and reliable characterization of ARD generating potential of mine waste material using precise and study testing methods is necessary. ARD prediction and characterization tools could be grouped in three categories i.e., analytical methods including mineralogical, physical, and chemical quantification; geochemical characterization using field or laboratory tests; and mathematical characterization models consisting of empirical, geochemical, and engineering models (Figure 2-5). A combined application of various selected ARD prediction tools designed for particular tests will increase the reliability of the results (Harrison *et al*., 2010; Hesketh *et al*., 2010).

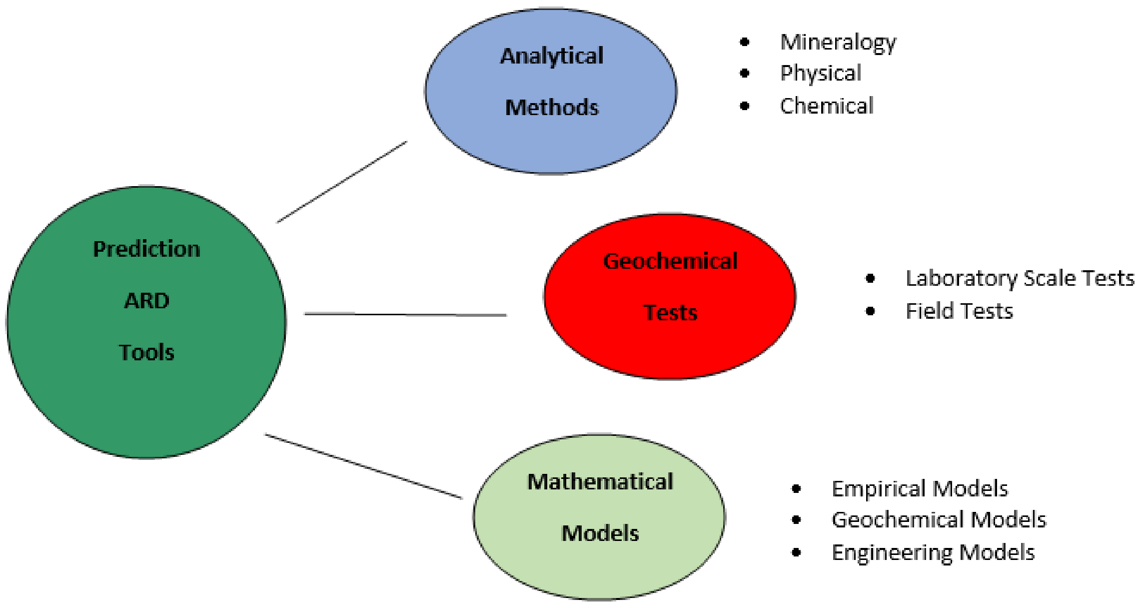

Figure 2-5: ARD prediction and characterization tools (Harrison et al., 2019)

Typically, the geochemical laboratory scale tests are used in predicting ARD generation potential and drainage quality (Karlsson, 2019; Karlsson *et al*., 2018). They include the conventional standard static and kinetic tests (Stewart *et al*., 2006; Weber *et al*., 2006) as well as the more recently developed biokinetic flask test (Hesketh *et al*., 2010). The static tests such as net acid generation (NAG) and acid base accounting (ABA) are used to indicate the overall potential to generate acid, however, they do not provide information on the relative rate of acidification and neutralization. They are used as an initial screen. The kinetic tests such as column leach and humidity cell tests are used to extract information on the relative rate of acid generating potential for a particular material and prediction of drainage quality over time. The major flaw of the kinetic tests is that they require substantial resources and time to generate significant data. The static and kinetic tests usually also ignore microbial activities in the generation of ARD, even though microbial colonization of material rich in sulphides is inevitable (Harrison *et al.*, 2010). In attempting to address the challenges associated with the standard static and conventional kinetic tests, the biokinetic tests was developed by Hesketh *et al*., (2010) to provide more information on ARD generation under microbial conditions as well as relative rates of acidification and neutralisation. In comparison to the conventional kinetic tests, this method is reasonably rapid, although like the static test, it is limited to finely milled samples. With ongoing refinement, standardization and application to varied waste materials, the biokinetic test has become a useful tool in characterizing ARD generation as well as validating results from static tests. The summary of the static, kinetic and biokinetic tests for ARD characterization is presented in Figure 2-6. The application of the biokinetic tests in conjunction with the static and kinetic tests aimed at enhancing understanding ARD generation and drainage quality within the common ARD characterization has not been extensively exploited (Harrison *et al*., 2019; Opitz *et al*., 2015; Parbhakar-Fox *et al.*, 2013).

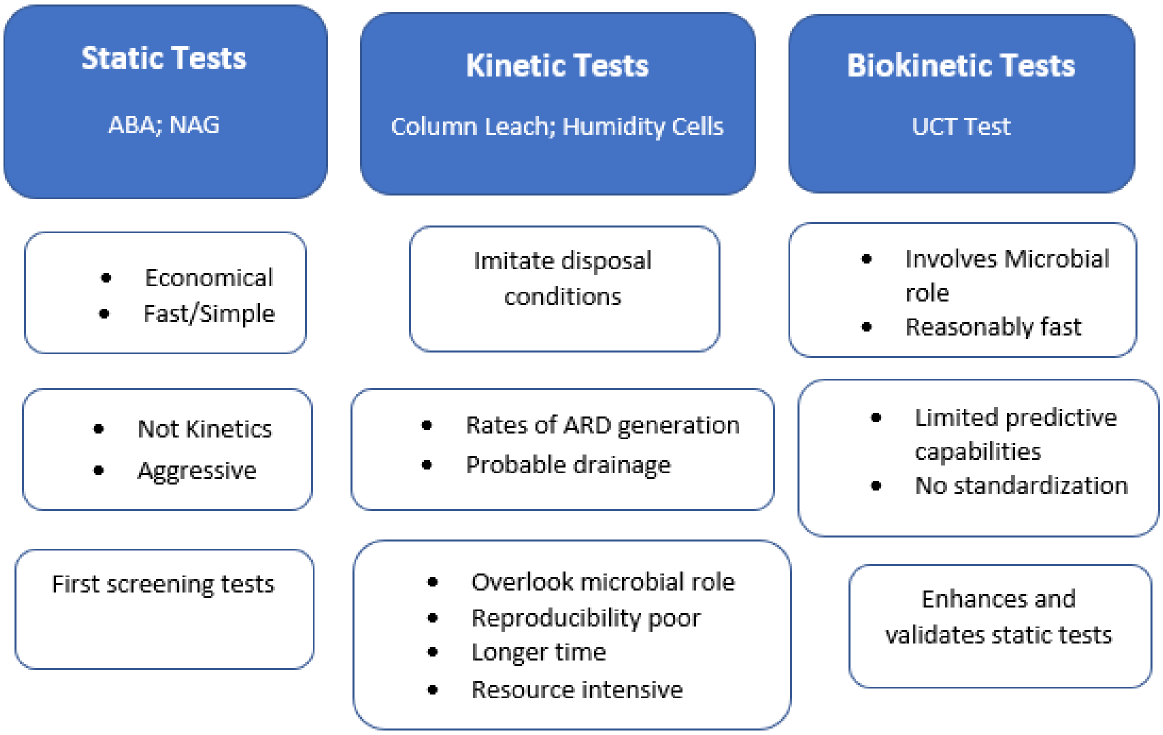

Figure 2-6: Comparison and summary of static, kinetic and biokinetic ARD characterization tools

The potential and rate of ARD formation in material is principally influenced by the minerals present within the waste deposit as well as contacting with oxygen and water. ARD generation from mine wastelands depends on the presence of sulphide minerals in the waste, their response to weathering and mineral oxidation (Becker *et al*., 2015; Kalin *et al*., 2018). Acid consuming minerals present in mine waste such as carbonates may neutralize acid produced from sulphide oxidation (Dold, 2014; Nyström *et al*., 2019). Furthermore, the rate at which minerals weather varies substantially from waste ore to waste ore; it is a function of mineral content, texture, liberation, particle size and grain size (Israeli and Emmanuel, 2018; Popov *et al.*, 2020). Weisener and Weber (2010) have observed that there was a difference in the oxidation rates between pyrite minerals under the same conditions that had different textures with framboidal pyrite reported to weather faster than euhedral pyrite. Additionally, trace minerals present within the crystal structure may increase mineral oxidation because of strains present in the crystal structure (Janzen *et al*., 2000). The processing and extraction methods used during the mining cycle influences the basic characteristics of the waste material. Mainly, the particle size and mineral liberation of the waste is the most affected (Dettrick *et al*., 2019; Wieszczycka, 2018); and mineral oxidation, liberation and permeability are often influenced by particle size (Farrokhpay and Fornasiero, 2017).

2.3.2. ARD Characterization and Metal Mobility

TFS material rich in sulphide minerals, can be a source of metal mobilization. Metal species in tailings storage facilities without buffering capacity and low organic matter content, tend to be easily released into the ambient environment (Heikkinen and Räisänen, 2009; Wang and Mulligan, 2009). As a result, managing these mine wastelands has become a subject of concern in recent times (Festin *et al.*, 2019). ARD generated in sulphide-bearing wastes is often characterised by low pH values, the acidic nature of the solution may result in mobilisation of metals within the mine waste (Nordstrom, 2011). The oxidation of sulphide minerals in the presence of water and oxygen, can further be accelerated by naturally occurring sulphur and iron oxidising organisms (Hesketh et al., 2010). ARD generation, release, mobility and attenuation is equally influenced by seasonal variations (Colombo *et al*., 2018; Vriens *et al*., 2019). Whether neutral mine drainage (NMD) or ARD enters the ambient environment depends largely on the source characteristics and pathways.

The drainage resulting from these reactions, promote metal species deportment from mineral waste in addition to affecting the ambient environment with high salinity and low pH. Elevated metal concentration transported into local aquifers may cause significant ecological damage. Additionally, uncontrolled metal release may also result in contamination of food crops, significant reduction in ecological stability, elimination of biological species, and high metal accumulation in fauna and flora (Briffa *et al*., 2020; Cimboláková *et al*., 2019). It is therefore important to understand the principal attenuation process controlling mobilization of metals from source. Understanding the ARD generation potential and release information of metals from mine waste, can effectively guide the practical pollution control (Fan *et al*., 2016). This is because mine waste materials have distinctive properties specific to mineralogy of the ore deposit, processing method and type of stacking adopted (Cappuyns *et al*., 2002; Carvalho *et al*., 2014). The distinctive properties of mine waste material might result into different release mechanisms of metals such as type of metals being released, main release period etc. A good understanding of mine wastelands characteristics might improve mine waste handling, monitoring of abiotic activities, effective disposal and management of wastelands (Lottermoser, 2010; Makgae, 2011). While it is recognised in the literature that detailed characterization is not always rigorously conducted, owing to expenses, time etc., it is essential to design effective and risk reduced disposal facilities. Implementation of preventive and mitigation methods largely depends on the type of deposit, mine development stage, climate regime, geochemistry, topography, geology, surface water, groundwater and terrestrial and aquatic ecosystems (Aznar-Sánchez *et al.*, 2018; Makgae, 2011). Other factors include land use, material availability, cost, risk, receptors, maintenance, regulatory and sustainability requirements.

2.3.3. Abiotic Monitoring

Physical and chemical parameters such as pH, turbidity, metal concentration etc., affect the abiotic characteristics of an aquatic ecosystem (Brysiewicz *et al*., 2020; Dabrowski *et al*., 2013). Water and sediment are the two most noticeable abiotic phases in aquatic ecosystems where these parameters can be measured (Maloney, 2019). Although sediment and water can be monitored individually to assess the ecosystem integrity, they have some shortfalls. One of the notable limitations is that most contaminants are known to have synergistic or antagonistic effects (Cabral *et al*., 2019; Coors and Meester, 2008), thus, individual assessment of water or sediment quality might lead to biased information, whether negative or positive. Owing to this, increased frequency of data collections from the affected water resources could improve the reliability of abiotic monitoring (Fu *et al*., 2020; Marcé *et al*., 2016). For instance, streams or rivers are influenced by flowing water that drain a specific catchment containing various land uses (Camara *et al*., 2019; Chapman *et al*., 1996), hence a single measurement increases the likelyhood of omitting the major contaminant drivers in a system. Studies have shown that monitoring water quality at low frequency can result in inaccurate and imprecise classification of the physical and chemical signature owing to sampling error (Chen and Han, 2017; Jung *et al*., 2020; Marcé *et al*., 2016). Another constraint associated with abiotic assessment is difficulties to differentiate between anthropogenically contributed pollutants and bakground values (Bartram et al., 1996; Reimann and Caritat, 2005). For instance, high enrichment factors (EF) are ofetn used in support of hypothesis that certain set of elements are induced by anthropogenic activities; on the contrary, EFs could be low or high for a variety of reasons, to which pollution is but one (Reimann and Caritat, 2005). Other factors such as biogeochemical processes that influence the redistribution of chemical elements in the environment, strongly influence EFs. As a result, it is possible that concentration of a particular suite of parameters could be influenced by natural processes such as geology (Khatri and Tyagi, 2015; Li *et al*., 2021). However, it is difficult to account for this.

2.3.3.1. *Assessment of Water Quality*

Water quality has direct effect on the biodiversity of an aquatic ecosystem (Amoatey and Baawain, 2019; Dallas and Day, 2004; Klimaszyk and Gołdyn, 2020). Physical and chemical parameters of water such as metals, nutrients, acidity, alkalinity, salts, and other contaminants influence its quality (Omer, 2019; Rahmanian et al., 2015). An imbalance in the physical and chemical parameters can lead to varying degree of deterioration of the aquatic ecosystem (Chapman *et al*., 1996; Issaka and Ashraf, 2017). Water quality is defined by its physical and chemical properties that regulate its range of uses and preservation of the integrity and health of aquatic ecosystems (Edokpayi *et al*., 2017). When the buffering capacity of the ecosystem is overwhelmed owing to the sustained introduction of several pollutants from nonpoint and point sources, pollution is actualized (Igbinosa and Okoh, 2009). Over time, many water quality monitoring methods have been developed, however, most of them have a biased approach to monitoring (e.g., selected physical endpoints). Inevitably, this kind of approach may result in certain shortcomings, thus increasing the chances of missing sporadic pulses of pollutants. An integrated approach to water quality monitoring which promotes a coordinated inclusiveness of impacts of land use activities, changes in physiochemical aspects, effects on habitat and aquatic life, as well as surrounding landscape, is necessary in order to achieve a comprehensive evaluation of water resources (Asadi et al., 2021; Salmiati et al., 2017)

2.3.3.2. *Assessing Sediment Quality*

The river or stream morphology, hydrology and sedimentology serve as the spinal column for the aquatic environment (Hauer *et al*., 2018; Maddock, 1999). Contamination or disturbance of the sedimentology is one of the principal stresses on the aquatic ecosystem (Dudgeon *et al*., 2006; Heim and Schwarzbauer, 2013). Disturbance to the sediment quality can alter the habitat composition of a stream/river and the ecosystem services provided for (Algül and Beyhan, 2020; Hauer *et al*., 2018; Kjelland *et al.*, 2015). In the recent past, monitoring the quality of sediment has received more attention (Chuan and Yunus, 2019) because sediments can be depository of a wide range of contaminants leading to an increase in contamination of overlying waters (Pobi *et al.*, 2019). If left unmitigated, sediment contamination could result in a various impacts on-site and occassionally off-site on social, economic and environmental values (Issaka and Ashraf, 2017; Rowlands, 2019). Monitoring the chemical content and physical composition of sediments such as metal elevation, pH, percentage of physical variables (clay, silt and sand) etc., may provide information on changes in the environment and anthropogenic activities linked to environmental change. Sediment profiles can be used to evaluate the historical pollution trends of an aquatic ecosystem over space and time (den Besten *et al*., 2003; Heim and Schwarzbauer, 2013). The distribution of metal concentration in the sediments, may be related to river function, accumulation of tailings in the river and discharge of mine waste water (Liu et al., 2020). Absorbed metals in sediments may cause secondary pollution which is difficult to control.

Some aquatic organisms are benthic and are in constant contact with sediments, hence contamination of sediments is detrimental to such organisms (Olayinka-Olagunju *et al.*, 2021; Pandiyan *et al.*, 2021). As a result, sediment contamination is more crucial to these aquatic organisms than elemental concentrations in surface water (De Jonge et al., 2010). Owing to the actuality that sediments normally consist of higher concentration of contaminants and are less influenced by variation compared to water, they provide a steadier monitoring platform (Ustaoğlu and Tepe, 2019; Van Damme *et al.*, 2008).

However, it is noteworthy that monitoring water and sediment parameters alone, might not be sufficient enough to predict the far-reaching impacts on ecological integrity, as a result, there is a need to integrate biological endpoints (Parmar *et al.*, 2016). The relationship between benthic invertebbrates and sediment quality measurement endpoints, can be used through statistical correlation analysis to evaluate specific habitat features influencing aquatic coummunity structures.

2.3.4. Biological Monitoring

2.3.4.1. *Potential of Bio-monitoring*

Studies have reported significant correlation between sediment quality and reduction in biological productivity of aquatic organisms (Koglin *et al.*, 2016; Maddock, 1999; Wenger *et al.*, 2017). Specifically, studies focused on macroinvertebrates and fish populations, have reported changes in compositional structures caused by sediment contamination (Fleeger *et al.*, 2006; Townsend *et al*., 2009; Yi *et al*., 2008).

Assessment of biota in aquatic ecosystems has been widely recognized as one of the reliable approaches for determining the health of aquatic ecosystems (Dickens & Graham, 2016). Several biomonitoring tools have been employed for various purposes to monitor aquatic ecosystems (Bain *et al*., 2000; Simon, 2000). These tools mainly focussed on assessing the integrity of the ecological infrastructure of respective ecosystems (Bae *et al*., 2005; Ferreira *et al.*, 2011; Growns *et al*., 1997; Resh *et al*., 1995). Aquatic organisms are normally used because of their constant and continuous exposure to the same environment (Gordon *et al.*, 2013; Jones *et al.*, 2012), and therefore would easily reflect the effects of exposure to environmental stressors in ecosystems (Bogardi *et al.*, 2020; Sun *et al*., 2019). For instance, studies by Gheorghe *et al*., (2017) on common Romanian Benthic invertebrates such as *V. viviparas, A. cygnea,* and *C. carpio* reported significant variations in bioaccumulation of selected metal species (Cd, As, Cu, Fe, Zn, Pb, Ni, Zr, Ti, Cr and Mn), indicating that the bioaccumulation was dependent on type of organism, metal, and location of sampling site. In Iran, a study by Aazami *et al*., (2015) on Tajan River, used macroinvertebrates and fish communities to map changes in water quality and ecological conditions from upstream to downstream. The study showed that biotic indices revealed better patterns with regards to changes of water quality scales compared to abiotic indices. This serves as an advantage over the limitations of conventional water and sediment quality surveillance tools (Harris and Silveira, 1999; Wepener, 2008; Zhou *et al*., 2008). Water and sediment monitoring tool mainly characterize the condition during sampling period, and as a result, making the chances of sporadic pulses of contaminant being missed to increase. Contamination may occur in exceedingly low concentrations as reported by Qu *et al.* (2010), and detecting such low concentrations necessitates tedious analyses with highly sensitive technologies at a prohibitive cost. Alternately, no matter how small, bioindicators provide a tolerance range of biologically meaningful levels of pollutants (Parmar *et al.*, 2016; Paustenbach and Galbraith, 2006). As a result, biomonitoring has become one of the effective monitoring techniques recommended to monitor ecological integrity (Dickens and Graham, 2002). Assessment tools for biological monitoring are built on assumption that anthropogenic will influence the composition, abundance, and diversity of these aquatic organisms (USEPA, 2002; DWAF, 2004).

Aquatic macroinvertebrates have been used widely to monitor stream conditions (Clements, 1994; Fierro *et al.*, 2017; He *et al.*, 2015; Perera and Wattavidanage, 2011). They are known for integrating precursor conditions from longer term changes to short term episodes with their varying life cycles from intra to inter annual (Mazor *et al.*, 2009; Mesa, 2012). In particular, each organism in a biological system indicates the healthiness of the surrounding environment and serves as a salient biosignature in evaluating aquatic ecosystem quality along with indication of contamination levels (Lomartire *et al.*, 2021; Parmar *et al*., 2016). A study using composition of macroinvertebrate communities by Kuzmanovic *et al.* (2017) on four different rivers, in Spain, conducted to assess the effects of pesticides and multiple stressors, showed that there was a significant relationship between macroinvertebrate assemblages and river contamination. The sites were separated according to the dominant environmental stressors. A high risk of egg mortality in sites impacted by pesticides was observed compared to other physical and chemical stressors. It is evident that the degree to which an aquatic ecosystem is contaminated can be predicted through the availability of bioindicators (Cornejo *et al*., 2019; Samiyappan, 2019). However, use of bioindicators ought to be undertaken carefully in order to discern the broad range of anthropogenic effects (Allen *et al.*, 1999). The disadvantage of biomonitoring, on the other hand, is its difficulty or inability to identify specific stressors that are influencing a specific system (Roux, 2001; Sumudumali and Jayawardana, 2021). In addition, biomonitoring tools require strong human element in conducting the assessment, thus this may permit subjectivity in the interpretation of results (Taylor *et al.*, 2007). It is noteworthy that the use of benthic macroinvertebrates to monitor biotic integrity may provide a complement to conventional methods. The choice of which assemblage of organisms to incoporate in biological monitoring depends on objective of the research and attributes of research area (Resh, 2008a; Scotti et al., 2019).

2.3.4.2. *Macroinvertebrates*

Macroinvertebrates are the most common and extensively used aquatic organisms in monitoring lotic systems (Buffagni *et al*., 2020; Collins and Fahrig, 2020; Dalu and Chauke, 2019). Many organisms such as phytoplankton, macrophytes, lichens, fish, phytobenthos, zooplankton and macroinvetebrates have been used as major monitoring tools for water quality. Notably, macroinvertebrates are more widely used in biomonitoring owing to their adaptability to habitat conditions as observed by many ecologists (Armitage *et al*., 1983; Dallas, 2004; Dixon *et al.*, 2002; Jones *et al.*, 2012). Their rich diversity, sedentary and sessile lifestyle, long lifespan and the ability for most of them to adapt to chemical and physical stressors support this (Hillman and Quinn, 2002; Rinne, 1990), and can indicate alterations as a result of anthropogenic disturbances (Dixon *et al.*, 2002; Moreyra and Padovesi-Fonseca, 2015). Although they may be sensitive to organic and other compounds (Anyanwu et al., 2019; Küçük, 2008), they easily adapt to the different environmental stressors during their lifetime (Hutchinson *et al*., 1998; Paisley *et al*., 2003; Rosenberg and Resh, 1993). Macroinvertebrates can be used by local people with minimum training as identification of taxonomy and sampling techniques is not difficult (Uherek and Pinto Gouveia, 2014), thus enabling citizen science.

Biomonitoring using macroinvertebrates has been extensively used to assess water quality and ecological status in Europe (Benetti et al., 2012; Escribano et al., 2018; Medupin, 2019; Munné and Prat, 2009; Resende et al., 2010; Tough et al., 2020), Asia (Kenney et al., 2009; Morse et al., 2007; Nguyen et al., 2014; Wang and Tan, 2017; Xu et al., 2014) and America (Desrosiers et al., 2020; Fierro et al., 2017; Mathuriau et al., 2011; Uherek and Pinto Gouveia, 2014). Following the 1960s Water Act in the UK, the development along with implementation of biotic indices accelerated the use of macroinvertebrates in Europe and North America (Daly et al., 2018; Eriksen et al., 2021). Over time, efficacious methods such as the Bilogical Monitoring Working Party index (BMWP), biotic index score, global biological normalised index (IBGN) etc., have been developed to respond to complexity of effluents owing to increase in industrial activities and intensified land-use (Birk et al., 2010). However, In Africa, studies on the applications of biomonitoring using macroinvertebrates remain limited (Li *et al*., 2010; Mangadze *et al*., 2019). The existing literature in African countries mainly comprises scholarly articles evaluating the ecological diversity of macroinvertebrates in particular areas (Resh, 2007). A small set of studies on the impact on macroinvertebrate community structures exist. For instance in Ethiopia, studies on macroinvertebrate assemblages reported a decline in species richness in selected sites of Kebena River and Borkena due to industrial and urban pollution related activities (Alemneh *et al.*, 2019; Alie, 2019; Beyene *et al*., 2009). The results indicated a decline in macroinvertebrates sensitive to pollution from families like

Ephemeroptera, Trichoptera, and *Plecoptera* owing to municipal and industrial discharges. Similarly in Kenya, macroinvertebrate assemblages have been utilised to evaluate the impacts of land use changes on water quality (Masese *et al.*, 2009; Oremo *et al.*, 2019). The influence of physical and chemical water variables such as pH, temperature, DO and selected metal species on macroinvertebrate community structures was investigated using multivariate analysis in regions dominated by coffee plantation and related processing activities. Observably, sensitive taxa from *Ephemeroptera, Coleoptera, Crabs* and *Trichopteran* families were found upstream in less impacted sites. In Uganda, studies by Kasangaki *et al.*, (2008, 2006) in high altitude rainforest streams reported high diversity and richness indices in comparison to grazed sites as well as cultivated parts. Particularly among the macroinvertebrates investigated, tolerant species from families of *Coleoptera, Hemiptera, Ephenemeroptera* and *Diptera* were dominant in sites impacted by agriculture activities. Several other studies showing similar response patterns have been conducted in various parts of Africa including Zimbabwe (Mwedzi *et al*., 2020; Nhiwatiwa *et al.*, 2017), Ghana (Thorne *et al.*, 2000; Thorne and Williams, 1997), Nigeria (Anyanwu *et al.*, 2019; Arimoro *et al.*, 2021), Gabon (Vinson *et al*., 2008), Botswana (Dallas and Mosepele, 2007; Kemosedile *et al*., 2020), Mozambique (Chilundo *et al*., 2008), Algeria (Baaloudj *et al*., 2020; Imène and Si Bachir, 2018), Morocco (Lahcen *et al.*, 2017; Souilmi *et al.*, 2021) and South Africa (Bredenhand, 2005; Niba and Sakwe, 2018). It is noteworthy that a considerable number of studies on biomonitoring using macroinvertebrates have been conducted in South Africa. Some of these studies have highlighted the impact of runoff from agriculture activities (Thiere and Schulz, 2004); effects of ARD on macroinvertebrate abundance (Dabrowski *et al.*, 2013; Steyn *et al*., 2019); impact of water hydrology on macroinvertebrate assemblages (Muller et al., 2012; Niba and Sakwe, 2018); influence of seasonality on macroinvertebrate community structures (Bollmohr and Schulz, 2009; Dallas, 2004); impact of biotope availability on macroinvertebrate assemblages (Bird *et al*., 2014; Dallas, 2006); and the growth of biological system and evaluation of natural fluctuation on macroinvertebrate communities (Dallas and Day, 2007).

Generally, two approaches have been used to assess the habitat conditions of the aquatic ecosystem using macroinvertebrates. These are either through the functional approach which focuses on the behaviour and morphological changes, or using the taxonomic approach which measures changes in the diversity of the community (Cummins *et al.,* 2005; Suriano *et al*., 2011). Disadvantages in using macroinvertebrates include their susceptibility to floods (Angradi, 1997; Calderon *et al*., 2017). Equally, the reproducibility and validity of results can be impacted by identification and sampling capabilities (Resh, 2008).

2.3.5. Assessing the Impact of Metal Deportment on Soils and Crops

Metal contamination of soils is a threat in agricultural production (Xiang *et al.*, 2021). With the rapid advancement of industries such as mining, metal contamination in soils has emerged as an environmental challenge (He *et al.*, 2021; Stewart, 2020). Soils can be substantially impacted by metal mobilization from point and non-point sources like chemical industries, gold mines, coal mines, copper mines, mine wastelands, processing plants agricultural runoff and sediment erosion (Rai *et al*., 2019). Food crops grown in soils contaminated by heavy metals may accumulate metals in edible parts of the plants (Sharma and Nagpal, 2020). Metal uptake via soil-crop system is a principal pathway to exposing humans to potential hazardous elements (Briffa *et al.*, 2020; Leila *et al.*, 2021; Rai *et al.*, 2019). Various health challenges such as cancer, skin ailments, respiratory illness, neurological disorder, weakening of bones, cardiovascular, improper functioning of endocrine glands etc. are linkable to the consumption of food crops laden with metals (Kasozi *et al.*, 2021; Khan *et al.*, 2013).

Uptake of metals by crops depends on the type of soil, concentration in soil as well as plant species (Balkhair and Ashraf, 2016; Grytsyuk *et al.*, 2006). Soil characteristics like organic matter, microbes, pH, Al and Fe oxides and redox potential affect the bioavailability of hazardous elements in soils (Wuana and Okieimen, 2011; Zhang *et al*., 2014). Bioavailability of metals and plant species influence the metals accumulated by plants over time (Chen *et al*., 2016). The effectiveness of plants in metal accumulation is influenced by the soil to plant transfer characteristics of particular metal species (Mirecki *et al.*, 2015). For instance, low lead levels in soils might limit plant processes like mitosis, photosynthesis, and hydrophilicity, resulting in toxicity symptoms like wilting of older leaves, stunted growth, dark green leaves etc., while high lead concentration in soils may reduce productivity of soils (Fahr *et al.*, 2013; Pourrut *et al.*, 2011). Metals are toxic and may increase chlorosis, feeble plant growth, poor yield, poor nutrient uptake and metabolism as well as reduction in the ability of leguminous plants to restore molecular nitrogen (Guala *et al.*, 2010).

An overview of global studies (Table 2.4) on metal contamination in food crops in relation to broad anthropogenic sources show elevated metal concentrations. Indeed, metal accumulation in food crops are of concern worldwide (Balkhair and Ashraf, 2016; Khalid *et al*., 2018; Rai et al., 2019). However, information on geographical trends may be helpful in understanding if the extent of their effect varies across sites, along with the source of contaminants, which has been reviewed scantly. Studies on the source, speciation, metal mobility, transformation, and fate of metals in soil and geochemical processes in soil-crop system are limited. A better understanding of metal mobility from source on to soil-food crop uptake is necessary for conceiving remediation measures. It is a conventional practice in developing nations to grow food crops along the banks of water resources traversing industrial and mining areas (Edogbo *et al*., 2020; Emmanuel *et al.*, 2018; Kapungwe, 2013; Kapwata *et al.*, 2020). Often such water resources have been observed to be contaminated by heavy metals (Attiogbe and Nkansah, 2017; Mcintyre *et al.*, 2018). Therefore, monitoring heavy metal contamination in soils and food crops irrigated by water resources traversing mining areas can give an indication of interactive relationship between mining activities, surface water resources and agro-ecosystem. Furthermore, soils like sediments are useful indicators because they normally have high concentration of contaminants with less variation, and resultantly provide a more dependable platform for monitoring ecological degradation

Table 2-4: Metal contamination in global foods from diverse sources

No	Food crops (cereals, fruits, vegetables etc)	Country where investigated	Sources of metal contamination	Metal concentration recorded	References
1	Brassica sp., Chenopodium sp., leafy and root vegatbles, grains	India	Sewage effluent	Cu 1.7-12.9 ppm, Pb 0.13 ppm, Zn 7.25-24.6 ppm, Cr 0.08-0.38 ppm	Rattan et al. (2005)
2	Maize, Cabbage, Brassica Juncea L, Radish (Raphanus sativus L), Turnip, Brassica napus, Spinach, Cauliflower and Lettuce	China	Sewage effluent (inadequately using a biological approach)	Cr 0.08-0.38 ppm, Pb 0.02-0.013 pmm, Cu 0.16-0.85 ppm, Zn 0.16-0.53 ppm	Khan et al. (2008)
3	Lettuce (Lactuca sativa); a leafy food crop/vegetable	Spain	Air (PM) from mining industries and vehicles	Ni <0.02 ppm, Hg <0.008 ppm, As <0.005 ppm, Cd <0.005 ppm	Ercilla-Montserrat et al. (2018)
4	Brassica sp., food grains, and leafy vegetables	China	Mine waste (from smelter) drained into river water used for irrigation	Cr 0.01-0.19 ppm, Pb 0.12-0.23 ppm, Cu 0.15-0.86 ppm, Zn 0.42-0.95 ppm	Liu et al. (2005)
5	Soyabean	Argentina	Industrial waste in soil	Metals (Pb & Zn) well above WHO/FAO permissible limits (Pb_0.3 ppm and Zn_100 ppm)	Rodriguez et al. (2014); Blanco et al. (2017)
6	Triticum aestivum (wheat), Lycopersicum esculentum L. (tomato), Raddish, Spinach, Brinjal, Carrot, Capsicum annum, Allium sativum (garlic), Coriandrum sativum (coriander), and Okra	Pakistan	Metal - contaminated ground water	Cr > 0.18 ppm, Pb 0.91-3.96 ppm	Khan et al. (2013)
7	Rice and other paddy crops and vegetables	Australia (food crops imported from Bangladesh, India, Pakistan, Thailand, Italy, Canada and Egypt)	Arsenic- and metal-contaminated groundwater	Rice: Cr 0.02-0.47 ppm, Pb 0.016-0.25 ppm, Cu 0.01-0.09 ppm, Zn 0.01-0.03 ppm, Cd 0.01-0.02 ppm, Co 0.01-0.04 ppm, Mn 0.06-0.36 ppm, Ni 0.06-0.04 ppm, Pb 0.67-16.5 ppm Vegetables: Cr0.03-0.77 ppm, Pb 0.04-0.5 ppm, Cu 1-29 ppm, Zn 17-183 ppm, Cd 0.00-0.37 ppm, Mn 0.00-0.14 ppm, Ni 0.15-10 ppm, Pb 0.04-0.5 ppm	Rahman et al. (2014) See also Islam et al. (2017), Yang et al. (2018)
8	Potato/other foodstuffs	Egypt	Inadequately treated mine wastewater	Cu 0.83 ppm, Pb 0.08 ppm, Cd 0.02 ppm, Zn 7.16 ppm, Cr nil	Radwan and Salama (2006); El-Kady and Abdel Wahhab (2018)
9	Lettuce (Lactuca sativa)	United States (Florida)	Metal contamination from mining activities	As 27.3 ppm	de Oliveira et al. (2017)
10	Cassava, Cocoyam, Yam and Plantain	Ghana	Metal contamination from mining activities	As <0.01-0.02 ppm, Cd <0.01 ppm, Pb 0.07-2.11 ppm, Hg 0.02-0.16 ppm, Mn 0.42-0.8 ppm	Adjei-Mensah et al. (2021)
11	Brassica oleracea (Chinese cabbage), Lycopersium esculentum (Tomato), Swiss chard, Pumpkin leaves, Bean leaves (Phaseolus vulgaris), Okra	Zambia	Metal contamination from mining activities	Cu 0.54-77.6 ppm, Pb 0.11-35.6 ppm, Co 1.27-6.38 ppm, Cr 0.12-51.6 ppm, Ni 0.03-12.9 ppm	Kapungwe (2013)
12	Okra	Saudi Arabia	Metal contamination on soil irrigated by treated wastewater	Ni 98% > limit (67.9 ppm), Pb 28% > limit (0.3 ppm), Cd 83% > limit (0.1 ppm), Cr 63% > limit (2.3 ppm) (WHO limit)	Balkhair and Ashraf (2016)

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2.4. Ecological Restoration of Mining Generated Wastelands

Ecological restoration of environments affected by mining and ore processing constitutes an important management intervention that mitigates against ecotoxicological risks associated with heavy metal(loids) contamination to ensure a well-functioning ecosystem (Pourret *et al.*, 2016). Attempts to re-vegetate without prior knowledge of suitable candidate plant species and their interaction with wasteland soils may prove abortive, but mostly uneconomical and unsustainable in the long run as the plant species used may require exogenous support for them to play the role of stabilization and/or extraction of the contaminants (Li, 2006; Pourret *et al.*, 2016). In re-vegetation of such lands, the use of native or indigenous plant species is preferable as such species tend to evolve site specific adaptation mechanisms, being adaptive and tolerant to the local environment (Cousins and Witkowski, 2015; (Gajić *et al*., 2018; Gibson *et al.*, 2016; Huang *et al*., 2012; Mok *et al.*, 2013); these ensure success of the re-vegetation programmes. Utilizing exotic species for re-vegetation purposes may result in an undesirable modification of the ecosystem that in some cases is manifested by loss of local plant diversity, thereby impacting on the ecosystem integrity (Alpert, 2006; Gornish *et al.*, 2016).

2.4.1. Re-vegetation of TSFs

Plant establishment on wastelands is challenging because of severe physical and chemical restrictions caused by soil contamination and low nutrient levels, particularly nitrogen and phosphorous, necessary for plant establishment and survival (Chen, 2018; Huang *et al.*, 2012). Additionally, the lack of organic material in mining generated soils reduces cation exchange capacities (CEC) of such soils (Kobina Mensah, 2015). Plants growing on metal contaminated sites normally experience oxidative stress upon exposure to heavy metals. This may lead to cellular damage thus inhibiting root growth (Yadav, 2010). In order to minimize the harmful effects of heavy metal accumulation and exposure, some plants have evolved their detoxification mechanisms based on subcellular and chelation compartmentalization (Bricker *et al.*, 2001). Plant species used for restoration of wastelands must therefore overcome a whole suite of physical, chemical, and biological constraints some of which may act synergistically (Gann *et al.*, 2019). Screening and selecting plant species based on their functional traits is the starting point for identifying candidate species for re-vegetation projects (Festin *et al*., 2019; Hasnaoui *et al*., 2020); understanding the functional traits of plants may help determine the type of restoration strategy suitable for each particular site. Restoration of mine wastelands have been carried out in China and other developed countries. For instance, massive ecological restoration projects in Chna were implemented on WHLD coal mine, LLG coal mine and JZT coal mine in Jungar Banner, Inner Mongolia Autonomous Region (Li et al., 2020). The studies revealed improved ecological stability, and that over time, it is possible to achieve recovery corresponding to pristine conditions. Although research on restoration of mine wastelands remains limited compared to substantial advances elsewhere, studies on progress (Table 2-5) have been reported by Festin *et al*., (2019).

Table 2-5: Studies on progress of restoration of mine wastelands in Africa (Festin et al., 2019)

Country	No. of studies	Resoration research and practices
DR Congo	6	Characterization of species naturally colonizing old mine sites for hyperaccumulation of copper and cobalt; evaluation of plant functional traits for identifying species suitable for phytoremediation of copper-mine wasteland; a field trial on the potential of soil amendments for catalysing autochthonous colonization and growth of planted species
South Africa	3	Survey of autochthonous colonizers on gold and uranium tailings dams and the adjacent polluted soils; evaluation of phytoremediation potential of five grasses species with application of fertilizer to restore lead/zinc mine tailings; Large-scale restoration of sand mine tailings using top soil addition and additional measures to assist natural colonization
Kenya	2	Large-scale restoration of exhausted limestone quarries using a mixture of trees species and litter decomposer; evaluating phytostabilization potential of four woody species, Acacia xanthophloea, Schinus molle, Casuarina equisetifolia and Grevillea robusta, for the restoration of limestone quarries
Ghana	3	Restoration of gold mine waste land using a combination of physical, chemical and biological methods; monitoring restoration progress based on soil quality indicators and possible improvements in the future
Zambia	2	Characterization of naturally colonizing species on lead/zinc mining-generated slag heaps and copper mine tailings
Zimbabwe	2	Identifying nickel hyperaccumulators; evaluating early growth performance of three indigenous Acacia sp. Established on nickel mine tailings amended with addition of top soil
Rwanda	1	Organic amendments of degraded Technosols on former Tantalum mining sites
Morocco	1	Evaluation of 25 species grown naturally on copper and polymetallic mining sites for their ability to accumulate copper, cadmium, lead and zinc

2.4.2. Characterizing Candidate Plant Species

Plants adapted to metalliferous sites have two key resistance strategies for heavy metals; namely, exclusion and accumulation (Baker, 1981); based on this, they are classified into two major categories namely excluders and accumulators (Ghaderian and Ravandi, 2012; Sainger *et al.*, 2011). Plants that prevent metal mobilization to above-ground biomass while maintaining relatively low concentration in below-ground biomass are known as excluders. They immobilize contaminants in below-ground tissues or favour metal complexation in their rhizospheres or both, thereby preventing metal release and, in turn, limiting the bioavailability of the metal to ambient environment (Ghosh, 2005; Girdhar et al., 2014; Mahar et al., 2016). In contrast, plants that translocate extraordinary high concentrations of specific elements into their shoots are called hyperaccumulators (Baker, 1981; Baker and Brooks, 1989; Masarovičová *et al.*, 2010; van der Ent *et al.*, 2013). High levels of foliar sequestration can be achieved by hyperaccumulator plants due to their enhanced metal uptake and translocation abilities (Baker, 1981, 1987; Baker and Brooks, 1989; Tognacchini *et al*., 2020).

Several studies based on functional traits (accumulation versus exclusion) of selected plants colonising contaminated environments have been reported from different regions of the world, including China (Deng *et al.*, 2004; Fu *et al.*, 2019), Europe (Kasowska *et al.*, 2018; Unterbrunner *et al*., 2007), South America (Boechat *et al.*, 2016; Gratão *et al.*, 2005) and Africa (Belford, 2017; Festin *et al.*, 2019; Schachtschneider *et al.*, 2017). Selection of plant materials for the purposes of re-vegetation of mine wastelands has been done extensively in mining regions (Mahar *et al.*, 2016). More attention has been placed on hyperaccumulator plants than excluders because of their potential for wider applications in rehabilitation of contaminated sites (Manara *et al.*, 2020; Suman *et al*., 2018). Currently, over 700 hyperaccumulator plants are known (Baker and Brooks, 1989; Reeves *et al.*, 2018; van der Ent *et al.*, 2018). Most research has focused on perennial herbaceous plant species with a large natural variation for metal hyperaccumulation. Plant species from the Asteraceae and Poaceae families have been found over a widespread area in Europe, Asia, America and Africa in metal contaminated areas (Dietterich *et al.*, 2017; Hasnaoui *et al.*, 2020; Pandey *et al.*, 2019).

The above-mentioned plant families have been observed to occur growing on a variety of different substrates from contaminated soils with a high concentration of elements such as Cu, Co, Ni, Au and Fe through to uncontaminated soils. Many populations of Asteraceae and Poaceae family such as *Chrysopogon zizanoides, Cymbopogon flexuosous, Cymbopogon martini, Matricaria sps* etc., can tolerate sites with high metal pollution including mine smelter sites and wastelands (Baker *et al.*, 2010; Rola, 2011; Woch *et al*., 2013). This ability by the plant species to show resilience to and to accumulate various elements, plausibly reflects particularity in element chelation and transport and suggests that these plants may be used in phytomining technologies (van der Ent *et al*., 2018, 2015, 2013). In spite of the contribution of many studies to illuminate metal hyperaccumulation (Baker and Brooks, 1989; Mok *et al.*, 2013; Suman *et al*., 2018; van der Ent *et al.*, 2013; Vara Prasad and de Oliveira Freitas, 2003), and increased understanding regarding rhizosphere processes (Kim *et al.*, 2010; Seshadri *et al.*, 2015), large-scale application of the technology remains limited..

2.4.3. Phytomining

Phytomining is an emerging technology where selected plant species are grown on metal enriched sites with the objective of metal recovery for commercial gain in addition to rehabilitation of mine wastelands (Sinha *et al.*, 2021). This novel approach of metal recovery from secondary resources using plant-based approach was developed by Chaney in 1983 (Li *et al.,* 2003). Recently, research on phytomining has increased with successful trials of nickel recovery from naturally enriched Ni soils (Chaney *et al.*, 2007; Nkrumah *et al.*, 2016). Phytomining field trials have been undertaken in Spain (Pardo *et al.*, 2018), Indonesia and Malaysia (van der Ent *et al.*, 2015), Mexico (Wilson-Corral *et al.*, 2011), Australia (Rosenkranz *et al.*, 2019, 2017) and Greece (Bani *et al.*, 2009; Kidd *et al.*, 2018). Successful examples showing the feasibility of plant species for phytomining include *Raphanus sativus, Berberis vulgaris, Daucus carota* and *Allium cepa*. When cultivated in silica sand containing 3.8 ppm of elemental gold, more than 200 ppm of gold concentration was reported in plant tissues of *R. sativus* (Msuya *et al.*, 2000). Similar trials on artificial gold bearing soils containing gold concentration of 5 ppm were conducted, in which *Brassica juncea, Berkheya roessler* and *Cichorium intybus* were grown. The results indicated that accumulation of gold in the leaves of *B. juncea* was as high as 326 ppm and in the stem and roots ranging from 46 – 88 ppm (Lamb *et al*., 2001). Equally, field trials on extracting Ni from plants *Odontarrhena chalcidica, Odontarrhena muralis sensu latu, Arenaria serpyllifolia, Alyssum betolonii, Bornmuellera tymphaea, Brownlowia emarginata, Berkheya coddii* and *Phyllanthus rufuschaneyi* have been conducted (Pardo *et al.*, 2018; Rosenkranz *et al.*, 2019; Španiel *et al.*, 2015). Studies by Chardot et al. (2005) have shown that plants can accumulate Ni about 2, 8 and 10 times, higher than concentrations present in soils Serp, Silt and Calc (Table 2-6).

Table 2-6: Ni accumulation in dry matter of plants on three soils (Chardot et al.,2005)

Soils	Plants	Ni concentration in aerial parts mg/kg	Total mass Ni mg	Transfer coefficient of Ni %
Calc	*Leptoplax emarginata*	13	0.6	0.7
	Bornmuellera tymphaea	130	0.3	0.3
	Thlaspi caerulescens	207	0.3	0.4
	Alyssum murale	111	0.3	0.4
Silt	*Leptoplax emarginata*	206	0.9	4.2
	Bornmuellera tymphaea	166	0.4	2.0
	Thlaspi caerulescens	104	0.03	1.2
	Alyssum murale	222	0.6	2.7
Serp	*Leptoplax emarginata*	4591	19.4	4.1
	Bornmuellera tymphaea	5595	17.2	3.7
	Thlaspi caerulescens	4808	14.6	3.1
	Alyssum murale	3671	13.3	2.8

Recently, studies assessing the potential of hyperaccumulation of Cu in plant species colonising mine wastelands have increased (Lam *et al*., 2018; Napoli *et al*., 2019; Rungwa *et al.*, 2013). Unlike natural soils, mine wastelands containing certain minerals of interest seldom demonstrate conditions that support growth of plants (Festin *et al*., 2019; Huang *et al*., 2015). Studies by Boisson *et al.* (2016) and Faucon *et al.* (2012) reported tolerance to Cu accumulation in plant species *Crotalaria colbalticola* and *Crepidorhopalon perennis* endemic to Cu contaminated sites. Similar studies by Festin *et al.* (2019) and Napoli *et al*. (2019) have reported plant species thriving on Cu contaminated wastelands. Notably, about 34 plant species have been observed to accumulate extraordinary Cu concentration in their plant tissues(Baker and Brooks, 1989; Chaney *et al.*, 1997; Lange *et al*., 2018; Sheoran *et al*., 2009; van der Ent *et al.*, 2013). Table 2-7 shows some of the plants that have been observed to be Cu-hyperaccumulators.

Table 2-7: Copper concentration in selected hyperaccumulator plants

Plant	Country	Above-ground	Growth conditions	Soil contamination	Mean Cu accumulation ppm	Reference
Elsholtzia splendens	DR Congo/China	Basal leaves	Polluted soils	1000	80	Lange et al., 2017; Peng et al., 2005
Cammelina communis	DR Congo/China	Flower stems	Naturally growing	4000-10000	>1000	Wang and Zhong, 2011
Geniosporum tenuiflorum	Sri Lanka	Leaves	Naturally growing	1350-9000	2299	Rajakaruna and Bohm, 2002
Aeolanthus biformifolius	Dr Congo	Flower stems	Naturally growing	1500-1800	3920	Baker and Brooks, 1989
Eleocharis acicularis	Japan	Shoots	Experimental pots	670	55	Nurfitri et al., 2017
Haumaniastrum katatangense	DR Congo/Zambia	Flower stems/Leaves	Naturally growing	200-7500	>1000	Sheoran et al., 2009; van der Ent *et al.* , 2013
Rumex acetosa	Nigeria	Shoots	Naturally growing	800	200	Barrutia et al., 2008
Anisopappus chinesis	DR Congo	Flower stems/Leaves	Naturally growing	953-13000	3-1335	Lange *et al* ., 2018
Conyza cordata	Zambia	Shoots	Naturally growing	1600	1284	van der Ent et al., 2015
Pseudognaphalium luteo-album	Zambia	Shoots	Naturally growing	2000	1042	van der Ent et al., 2015
Ipomoea alpina	DR Congo/Zambia	Leaves	Naturally growing	500-12300	>1000	Brooks et al., 1980

Metal accumulation can fluctuate widely depending on physiology of plants and certain environmental factors (Lange *et al.*, 2016; Lou *et al.*, 2004). In many plants, increased tolerance of metals is synonymous with decline in metal accumulation in shoots due to exclusion strategies that restrict metal translocation (Gonnelli *et al*., 2001). Contrary to this, studies by Faucon *et al*. (2012) and Peng *et al*. (2012) reported increased tolerance in metallicolous populations of plants *C. tenuis* and *H. katangense*, coupled with high Cu concentration in shoots. Most plant species with such tendencies belong to the *Asteraceae* and *Poaceae* families (Lange *et al.*, 2018).

Identification of species with metal-accumulating propensity is extremely valuable for phytomining. They also contribute to developing understanding of the mechanisms underlying their adaptation to highly contaminated environments (Perlatti *et al.*, 2015; Mendez and Maier, 2008; Sheoran *et al.*, 2009). Phytomining has potential economic advantages, due to the possibility of generating income from residual metals and creating employment opportunities in post-mining regions. Although large scale commercial application of phytomining has not yet happened, experimental studies (Table 2-8) to provide attainable yields have been setup in selected countries (Akinbile et al., 2021). Suitability of phytomining depends on the tolerance to target metals, accumulation rate and biomass production (Anderson *et al.*, 1999). Despite the recent increase in global research on phytomining research and practice remain sluggish in Africa. The large areas of metal-enriched mine wastelands with a potential for occurrence of hyperaccumulator plant accumulating cobalt and copper makes a compelling case for exploiting phytomining technologies in Zambia (Festin *et al.*, 2019; van der Ent *et al.*, 2013).

Table 2-8: List of countries that have undertaken phytomining trials (Akinbile et al., 2021)

Country	Demonstrated phytomining	Potential phytominng sites	Recovered metals
Albania	Yes	Vertisol mine site	Nickel
Australia	Yes	Stawell Gold Mine Victoria, Eastern Goldfields area, Western Australia and PGE-Ni-Cu gossan in Broken Hill mineral complex	Gold, Nickel
Brazil	Yes	Fazenda Brasileiro mine site.	Cobalt
Canada	Yes	Coldwell Complex in northwest Ontario (Stillwater, the Marathon PGM-Copper project); Lac des Iies Intrusive Complex near Thunder Bay (North American Palladium Ltd); area Sudbury	Nickel
Italy	Yes	Varenche mine	Nickel
Mexico	Yes	El Magistral mine	Gold
New Zealand	Yes	West Coast, Coromandel Peninsula	Gold
New Caledonia	Yes	Camp des Sapins mine, Thio	Nickel
U.S.A	Yes	Agnes mine, Bushveld Complex, Great Dyke, Duluth Complex in Minnesota (Twin Metals Minnesota LLC), Stillwater and East Boulder mines	Nickel
Zambia	No		

2.5. Conclusion

Significant amount of copper has been and are still being mined within the Kafue River catchment in Zambia. For decades, these mine activities have been ongoing in this catchment, pausing a persistent risk on natural resources. Urgent adoption of more sustainable management and monitoring practices are required. This need has led to growing interest to invest on monitoring systems for assessing the impacts of mine activities and tracking conditions of natural resources. For instance, to understand the impacts of mine wastes, metal release mechanisms must be understood (Fan et al., 2016). Assessing the ARD generation potential and associated mobilization of metals is one of the effective monitoring tools. The development of the UCT Biokinetic test (Hesketh et al., 2010) and integration of the column bioleach test can be a useful assessment system for ecological risk of streams and rivers throughout mining regions. This approach can be consolidated by increased abiotic and biotic monitoring of aquatic ecosystems and agroecosystems, to provide a comprehensive understanding of current and future impacts of mine wastes.

Much attention must be given in the conservation of water resources by applying abiotic, biotic monitoring and management schemes. Abiotic monitoring programs can mainly be managed using water and sediment quality, while benthic macroinvertebrates can be applied for biomonitoring, to provide basic data for water quality. This combined approach is needed to enhance understanding of water quality. In most developing countries, expertise about abiotic and biotic monitoring is present, making it easy to simultaneously use these monitoring tools for surface water resources.

One of the challenges associated with water contamination in mining regions is accumulation of metals in food crops through irrigation. To mitigate this, studies on the source, metal mobility, transformation, and fate of metals in soil and geochemical processes in soil-crop are necessary. This may require a rigorous approach such as a combined usage of ARD characterization tools, assessment of metal mobility, monitoring of aquatic ecosystems and agroecosystems to understand metal mobility from source on to soil-food crop uptake, in order to conceive appropriate remediation measures. Such environmental issues are overlooked in most studies. This may help policy makers and managers address environmental issues like environmental degradation by pollution.

Preventing pollution and environmental degradation should be a primary issue in mining regions. In this regard, application of appropriate remediation techniques such as phytomining could help mitigate pollution from mine waste. Studying the functional traits of native plants thriving on metal contaminated sites could help select suitable plants for phytomining and contribute to a reduction of environmental degradation. Since there are many mine waste sites in the Kafue River catchment, comparative assessment of metal accumulation in native plants from different sites may help select suitable plants for phytomining and create common rehabilitation programme in accordance with the tenets of circular economy and industrial ecology.

2.6. References

Aazami, J., Esmaili-Sari, A., Abdoli, A., Sohrabi, H., Van den Brink, P.J., 2015. Monitoring and assessment of water health quality in the Tajan River, Iran using physicochemical, fish and macroinvertebrates indices. Journal of Environmental Health Science and Engineering 13. https://doi.org/10.1186/s40201-015-0186-y

Abebe, B.H., Triest, L., 2009. Urban impact on ecological integrity of nearby rivers in developing countries: the Borkena river in Highland Ethiopia. Environmental Monitoring and Assessment 153, 461–476.

Adjei-Mensah, R., Ofori, H., Tortoe, C., Torgbor Johnson, P.-N., Aryee, D., Kofi Frimpong, S., 2021. Effect of home processing methods on the levels of heavy metal contaminants in four food crops grown in and around two mining towns in Ghana. Toxicology Reports 8, 1830–1838. https://doi.org/10.1016/j.toxrep.2021.11.001

Akayombokwa, I.M., van Koppen, B., Matete, M., 2015. Trends and Outlook: Agricultural Water Management in southern Africa. Country report - Zambia. [Project report submitted to United States Agency for International Development's (USAID's) Feed the Future Program].

Akinbile, B.J., Makhubela, B.C.E., Ambushe, A.A., 2021. Phytomining of valuable metals: status and prospective-a review. International Journal of Environmental Analytical Chemistry 0, 1–21. https://doi.org/10.1080/03067319.2021.1917557

Alemneh, T., Ambelu, A., Zaitchik, B.F., Bahrndorff, S., Mereta, S.T., Pertoldi, C., 2019. A macroinvertebrate multi-metric index for Ethiopian highland streams. Hydrobiologia 843, 125–141. https://doi.org/10.1007/s10750-019-04042-x

Algül, F., Beyhan, M., 2020. Concentrations and sources of heavy metals in shallow sediments in Lake Bafa, Turkey. Scientific Reports 10, 11782. https://doi.org/10.1038/s41598-020-68833-2

Al-Hwaiti, M.S., Brumsack, H.J., Schnetger, B., 2016. Suitability assessment of phosphate mine waste water for agricultural irrigation: an example from Eshidiya Mines, South Jordan. Environmental Earth Science 75, 276. https://doi.org/10.1007/s12665-015-4850-4

Alie, M.S., 2019. Influence Of Urbanization on Ecological Status of River In Amhara Region, Ethiopia 2019. BioRxiv 2019.12.28.889949. https://doi.org/10.1101/2019.12.28.889949

Allen, A., Whittier, T., Larsen, D., Kaufmann, P., O'Connor, R., Hughes, R., Stemberger, R., Dixit, S., Brinkhurst, R., Herlihy, A., Paulsen, S., 1999. Concordance of Taxonomic Composition Patterns Across Multiple Lake Assemblages: Effects of Scale, Body Size, and Land Use. Canadian Journal of Fisheries and Aquatic Sciences 56, 2029–2040. https://doi.org/10.1139/cjfas-56-11-2029

Alpert, P., 2006. The advantages and disadvantages of being introduced. Biol Invasions 8, 1523–1534. https://doi.org/10.1007/s10530-005-5844-z

Amoatey, P., Baawain, M.S., 2019. Effects of pollution on freshwater aquatic organisms. Water Environment Research 91, 1272–1287. https://doi.org/10.1002/wer.1221

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R., Stewart, R.B., 1999. Phytomining for nickel, thallium and gold. Journal of Geochemical Exploration 67, 407–415. https://doi.org/10.1016/S0375-6742(99)00055-2

Anderson, C.W.N., Brooks, R.R., Stewart, R.B., Simcock, R. (Department of S.S., 1998. Harvesting a crop of gold in plants. Nature 395, 553-554

Angradi, T.R., 1997. Hydrologic Context and Macroinvertebrate Community Response to Floods in an Appalachian Headwater Stream. The American Midland Naturalist 138, 371–386. https://doi.org/10.2307/2426829

Antonio, C., Meyerson, L., 2002. Exotic Plant Species as Problems and Solutions in Ecological Restoration: A Synthesis. DECEMBER 2002 Restoration Ecology 10, 703–713. https://doi.org/10.1046/j.1526-100X.2002.01051.x

Anyanwu, E., Okorie, M., Odo, S., 2019. RESEARCHPAPER Macroinvertebrates as bioindicators of Water Quality of Effluent- receiving Ossah River, Umuahia, Southeast Nigeria. Journal of Applied Sciences 31, 9–17. http://dx.doi.org/10.21271/ZIPAS.31.5.2

Apostolaki, S., Akinsete, E., Koundouri, P., Samartzis, P., 2020. Freshwater: The Importance of Freshwater for Providing Ecosystem Services, in: Goldstein, M.I., DellaSala, D.A. (Eds.), Encyclopedia of the World's Biomes. Elsevier, Oxford, pp. 71–79. https://doi.org/10.1016/B978-0-12-409548-9.12117-7

Appleton, J.D., Weeks, J.M., Calvez, J.P.S., Beinhoff, C., 2006. Impacts of mercury contaminated mining waste on soil quality, crops, bivalves, and fish in the Naboc River area, Mindanao, Philippines. Science of The Total Environment 354, 198–211.

Arimoro, F.O., Abubakar, M.D., Obi-Iyeke, G.E., Keke, U.N., 2021. Achieving sustainable river water quality for rural dwellers by prioritizing the conservation of macroinvertebrates biodiversity

in two Afrotropical streams. Environmental and Sustainability Indicators 10, 100103. https://doi.org/10.1016/j.indic.2021.100103
Armitage, P.D., Moss, D., Wright, J.F., Furse, M.T., 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water Research 17, 333–347. https://doi.org/10.1016/0043-1354(83)90188-4
Asadi, A., Moghaddam Nia, A., Bakhtiari Enayat, B., Alilou, H., Ahmadisharaf, E., Kimutai Kanda, E., Chessum Kipkorir, E., 2021. An integrated approach for prioritization of river water quality sampling points using modified Sanders, analytic network process, and hydrodynamic modeling. Environmental Monitoring and Assessment 193, 482. https://doi.org/10.1007/s10661-021-09272-y
Attiogbe, F., Nkansah, A., 2017. The Impact of Mining on the Water Resources in Ghana: Newmont Case Study at Birim North District (New Abirem). Energy and Environment Research 7, p27. https://doi.org/10.5539/eer.v7n2p27
Aznar-Sánchez, J.A., García-Gómez, J.J., Velasco-Muñoz, J.F., Carretero-Gómez, A., 2018. Mining Waste and Its Sustainable Management: Advances in Worldwide Research. Minerals 8, 284. https://doi.org/10.3390/min8070284
Baaloudj, A., Samia, O., Ahmed, K., Bouriach, M., Hussein, A., Hammana, C., N'Diaye, D., 2020. Use of macro invertebrates to assess the quality of Seybouse River (North-East of Algeria). Ukrainian Journal of Ecology 10, 60–66. https://doi.org/10.15421/2020_168
Bae, Y.J., Kil, H.K., Bae, K.S., 2005. Benthic macroinvertebrates for uses in stream biomonitoring and restoration. KSCE Journal of Civil Engineering 9, 55–63. https://doi.org/10.1007/BF02829098
Bailey, B.L., Blowes, D.W., Smith, L., Sego, D.C., 2015. The Diavik Waste Rock Project: Geochemical and microbiological characterization of drainage from low-sulfide waste rock: Active zone field experiments. Applied Geochemistry, A Special Issue to Honor the Geochemical Contributions of D. Kirk Nordstrom 62, 18–34. https://doi.org/10.1016/j.apgeochem.2015.02.014
Bain, M.B., Harig, A.L., Loucks, D.P., Goforth, R.R., Mills, K.E., 2000. Aquatic ecosystem protection and restoration: advances in methods for assessment and evaluation. Environmental Science and Policy 3, 89–98. https://doi.org/10.1016/S1462-9011(00)00029-0
Baker, A., 1981. Accumulators and Excluders Strategies in Response of Plants to Heavy Metals. Journal of Plant Nutrition 3, 643–654. https://doi.org/10.1080/01904168109362867
Baker, A., Brooks, R., 1989. Terrestrial Higher Plants Which Hyperaccumulate Metallic Elements, A Review of Their Distribution, Ecology and Phytochemistry. Biorecovery 1, 81-126.
Baker, A., Ernst, W., Ent, A., Malaisse, F., Ginocchio, R., 2010. Metallophytes: the unique biological resource, its ecology and conservational status in Europe, Central Africa and Latin America, in: Ecology of Industrial Pollution 7–40. https://doi.org/10.1017/CBO9780511805561.003
Baker, A.J.M., 1987. Metal Tolerance. New Phytologist 106, 93–111. https://doi.org/10.1111/j.1469-8137.1987.tb04685.x
Balkhair, K.S., Ashraf, M.A., 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. Saudi Journal of Biological Sciences, SI: Recent Topics in Life Sciences 23, S32–S44. https://doi.org/10.1016/j.sjbs.2015.09.023
Banda, W., Chanda, E.K., 2021. A proposed cooperatives strategy for artisanal and small-scale gold mining sector in Zambia. Resources Policy 70, 101909. https://doi.org/10.1016/j.resourpol.2020.101909
Bani, A., Echevarria, G., Mullaj, A., Reeves, R., Morel, J.-L., Sulejman, S., 2009. Nickel Hyperaccumulation by Brassicaceae in Serpentine Soils of Albania and Northwestern Greece. Northeastern Naturalist 16, 385–404. https://doi.org/10.1656/045.016.0528
Barbour, M., Gerritsen, J., Snyder, B., Stribling, J., 1999. Rapid bioassessment protocols foruse in streams and wadable rivers: Periphyton, benthic invertebrates and fish.
Barrutia, O., Epelde, L., Garcia Plazaola, J.I., Garbisu, C., Becerril, J., 2008. Phytoextraction potential of two Rumex acetosa L. accessions collected from metalliferous and non-metalliferous sites:

Effect of fertilization. Chemosphere 74, 259–64. https://doi.org/10.1016/j.chemosphere.2008.09.036

Bartram, J., Ballance, R., Organization, W.H., Programme, U.N.E., 1996. Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programs. London: E & FN Spon.

Beauchemin, S., Fiset, J.-F., Poirier, G., Ablett, J., 2010. Arsenic in an alkaline AMD treatment sludge: Characterization and stability under prolonged anoxic conditions. Applied Geochemistry 25, 1487–1499. https://doi.org/10.1016/j.apgeochem.2010.07.004

Bech, J., Poschenrieder, C., Barceló, J., Lansac, A., 2002. Plants from Mine Spoils in the South American Area as Potential Sources of Germplasm for Phytoremediation Technologies. Acta Biotechnologica 22, 5–11. https://doi.org/10.1002/1521-3846(200205)22:1/2<5::AID-ABIO5>3.0.CO;2-B

Becker, M., Dyantyi, N., Broadhurst, J.L., Harrison, S.T.L., Franzidis, J.-P., 2015. A mineralogical approach to evaluating laboratory scale acid rock drainage characterisation tests. Minerals Engineering 80, 33–36. https://doi.org/10.1016/j.mineng.2015.06.015

Belford, E., 2017. Heavy Metals Accumulation by Indigenous Plants Growing in Contaminated Soil in a Gold Mining Area in Ghana. Journal of Natural Sciences Research 7, 41–47 www.iiste.org.

Benetti, C., Pérez-Bilbao, A., Garrido, J., 2012. Macroinvertebrates as Indicators of Water Quality in Running Waters: 10 Years of Research in Rivers with Different Degrees of Anthropogenic Impacts. Ecological Water Quality – Water Treatment and Reuspp. 95–122. https://doi.org/10.5772/32115

Besley, C.H., Chessman, B.C., 2008. Rapid biological assessment charts the recovery of stream macroinvertebrate assemblages after sewage discharges cease. Ecological Indicators 8, 625–638. https://doi.org/10.1016/j.ecolind.2007.09.003

Beyene, A., Addis, T., Kifle, D., Legesse, W., Kloos, H., Triest, L., 2009. Comparative study of diatoms and macroinvertebrates as indicators of severe water pollution: Case study of the Kebena and Akaki rivers in Addis Ababa, Ethiopia. Ecological Indicators 9, 381–392. https://doi.org/10.1016/j.ecolind.2008.05.001

Bird, M.S., Day, J.A., Malan, H.L., 2014. The influence of biotope on invertebrate assemblages in lentic environments: A study of two perennial alkaline wetlands in the Western Cape, South Africa. Limnologica 48, 16–27. https://doi.org/10.1016/j.limno.2014.05.002

Blanco, A., Salazar, M., Vergara Cid, C., Pignata, M., Rodriguez, J., 2017. Accumulation of lead and associated metals (Cu and Zn) at different growth stages of soybean crops in lead-contaminated soils: food security and crop quality implications. Environmental Earth Sciences 76. https://doi.org/10.1007/s12665-017-6508-x

Boechat, C.L., Pistóia, V.C., Gianelo, C., Camargo, F.A. de O., 2016. Accumulation and translocation of heavy metal by spontaneous plants growing on multi-metal-contaminated site in the Southeast of Rio Grande do Sul state, Brazil. Environmental Science and Pollution Research 23, 2371–2380. https://doi.org/10.1007/s11356-015-5342-5

Bogardi, J.J., Leentvaar, J., Sebesvári, Z., 2020. Biologia Futura: integrating freshwater ecosystem health in water resources management. Biologia Futura 71, 337–358. https://doi.org/10.1007/s42977-020-00031-7

Boisson, S., Séleck, M., Stradic, S.L., Collignon, J., Garin, O., Malaisse, F., Shutcha, M.N., Mahy, G., 2018. Using phytostabilisation to conserve threatened endemic species in southeastern Democratic Republic of the Congo. Ecological Research 33, 789–798. https://doi.org/10.1007/s11284-018-1604-2Boisson, S., Séleck, M., Stradic, S.L., Collignon, J., Garin, O., Malaisse, F., Shutcha, M.N., Mahy, G., 2018. Using phytostabilisation to conserve threatened endemic species in southeastern Democratic Republic of the Congo. Ecological Research 33, 789–798. https://doi.org/10.1007/s11284-018-1604-2

Bollmohr, S., Schulz, R., 2009. Seasonal changes of macroinvertebrate communities in a Western cape river, South Africa, receiving nonpoint-source insecticide pollution. Environ Toxicol Chem 28, 809–817. https://doi.org/10.1897/08-228R.1

Bredenhand, E., 2005. Evaluation of macro-invertebrates as bio-indicators of water quality and the assessment of the impact of the Klein Plaas dam on the Eerste River (Thesis). Stellenbosch : University of Stellenbosch.

Bricker, T.J., Pichtel, J., Brown, H.J., Simmons, M., 2001. Phytoextraction of Pb and Cd from a suspended soil: effects of amendments and croppings. Journal of Environmental Science and Health, Part A 36, 1597–1610. https://doi.org/10.1081/ESE-100106245

Briffa, J., Sinagra, E., Blundell, R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6, e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Brooks, R.R., Chambers, M.F., Nicks, L.J., Robinson, B.H., 1998. Phytomining. Trends in Plant Science 3, 359–362. https://doi.org/10.1016/S1360-1385(98)01283-7

Brooks, R., Reeves, R., Morrison, R., Malaisse, F., 1980. Hyperaccumulation of copper and cobalt - A review. Bulletin de la Société Royale de Botanique de Belgique / Bulletin van de Koninklijke Belgische Botanische Vereniging 113, 166–172. https://doi.org/10.2307/20793840

Broughton, D.W., 2013. Geology and ore deposits of the Central African Copperbelt (Thesis). Colorado School of Mines.

Brysiewicz, A., Czerniejewski, P., Bonisławska, M., 2020. Effect of Diverse Abiotic Conditions on the Structure and Biodiversity of Ichthyofauna in Small, Natural Water Bodies Located on Agricultural Lands. Water 12, 2674. https://doi.org/10.3390/w12102674

Buffagni, A., Erba, S., Cazzola, M., Barca, E., Belfiore, C., 2020. The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics used in southern Europe for ecological status classification. Ecological Indicators 117, 106563. https://doi.org/10.1016/j.ecolind.2020.106563

Bull, S., Selley, D., Broughton, D., Hitzman, M., Cailteux, J., Large, R., McGoldrick, P., 2011. Sequence and carbon isotopic stratigraphy of the Neoproterozoic Roan Group strata of the Zambian copperbelt. Precambrian Research 190, 70–89. https://doi.org/10.1016/j.precamres.2011.07.021

Cabral, H., Fonseca, V., Sousa, T., Costa Leal, M., 2019. Synergistic Effects of Climate Change and Marine Pollution: An Overlooked Interaction in Coastal and Estuarine Areas. International Journal of Environmental Research and Public Health 16. https://doi.org/10.3390/ijerph16152737

Calderon, M., Baldigo, B., Smith, A., Endreny, T., 2017. Effects of extreme floods on macroinvertebrate assemblages in tributaries to the Mohawk River, New York, USA. River Research and Applications 33. https://doi.org/10.1002/rra.3158

Camara, M., Jamil, N.R., Abdullah, A.F.B., 2019. Impact of land uses on water quality in Malaysia: a review. Ecological Processes 8, 10. https://doi.org/10.1186/s13717-019-0164-x

Çelebi, E.E., Öncel, M.S., 2016. Determination of acid forming potential of massive sulfide minerals, and the tailings situated in lead/zinc mining district of Balya (NW Turkey). Journal of African Earth Sciences 124, 487–496. https://doi.org/10.1016/j.jafrearsci.2016.09.014

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. Journal of Environmental. Quality 36, 1429–1443. https://doi.org/10.2134/jeq2006.0514

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., Baker, A.J., 1997. Phytoremediation of soil metals. Current Opinion in Biotechnology 8, 279–284. https://doi.org/10.1016/s0958-1669(97)80004-3

Chapman, D.V., Organization, W.H., Unesco, Programme, U.N.E., 1996. Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. London: E & FN Spon.
Chardot, V., Massoura, S.T., Echevarria, G., Reeves, R.D., Morel, J.-L., 2005. Phytoextraction Potential of the Nickel Hyperaccumulators Leptoplax emarginata and Bornmuellera tymphaea. International Journal of Phytoremediation 7, 323–335. https://doi.org/10.1080/16226510500327186
Chen, H., Yuan, X., Li, T., Hu, S., Ji, J., Wang, C., 2016. Characteristics of heavy metal transfer and their influencing factors in different soil–crop systems of the industrialization region, China. Ecotoxicology and Environmental Safety 126, 193–201. https://doi.org/10.1016/j.ecoenv.2015.12.042
Chen, K., 2018. Research of mining wasteland reclamation and regeneration modes in Shendong Mining Region. IOP Conference Series: Earth and Environmental Science 185, 012027. https://doi.org/10.1088/1755-1315/185/1/012027
Chen, Y., Han, D., 2017. Explore the advantage of High-frequency Water Quality Data in Urban Surface Water: A Case Study in Bristol, UK. AGU Fall Meeting Abstracts 31. https://ui.adsabs.harvard.edu › abs › abstract
Chen, M., Lu, G., Wu, J., Yang, C., Niu, X., Tao, X., Shi, Z., Yi, X., Dang, Z., 2018. Migration and fate of metallic elements in a waste mud impoundment and affected river downstream: A case study in Dabaoshan Mine, South China. Ecotoxicology and Environmental Safety 164, 474–483. https://doi.org/10.1016/j.ecoenv.2018.08.063
Chen, Y., Han, D., 2017. Explore the advantage of High-frequency Water Quality Data in Urban Surface Water: A Case Study in Bristol, UK. AGU Fall Meeting Abstracts 31.
Chibuye, L., Buitendag, J., 2020. The indigenisation of eco-theology: The case of the Lamba people of the Copperbelt in Zambia. HTS Theological Studies 76, 1–8. https://doi.org/10.4102/hts.v76i1.6067
Chileshe, M.N., Syampungani, S., Festin, E.S., Tigabu, M., Daneshvar, A., Odén, P.C., 2020. Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. Journal of Forest Research 31, 1283–1293. https://doi.org/10.1007/s11676-019-00921-0
Chilundo, M., Kelderman, P., O´keeffe, J.H., 2008. Design of a water quality monitoring network for the Limpopo River Basin in Mozambique. Physics and Chemistry of the Earth, Parts A/B/C, Integrated Water Resources Management - From Concept to Practice 33, 655–665. https://doi.org/10.1016/j.pce.2008.06.055
Chuan, O.M., Yunus, K., 2019. Sediment and Organisms as Marker for Metal Pollution. Monitoring of Marine Pollution. https://doi.org/10.5772/intechopen.85569
Chungu, D., Mwanza, A., Ng'andwe, P., Chungu, B.C., Maseka, K., 2019. Variation of heavy metal contamination between mushroom species in the Copperbelt province, Zambia: are the people at risk? Journal of the Science of Food and Agriculture 99, 3410–3416. https://doi.org/10.1002/jsfa.9558
Cimboláková, I., Uher, I., Laktičová, K.V., Vargová, M., Kimáková, T., Papajová, I., 2019. Heavy Metals and the Environment, Environmental Factors Affecting Human Health. IntechOpen. https://doi.org/10.5772/intechopen.86876
Clements, W.H., 1994. Benthic Invertebrate Community Responses to Heavy Metals in the Upper Arkansas River Basin, Colorado. Journal of the North American Benthological Society 13, 30–44. https://doi.org/10.2307/1467263
Collins, S.J., Fahrig, L., 2020. Are macroinvertebrate traits reliable indicators of specific agrichemicals? Ecological Indicators 111, 105965. https://doi.org/10.1016/j.ecolind.2019.105965
Colombo, N., Gruber, S., Martin, M., Malandrino, M., Magnani, A., Godone, D., Freppaz, M., Fratianni, S., Salerno, F., 2018. Rainfall as primary driver of discharge and solute export from rock

glaciers: The Col d'Olen Rock Glacier in the NW Italian Alps. Science of The Total Environment 639, 316–330. https://doi.org/10.1016/j.scitotenv.2018.05.098

Coors, A., Meester, L.D., 2008. Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in Daphnia magna. Journal of Applied Ecology 45, 1820–1828. https://doi.org/10.1111/j.1365-2664.2008.01566.x

Cornejo, A., Tonin, A.M., Checa, B., Tuñon, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PLoS One 14. https://doi.org/10.1371/journal.pone.0220528

Cousins, S., Witkowski, E., 2015. Indigenous Plants: Key Role Players in Community Horticulture Initiatives. Human Ecology Review 21, 59-86. DOI:10.22459/HER.21.01.2015.03

Cummins, K.W., Merritt, R.W., Andrade, P.C., 2005. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. Studies on Neotropical Fauna and Environment 40, 69–89. https://doi.org/10.1080/01650520400025720

Dabrowski, J., Oberholster, P.J., Dabrowski, J.M., Brasseur, J.L., Gieskes, J., 2013. Chemical characteristics and limnology of Loskop Dam on the Olifants River (South Africa), in light of recent fish and crocodile mortalities. Water SA 39, 675–686. https://doi.org/10.4314/wsa.v39i5.12

Dallas, H., 2006. The influence of biotope availability on macroinvertebrate assemblages in South African rivers: Implications for aquatic bioassessment. Freshwater Biology 52, 370–380. https://doi.org/10.1111/j.1365-2427.2006.01684.x

Dallas, H., Day, J., 2007. Natural variation in macroinvertebrate assemblages and the development of a biological banding system for interpreting bioassessment data - A preliminary evaluation using data from upland sites in the south-western Cape, South Africa. Hydrobiologia 575, 231–244. https://doi.org/10.1007/s10750-006-0374-y

Dallas, H., Day, J., 2004. The Effect of Water Quality Variables on Aquatic Ecosystems: a Review.

Dallas, H.F., 2004. Seasonal variability of macroinvertebrate assemblages in two regions of South Africa: implications for aquatic bioassessment. African Journal of Aquatic Science 29, 173–184. https://doi.org/10.2989/16085910409503808

Dallas, H.F., Mosepele, B., 2007. A preliminary survey and analysis of the spatial distribution of aquatic invertebrates in the Okavango Delta, Botswana. African Journal of Aquatic Science 32, 1–11. https://doi.org/10.2989/AJAS.2007.32.1.1.138

Dalu, T., Chauke, R., 2019. Assessing macroinvertebrate communities in relation to environmental variables: the case of Sambandou wetlands, Vhembe Biosphere Reserve. Applied Water Science 10, 16. https://doi.org/10.1007/s13201-019-1103-9

Daly, A.J., Baetens, J.M., De Baets, B., 2018. Ecological Diversity: Measuring the Unmeasurable. Mathematics 6, 119. https://doi.org/10.3390/math6070119

Davey, J., Roberts, S., Wilkinson, J., 2020. Copper- and cobalt-rich, ultrapotassic bittern brines responsible for the formation of the Nkana-Mindola deposits, Zambian Copperbelt. Geology 49. https://doi.org/10.1130/G48176.1

de Castro Pena, J.C., Goulart, F., Wilson Fernandes, G., Hoffmann, D., Leite, F.S.F., Britto dos Santos, N., Soares-Filho, B., Sobral-Souza, T., Humberto Vancine, M., Rodrigues, M., 2017. Impacts of mining activities on the potential geographic distribution of eastern Brazil mountaintop endemic species. Perspectives in Ecology and Conservation 15, 172–178. https://doi.org/10.1016/j.pecon.2017.07.005

de Jonge, M., Blust, R., Bervoets, L., 2010. The relation between Acid Volatile Sulfides (AVS) and metal accumulation in aquatic invertebrates: Implications of feeding behavior and ecology. Environmental Pollution 158, 1381–1391. https://doi.org/10.1016/j.envpol.2010.01.001

de la Rey, P.A., Roux, H., van Rensburg, L., Vosloo, A., 2008. On the use of diatom-based biological monitoring Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation. Water SA 34, 61–70.

de Oliveira, L., Das, S., Siddiqui, A., Rathinasabapathi, B., 2017. Arsenic uptake by lettuce from As-contaminated soil remediated with Pteris vittata and organic amendment. Chemosphere 176, 249–254.

Dejun, Y., Zhengfu, B., Shaogang, L., 2016. Impact on soil physical qualities by the subsidence of coal mining: a case study in Western China. Environmental Earth Sciences 75, 652. https://doi.org/10.1007/s12665-016-5439-2

Deluca, T., Aplet, G., Wilmer, B., Burchfield, J., 2010. The Unknown Trajectory of Forest Restoration: A Call for Ecosystem Monitoring. Journal of Forestry 108, 288–295.

den Besten, P.J., de Deckere, E., Babut, M.P., Power, B., DelValls, T.A., Zago, C., Oen, A.M.P., Heise, S., 2003. Biological effects-based sediment quality in ecological risk assessment for European waters. Journal of Soils and Sediments 3, 144. https://doi.org/10.1065/jss2003.08.084

Deng, H., Ye, Z.H., Wong, M.H., 2004. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environmental Pollution 132, 29–40. https://doi.org/10.1016/j.envpol.2004.03.030

Desrosiers, M., Pinel-Alloul, B., Spilmont, C., 2020. Selection of Macroinvertebrate Indices and Metrics for Assessing Sediment Quality in the St. Lawrence River (QC, Canada). Water 12, 3335. https://doi.org/10.3390/w12123335

Dettrick, D., Bourgeot, N., Costelloe, J., Yuen, S., Arora, M., 2019. The Effect of Particle Size on Mine Waste Sulfide Oxidation Rates and Conceptual Treatment Costs. Mine Water Environment 38, 735–745. https://doi.org/10.1007/s10230-019-00641-1

Dickens, C.W., Graham, P.M., 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27, 1–10. https://doi.org/10.2989/16085914.2002.9626569

Dietterich, L.H., Gonneau, C., Casper, B.B., 2017. Arbuscular mycorrhizal colonization has little consequence for plant heavy metal uptake in contaminated field soils. Ecological Applications 27, 1862–1875. https://doi.org/10.1002/eap.1573

Dixon, D.R., Pruski, A.M., Dixon, L.R.J., Jha, A.N., 2002. Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis 17, 495–507. https://doi.org/10.1093/mutage/17.6.495

Dold, B., 2014. Evolution of Acid Mine Drainage Formation in Sulphidic Mine Tailings. Minerals 4, 621–641. https://doi.org/10.3390/min4030621

Dong, Y., Lin, H., Xu, X., Zhang, Y., Gao, Y., Zhou, S., 2013. Comparative study on the bioleaching, biosorption and passivation of copper sulfide minerals. International Biodeterioration & Biodegradation 84, 29–34. https://doi.org/10.1016/j.ibiod.2013.05.024

Duda, A., Nawar, M., 1996. Implementing the World Bank’s water resources management policy: A priority on toxic substances from nonpoint sources. Water Science and Technology, Diffuse Pollution ’95 33, 45–51. https://doi.org/10.1016/0273-1223(96)00213-2

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Soc 81, 163–182. https://doi.org/10.1017/S1464793105006950

Edogbo, B., Okolocha, E., Maikai, B., Aluwong, T., Uchendu, C., 2020. Risk analysis of heavy metal contamination in soil, vegetables and fish around Challawa area in Kano State, Nigeria. Scientific African 7, e00281. https://doi.org/10.1016/j.sciaf.2020.e00281

Edokpayi, J., Odiyo, J., Popoola, O., Msagati, T.A.M., 2016. Assessment of Trace Metals Contamination of Surface Water and Sediment: A Case Study of Mvudi River, South Africa. Sustainability 8, 135. https://doi.org/10.3390/su8020135

Edokpayi, J.N., Odiyo, J.O., Durowoju, O.S., 2017. Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. Water Quality 65, 401-416. https://doi.org/10.5772/66561

Elghali, A., Benzaazoua, M., Bussière, B., Genty, T., 2019. Spatial Mapping of Acidity and Geochemical Properties of Oxidized Tailings within the Former Eagle/Telbel Mine Site. Minerals 9, 180. https://doi.org/10.3390/min9030180

El-Kady, A.A., Abdel-Wahhab, M.A., 2018. Occurrence of trace metals in foodstuffs and their health impact. Trends in food science and technology 75, 36-45.

Ellenbroek, G.A., 1987. Ecology and productivity of an African wetland system: The Kafue Flats, Zambia, Geobotany. Springer Netherlands. https://doi.org/10.1007/978-94-009-4051-2

Emmanuel, A.Y., Jerry, C.S., Dzigbodi, D.A., 2018. Review of Environmental and Health Impacts of Mining in Ghana. Journal of Health and Pollution 8, 43–52. https://doi.org/10.5696/2156-9614-8.17.43

Ercilla-Montserrat, M., Muñoz, P., Montero, J.I., Gabarrell, X., Rieradevall, J., 2018. A study on air quality and heavy metals content of urban food produced in a Mediterranean city (Barcelona). Journal of Cleaner Production 195, 385–395. https://doi.org/10.1016/j.jclepro.2018.05.183

Eriksen, T.E., Brittain, J.E., Søli, G., Jacobsen, D., Goethals, P., Friberg, N., 2021. A global perspective on the application of riverine macroinvertebrates as biological indicators in Africa, South-Central America, Mexico and Southern Asia. Ecological Indicators 126, 107609. https://doi.org/10.1016/j.ecolind.2021.107609

Escribano, N., Oscoz, J., Galicia, D., Cancellario, T., Durán, C., Navarro, P., Ariño, A.H., 2018. Freshwater macroinvertebrate samples from a water quality monitoring network in the Iberian Peninsula. Scientific Data 5, 180108. https://doi.org/10.1038/sdata.2018.108

Fahr, M., Laplaze, L., Bendaou, N., Hocher, V., El Mzibri, M., Bogusz, D., Smouni, A., 2013. Effect of lead on root growth. Frontiers in Plant Science. 4. https://doi.org/10.3389/fpls.2013.00175

Fallon, E.K., Petersen, S., Brooker, R.A., Scott, T.B., 2017. Oxidative dissolution of hydrothermal mixed-sulphide ore: An assessment of current knowledge in relation to seafloor massive sulphide mining. Ore Geology Reviews 86, 309–337. https://doi.org/10.1016/j.oregeorev.2017.02.028

Fan, L., Zhou, X., Luo, H., Deng, J., Dai, L., Ju, Z., Zhu, Z., Zou, L., Ji, L., Li, B., Cheng, L., 2016. Release of Heavy Metals from the Pyrite Tailings of Huangjiagou Pyrite Mine: Batch Experiments. Sustainability 8, 96. https://doi.org/10.3390/su8010096

Farrokhpay, S., Fornasiero, D., 2017. Flotation of coarse composite particles: Effect of mineral liberation and phase distribution. Advanced Powder Technology 28, 1849–1854. https://doi.org/10.1016/j.apt.2017.03.012

Faucon, M.-P., Chipeng, F., Verbruggen, N., Mahy, G., Colinet, G., Shutcha, M., Pourret, O., Meerts, P., 2012. Copper tolerance and accumulation in two cuprophytes of South-Central Africa: Crepidorhopalon perennis and C. tenuis (Linderniaceae). Environmental and Experimental Botany 84, 11–16. https://doi.org/10.1016/j.envexpbot.2012.04.012

Ferreira, W.R., Paiva, L.T., Callisto, M., 2011. Development of a benthic multimetric index for biomonitoring of a neotropical watershed. Brazilian Journal of Biology 71, 15–25. https://doi.org/10.1590/S1519-69842011000100005

Festin, E.S., Tigabu, M., Chileshe, M.N., Syampungani, S., Odén, P.C., 2019. Progresses in restoration of post-mining landscape in Africa. Journal of Forest Research 30, 381–396. https://doi.org/10.1007/s11676-018-0621-x

Fierro, P., Valdovinos, C., Vargas-Chacoff, L., Bertrán, C., Arismendi, I., 2017. Macroinvertebrates and Fishes as Bioindicators of Stream Water Pollution. pp. 23–38. https://doi.org/10.5772/65084

Fleeger, J.W., Tita, G., Carman, K.R., Millward, R.N., Moser, E.B., Portier, R.J., Gambrell, R.P., 2006. Does bioturbation by a benthic fish modify the effects of sediment contamination on saltmarsh benthic microalgae and meiofauna? Journal of Experimental Marine Biology and Ecology 330, 180–194. https://doi.org/10.1016/j.jembe.2005.12.026

Fosso-Kankeu, E., Manyatshe, A., Waanders, F., 2017. Mobility potential of metals in acid mine drainage occurring in the Highveld area of Mpumalanga Province in South Africa: Implication of sediments and efflorescent crusts. International Biodeterioration & Biodegradation, Environmental Biotechnologies for Sustainable Development (EBSuD) 119, 661–670. https://doi.org/10.1016/j.ibiod.2016.09.018

Fu, B., Horsburgh, J.S., Jakeman, A.J., Gualtieri, C., Arnold, T., Marshall, L., Green, T.R., Quinn, N.W.T., Volk, M., Hunt, R.J., Vezzaro, L., Croke, B.F.W., Jakeman, J.D., Snow, V., Rashleigh, B., 2020. Modeling Water Quality in Watersheds: From Here to the Next Generation. Water Resources Research 56, e2020WR027721. https://doi.org/10.1029/2020WR027721

Fu, S., Wei, C., Xiao, Y., Li, L., Wu, D., 2019. Heavy metals uptake and transport by native wild plants: implications for phytoremediation and restoration. Environmental Earth Sciences 78, 103. https://doi.org/10.1007/s12665-019-8103-9

Gann, G.D., McDonald, T., Walder, B., Aronson, J., Nelson, C.R., Jonson, J., Hallett, J.G., Eisenberg, C., Guariguata, M.R., Liu, J., Hua, F., Echeverría, C., Gonzales, E., Shaw, N., Decleer, K., Dixon, K.W., 2019. International principles and standards for the practice of ecological restoration. Second edition. Restoration Ecology 27, S1–S46. https://doi.org/10.1111/rec.13035

García, C., Ballester, A., González, F., Blázquez, M.L., 2005. Pyrite behaviour in a tailings pond. Hydrometallurgy 76, 25–36. https://doi.org/10.1016/j.hydromet.2004.07.010

GBR - Copperbelt Mining 2014 E&MJ Release [WWW Document], n.d. URL https://www.gbreports.com/publication/copperbelt-mining-2014-emj-release (accessed 7.23.21).

Ghaderian, S.M., Ghotbi Ravandi, A.A., 2012. Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. Journal of Geochemical Exploration, Phytoremediation of polluted soils 123, 25–32. https://doi.org/10.1016/j.gexplo.2012.06.022

Gheorghe, S., Stoica, C., Vasile, G.G., Nita-Lazar, M., Stanescu, E., Lucaciu, I.E., 2017. Metals Toxic Effects in Aquatic Ecosystems: Modulators of Water Quality, Water Quality. IntechOpen. https://doi.org/10.5772/65744

Ghosh, M., 2005. A REVIEW ON PHYTOREMEDIATI ON OF HEAVY METALS AND UTILIZATION OF ITS BYPRODUCTS. Applied Ecology and Environmental Research 3, 1–18. https://doi.org/10.15666/aeer/0301_001018

Gibson, A.L., Espeland, E.K., Wagner, V., Nelson, C.R., 2016. Can local adaptation research in plants inform selection of native plant materials? An analysis of experimental methodologies. Evolutionary Applications 9, 1219–1228. https://doi.org/10.1111/eva.12379

Girdhar, M., Sharma, N.R., Rehman, H., Kumar, A., Mohan, A., 2014. Comparative assessment for hyperaccumulatory and phytoremediation capability of three wild weeds. Biotechnology 4, 579–589. https://doi.org/10.1007/s13205-014-0194-0

Gonnelli, C., Galardi, F., Gabbrielli, R., 2001. Nickel and copper tolerance and toxicity in three Tuscan populations of Silene paradoxa. Physiologia Plantarum 113, 507–514. https://doi.org/10.1034/j.1399-3054.2001.1130409.x

Gordon, A., Niedballa, J., Palmer, C., 2013. Sediment as a Physical Water Quality Stressor on Macro-Invertebrates: A Contribution to the Development of a Water Quality Guideline for Suspended Solids Report to the Water Research Commission. Water Research Commission Report: 2040/1/13

Gornish, E., Brusati, E., Johnson, D., 2016. Practitioner perspectives on using nonnative plants for revegetation. California Agriculture 70, 194–199.

Gratão, P.L., Prasad, M.N.V., Cardoso, P.F., Lea, P.J., Azevedo, R.A., 2005. Phytoremediation: green technology for the clean-up of toxic metals in the environment. Brazilian Journal of Plant Physiology 17, 53–64. https://doi.org/10.1590/S1677-04202005000100005

Growns, J.E., Chessman, B.C., Jackson, J.E., Ross, D.G., 1997. Rapid Assessment of Australian Rivers Using Macroinvertebrates: Cost and Efficiency of 6 Methods of Sample Processing. Journal of the North American Benthological Society 16, 682–693. https://doi.org/10.2307/1468153

Grytsyuk, N., Arapis, G., Perepelyatnikova, L., Ivanova, T., Vynograds'ka, V., 2006. Heavy metals effects on forage crops yields and estimation of elements accumulation in plants as affected by soil. Science of the Total Environment 354, 224–231. https://doi.org/10.1016/j.scitotenv.2005.01.007

Guala, S.D., Vega, F.A., Covelo, E.F., 2010. The dynamics of heavy metals in plant–soil interactions. Ecological Modelling 221, 1148–1152. https://doi.org/10.1016/j.ecolmodel.2010.01.003

Guo, Y., Huang, P., Zhang, W., Yuan, X., Fan, F., Wang, H., Liu, J., Wang, Z., 2013a. Leaching of heavy metals from Dexing copper mine tailings pond. Transactions of Nonferrous Metals Society of China 23, 3068–3075. https://doi.org/10.1016/S1003-6326(13)62835-6

Hachigonta, S., Reason, C.J.C., Tadross, M., 2008. An analysis of onset date and rainy season duration over Zambia. Theoretical and Applied Climatology 91, 229–243. https://doi.org/10.1007/s00704-007-0306-4

Hampwaye, G., Rogerson, C.M., 2010. Economic Restructuring in the Zambian Copperbelt: Local Responses in Ndola. Urban Forum 21, 387–403. https://doi.org/10.1007/s12132-010-9096-3

Hanberry, B.B., Noss, R.F., Safford, H.D., Allison, S.K., Dey, D.C., 2015. Restoration Is Preparation for the Future. Journal of Forestry 113, 425–429. https://doi.org/10.5849/jof.15-014

Harris, J., Silveira, R., 1999. Large-scale assessments of river health using an Index of Biotic Integrity with low-diversity fish communities. Freshwater Biology 41, 235–252. https://doi.org/10.1046/j.1365-2427.1999.00428.x

Harrison, S., Broadhurst, J.L., Opitz, A., Fundikwa, B., Stander, H.-M., Mostert, L., Juarez, Amaral Filho, J., Kotsiopoulos, A., 2019. An Industrial Ecology Approach to Sulphide-containing Mineral Wastes to Minimise ARD Formation Part 2: Design for Disposal and Extraction of Products of Value. Water Research Commission Report: K5/2231 November 201. Pp145.

Harrison, S.T., Broadhurst, J.L., Van Hille, R., Oyekola, O., Bryan, C., Hesketh, A., Opitz, A., 2010. A systematic approach to sulphidic waste rock and tailings management to minimise acid rock drainage formation. Water Research Commission Report: 1831/1/10.

Hasnaoui, S.E., Fahr, M., Keller, C., Levard, C., Angeletti, B., Chaurand, P., Triqui, Z.E.A., Guedira, A., Rhazi, L., Colin, F., Smouni, A., 2020. Screening of Native Plants Growing on a Pb/Zn Mining Area in Eastern Morocco: Perspectives for Phytoremediation. Plants 9, 1458. https://doi.org/10.3390/plants9111458

Hauer, C., Leitner, P., Unfer, G., Pulg, U., Habersack, H., Graf, W., 2018. The Role of Sediment and Sediment Dynamics in the Aquatic Environment, in: Schmutz, S., Sendzimir, J. (Eds.), Riverine Ecosystem Management: Science for Governing Towards a Sustainable Future, Aquatic Ecology Series. Springer International Publishing 151–169. https://doi.org/10.1007/978-3-319-73250-3_8

He, B., Wang, W., Geng, R., Ding, Z., Luo, D., Qiu, J., Zheng, G., Fan, Q., 2021. Exploring the fate of heavy metals from mining and smelting activities in soil-crop system in Baiyin, NW China. Ecotoxicology and Environmental Safety 207, 111234. https://doi.org/10.1016/j.ecoenv.2020.111234

He, F., Jiang, W., Tang, T., Cai, Q., 2015. Assessing impact of acid mine drainage on benthic macroinvertebrates: can functional diversity metrics be used as indicators? Journal of Freshwater Ecology 30, 513–524. https://doi.org/10.1080/02705060.2014.998730

Heikkinen, P.M., Räisänen, M.L., 2009. Trace metal and As solid-phase speciation in sulphide mine tailings – Indicators of spatial distribution of sulphide oxidation in active tailings impoundments. Applied Geochemistry 24, 1224–1237. https://doi.org/10.1016/j.apgeochem.2009.03.007

Heim, S., Schwarzbauer, J., 2013. Pollution history revealed by sedimentary records: A review. Environmental Chemistry Letters 11. https://doi.org/10.1007/s10311-013-0409-3

Hesketh, A.H., Broadhurst, J.L., Bryan, C.G., van Hille, R.P., Harrison, S.T.L., 2010. Biokinetic test for the characterisation of AMD generation potential of sulfide mineral wastes. Hydrometallurgy,

18th International Biohydrometallurgy Symposium, IBS2009, Bariloche-Argentina, 13-17 September 2009 104, 459–464. https://doi.org/10.1016/j.hydromet.2010.01.015
Hillman, T.J., Quinn, G.P., 2002. Temporal changes in macroinvertebrate assemblages following experimental flooding in permanent and temporary wetlands in an Australian floodplain forest. River Research and Applications 18, 137–154. https://doi.org/10.1002/rra.628
Hilson, G., 2020. The 'Zambia Model': A blueprint for formalizing artisanal and small-scale mining in sub-Saharan Africa? Resources Policy 68, 101765. https://doi.org/10.1016/j.resourpol.2020.101765
Huang, L., Baumgartl, T., Mulligan, D., 2012. Is rhizosphere remediation sufficient for sustainable revegetation of mine tailings? Annals of Botany 110, 223–238. https://doi.org/10.1093/aob/mcs115
Huang, Z., Xiang, W., Ma, Y., Lei, P., Tian, D., Deng, X., Yan, W., Fang, X., 2015. Growth and Heavy Metal Accumulation of Koelreuteria Paniculata Seedlings and Their Potential for Restoring Manganese Mine Wastelands in Hunan, China. International Journal of Environmental Research and Public Health 12, 1726–1744. https://doi.org/10.3390/ijerph120201726
Hudson-Edwards, K.A., Schell, C., Macklin, M.G., 1999. Mineralogy and geochemistry of alluvium contaminated by metal mining in the Rio Tinto area, southwest Spain. Applied Geochemistry 14, 1015–1030. https://doi.org/10.1016/S0883-2927(99)00008-6
Hutchinson, T.H., Solbe, J., Kloepper-Sams, P.J., 1998. Analysis of the ecetoc aquatic toxicity (EAT) database III — Comparative toxicity of chemical substances to different life stages of aquatic organisms. Chemosphere 36, 129–142. https://doi.org/10.1016/S0045-6535(97)10025-X
Igbinosa, E., Okoh, A., 2009. Impact of Discharge Wastewater Effluents on the Physicochemical Qualities of a Receiving Watershed in a Typical Rural Community. International Journal of Environmental Science and Technology 6. https://doi.org/10.1007/BF03327619
Imène, B., Si Bachir, A., 2018. Diversity Of Benthic Macroinvertebrates and Streams Quality In The National Park of Belezma (Northern-East, Algeria). International Journal of Health and Life-Sciences 4, 01–18. https://doi.org/10.20319/lijhls.2018.41.0118
Islam, S., Rahman, M.M., Rahman, M.A., Naidu, R., 2017. Inorganic arsenic in rice and rice-based diets: Health risk assessment. Food Control 82, 196–202. https://doi.org/10.1016/j.foodcont.2017.06.030
Israeli, Y., Emmanuel, S., 2018. Impact of grain size and rock composition on simulated rock weathering. Earth Surface Dynamics Discussions 1–16. https://doi.org/10.5194/esurf-2018-1
Issaka, S., Ashraf, M.A., 2017. Impact of soil erosion and degradation on water quality: a review. Geology, Ecology, and Landscapes 1, 1–11. https://doi.org/10.1080/24749508.2017.1301053
Jamieson, H.E., Walker, S.R., Parsons, M.B., 2015. Mineralogical characterization of mine waste. Applied Geochemistry, Environmental Geochemistry of Modern Mining 57, 85–105. https://doi.org/10.1016/j.apgeochem.2014.12.014
Janzen, M., Nicholson, R., Scharer, J., 2000. Pyrrhotite reaction kinetics: Reaction rates for oxidation by oxygen, ferric iron, and for nonoxidative dissolution. Geochimica et Cosmochimica Acta 64, 1511–1522. https://doi.org/10.1016/S0016-7037(99)00421-4
Jhariya, D., Khan, R., Thakur, G.S., 2016. Impact of Mining Activity on Water Resource: An Overview study. Conference: National Seminar on Recent Practices & innovations in Mining Industry (RPIMI 2016)
Jiang, X., Liu, W., Xu, H., Cui, X., Li, J., Chen, J., Zheng, B., 2021. Characterizations of heavy metal contamination, microbial community, and resistance genes in a tailing of the largest copper mine in China. Environmental Pollution 116947. https://doi.org/10.1016/j.envpol.2021.116947
Jones, J., Murphy, J., Collins, A., Sear, D., Naden, P.S., Armitage, P., 2012. The Impact of Fine Sediment on Macro-Invertebrates. River Research and Applications 28. https://doi.org/10.1002/rra.1516

Jung, H., Senf, C., Jordan, P., Krueger, T., 2020. Benchmarking inference methods for water quality monitoring and status classification. Environmental Monitoring and Assessment 192, 261. https://doi.org/10.1007/s10661-020-8223-4

Kalin, M., Wheeler, W.N., Bellenberg, S., 2018. Acid Rock Drainage or Not—Oxidative vs. Reductive Biofilms—A Microbial Question. Minerals 8, 199. https://doi.org/10.3390/min8050199

Kalumba, M., Nyirenda, E., 2017. River flow availability for environmental flow allocation downstream of hydropower facilities in the Kafue Basin of Zambia. Physics and Chemistry of the Earth, Parts A/B/C 102, 21–30. https://doi.org/10.1016/j.pce.2017.07.003

Kambole, M.S., 2003. Managing the water quality of the Kafue River. Physics and Chemistry of the Earth, Parts A/B/C 28, 1105–1109. https://doi.org/10.1016/j.pce.2003.08.031

Kapungwe, E., 2012. Traditional farming practices and wastewater irrigation farming in peri urban Zambia. The Indonesian journal of geography 44, 104–120. https://doi.org/10.22146/indo.j.geog,2393

Kapungwe, E., 2011. Industrial Land Use and Heavy Metal Contaminated Wastewater Used for Irrigation in Periurban Zambia. Singapore Journal of Tropical Geography 32, 71–84. https://doi.org/10.1111/j.1467-9493.2011.00422.x

Kapungwe, E.M., 2013. Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. Journal of Geography and Geology 5, 55. https://doi.org/10.5539/jgg.v5n2p55

Kapwata, T., Mathee, A., Sweijd, N., Minakawa, N., Mogotsi, M., Kunene, Z., Wright, C.Y., 2020. Spatial assessment of heavy metals contamination in household garden soils in rural Limpopo Province, South Africa. Environmental Geochemistry and Health 42, 4181–4191. https://doi.org/10.1007/s10653-020-00535-0

Karaca, O., Cameselle, C., Reddy, K.R., 2018. Mine tailing disposal sites: contamination problems, remedial options and phytocaps for sustainable remediation. Reviews in Environmental Science and Biotechnology 17, 205–228. https://doi.org/10.1007/s11157-017-9453-y

Karlsson, T., 2019. Geochemical and mineralogical laboratory methods in waste rock drainage quality prediction (PhD Thesis, Lulea University of Technology, Sweden).

Karlsson, T., Räisänen, M.L., Lehtonen, M., Alakangas, L., 2018. Comparison of static and mineralogical ARD prediction methods in the Nordic environment. Environmental Monitoring and Assessment 190, 719. https://doi.org/10.1007/s10661-018-7096-2

Kasangaki, A., Babaasa, D., Efitre, J., McNeilage, A., Bitariho, R., 2006. Links Between Anthropogenic Perturbations and Benthic Macroinvertebrate Assemblages in Afromontane Forest Streams in Uganda. Hydrobiologia 563, 231–245. https://doi.org/10.1007/s10750-005-0009-8

Kasangaki, A., Chapman, L., Balirwa, J., 2008. Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. Freshwater Biology 53, 681–697. https://doi.org/10.1111/j.1365-2427.2007.01925.x

Kasowska, D., Gediga, K., Spiak, Z., 2018. Heavy metal and nutrient uptake in plants colonizing post-flotation copper tailings. Environmental Science and Pollution Research 25, 824–835. https://doi.org/10.1007/s11356-017-0451-y

Kasozi, K.I., Otim, E.O., Ninsiima, H.I., Zirintunda, G., Tamale, A., Ekou, J., Musoke, G.H., Muyinda, R., Matama, K., Mujinya, R., Matovu, H., Ssempijja, F., Eze, E.D., Atino, M., Udechukwu, B., Kayima, R., Etiang, P., Ayikobua, E.T., Kembabazi, S., Usman, I.M., Sulaiman, S.O., Natabo, P.C., Kyeyune, G.N., Batiha, G.E.-S., Otim, O., 2021. An analysis of heavy metals contamination and estimating the daily intakes of vegetables from Uganda. Toxicology Research and Application 5, 2397847320985255. https://doi.org/10.1177/2397847320985255

Kemosedile, T., Khaneguba, W., Mothobi, R., Mogojwa, B., Mosie, I., Makati, K., Murray-Hudson, M., n.d. Long Term Time-Series Data on Aquatic Macro-invertebrates Monitoring by Okavango Research Institute, Botswana. https://doi.org/10.15468/kcjznw

Kenney, M., Sutton-Grier, A., Smith, R., Gresens, S., 2009a. Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. Terrestrial Arthropod Reviews 2, 99–128. https://doi.org/10.1163/187498209X12525675906077

Khalid, S., Shahid, M., Natasha, Bibi, I., Sarwar, T., Shah, A.H., Niazi, N.K., 2018. A Review of Environmental Contamination and Health Risk Assessment of Wastewater Use for Crop Irrigation with a Focus on Low and High-Income Countries. International Journal of Environmental Research and Public Health 15. https://doi.org/10.3390/ijerph15050895

Khan, K., Lu, Y., Khan, H., Ishtiaq, M., Khan, S., Waqas, M., Wei, L., Wang, T., 2013. Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. Food and Chemical Toxicology 58, 449–458. https://doi.org/10.1016/j.fct.2013.05.014

Khan, S., Aijun, L., Zhang, S., Hu, Q., Zhu, Y.-G., 2008. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. J Hazard Mater 152, 506–515. https://doi.org/10.1016/j.jhazmat.2007.07.014

Khatri, N., Tyagi, S., 2015. Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Frontiers in Life Science 8, 23–39. https://doi.org/10.1080/21553769.2014.933716

Khoeurn, K., Sakaguchi, A., Tomiyama, S., Igarashi, T., 2019. Long-term acid generation and heavy metal leaching from the tailings of Shimokawa mine, Hokkaido, Japan: Column study under natural condition. Journal of Geochemical Exploration 201, 1–12. https://doi.org/10.1016/j.gexplo.2019.03.003

Kidd, P.S., Bani, A., Benizri, E., Gonnelli, C., Hazotte, C., Kisser, J., Konstantinou, M., Kuppens, T., Kyrkas, D., Laubie, B., Malina, R., Morel, J.-L., Olcay, H., Pardo, T., Pons, M.-N., Prieto-Fernández, Á., Puschenreiter, M., Quintela-Sabarís, C., Ridard, C., Rodríguez-Garrido, B., Rosenkranz, T., Rozpądek, P., Saad, R., Selvi, F., Simonnot, M.-O., Tognacchini, A., Turnau, K., Ważny, R., Witters, N., Echevarria, G., 2018. Developing Sustainable Agromining Systems in Agricultural Ultramafic Soils for Nickel Recovery. Frontiers in Environmental Science. 6. https://doi.org/10.3389/fenvs.2018.00044

Kim, K.R., Owens, G., Naidu, R., 2010. Effect of Root-Induced Chemical Changes on Dynamics and Plant Uptake of Heavy Metals in Rhizosphere Soils. Pedosphere 20, 494–504. https://doi.org/10.1016/S1002-0160(10)60039-2

Kimball, B.A., Callender, E., Axtmann, E.V., 1995. Effects of colloids on metal transport in a river receiving acid mine drainage, upper Arkansas River, Colorado, U.S.A. Applied Geochemistry 10, 285–306. https://doi.org/10.1016/0883-2927(95)00011-8

Kimball, B., Runkel, R., Walton-Day, K., Bencala, K., 2002. Assessment of Metal Loads in Watersheds Affected by Acid Mine Drainage by Using Tracer Injection and Synoptic Sampling: Cement Creek, Colorado, USA. Applied Geochemistry 17, 1183–1207. https://doi.org/10.1016/S0883-2927(02)00017-3

Klimaszyk, P., Gołdyn, R., 2020. Water Quality of Freshwater Ecosystems in a Temperate Climate. Water 12, 2643. https://doi.org/10.3390/w12092643

Kobina Mensah, A., 2015. Role of revegetation in restoring fertility of degraded mined soils in Ghana: A review. International Journal of Biodiversity and Conservation 7, 57–80. https://doi.org/10.5897/IJBC2014.0775

Koglin, S., Kammann, U., Eichbaum, K., Reininghaus, M., Eisner, B., Wiseman, S., Hecker, M., Buchinger, S., Reifferscheid, G., Hollert, H., Brinkmann, M., 2016. Toward understanding the impacts of sediment contamination on a native fish species: transcriptional effects, EROD activity, and biliary PAH metabolites. Environmental Sciences Europe 28. https://doi.org/10.1186/s12302-016-0096-3

Kolala, C., Umar, B.B., 2019. National benefits, local costs? Local residents' views on environmental and social effects of large-scale mining in Chingola, Zambia. Natural Resources Forum 43, 205–217. https://doi.org/10.1111/1477-8947.12182

Kotsiopoulos, A., Harrison, S.T.L., 2018. Co-disposal of benign desulfurised tailings with sulfidic waste rock to mitigate ARD generation: Influence of flow and contact surface. Minerals Engineering 116, 62–71. https://doi.org/10.1016/j.mineng.2017.03.003

Kramer, P., Zabowski, D., Scherer, G., Everett, R., 2000. Native Plant Restoration of Copper Mine Tailings: I. Substrate Effect on Growth and Nutritional Status in a Greenhouse Study. Journal of Environmental Quality - Journal of Environmental Quality 29. https://doi.org/10.2134/jeq2000.00472425002900060005x

Küçük, S., 2008. The effect of organic pollution on benthic macroinvertebrate fauna in the Kirmir creek in the Sakarya basin. Sakarya havzası Kirmir Çayı ’nın bentik makroomurgasız faunası üzerine organik kirliğin etkisi. Journal of Adnan Menderes University Agricultural Faculty 5, 5-12

Kumar, S., Talan, A., Boyle, K., Ormeci, B., Drogui, P., Tyagi, R.D., 2021. 4 - Water Recycling: Economic and Environmental Benefits, in: Pandey, A., Tyagi, R.D., Varjani, S. (Eds.), Biomass, Biofuels, Biochemicals. Elsevier, pp. 91–120. https://doi.org/10.1016/B978-0-12-821878-5.00015-5

Kuzmanovic, M., Dolédec, S., de Castro-Catala, N., Ginebreda, A., Sabater, S., Muñoz, I., Barceló, D., 2017. Environmental stressors as a driver of the trait composition of benthic macroinvertebrate assemblages in polluted Iberian rivers. Environmental Research 156, 485–493. https://doi.org/10.1016/j.envres.2017.03.054

Lahcen, K., Chahlaoui, A., Essahale, A., 2017. Anthropogenic Impacts on the Distribution and Biodiversity of Benthic Macroinvertebrates and Water Quality of the Boufekrane River, Meknes, Morocco. Journal of Geoscience and Environment Protection 05, 173–195. https://doi.org/10.4236/gep.2017.57014

Lam, E.J., Keith, B.F., Montofré, Í.L., Gálvez, M.E., 2018. Copper Uptake by Adesmia atacamensis in a Mine Tailing in an Arid Environment. Air, Soil and Water Research 11, 1178622118812462. https://doi.org/10.1177/1178622118812462

Lamb, A.E., Anderson, C.W.N., Haverkamp, R.G., 2001. The Induced Accumulation of Gold in The Plants Brassica juncea, Berkheya coddii and Chicory. Chemistry in New Zealand Journal. http://hdl.handle.net/10179/9608

Lange, B., Delhaye, G., Boisson, S., Verbruggen, N., Meerts, P., Faucon, M.-P., 2018. Variation in copper and cobalt tolerance and accumulation among six populations of the facultative metallophyte Anisopappus chinensis (Asteraceae). Environmental and Experimental Botany 153, 1–9. https://doi.org/10.1016/j.envexpbot.2018.04.009

Lange, B., Ent, A. van der, Baker, A.J.M., Echevarria, G., Mahy, G., Malaisse, F., Meerts, P., Pourret, O., Verbruggen, N., Faucon, M.-P., 2017. Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytologist 213, 537–551. https://doi.org/10.1111/nph.14175

Lange, B., Pourret, O., Meerts, P., Jitaru, P., Cancès, B., Grison, C., Faucon, M.-P., 2016. Copper and cobalt mobility in soil and accumulation in a metallophyte as influenced by experimental manipulation of soil chemical factors. Chemosphere 146, 75–84. https://doi.org/10.1016/j.chemosphere.2015.11.105

Langman, J.B., Sinclair, S., Amos, R.T., Wilson, D., Ptacek, C.J., Sego, D.C., Smith, L., Blowes, D.W., 2019. Alkalinity generation from weathering of accessory calcite and apatite and acid drainage neutralization in an Archean granitoid waste rock. Journal of Geochemical Exploration 205, 106341. https://doi.org/10.1016/j.gexplo.2019.106341

Larson, A., Belote, R.T., Williamson, M., Aplet, G., 2013. Making Monitoring Count: Project Design for Active Adaptive Management. https://doi.org/10.5849/JOF.13-021

Leila, B., Loubna, B., Ali, B., 2021. Impacts of mining activities on soil properties: case studies from Morocco mine sites. Soil Science Annual 71, 395–407. https://doi.org/10.37501/soilsa/133011

Li, L., Zheng, B., Liu, L., 2010. Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends. Procedia Environmental Sciences, International Conference on

Ecological Informatics and Ecosystem Conservation (ISEIS 2010) 2, 1510–1524. https://doi.org/10.1016/j.proenv.2010.10.164

Li, P., Karunanidhi, D., Subramani, T., Srinivasamoorthy, K., 2021. Sources and Consequences of Groundwater Contamination. Archives of Environmental Contamination and Toxicology 80, 1–10. https://doi.org/10.1007/s00244-020-00805-z

Li, X., Lei, S., Liu, F., Wang, W., 2020. Analysis of Plant and Soil Restoration Process and Degree of Refuse Dumps in Open-Pit Coal Mining Areas. Int J Environ Res Public Health 17, 1975. https://doi.org/10.3390/ijerph17061975

Libanda, B., Bwalya, K., Nkolola, N.B., Chilekana, N., 2020. Quantifying long-term variability of precipitation and temperature over Zambia. Journal of Atmospheric and Solar-Terrestrial Physics 198, 105201. https://doi.org/10.1016/j.jastp.2020.105201

Libanda, B., Ngonga, C., 2018. Projection of frequency and intensity of extreme precipitation in Zambia: a CMIP5 study. https://doi.org/10.3354/CR01528

Liu, J., Wu, J., Feng, W., Li, X., 2020. Ecological Risk Assessment of Heavy Metals in Water Bodies around Typical Copper Mines in China. International Journal of Environmental Research and Public Health 17, 4315. https://doi.org/10.3390/ijerph17124315

Liu, W., Zhao, J., Ouyang, Z., Söderlund, L., Liu, G., 2005. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environment International 31, 805–812. https://doi.org/10.1016/j.envint.2005.05.042

Lomartire, S., Marques, J.C., Gonçalves, A.M.M., 2021. Biomarkers based tools to assess environmental and chemical stressors in aquatic systems. Ecological Indicators 122, 107207. https://doi.org/10.1016/j.ecolind.2020.107207

Loska, K., Cebula, J., Pelczar, J., Wiechuła, D., Kwapuliński, J., 1997. Use of Enrichment, and Contamination Factors Together with Geoaccumulation Indexes to Evaluate the Content of Cd, Cu, and Ni in the Rybnik Water Reservoir in Poland. Water, Air, & Soil Pollution 93, 347–365. https://doi.org/10.1023/A:1022121615949

Lottermoser, B., 2010. Mine Wastes: Characterization, Treatment and Environmental Impacts, Mine Wastes (Third Edition): Characterization, Treatment and Environmental Impacts. DOI:10.1007/978-3-540-48630-5

Lou, L., Shen, Z., Li, X., 2004. The copper tolerance mechanisms of Elsholtzia haichowensis, a plant from copper-enriched soils. Environmental and Experimental Botany 51, 111–120. https://doi.org/10.1016/j.envexpbot.2003.08.002

Lyu, Z., Chai, J., Xu, Z., Qin, Y., Cao, J., 2019. A Comprehensive Review on Reasons for Tailings Dam Failures Based on Case History [WWW Document]. Advances in Civil Engineering. https://doi.org/10.1155/2019/4159306

Macharia, A., Ndiritu, G., Gichuki, N., Wamicha, W., 2004. Impact of water quality on macroinvertebrate assemblages along a tropical stream in Kenya. African Journal of Ecology 42, 208–216. https://doi.org/10.1111/j.1365-2028.2004.00516.x

Maddock, I., 1999. The importance of physical habitat assessment for evaluating river health. Freshwater Biology 41, 373–391. https://doi.org/10.1046/j.1365-2427.1999.00437.x

Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R., Zhang, Z., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotoxicology and Environmental Safety 126, 111–121. https://doi.org/10.1016/j.ecoenv.2015.12.023

Makgae, M., 2011. Key Areas in Waste Management: A South African Perspective, Integrated Waste Management - Volume II. IntechOpen. https://doi.org/10.5772/18023

Malmström, M.E., Gleisner, M., Herbert, R.B., 2006. Element discharge from pyritic mine tailings at limited oxygen availability in column experiments. Applied Geochemistry 21, 184–202. https://doi.org/10.1016/j.apgeochem.2005.09.006

Maloney, E.M., 2019. How do we take the pulse of an aquatic ecosystem? Current and historical approaches to measuring ecosystem integrity. Environmental Toxicology and Chemistry 38, 289–301. https://doi.org/10.1002/etc.4308

Manara, A., Fasani, E., Furini, A., DalCorso, G., 2020. Evolution of the metal hyperaccumulation and hypertolerance traits. Plant, Cell & Environment 43, 2969–2986. https://doi.org/10.1111/pce.13821

Mangadze, T., Dalu, T., Froneman, P.W., 2019. Biological monitoring in southern Africa: A review of the current status, challenges and future prospects. Science of the Total Environment 648, 1492–1499. https://doi.org/10.1016/j.scitotenv.2018.08.252

Manolaki, P., Chourabi, S., Vogiatzakis, I.N., 2021. A rapid qualitative methodology for ecological integrity assessment across a Mediterranean island's landscapes. Ecological Complexity 46, 100921. https://doi.org/10.1016/j.ecocom.2021.100921

Marcé, R., George, G., Buscarinu, P., Deidda, M., Dunalska, J., de Eyto, E., Flaim, G., Grossart, H.-P., Istvanovics, V., Lenhardt, M., Moreno-Ostos, E., Obrador, B., Ostrovsky, I., Pierson, D.C., Potužák, J., Poikane, S., Rinke, K., Rodríguez-Mozaz, S., Staehr, P.A., Šumberová, K., Waajen, G., Weyhenmeyer, G.A., Weathers, K.C., Zion, M., Ibelings, B.W., Jennings, E., 2016. Automatic High Frequency Monitoring for Improved Lake and Reservoir Management. Environmental Science and Technology. 50, 10780–10794. https://doi.org/10.1021/acs.est.6b01604

Masarovičová, E., Kráľová, K., Kummerová, M., 2010. Principles of classification of medicinal plants as hyperaccumulators or excluders. Acta Physiologiae Plantarum 32, 823–829. https://doi.org/10.1007/s11738-010-0474-1

Masese, F., Muchiri, M., Raburu, P., 2009. Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. African Journal of Aquatic Science 34, 15–26. https://doi.org/10.2989/AJAS.2009.34.1.2.727

Mathuriau, C., Mercado-Silva, N., Lyons, J., Martinez Rivera, L., 2011. Fish and Macroinvertebrates as Freshwater Ecosystem Bioindicators in Mexico: Current State and Perspectives. Hexagon Series on Human and Environmental Security and Peace pp. 251–261. https://doi.org/10.1007/978-3-642-05432-7_19

Mattson, K.M., Angermeier, P.L., 2007. Integrating Human Impacts and Ecological Integrity into a Risk-Based Protocol for Conservation Planning. Environmental Management 39, 125–138. https://doi.org/10.1007/s00267-005-0238-7

Mazor, R.D., Purcell, A.H., Resh, V.H., 2009. Long-Term Variability in Bioassessments: A Twenty-Year Study from Two Northern California Streams. Environmental Management 43, 1269–1286. https://doi.org/10.1007/s00267-009-9294-8

Mbewe, G., Mutondo, M., Maseka, K., Sichilongo, K., 2016. Assessment of Heavy-Metal Pollution in Sediments and Tilapia Fish Species in Kafue River of Zambia. Archives of Environmental Contamination and Toxicology 71, 383–393. https://doi.org/10.1007/s00244-016-0295-3

McGowan, R.R., Roberts, S., Boyce, A.J., 2005. Origin of the Nchanga copper–cobalt deposits of the Zambian Copperbelt. Mineralium Deposita 40, 617. https://doi.org/10.1007/s00126-005-0032-8

McGrath, S.P., Zhao, F.J., Lombi, E., 2001. Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant and Soil 232, 207–214. https://doi.org/10.1023/A:1010358708525

Mcintyre, N., Angarita, M., Fernández, N., Camacho-Botero, L., Pearse, J., Huguet, C., Restrepo, O., Ossa Moreno, J., 2018. A Framework for Assessing the Impacts of Mining Development on Regional Water Resources in Colombia. Water 10, 268. https://doi.org/10.3390/w10030268

Medupin, C., 2019. Distribution of benthic macroinvertebrate communities and assessment of water quality in a small UK river catchment. SN Applied Science 1, 544. https://doi.org/10.1007/s42452-019-0464-x

Mendez, M.O., Maier, R.M., 2008. Phytostabilization of Mine Tailings in Arid and Semiarid Environments—An Emerging Remediation Technology. Environ Health Perspectives 116, 278–283. https://doi.org/10.1289/ehp.10608

Mesa, L.M., 2012. Interannual and seasonal variability of macroinvertebrates in monsoonal climate streams. Brazilian Archives of Biology and Technology 55, 403–410. https://doi.org/10.1590/S1516-89132012000300011

Metzeling, L., Chessman, B., Hardwick, R., Wong, V., 2003. Rapid assessment of rivers using macroinvertebrates: the role of experience, and comparisons with quantitative methods. Hydrobiologia 510, 39–52. https://doi.org/10.1023/B:HYDR.0000008500.34301.a0

Mirecki, N., Agic, R., Šunić, L., Milenkovic, L., Ilic, Z., 2015. Transfer factor as indicator of heavy metals content in plants. Fresenius Environmental Bulletin 24, 4212–4219.

M'kandawire, E., Choongo, K., Yabe, J., Mwase, M., Saasa, N., Nakayama, S.M.M., Bortey-Sam, N., Blindauer, C.A., 2017. Sediment Metal Contamination in the Kafue River of Zambia and Ecological Risk Assessment. Bulletin of Environmental Contamination and Toxicology 99, 108–116. https://doi.org/10.1007/s00128-017-2089-3

Mok, H.-F., Majumder, R., Laidlaw, W.S., Gregory, D., Baker, A.J.M., Arndt, S.K., 2013. Native Australian Species are Effective in Extracting Multiple Heavy Metals from Biosolids. International Journal of Phytoremediation 15, 615–632. https://doi.org/10.1080/15226514.2012.723063

Moreyra, A.K., Padovesi-Fonseca, C., 2015. Environmental effects and urban impacts on aquatic macroinvertebrates in a stream of central Brazilian Cerrado. Sustainable Water Resources Management 1, 125–136. https://doi.org/10.1007/s40899-015-0013-8

Morse, J., Bae, Y., Munkhjargal, G., Sangpradub, N., Tanida, K., Vshivkova, T., Wang, B., Yang, L., Yule, C., 2007. Freshwater biomonitoring with macroinvertebrates in East Asia. Frontiers in Ecology and The Environment 5, 33–42. https://doi.org/10.1890/1540-9295(2007)5[33:FBWMIE]2.0.CO;

Motevalli, A., Naghibi, S.A., Hashemi, H., Berndtsson, R., Pradhan, B., Gholami, V., 2019. Inverse method using boosted regression tree and k-nearest neighbor to quantify effects of point and non-point source nitrate pollution in groundwater. Journal of Cleaner Production 228, 1248–1263. https://doi.org/10.1016/j.jclepro.2019.04.293

Msuya, F.A., Brooks, R.R., Anderson, C.W.N., 2000. Chemically induced uptake of gold by root crops: Its significance for phytomining. Gold Bull 33, 134–137. https://doi.org/10.1007/BF03215491

Muchez, Ph., Brems, D., Clara, E., De Cleyn, A., Lammens, L., Boyce, A., De Muynck, D., Mukumba, W., Sikazwe, O., 2010. Evolution of Cu–Co mineralizing fluids at Nkana Mine, Central African Copperbelt, Zambia. Journal of African Earth Sciences 58, 457–474. https://doi.org/10.1016/j.jafrearsci.2010.05.003

Mukosa, F., Katebe, M., Sakyi, K., Mweemba, B., Mbewe, S., Sikazwe, W., 2020. The Impact of Youth Unemployment on the Zambian Economy. Journal of Economics and Sustainable Development 11, 81.

Mulenga, C., 2017. Foreign direct investment in Zambian mining sector: the need for environmental protection and human rights (Thesis). University of Pretoria.

Müller, F., Hoffmann-Kroll, R., Wiggering, H., 2000. Indicating ecosystem integrity — theoretical concepts and environmental requirements. Ecological Modelling 130, 13–23. https://doi.org/10.1016/S0304-3800(00)00210-6

Muller, W., Arimoro, F., Palmer, C., 2012. The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River, South Africa: a multimetric approach. African Journal of Aquatic Science 37, 191–200. https://doi.org/10.2989/16085914.2012.670613

Mulonga, N.G., 2017. Mining induced heavy metal soil and crop contamination in Chililabombwe on the Copperbelt of Zambia (Thesis). The University of Zambia.

Muma, D., Besa, B., Manchisi, J., Banda, W., 2020. Effects of mining operations on air and water quality in Mufulira district of Zambia: A case study of Kankoyo Township. Journal of the Southern

African Institute of Mining and Metallurgy 120, 287–298. https://doi.org/10.17159/2411-9717/952/2020
Munné, A., Prat, N., 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: An intercalibration approach with the IBMWP index. Hydrobiologia 628, 203–225. https://doi.org/10.1007/s10750-009-9757-1
Mwaanga, P., Silondwa, M., Kasali, G., Banda, P.M., 2019. Preliminary review of mine air pollution in Zambia. Heliyon 5. https://doi.org/10.1016/j.heliyon.2019.e02485
Mwedzi, T., Siziba, N., Odume, O.N., Nyamazana, E., Mabika, I., 2020. Responses of macroinvertebrate community metrics to urban pollution in semi-arid catchments around the city of Bulawayo, Zimbabwe. Water SA 46, 583–592.
Nalishuwa, L., 2015. Investigation on copper levels in and around fish farms in Kitwe, copperbelt province, Zambia (Thesis). Sokoine University of Agriculture.
Napoli, M., Cecchi, S., Grassi, C., Baldi, A., Zanchi, C.A., Orlandini, S., 2019. Phytoextraction of copper from a contaminated soil using arable and vegetable crops. Chemosphere 219, 122–129. https://doi.org/10.1016/j.chemosphere.2018.12.017
Ngubo, N., 2016. Contemporary insights into the dynamics of foreign direct investment in Zambia. (Thesis) University of Cape Town
Nguyen, H.H., Everaert, G., Gabriels, W., Hoang, T.H., Goethals, P.L.M., 2014. A multimetric acroinvertebrate index for assessing the water quality of the Cau river basin in Vietnam. Limnologica 45, 16–23. https://doi.org/10.1016/j.limno.2013.10.001
Nhiwatiwa, T., Dalu, T., Sithole, T., 2017. Assessment of river quality in a subtropical Austral River system: a combined approach using benthic diatoms and macroinvertebrates. Applied Water Science 7, 4785–4792. https://doi.org/10.1007/s13201-017-0599-0
Niba, A., Sakwe, S., 2018. Turnover of benthic macroinvertebrates along the Mthatha River, Eastern Cape, South Africa: implications for water quality biomonitoring using indicator species. Journal of Freshwater Ecology 33, 157–171. https://doi.org/10.1080/02705060.2018.1431969
Nikolic, M., Stevovic, S., 2015. Family Asteraceae as sustainable planning tool in phytoremediation and its relevance in urban areas. Urban Forestry & Urban Greening 14. https://doi.org/10.1016/j.ufug.2015.08.002
Nkrumah, P.N., Baker, A.J.M., Chaney, R.L., Erskine, P.D., Echevarria, G., Morel, J.L., van der Ent, A., 2016. Current status and challenges in developing nickel phytomining: an agronomic perspective. Plant Soil 406, 55–69. https://doi.org/10.1007/s11104-016-2859-4
Nkwunonwo, U.C., Odika, P.O., Onyia, N.I., 2020. A Review of the Health Implications of Heavy Metals in Food Chain in Nigeria. ScientificWorldJournal 2020. https://doi.org/10.1155/2020/6594109
Nordstrom, D., 2011. Hydrogeochemical processes governing the origin, transport and fate of major and trace elements from mine wastes and mineralized rock to surface waters. Applied Geochemistry 26, 1777–1791. https://doi.org/10.1016/j.apgeochem.2011.06.002
Norrgren, L., Pettersson, U., Örn, S., Bergqvist, P.-A., 2000. Environmental Monitoring of the Kafue River, Located in the Copperbelt, Zambia. Archives Environmental Contamination and Toxicology 38, 334–341. https://doi.org/10.1007/s002449910044
Ntengwe, F.W., 2005. An overview of industrial wastewater treatment and analysis as means of preventing pollution of surface and underground water bodies—the case of Nkana Mine in Zambia. Physics and Chemistry of the Earth, Parts A/B/C, Integrated Water Resources Management (IWRM) and the Millennium Development Goals: Managing Water for Peace and Prosperity 30, 726–734. https://doi.org/10.1016/j.pce.2005.08.014
Ntengwe, F.W., Maseka, K.K., 2006. The impact of effluents containing zinc and nickel metals on stream and river water bodies: The case of Chambishi and Mwambashi streams in Zambia. Physics and Chemistry of the Earth 31, 814–820. https://doi.org/10.1016/j.pce.2006.08.027

Ntshane, B.C., Gambiza, J., 2016. Habitat assessment for ecosystem services in South Africa. International Journal of Biodiversity Science, Ecosystem Services & Management 12, 242–254. https://doi.org/10.1080/21513732.2016.1217935

Nurfitri, A.G., Masayuki, S., Koichiro, S., 2017. Phytoremediation Of Heavy Metal-polluted Mine Drainage By Eleocharis Acicularis. Environmental Science: An Indian Journal 13, 131.

Nyström, E., Kaasalainen, H., Alakangas, L., 2019. Prevention of sulfide oxidation in waste rock by the addition of lime kiln dust. Environmental Science and Pollution Research 26, 25945–25957. https://doi.org/10.1007/s11356-019-05846-z

Oberholster, P.J., Botha, A.-M., Cloete, T.E., 2008. Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution 156, 184–192. https://doi.org/10.1016/j.envpol.2007.12.028

Oberholster, P.J., Myburgh, J.G., Ashton, P.J., Botha, A.-M., 2010. Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 73, 326–335. https://doi.org/10.1016/j.ecoenv.2009.08.011

Obiora, S.C., Chukwu, A., Davies, T.C., 2016. Heavy metals and health risk assessment of arable soils and food crops around Pb–Zn mining localities in Enyigba, southeastern Nigeria. Journal of African Earth Sciences 116, 182–189. https://doi.org/10.1016/j.jafrearsci.2015.12.025

Ochieng, G., Seanego, E., Nkwonta, O., 2010. Impacts of mining on water resources in South Africa: A review. Scientific Research and Essays 5, 3351–3357.

Olayinka-Olagunju, J.O., Dosumu, A.A., Olatunji-Ojo, A.M., 2021. Bioaccumulation of Heavy Metals in Pelagic and Benthic Fishes of Ogbese River, Ondo State, South-Western Nigeria. Water, Air, Soil and Pollution 232, 44. https://doi.org/10.1007/s11270-021-04987-7

Omar, W.M.W., 2010. Perspectives on the Use of Algae as Biological Indicators for Monitoring and Protecting Aquatic Environments, with Special Reference to Malaysian Freshwater Ecosystems. Trop Life Sci Res 21, 51–67.

Omer, N.H., 2019. Water Quality Parameters. Water Quality - Science, Assessments and Policy. https://doi.org/10.5772/intechopen.89657

Opitz, A.K.B., Broadhurst, J.L., Harrison, S.T.L., 2015. Assessing Environmental Risks Associated with Ultrafine Coal Wastes Using Laboratory-Scale Tests [WWW Document]. Advanced Materials Research. https://doi.org/10.4028/www.scientific.net/AMR.1130.635

Oremo, J., Orata, F., Owino, J., Shivoga, W., 2019. Assessment of heavy metals in benthic macroinvertebrates, water and sediments in River Isiukhu, Kenya. Environmental Monitoring and Assessment 191, 646. https://doi.org/10.1007/s10661-019-7858-5

Paisley, M., Walley, W.J., Nikhade, J., Dils, R., 2003. Identification of the Key Biological Indicators of Nutrient Enrichment in Rivers for Use in Predictive / Diagnostic Models [WWW Document]. URL /paper/Identification-of-the-Key-Biological-Indicators-of-Paisley-Walley/7fd953db9a045b0fa8cc8a661a3fa58a856a22e4 (accessed 4.30.21).

Pandey, J., Verma, R.K., Singh, S., 2019. Suitability of aromatic plants for phytoremediation of heavy metal contaminated areas: a review. International Journal of Phytoremediation 21, 405–418. https://doi.org/10.1080/15226514.2018.1540546

Pandiyan, J., Mahboob, S., Govindarajan, M., Al-Ghanim, K.A., Ahmed, Z., Al-Mulhm, N., Jagadheesan, R., Krishnappa, K., 2021. An assessment of level of heavy metals pollution in the water, sediment and aquatic organisms: A perspective of tackling environmental threats for food security. Saudi Journal of Biological Sciences 28, 1218–1225. https://doi.org/10.1016/j.sjbs.2020.11.072

Parbhakar-Fox, A., Lottermoser, B., Bradshaw, D., 2013. Evaluating waste rock mineralogy and microtexture during kinetic testing for improved acid rock drainage prediction. Minerals Engineering, Process Mineralogy 52, 111–124. https://doi.org/10.1016/j.mineng.2013.04.022

Pardo, T., Rodríguez-Garrido, B., Saad, R.F., Soto-Vázquez, J.L., Loureiro-Viñas, M., Prieto-Fernández, Á., Echevarria, G., Benizri, E., Kidd, P.S., 2018. Assessing the agromining potential of

Mediterranean nickel-hyperaccumulating plant species at field-scale in ultramafic soils under humid-temperate climate. Science of The Total Environment 630, 275–286. https://doi.org/10.1016/j.scitotenv.2018.02.229
Parmar, T.K., Rawtani, D., Agrawal, Y.K., 2016. Bioindicators: the natural indicator of environmental pollution. Frontiers in Life Science 9, 110–118. https://doi.org/10.1080/21553769.2016.1162753
Parrish, J.D., Braun, D.P., Unnasch, R.S., 2003. Are We Conserving What We Say We Are? Measuring Ecological Integrity within Protected Areas. BioScience 53, 851–860. https://doi.org/10.1641/0006-3568(2003)053[0851: AWCWWS]2.0.CO;2
Paustenbach, D., Galbraith, D., 2006. Biomonitoring and Biomarkers: Exposure Assessment Will Never Be the Same. Environmental Health Perspectives 114, 1143–1149. https://doi.org/10.1289/ehp.8755
Peng, H., Wang-Mueller, Q., Witt, T., Malaisse, F., Küpper, H., 2012. Differences in copper accumulation and copper stress between eight populations of Haumaniastrum katangense. Environmental and Experimental Botany 79, 58–65. https://doi.org/10.1016/j.envexpbot.2011.12.015
Peng, H. Y., Yang, X.-E., Tian, S., 2005. Accumulation and ultrastructural distribution of copper in Elsholtzia splendens. Journal of Zhejiang University. Science. B 6, 311–8. https://doi.org/10.1631/jzus.2005.B0311
Perera, L.G.R., Wattavidanage, J., 2011. Aquatic Macroinvertebrates as Bioindicators for stream and wetland health monitoring. Proceedings of the National Mini Symposium on Bioindicators of Environment Health and Biodiversity Indices. https://www.researchgate.net/publication/301549341
Perlatti, F., Ferreira, T.O., Romero, R.E., Costa, M.C.G., Otero, X.L., 2015. Copper accumulation and changes in soil physical–chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: Implications for restoration of mine sites. Ecological Engineering 82, 103–111. https://doi.org/10.1016/j.ecoleng.2015.04.085
Petersen-Perlman, J.D., 2016. Water Conflict/Cooperation Case Study: Zambezi River Basin☆, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-409548-9.09448-3
Pettersson, U., Ingri, J., 2001. The geochemistry of Co and Cu in the Kafue River as it drains the Copperbelt mining area, Zambia. Chemical Geology 177, 399–414. https://doi.org/10.1016/S0009-2541(00)00422-8
Pobi, K.K., Satpati, S., Dutta, S., Nayek, S., Saha, R.N., Gupta, S., 2019. Sources evaluation and ecological risk assessment of heavy metals accumulated within a natural stream of Durgapur industrial zone, India, by using multivariate analysis and pollution indices. Applied Water Science 9, 58. https://doi.org/10.1007/s13201-019-0946-4
Podolský, F., Ettler, V., Šebek, O., Ježek, J., Mihaljevič, M., Kříbek, B., Sracek, O., Vaněk, A., Penížek, V., Majer, V., Mapani, B., Kamona, F., Nyambe, I., 2015. Mercury in soil profiles from metal mining and smelting areas in Namibia and Zambia: distribution and potential sources. Journal of Soils and Sediments 15, 648–658. https://doi.org/10.1007/s11368-014-1035-9
Popov, O., Talovina, I., Lieberwirth, H., Duriagina, A., 2020. Quantitative Microstructural Analysis and X-ray Computed Tomography of Ores and Rocks—Comparison of Results. Minerals 10, 129. https://doi.org/10.3390/min10020129
Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E., 2011. Lead uptake, toxicity, and detoxification in plants. Reviews of Environmental Contamination and Toxicology 213, 113–136. https://doi.org/10.1007/978-1-4419-9860-6_4
Qu, X., Wu, N., Tang, T., Cai, Q., Park, Y.-S., 2010. Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams. Annales de Limnologie - International Journal of Limnology. 46, 291–302. https://doi.org/10.1051/limn/2010027

Qureshi, A., Maurice, C., Öhlander, B., 2016. Potential of coal mine waste rock for generating acid mine drainage. Journal of Geochemical Exploration 160, 44–54. https://doi.org/10.1016/j.gexplo.2015.10.014

Radwan, M., Salama, A., 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology 44, 1273–1278.

Rahman, M.A., Rahman, M.M., Reichman, S.M., Lim, R.P., Naidu, R., 2014. Heavy metals in Australian grown and imported rice and vegetables on sale in Australia: health hazard. Ecotoxicology and Environmental Safety 100, 53–60. https://doi.org/10.1016/j.ecoenv.2013.11.024

Rahmanian, N., Ali, S.H.B., Homayoonfard, M., Ali, N.J., Rehan, M., Sadef, Y., Nizami, A.S., 2015. Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia [WWW Document]. Journal of Chemistry. https://doi.org/10.1155/2015/716125

Rajakaruna, N., Bohm, B., 2002. Serpentine and its vegetation: A preliminary study from Sri Lanka. Journal of Applied Botany 76, 20–28.

Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F., Kim, K.-H., 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment International 125, 365–385. https://doi.org/10.1016/j.envint.2019.01.067

Rainaud, C., Master, S., Armstrong, R.A., Robb, L.J., 2005. Geochronology and nature of the Palaeoproterozoic basement in the Central African Copperbelt (Zambia and the Democratic Republic of Congo), with regional implications. Journal of African Earth Sciences 42, 1–31. https://doi.org/10.1016/j.jafrearsci.2005.08.006

Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K., 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. Agriculture, Ecosystems & Environment 109, 310–322. https://doi.org/10.1016/j.agee.2005.02.025

Reeves, R.D., Baker, A.J.M., Jaffré, T., Erskine, P.D., Echevarria, G., Ent, A. van der, 2018. A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytologist 218, 407–411. https://doi.org/10.1111/nph.14907

Reimann, C., Caritat, P. de, 2005. Distinguishing between natural and anthropogenic sources of element in the environment: regional geochemical surveys versus enrichment factors. The Science of the total environment 337, 91–107. https://doi.org/10.1016/j.scitotenv.2004.06.011

Resende, P.C., Resende, P., Pardal, M., Almeida, S., Azeiteiro, U., 2010. Use of biological indicators to assess water quality of the Ul River (Portugal). Environ Monit Assess 170, 535–544. https://doi.org/10.1007/s10661-009-1255-4

Resh, V.H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environmental Monitoring and Assessment 138, 131–138. https://doi.org/10.1007/s10661-007-9749-4

Resh, V.H., 2007. Multinational, Freshwater Biomonitoring Programs in the Developing World: Lessons Learned from African and Southeast Asian River Surveys. Environmental Management 39, 737–748. https://doi.org/10.1007/s00267-006-0151-8

Resh, V.H., Norris, R.H., Barbour, M.T., 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Australian Journal of Ecology 20, 108–121. https://doi.org/10.1111/j.1442-9993.1995.tb00525.x

Rimawi, O., Jiries, A., al-zubi, Y., El-Naqa, A., 2009. Reuse of mining wastewater in agricultural activities in Jordan. Environment Development and Sustainability 11, 695–703. https://doi.org/10.1007/s10668-007-9137-9

Rinne, J.N., 1990. The utility of stream habitat and biota for identifying potential conflicting forest land uses: Montane riparian areas. Forest Ecology and Management 33–34, 363–383. https://doi.org/10.1016/0378-1127(90)90204-O

Rodriguez, J., Salazar, M., Steffan, L., Pignata, M.L., Franzaring, J., Klumpp, A., Fangmeier, A., 2014. Assessment of Pb and Zn contents in agricultural soils and soybean crops near to a former battery recycling plant in C??rdoba, Argentina. Journal of Geochemical Exploration 145. https://doi.org/10.1016/j.gexplo.2014.05.025

Rola, K., 2011. Vascular flora of sites contaminated with heavy metals on the example of two post-industrial spoil heaps connected with manufacturing of zinc and lead products in upper silesia. Archives of Environmental Protection 37, 57–74.

Rosenberg, D.M., Resh, V.H. (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Springer US.

Rosenkranz, T., Hipfinger, C., Ridard, C., Puschenreiter, M., 2019. A nickel phytomining field trial using Odontarrhena chalcidica and Noccaea goesingensis on an Austrian serpentine soil. Journal of Environmental Management 242, 522–528. https://doi.org/10.1016/j.jenvman.2019.04.073

Rosenkranz, T., Kisser, J., Wenzel, W.W., Puschenreiter, M., 2017. Waste or substrate for metal hyperaccumulating plants — The potential of phytomining on waste incineration bottom ash. Science of The Total Environment 575, 910–918. https://doi.org/10.1016/j.scitotenv.2016.09.144

Roux, D.J., 2001. Strategies used to Guide the Design and Implementation of a National River Monitoring Programme in South Africa. Environmental Monitoring and Assessment 69, 131–158. https://doi.org/10.1023/A:1010793505708

Rowlands, L., 2019. Chapter 8 - Erosion and Sediment Control—WSUD During the Construction Phase of Land Development, in: Sharma, A.K., Gardner, T., Begbie, D. (Eds.), Approaches to Water Sensitive Urban Design. Woodhead Publishing, pp. 163–176. https://doi.org/10.1016/B978-0-12-812843-5.00008-3V

Sağlam, E.S., Akçay, M., 2016. Chemical and mineralogical changes of waste and tailings from the Murgul Cu deposit (Artvin, NE Turkey): implications for occurrence of acid mine drainage. Environmental Science and Pollution Research 23, 6584–6607. https://doi.org/10.1007/s11356-015-5835-2

Sainger, P.A., Dhankhar, R., Sainger, M., Kaushik, A., Singh, R.P., 2011. Assessment of heavy metal tolerance in native plant species from soils contaminated with electroplating effluent. Ecotoxicology and Environmental Safety 74, 2284–2291. https://doi.org/10.1016/j.ecoenv.2011.07.028

Saintilan, N.J., Selby, D., Creaser, R.A., Dewaele, S., 2018. Sulphide Re-Os geochronology links orogenesis, salt and Cu-Co ores in the Central African Copperbelt. Scientific Reports 8. https://doi.org/10.1038/s41598-018-33399-7

Sakakibara, M., Ohmori, Y., Ha, N.T.H., Sano, S., Sera, K., 2011. Phytoremediation of heavy metal-contaminated water and sediment by Eleocharis acicularis. CLEAN – Soil, Air, Water 39, 735–741. https://doi.org/10.1002/clen.201000488

Salmiati, Arman, N.Z., Salim, M.R., 2017. Integrated Approaches in Water Quality Monitoring for River Health Assessment: Scenario of Malaysian River, Water Quality. IntechOpen. https://doi.org/10.5772/65703

Samiyappan, M., 2019. Bioindicators in Aquatic Environment and their significance. https://doi.org/10.32381/JAT.2019.34.01.6

Schachtschneider, K., Chamier, J., Somerset, V., 2017. Phytostabilization of metals by indigenous riparian vegetation. Water SA 43, 177–185. https://doi.org/10.4314/wsa.v43i2.01

Schelle, P., Pittock, J., 2006. Restoring the Kafue Flats: A Partnership Approach to Environmental Flows in Zambia. Proceedings of the 8th International River symposium. Brisbane, Australia. Available from: www.riversymposium.com [Accessed 14 January 2021]

Scotti, A., Tappeiner, U., Bottarin, R., 2019. Stream benthic macroinvertebrates abundances over a 6-year monitoring period of an Italian glacier-fed stream. Biodiversity Data Journal 7, e33576. https://doi.org/10.3897/BDJ.7.e33576

Seshadri, B., Bolan, N.S., Naidu, R., 2015. Rhizosphere-induced heavy metal(loid) transformation in relation to bioavailability and remediation. Journal of soil science and plant nutrition 15, 524–548. https://doi.org/10.4067/S0718-95162015005000043

Shan, Q., Liu, X., Zhang, J., Chen, G., Liu, S., Zhang, P., Wang, Y., 2011. Analysis on the Tolerance of Four Ecotype Plants Against Copper Stress in Soil. Procedia Environmental Sciences, 2011 3rd International Conference on Environmental Science and Information Application Technology ESIAT 2011 10, 1802–1810. https://doi.org/10.1016/j.proenv.2011.09.282

Sharma, A., Nagpal, A.K., 2020. Contamination of vegetables with heavy metals across the globe: hampering food security goal. Journal of Food Science and Technology 57, 391–403. https://doi.org/10.1007/s13197-019-04053-5

Sheoran, V., S.Sheoran, A., Poonia, P., 2013. Phytomining of gold: A review. Journal of Geochemical Exploration 128, 42–50. https://doi.org/10.1016/j.gexplo.2013.01.008

Sheoran, V., Sheoran, A. s, Poonia, P., 2011. Role of Hyperaccumulators in Phytoextraction of Metals from Contaminated Mining Sites: A Review. Critical Reviews in Environmental Science and Technology 41, 168–214. https://doi.org/10.1080/10643380902718418

Sheoran, V., Sheoran, A.S., Poonia, P., 2009. Phytomining: A review. Minerals Engineering 22, 1007–1019. https://doi.org/10.1016/j.mineng.2009.04.001

Sherlock, E.J., Lawrence, R.W., Poulin, R., 1995. On the neutralization of acid rock drainage by carbonate and silicate minerals. Geoforum 25, 43–54. https://doi.org/10.1007/BF01061829

Shimaponda-Mataa, N.M., Tembo-Mwase, E., Gebreslasie, M., Achia, T.N.O., Mukaratirwa, S., 2017. Modelling the influence of temperature and rainfall on malaria incidence in four endemic provinces of Zambia using semiparametric Poisson regression. Acta Tropica 166, 81–91. https://doi.org/10.1016/j.actatropica.2016.11.007

Shu, X.-H., Zhang, Q., Lu, G.-N., Yi, X.-Y., Dang, Z., 2018. Pollution characteristics and assessment of sulfide tailings from the Dabaoshan Mine, China. International Biodeterioration & Biodegradation, Remediation and Restoration of Polluted Mining Areas 128, 122–128. https://doi.org/10.1016/j.ibiod.2017.01.012

Sikamo, J., Mwanza, A., Mweemba, C., 2016. Copper mining in Zambia - history and future. Journal of the Southern African Institute of Mining and Metallurgy 116, 491–496. https://doi.org/10.17159/2411-9717/2016/v116n6a1

Sillitoe, R., Perelló, J., Garcia, A., 2011. Sulfide-Bearing Veinlets Throughout the Stratiform Mineralization of the Central African Copperbelt: Temporal and Genetic Implications. Economic Geology 105, 1361–1368. https://doi.org/10.2113/econgeo.105.8.1361

Simate, G.S., Ndlovu, S., 2014. Acid mine drainage: Challenges and opportunities. Journal of Environmental Chemical Engineering 2. https://doi.org/10.1016/j.jece.2014.07.021

Simon, T.P., 2000. The use of biological criteria as a tool for water resource management. Environmental Science & Policy 3, 43–49. https://doi.org/10.1016/S1462-9011(00)00026-5

Singh, A., Prasad, S., 2014. Remediation of heavy metal contaminated ecosystem: an overview on technology advancement. International Journal of Environmental Science and Technology 12, 353–366. https://doi.org/10.1007/s13762-014-0542-y

Singh, K.P., Mohan, D., Sinha, S., Dalwani, R., 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. Chemosphere 55, 227–255. https://doi.org/10.1016/j.chemosphere.2003.10.050

Sinha, R., Singh, A.K., Bauddh, K., Sharma, T.R., Sharma, P., 2021. Chapter 21 - Phytomining: a sustainable approach for recovery and extraction of valuable metals, in: Bauddh, K., Korstad, J., Sharma, P. (Eds.), Phytorestoration of Abandoned Mining and Oil Drilling Sites. Elsevier 487–506. https://doi.org/10.1016/B978-0-12-821200-4.00013-3

Sonter, L.J., Ali, S.H., Watson, J.E.M., 2018. Mining and biodiversity: key issues and research needs in conservation science. Proceedings: Biological Sciences 285. https://doi.org/10.1098/rspb.2018.1926

Souilmi, F., Ghedda, K., Fahde, A., el Fihri, F.Z., Tahraoui, S., Elasri, F., Malki, M., 2021. Taxonomic diversity of benthic macroinvertebrates along the Oum Er Rbia River (Morocco): implications for water quality biomonitoring using indicator species. West African Journal of Applied Ecology 27, 137–149.

Španiel, S., Kempa, M., Salmerón-Sánchez, E., Fuertes-Aguilar, J., Mota, J.F., Al-Shehbaz, I.A., German, D.A., Olšavská, K., Šingliarová, B., Zozomová-Lihová, J., Marhold, K., 2015. AlyBase: database of names, chromosome numbers, and ploidy levels of Alysseae (Brassicaceae), with a new generic concept of the tribe. Plant Systematics and Evolution 301, 2463–2491. https://doi.org/10.1007/s00606-015-1257-3

Sracek, O., 2015. Formation of secondary hematite and its role in attenuation of contaminants at mine tailings: review and comparison of sites in Zambia and Namibia. Frontiers in Environmental Science 2. https://doi.org/10.3389/fenvs.2014.00064

Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Stanley Rungwa, Arpa, G., Sakulas, H., Harakuwe, A., Timi, D., 2013. Phytoremediation – An Eco-friendly and Sustainable Method of Heavy Metal Removal from Closed Mine Environments in Papua New Guinea. Procedia Earth and Planetary Science 6, 269–277. https://doi.org/10.1016/j.proeps.2013.01.036

Stewart, A.G., 2020. Mining is bad for health: a voyage of discovery. Environ Geochem Health 42, 1153–1165. https://doi.org/10.1007/s10653-019-00367-7

Stewart, W., Miller, S., Smart, R., 2006. Advances in Acid Rock Drainage (ARD) Characterisation of Mine Wastes 1. 7th International Conference on Acid Rock Drainage (ICARD), March 26-30, 2006, St. Louis MO. R.I. Barnhisel (ed.) Published by the American Society of Mining and Reclamation (ASMR), 3134 Montavesta Road, Lexington, KY 40502.

Steyn, M., Oberholster, P.J., Botha, A.M., Genthe, B., van den Heever-Kriek, P.E., Weyers, C., 2019. Treated acid mine drainage and stream recovery: Downstream impacts on benthic macroinvertebrate communities in relation to multispecies toxicity bioassays. Journal of Environmental Management 235, 377–388. https://doi.org/10.1016/j.jenvman.2019.01.051

Suman, J., Uhlik, O., Viktorova, J., Macek, T., 2018. Phytoextraction of Heavy Metals: A Promising Tool for Clean-Up of Polluted Environment? Frontiers in Plant Science 9. https://doi.org/10.3389/fpls.2018.01476

Sumudumali, R.G.I., Jayawardana, J.M.C.K., 2021. A Review of Biological Monitoring of Aquatic Ecosystems Approaches: with Special Reference to Macroinvertebrates and Pesticide Pollution. Environmental Management 67, 263–276. https://doi.org/10.1007/s00267-020-01423-0

Sun, Z., Sokolova, E., Brittain, J.E., Saltveit, S.J., Rauch, S., Meland, S., 2019. Impact of environmental factors on aquatic biodiversity in roadside stormwater ponds. Scientific Reports 9, 5994. https://doi.org/10.1038/s41598-019-42497-z

Suresh, G., Ramasamy, V., Meenakshisundaram, V., Venkatachalapathy, R., Ponnusamy, V., 2011. Influence of mineralogical and heavy metal composition on natural radionuclide concentrations in the river sediments. Applied Radiation and Isotopes 69, 1466–1474. https://doi.org/10.1016/j.apradiso.2011.05.020

Suriano, M.T., Fonseca-Gessner, A.A., Roque, F.O., Froehlich, C.G., 2011. Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. Environmental Monitoring and Assessment 175, 87–101. https://doi.org/10.1007/s10661-010-1495-3

Suruchi, Khanna, P., 2011. Assessment of Heavy Metal Contamination in Different Vegetables Grown in and Around Urban Areas. Research Journal of Environmental Toxicology 5, 162–179. https://doi.org/10.3923/rjet.2011.162.179

Taylor, J.C., Prygiel, J., Vosloo, A., de la Rey, P.A., van Rensburg, L., 2007. Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area. Hydrobiologia 592, 455–464. https://doi.org/10.1007/s10750-007-0788-1
Thiere, G., Schulz, R., 2004. Runoff-related agricultural impact in relation to macroinvertebrate communities of the Lourens River, South Africa. Water Research 38, 3092–3102. https://doi.org/10.1016/j.watres.2004.04.045
Thorne, R., Williams, P., 1997. The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment. Freshwater Biology 37, 671–686. https://doi.org/10.1046/j.1365-2427.1997.00181.x
Thorne, R., Williams, W., Gordon, C., 2000. The Macroinvertebrates of a Polluted Stream in Ghana. Journal of Freshwater Ecology 15, 209–217. https://doi.org/10.1080/02705060.2000.9663738
Tognacchini, A., Salinitro, M., Puschenreiter, M., van der Ent, A., 2020. Root foraging and avoidance in hyperaccumulator and excluder plants: a rhizotron experiment. Plant Soil 450, 287–302. https://doi.org/10.1007/s11104-020-04488-2
Tough, L., Böhm, M., Pecorelli, J., McRae, L., England, J., 2020. Changes in London’s freshwater macroinvertebrates over time (1990-2019) and their responses to water quality, flow and the work of citizen scientists.
Townsend, K., PettigroveA, V., Carew, M., HoffmannA, A., 2009. The effects of sediment quality on benthic macroinvertebrates in the River Murray, Australia. Marine and Freshwater Research 60. https://doi.org/10.1071/MF08121
Uherek, C.B., Pinto Gouveia, F.B., 2014. Biological Monitoring Using Macroinvertebrates as Bioindicators of Water Quality of Maroaga Stream in the Maroaga Cave System, Presidente Figueiredo, Amazon, Brazil. International Journal of Ecology 2014, e308149. https://doi.org/10.1155/2014/308149
Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustos, P., Zupan, M., Wenzel, W.W., 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environmental Pollution 148, 107–114. https://doi.org/10.1016/j.envpol.2006.10.035
Ustaoğlu, F., Tepe, Y., 2019. Water quality and sediment contamination assessment of Pazarsuyu Stream, Turkey using multivariate statistical methods and pollution indicators. International Soil and Water Conservation Research 7, 47–56. https://doi.org/10.1016/j.iswcr.2018.09.001
van Damme, P.A., Hamel, C., Ayala, A., Bervoets, L., 2008. Macroinvertebrate community response to acid mine drainage in rivers of the High Andes (Bolivia). Environmental Pollution 156, 1061–1068. https://doi.org/10.1016/j.envpol.2008.04.018
van der Ent, A., Baker, A.J.M., Reeves, R.D., Chaney, R.L., Anderson, C.W.N., Meech, J.A., Erskine, P.D., Simonnot, M.-O., Vaughan, J., Morel, J.L., Echevarria, G., Fogliani, B., Rongliang, Q., Mulligan, D.R., 2015. Agromining: farming for metals in the future? Environmental Science and Technology 49, 4773–4780. https://doi.org/10.1021/es506031u
van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3
van der Ent, A., Erskine, P., 2015. The potential of Zambian copper-cobalt hyper-accumulator plants for phytoremediation of polluted (mining/smelter) soils. IM4DC action research report.
van der Ent, A., Mak, R., de Jonge, M.D., Harris, H.H., 2018. Simultaneous hyperaccumulation of nickel and cobalt in the tree Glochidion cf. sericeum (Phyllanthaceae): elemental distribution and chemical speciation. Scientific Reports 8, 9683. https://doi.org/10.1038/s41598-018-26891-7
Vara Prasad, M.N., de Oliveira Freitas, H.M., 2003. Metal hyperaccumulation in plants: Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6, 285–321.

Vinson, M., Dinger, E., Kotynek, J., Dethier, M., 2008. Effects of oil pollution on aquatic macroinvertebrate assemblages in Gabon wetlands. African Journal of Aquatic Science 33, 261–268. https://doi.org/10.2989/AJAS.2008.33.3.9.621
von der Heyden, C.J., New, M.G., 2004. Sediment chemistry: a history of mine contaminant remediation and an assessment of processes and pollution potential. Journal of Geochemical Exploration 82, 35–57. https://doi.org/10.1016/j.gexplo.2003.11.001
Vriens, B., Smith, L., Mayer, K.U., Beckie, R.D., 2019. Poregas distributions in waste-rock piles affected by climate seasonality and physicochemical heterogeneity. Applied Geochemistry 100, 305–315. https://doi.org/10.1016/j.apgeochem.2018.12.009
Wang, H., Zhong, G., 2011. Effect of Organic Ligands on Accumulation of Copper in Hyperaccumulator and Non-Accumulator Commelina communis. Biological trace element research 143, 489–99. https://doi.org/10.1007/s12011-010-8850-7
Wang, S., Mulligan, C.N., 2009. Enhanced mobilization of arsenic and heavy metals from mine tailings by humic acid. Chemosphere 74, 274–279. https://doi.org/10.1016/j.chemosphere.2008.09.040
Wang, X., Tan, X., 2017. Macroinvertebrate community in relation to water quality and riparian land use in a substropical mountain stream, China. Environmental Science and Pollution Research 24. https://doi.org/10.1007/s11356-017-9042-1
Watts, G., Jenkins, A., Hess, T., Humble, A., Olbert, C., Kay, M., Pope, V., Stannard, T., Storey, M., Meacham, T., Benton, T., Noble, A., 2015. Agriculture's impacts on water availability. https://doi.org/10.13140/RG.2.2.25578.95681
Weber, P., Hughes, J., Conner, L.B., Lindsay, P., St, R., Smart, C., 2006. Short-Term Acid Rock Drainage Characteristics Determined by Paste pH And Kinetic NAG Testing: Cypress Prospect, New Zealand 1. Journal of the American Society of Mining and Reclamation 2006, 2289-2310. DOI:10.21000/JASMR06022289
Weisener, C.G., Weber, P.A., 2010. Preferential oxidation of pyrite as a function of morphology and relict texture. New Zealand Journal of Geology and Geophysics 53, 167–176. https://doi.org/10.1080/00288306.2010.499158
Wenger, A.S., Harvey, E., Wilson, S., Rawson, C., Newman, S.J., Clarke, D., Saunders, B.J., Browne, N., Travers, M.J., Mcilwain, J.L., Erftemeijer, P.L.A., Hobbs, J.-P.A., Mclean, D., Depczynski, M., Evans, R.D., 2017. A critical analysis of the direct effects of dredging on fish. Fish and Fisheries 18, 967–985. https://doi.org/10.1111/faf.12218
Wepener, V., 2008. Application of active biomonitoring within an integrated water resources management framework in South Africa. South African Journal of Science 104, 367–373.
Westra, L., 2000. Peter Miller, Forestry and the Ethics of Integrity. Business & Professional Ethics Journal 19, 91–95.
Wieszczycka, K., 2018. Wastes generated by mineral extraction industries. Physical Sciences Reviews 3, 2018-0026. https://doi.org/10.1515/psr-2018-0026
Wilderode, J., El Desouky, H., Elburg, M., Vanhaecke, F., Muchez, P., 2014. Metal sources for the Katanga Copperbelt deposits (DRC): Insights from Sr and Nd isotope ratios. Geologica Belgica 17, 137–147.
Wilson-Corral, V., Anderson, C., Rodriguez-Lopez, M., Arenas-Vargas, M., Lopez-Perez, J., 2011. Phytoextraction of gold and copper from mine tailings with Helianthus annuus L. and Kalanchoe serrata L. Minerals Engineering 24, 1488–1494. https://doi.org/10.1016/j.mineng.2011.07.014
Woch, M., Radwańska, M., Stefanowicz, A., 2013. Flora of spoil heaps after hard coal mining in Trzebinia (southern Poland): Effect of substratum properties. Acta Botanica Croatica 72. https://doi.org/10.2478/v10184-012-0020-x
Worlanyo, A.S., Jiangfeng, L., 2021. Evaluating the environmental and economic impact of mining for post-mined land restoration and land-use: A review. Journal of Environmental Management 279, 111623. https://doi.org/10.1016/j.jenvman.2020.111623

Wuana, R.A., Okieimen, F.E., 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation [WWW Document]. ISRN Ecology. International Scholarly Research Notices 2011. https://doi.org/10.5402/2011/402647

Wurtzebach, Z., Schultz, C., 2016. Measuring Ecological Integrity: History, Practical Applications, and Research Opportunities. BioScience 66, 446–457. https://doi.org/10.1093/biosci/biw037

Xiang, M., Li, Yan, Yang, J., Lei, K., Li, Yi, Li, F., Zheng, D., Fang, X., Cao, Y., 2021. Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. Environmental Pollution 278, 116911. https://doi.org/10.1016/j.envpol.2021.116911

Xu, M., Wang, Z., Duan, X., Pan, B., 2014. Effects of pollution on macroinvertebrates and water quality bio-assessment. Hydrobiologia 729, 247-259

Yabe, J., Ishizuka, M., Umemura, T., 2010. Current levels of heavy metal pollution in Africa. Journal of Veterinary Medical Science 72, 1257–1263. https://doi.org/10.1292/jvms.10-0058

Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany 76, 167–179. https://doi.org/10.1016/j.sajb.2009.10.007

Yang, J., Ma, S., Zhou, J., Song, Y., Li, F., 2018. Heavy metal contamination in soils and vegetables and health risk assessment of inhabitants in Daye, China. Journal of International Medical Research 46, 3374–3387. https://doi.org/10.1177/0300060518758585

Yang, Y., Li, Y., Sun, Q., 2014. Archaeal and bacterial communities in acid mine drainage from metal-rich abandoned tailing ponds, Tongling, China. Transactions of Nonferrous Metals Society of China 24, 3332–3342. https://doi.org/10.1016/S1003-6326(14)63474-9

Yi, Y., Wang, Z., Zhang, K., Yu, G., Duan, X., 2008. Sediment pollution and its effect on fish through food chain in the Yangtze River. International Journal of Sediment Research 23, 338–347. https://doi.org/10.1016/S1001-6279(09)60005-6

Young, P.L., 1997. The longevity of minewater pollution: a basis for decision-making. Science of The Total Environment 194–195, 457–466. https://doi.org/10.1016/S0048-9697(96)05383-1

Yoshimura, C., Tockner, K., Omura, T., Moog, O., 2006. Species diversity and functional assessment of macroinvertebrate communities in Austrian rivers. Limnology 7, 63–74. https://doi.org/10.1007/s10201-006-0170-4

Yuan, Q., Wang, P., Wang, C., Chen, J., Wang, X., Liu, S., 2021. Indicator species and co-occurrence pattern of sediment bacterial community in relation to alkaline copper mine drainage contamination. Ecological Indicators 120, 106884. https://doi.org/10.1016/j.ecolind.2020.106884

Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606, 135–50. https://doi.org/10.1016/j.aca.2007.11.018

Zhou, Z., Chen, Z., Pan, H., Sun, B., Zeng, D., He, L., Yang, R., Zhou, G., 2018. Cadmium contamination in soils and crops in four mining areas, China. Journal of Geochemical Exploration 192, 72–84. https://doi.org/10.1016/j.gexplo.2018.06.003

Zia, H., Harris, N.R., Merrett, G.V., Rivers, M., Coles, N., 2013. The impact of agricultural activities on water quality: A case for collaborative catchment-scale management using integrated wireless sensor networks. Computers and Electronics in Agriculture 96, 126–138. https://doi.org/10.1016/j.compag.2013.05.001

CHAPTER 3: ASSESSING ECOLOGICAL RISKS ASSOCIATED WITH TAILINGS STORAGE FACILITIES ON THE ZAMBIAN COPPERBELT

Acid rock drainage (ARD) and associated metal mobilization represents a major potential environmental impact of mining sulphidic minerals. To mitigate its impact, appropriate characterization of hard rock tailings, waste rock, fine coal waste etc., is required. In this study, we have addressed both the ARD generating potential of copper tailing materials and ecological risks associated with metal mobilization from the waste. Our work is informed by the over-arching premise that understanding the risks posed by the mineral waste will provide potential for improved monitoring of water quality and allow us to develop mitigation strategies in regions associated with mining activities. Using the standard ARD and biokinetic characterization tests, we explore the potential for ARD formation. Thereafter, we extend the analysis using the column bioleach experiments to assess the ecological burden and shed light on metal mobility under conditions ranging from neutral through varying levels of acidic conditions. Through these approaches, an appropriate environment can be created to simulate potential ARD generation, mobilization of metals and ecological burden. This suite of tests is critical for improved monitoring and mitigation strategies of ecosystems, an area of increasing importance as we work towards environmental sustainability.

3.1. Introduction

One of the largest environmental problems arising from the mining activities is the contamination of associated water bodies through the formation of acid rock drainage (ARD) and mobilisation of contaminants, especially metal mobilisation (Masindi et al., 2018; Naidu *et al*., 2019; Neculita and Rosa, 2019). ARD from the mining related materials, such as waste rock dumps and tailings storage facilities (TSF), is primarily a function of the availability of oxygen and water, and the mineralogy of the rock material, specifically the presence of metal sulphides (Larsson *et al*., 2018; Madzivire *et al*., 2019). Mineralogy and other factors contributing to the formation of ARD vary from site to site, and the availability or abundance of naturally occurring iron- and sulphur-oxidizing microorganisms and iron in the ambient environment accelerates the generation of ARD in mine wastelands (Nordstrom *et al*., 2015). Equally, underground pits and surface excavations associated with subgrade ore have potential for ARD generation and metal mobilisation. ARD is normally characterised by the low pH (pH 2 to 3) of surface and groundwater, high salinity and sustained metal dissolution in the ambient environment. The acidity aggravates metal dissolution. ARD can persist for centuries post mining (Fan *et al.*, 2016; Larsson *et al.*, 2018). Metal loads can cause damage to the ecosystem services and negatively impact human health (Christophoridis *et al.*, 2019; Korkmaz *et al.*, 2019; Mao *et al.*, 2019). Mine wastes without buffering capacity and with oxidisable material and low organic matter are most likely to release metals into the environment (Carvalho *et al*., 2014; Heikkinen and Räisänen, 2009). The strong focus on ARD generation potential has resulted in limited information on the mobility of elements from mine waste material and associated environmental degradation from elemental concentrations in neutral drainage (Opitz *et al.*, 2016; Plante *et al*., 2011a, 2011b). This is because samples classified as non-acid generating are seldom subjected to further tests. The possibility of metal mobilization with associated environmental degradation can also occur in the absence of ARD. Weathering of high carbonate rock may result in the formation of drainage that is circumneutral for years, with lower concentration of metals (Vriens et al., 2019).

In the recent past, management and control of metal pollution from mine wastes have become important aspects of environmental management (Ben Ali *et al.*, 2019; Fernando *et al.*, 2018; Masindi *et al.*, 2018). Studies on the application of physical, chemical and biological methods to control metal pollution have been undertaken in many mining regions (Carvalho *et al*., 2014; Jamieson *et al.*, 2015; Wang *et al.*, 2019). To use any of these methods to mitigate metal mobilization from tailings effectively, release information of the metals must be understood. This is because tailings properties are distinct in different mines due to compositional differences in the mineralogy of the ore as well as diverse processing and dumping approaches (Jamieson *et al*., 2015; Wang *et al.*, 2019). The distinct tailing properties can affect release information of metals from tailings such as the release period and type of metals released. Understanding the release information of metals from tailings can be used to select appropriate approaches to control pollution of ambient environment. The ambient environment is affected by the drainage from the mine wastes due to its low or elevated pH and its salinity, coupled with the mobilization of metal species from the mineral wastes. Over time, drainage from mine waste can cause significant ecological damage.

In Zambia, studies related to the potential for ARD and metal mobilization from mine waste are limited, despite the prevalence of mineral sulphides such as pyrite, carrolite, chalcocite and chalcopyrite (Broughton, 2013; Davey *et al*., 2020). Mine waste has been shown as a major source of metal contamination in the Kafue River catchment (Chileshe *et al*., 2020; Mbewe *et al.*, 2016; Sracek *et al*., 2012). Investigating the ecological risks in the Kafue River catchment in the Copperbelt Province of Zambia that are linked with copper tailing storage facilities in the region provides an opportunity to understand the potential ecological implication of ARD and metal mobilization on the aquatic ecosystem and, where necessary, propose mitigation approaches. In this component of the study, the potential for ARD generation from Chibuluma TSF (active), TSF14 (passive) and TSF15A (active) material is assessed and associated metal mobilization from the TSFs. The selection process for these TSFs was influenced by close proximity to aquatic ecosystem and arable land. The results generated were compared to the influence of TSFs on the ecosystem in subsequent chapters to enhance understanding of these relationships and insight into potential mitigation approaches.

3.2. Materials and Methods

3.2.1. Description of Study Area and Sample Selection

TSF samples were collected from three copper tailing storage facilities (Chibuluma TSF, TSF15A and TSF14 respectively) sourced from the Copperbelt in Zambia (Figure 3-1). From each TSF, 30 kg of tailing material was collected from several sampling points. Selection of study sites was influenced by similarities in geology of tailings, geographical location, proximity to water resources and agricultural lands, thus permitting a precise comparative evaluation of the impacts of TSFs on aquatic and agro ecosystems.

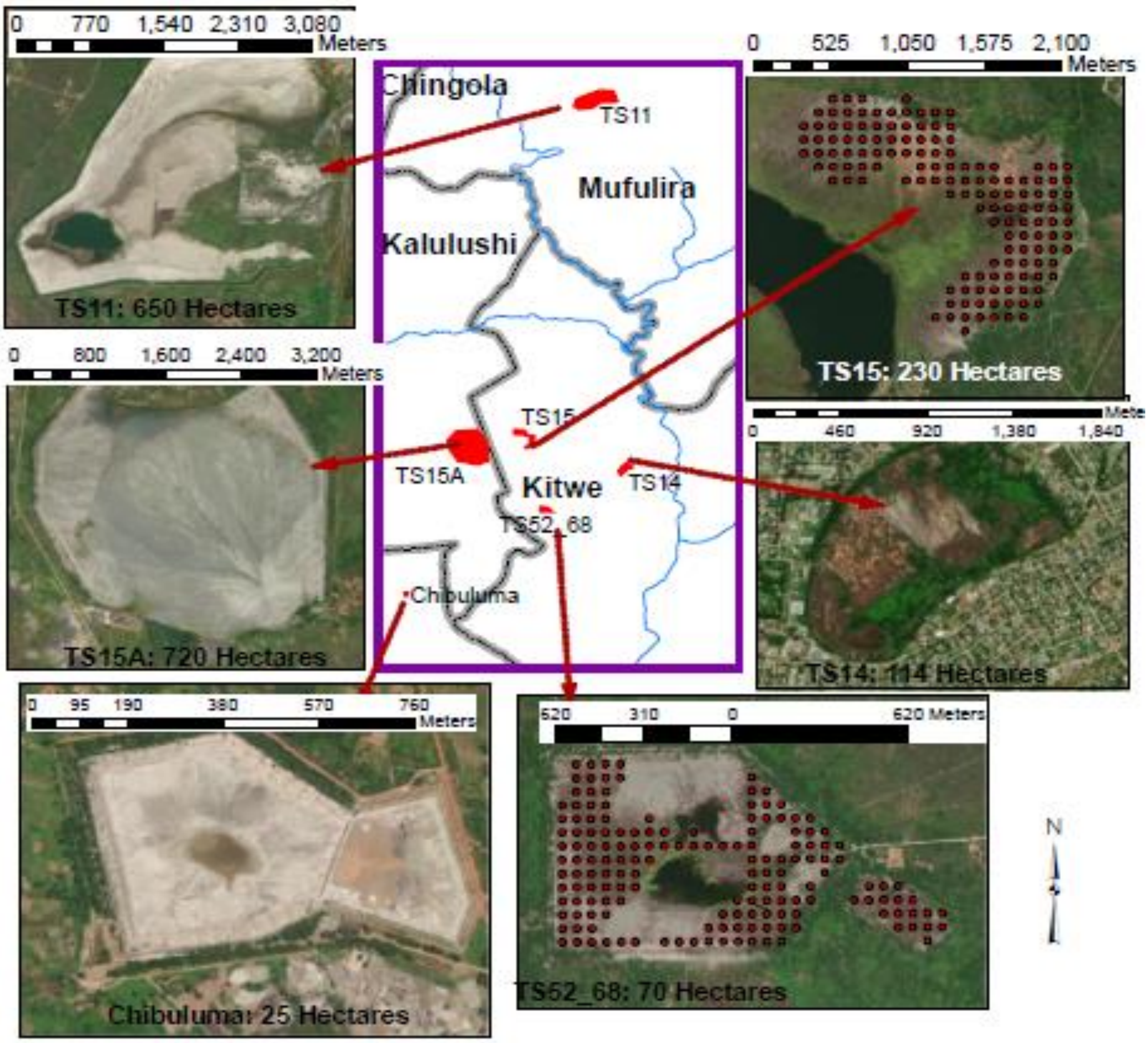


Figure 3-1: Shows the selected TSF within the Kafue River catchment. (tailing samples from Chibuluma TSF, TSF14 and TSF15A have been used in this study)

Since individual samples were used to test and classify larger volumes of waste, it is important to ensure best representativity of samples collected. Compositing, a common practice used to sample large volumes of material (Lovison *et al*., 1994; Patil, 1995), was adopted. Twenty (20) sampling points were selected in each tailing storage facility reservoir following a systematic random pattern (Singh and Mangat, 1996). At each sampling point, approximately 2.5 kg of tailings material were collected at 1 m, 1.5 m, and 2 m depth respectively.

The collected samples were air-dried and passed through a 150 µm sieve for the purposes of homogenization and removal of large particles. Less than 20% of samples were above 150 µm. The samples were split using a Dickie and Stockler rotary splitter; the resulting samples were used for mineralogy and geochemical characterization, LECO analysis, ARD, and column leach experiments.

3.2.2. Mineralogical and Geochemical Characterisation

The mineralogical composition of a representative fraction of the tailing material was analysed using X-ray diffraction (XRD)A (Department of Geology, University of Cape Town). The total sulphur content was analysed using thermal decomposition combustion infrared spectrophotometry by way of a LECO S632 Sulphur analyser. The metal content (Cu, Fe, Co, Mn, Zn, Pb, Ni, Cd, As, Ca, Mg, Al) in the tailings was determined by acid digestion (HNO_3/ $HClO_4$/HF/HCl) followed by inductively coupled plasma atomic emission spectrometry (ICP-AES IRIS Intrepid, Thermo Electron Corporation). These analyses were conducted in the analytical laboratory in the Department of Chemical Engineering at the University of Cape Town.

3.2.2.1. XRD Test

The XRD analysis was done in duplicate for the three copper tailing samples. The samples were spiked with 10 % corundum standard in order to identify the amorphous phase's percentage in the samples. The samples were first mixed with the corundum then the mixture was micronized to 10 µm allowing intimate mixing and liberation of particles. After micronizing, the samples were dried by placing under drying lamps. The samples were then analysed by a powder diffractometric XRD, Bruker D8 equipped with Vantec detector. The detector has a fixed divergence and receiving slits with Co-K radiation. The Bruker Topas 4.1

software was used to identify the phases which were then calculated by Rietveld method to their respective percentages. The QXRD analysis provides a preliminary mineralogical assessment of the samples

3.2.3. ARD Characterisation Tests

Acid-base accounting was used to characterise the overall ARD potential for the copper tailings samples using the difference in the inherent potential to generate and neutralise acidity. The Net Acid Producing Potential (NAPP) represented the estimation of ARD potential. The NAPP was quantified as the difference between maximum potential acid generation (MPA) (Weber et al., 2006) and acid neutralizing capability (ANC) (Smart *et al*., 2002), expressed as kg H_2SO_4/ton of tailing material. A positive NAPP indicates that the sample is acid generating whilst a negative value indicates that the ANC is sufficient to neutralize the acid generated, provided their rates of generation are matched in an open system.

In conjunction with the standard acid-base accounting (ABA), the net acid generation (NAG) tests supplemented the classification of ARD generation potential of tailings samples. The NAG test was assessed using a progression of sequential NAG tests until stabilization of pH at pH 4.5. As suggested by Stewart *et al*. (2006), using both the NAG and NAPP methods for ARD characterization might outline potential issues in the MPA and ANC estimations, and as a result, the likelihood of mis-classification of samples may be reduced.

To assess the potential of ARD under microbial conditions and provide a time correlated attestation on the acid generating and acid neutralizing behaviour, the biokinetic tests were conducted using the procedure outlined by Hesketh et al. (2010). The standard ABA protocols frequently used in ARD classification of solid wastes do not account for the influence of microbial activities, the presence of organic sulphur in ARD generation or the relative rates of acid generation and neutralisation. Following inoculation with a defined microbial consortium, the biokinetic tests were conducted to assess the potential generation of ARD under biotic conditions and congregate information on the rate at which acidification and neutralision occurs. The biokinetic tests were expanded to two sets of test conditions i.e., inoculated and pH controlled (pH 2), and inoculated without pH control. In summary, a 7.5g of sample of tailings material (100 per cent < 150 µm) was suspended in 150 mL autotrophic basal salt media and inoculated with 1×10^9 cells of a mesophilic mixed culture dominated by the iron-oxidiser Leptospirillum ferriphilum (ATC 49881) and 1×10^9 cells of an Acidithiobacillus caldus (DSM 8485) culture (a sulphur-oxidiser) obtained from stock cultures maintained at the Centre for Bioprocess Engineering Research (CeBER) at UCT. The flasks were maintained in an orbital shaker (150 rpm) at 37 ^oC for 90 days. Samples were taken for measurement of pH, redox potential, ferrous and total iron concentrations as per Hesketh et al. (2010). The Metrohm 713 pH meter was employed to measure pH, while the Metrohm 704 Eh meter was used to measure redox potential. Ferrous and total iron concentration were quantified colorimetrically using a 1-10 phenanthroline method. The absorbance readings were measured at a wavelength of 510 nm using a Helios UV-Vis spectrophotometer.

3.2.4. Column Bioleach Tests

To simulate conditions in the TSFs, laboratory scale column leach reactors (Figure 3-2) were operated under three conditions. While not a full representation of TSFs conditions, these conditions simulated both neutral irrigation and conditions that aggravate ARD formation, thus allowing the experiments to be conducted within a feasible timeframe. ARD formation, salinity and metal deportment were considered. Samples from Chibuluma TSF, TSF14 and TSF15A were selected to explore simulated metal mobilization under varying conditions (Table 3-1). Through this approach, the ecological burden associated with metal mobility can be used for improved monitoring and mitigation of water quality.

3.2.4.1. *Experimental design*

The ARD generation and deportment of heavy metals from the TSFs was investigated in six bioleach columns. Column 1 to 4 were used to investigate the leaching of the Chibuluma TSF under different conditions. Columns 5 and 6 were used to study the tailings from TSF14 and TSF15A. Column 1 (inoculated) was employed to investigate the release behaviour under neutral irrigation conditions, using deionised H_2O. Columns 2 to 6 investigated release behaviour under acid conditions by irrigating with acidified H_2O at pH 1.5 using different irrigation regimes. Columns 2 and 3 investigated Chibuluma tailings under acidified conditions following inoculation with a Fe- and S-oxidising microbial consortium under continuous and intermittent irrigation. Column 4 formed the un-inoculated control for Chibuluma tailings under continually irrigated acid conditions. In Column 5 and 6, the conditions of acidified continuous irrigation and inoculation used in Column 2 were used for TSF14 and TSF15A. Details of the bioleach column conditions are presented in Table 3-1.

3.2.4.2. *Column Design*

The column bioleach experiment method have been used to investigate mobilization of metals from solid material This method can be used to simulate waste dump conditions and a long-term release process of metals from solid waste, although this is not fully representative of dump conditions (Fan et al., 2016; Guo et al., 2013; Opitz, 2013).

Polyvinyl chloride (PVC) laboratory scale column reactors (Figure 3-2) were used in this study. The height of the reactors was 50 cm with an internal diameter of 10 cm. Temperature in the column was controlled to 26^{o}C, using an external heating coil; sensors for temperature were located internally within the ore bed and externally on the surface of the columns. Insulation was used to promote heat retention.

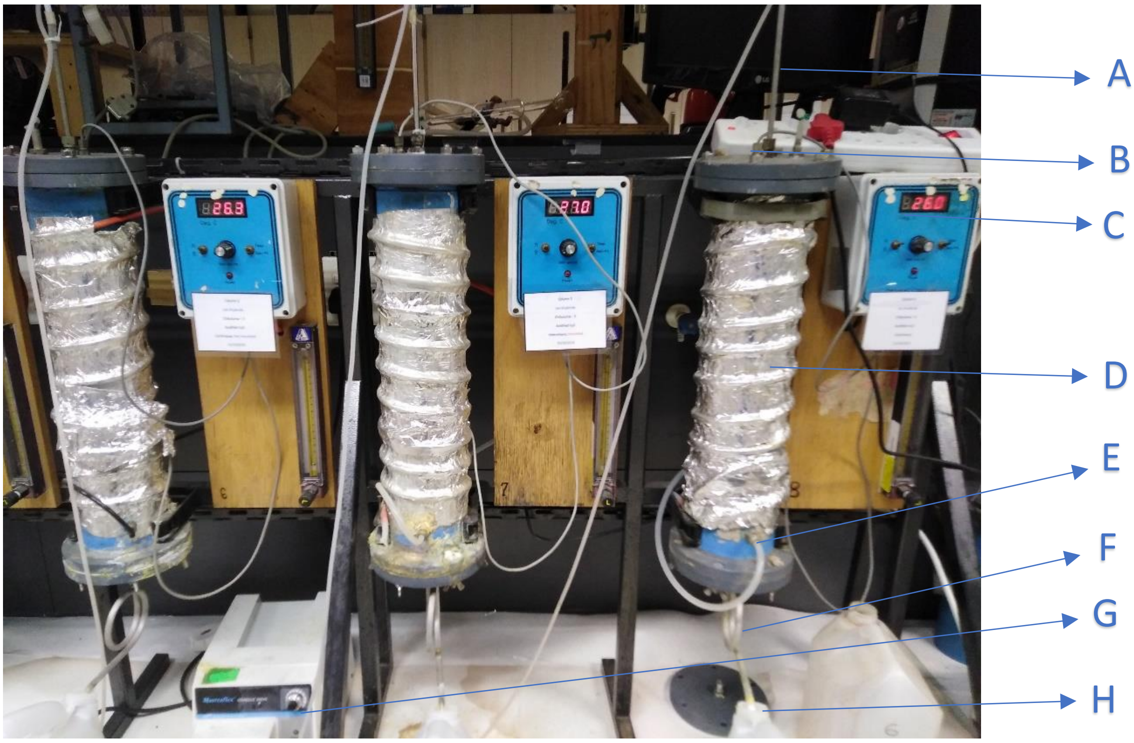


Figure 3-2: Bioleach column reactor system. A (feeding inlet), B (inside temperature sensor), C (temperature control unit), D (outside temperature sensor), E (air inlet), G (pump) and H (collection of leachates)

3.2.4.3. *Column Loading*

The column experiments conducted over six months are detailed in Table 3-1. Columns 1 to 4 contain Chibuluma tailings, Column 5 tailings from TSF 14 and Column 6 tailings from TSF

15A. In each experiment, 1 kg of tailings material (100% passing 150 μm) was agglomerated with 50 ml of H_2O/kg and 3.7 ml H_2SO_4/kg. The mixture was coated onto gray wacke rocks (8 – 10 mm diameter) at a mass ratio of 1 (tailings sample) to 3 (support material) in a manner similar to the GEOCoat™ process. The gray wacke coated with agglomerated material was packed in the column with 4 kg at the ratio of 1 to 3 in each column. Prior to these tests, the gray wacke was subjected to the leach test and slake durability test and was observed not to leach nor disintegrate. The packing was placed onto two layers of marbles to ensure good drainage of the unsaturated ore bed. On completion, two layers of marbles were placed on top of the bed to ensure distribution of irrigant. Masses of all elements were recorded throughout the packing process.

3.2.4.4. *Column Operation and Analysis*

The columns were irrigated at flow rates of 40 mL/h. Column 1 was irrigated with deionised water. The feed solution for columns 2 to 6 was comprised of 2g/L ferrous sulphate, 183.3 mg/L $(NH_4)_2SO_4$, 60.5 mg/L $NH_4H_2PO_4$, and 111.2 mg/L K_2SO_4 with pH adjusted to 1.5 using concentrated H_2SO_4. This provided nutrients equivalent to 50 mg/L each of NH_4, PO_4, and K respectively. With the exception of column 3, the bioleach columns were irrigated continuously at 40 mL/h using Masterflex peristaltic pumps. Intermittent irrigation was applied to column 3 to compare metal dissolution to continuous irrigation. The same flow rate was used, the column was fed 3 days/per week. Compressed air was introduced into the bottom of the bioleach columns at a flowrate of 12 L/min to aerate the columns. The leachate from the columns was collected from the bottom of the columns into 5 L sample containers with its volume recorded daily. Approximately 940 mL of leachate solution was collected over a 24-hour period. A 20 mL fresh sample in the hour preceding sampling was used for redox potential, pH, and iron analyses, while another 20 mL from the bulk collection was stored for further elemental analyses.

The microbial activity within the column leaching system was indicated by the redox potential, a measure of the ferrous to ferric ratio owing to the catalytic acceleration of ferrous iron oxidation. While all the columns were inoculated with identical cultures, except column 2 which remained un-inoculated, any subsequent change in microbial community was not investigated.

Table 3-1: Summary of the experimental conditions for the bioleach column tests

Parameter	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Filling material	1 kg tailings / 3 kg GW	1 kg tailings / 3 kg GW	1 kg tailings / 3 kg GW	1 kg tailings / 3 kg GW	1 kg tailings / 3 kg GW	1 kg tailings / 3 kg GW
Sample type	Chibuluma TSF	Chibuluma TSF	Chibuluma TSF	Chibuluma TSF	TSF14	TSF15A
Sampling interval	Daily	Daily	3 times/week	Daily	Daily	Daily
Filled volume, cm^3	2775.91	2762.03	2796.73	2782.85	2741.21	2803.67
Column equilibration time, h	24	24	24	24	24	24
Microbial consortium	Inoculated	Inoculated	Inoculated	Not Inoculated	Inoculated	Inoculated
Column feeding solution	Deionised H_2O	Acidified H_2O	Acidified H_2O	Acidified H_2O	Acidified H_2O	Acidified H_2O
Feeding frequency	Continuous	Continuous	Intermittent	Continuous	Continuous	Continuous

3.2.5. Analysis of Metal Mobility and Characterization of Ecological Risk

The characterization of the ecological risk associated with tailing storage facilities was investigated through the analysis of the drainage quality from the column experiments. Leachate solutions from the column tests were analysed to assess the mobility of major metal species associated with copper tailings under disposal conditions. The leachate solution was quantified using the inductively coupled plasma-atomic emission spectrometry (Varian ES 730 ICP-OES), at the analytical laboratory in the Department of Chemical Engineering, UCT. Using protocols developed by Broadhurst and Petrie (2010), the potential ecological risk associated with deportment of metals under column leach test conditions was assessed to determine the major metals associated with mobility from the tailings. The concentration of soluble metals in the leachates are used in conjunction with permissible limits for water quality (IRMA standards) and natural background concentration (from upstream samples) for each elemental species to compute risk potential factors and hazard potential factors. Using the Broadhurst and Petrie (2010) ranking system, these factors are rated to ascertain which metal species pose a risk to the environment (Table 3-2). The effective and enrichment factors are used to quantitively express the risk, where:

$$EF_i = TC_i/ARC_i \quad (1)$$

$$EnF_i = TC_i/BC_i \quad (2)$$

where EF_i and EnF_i represents the effect and enrichment factors for constituent i, respectively; TC_i the total concentration (ppm); ARC_i the environmentally acceptable concentration (ppm); and BC_i the natural background concentration (ppm)

Table 3-2: Generic ranking of environmentally significant concentration levels for waste constituents on the basis of their hazard potentials

Group description		Estimated environmentally significant concentration levels (mg/kg)
I	Potential for environmental rist if present at low (trace) available concentration levels	<10
II	Potential for environmental risk present at low (minor) available concentration	10 - 100
III	Potential for environmental risk if present at moderate available concentration levels	100 - 1000
IV	Potential for environmental risk only if present at relatively high available concentration levels	A: 1000 - 10000
		B: >10000

3.3. Results

3.3.1. Characteristics of Tailings Material

The mineralogical composition of the tailing samples, determined by XRD, is presented in Table 3-3. Acid neutralising minerals present in high quantities dominated all three samples. The samples mainly consisted of quartz, muscovite, and feldspar, with trace dolomite gibbsite, and chlorite in TSF14 and TSF15A respectively, while kaolinite, amphibole, and calcite in Chibuluma TSF and TSF14 samples respectively. However, not all the characteristic peaks for Cu and Fe oxides were identified even though they were abundant in tailing samples as shown by elemental analysis (Table 3-4), possibly due to their occurrences in the non-crystalline structure or relative low content in mine tailings or interference and coverage with abundance Ca and Al. The results of elemental analysis showed that at least 10 different metals were contained in the tailing samples and that the tailings were rich in the metals Fe, Cu, Mn, As and Co.

Table 3-3: Mineralogical Composition (%) of the Tailing Samples used in the Completion of this Study Following XRD Analysis

Mineral		Chibuluma Tailings	TSF14 Tailings	TSF15A Tailings	Group
Quartz	SiO_2	69	38,4	27,8	Inert
Muscovite	$KAl_2(F, OH)_2$	8,5	13,3	24,9	Very slow weathering
Feldspar	$KAlSi_3O_8 – NaAlSi_3O_8 – CaAl_2Si_2O_8$	8,7	15,5	16,6	Very slow weathering
Amphibole	$Mg_7Si_8O_{22}(OH)_2$	4,1	5,7	-	Dissolving
Chlorite	$(Mg,Fe,Al)_6(Si,Al)_4O_{10}(OH)_8$	-	6	3,4	Intermediate weathering
Kaolinite	$Al_2Si_2O_5(OH)_4$	1,2	1,5	-	slow weathering
Montmorillonite	$(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2n(H_2O)$	-	1,9	1,6	slow weathering
Sepiolite	$Mg_4Si_6O_{15}(OH)_2 \bullet 6(H_2O)$	0,6	0,3	-	Intermediate weathering
Calcite	$CaCO_3$	1	4,7	-	Dissolving
Dolomite	$MgCO_3 \cdot CaCO_3$	-	4,8	24,2	Dissolving
Goethite	$FeO(OH)$	-	0,8	0,1	slow weathering
Gibbsite	$Al(OH)_3$	4,9	3,9	-	slow weathering
Anatase	TiO_2	2,1	3,2	-	
Jarosite	$KFe_3(SO_4)_2(OH)_6$	-	-	1,5	
Total		100	100	100	

Table 3-4: Metal concentration in samples in wt%

Sample	Al	As	Ca	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Chibuluma	5,73	0,04	1,74	0,02	1,3	2,63	2,52	0,12	0,05	NQ	NQ
TSF14	7,38	0,03	4,82	0,05	0,45	2,45	3,88	0,18	0,04	NQ	0,01
TSF15A	6,03	0,02	7,52	0,03	0,14	1,91	4,76	0,21	0,04	NQ	0,01

3.3.2. Standard ARD Characterisation

The static ARD characterization tests using the acid-base accounting are reported in Table 3-5 and Figure 3-3 for the Chibuluma, TSF14 and TSF15A waste samples. The samples were categorised as potentially non-acid forming, with high neutralising capacities observed for all tailing samples. This was expected given the abundance of neutralising minerals reported (Table 3-2). The ANC values obtained for the samples was relatively high (113,5 H_2SO_4 /ton Chibuluma, 229,3 H_2SO_4 /ton TSF14 and 411 H_2SO_4 /ton TSF15A respectively). This could be due to the presence of fast and slow weathering minerals such as dolomite, calcite, mica, and feldspar which contribute to neutralisation. Notably, low sulphur content in the samples was reported (sulphur content was observed to be <0,001%, <0,001% and 0,067% for Chibuluma, TSF14 and TSF15A, respectively), impacting the NAPP values. As a result, all samples had significantly negative NAPP values of -113, -229 and -408 Kg H_2SO_4 /ton, thus indicating high neutralising potential of tailings samples and their non-acid forming classification.

Further ARD classification by combining ABA and NAG_{pH} are presented graphically in Figure 3-3. The NAG_{pH} values were measured following a complete reaction of the sample with a familiar volume of 15% H_2O_2 while the NAPP value plotted on the x-axis were computed using results from the ABA analysis. The resulting pH values from the NAG tests hold up the non-acid forming (NAF) categorization as presented by the ABA test protocol. Following the reaction of tailing samples with 15% H_2O_2, the pH of the solutions remained above pH 6.5.

Observably, the pH after reaction remained above pH 6.5, indicating a non-acid classification for the copper tailing samples. The NAG pH was found to be pH ≈6.77, pH ≈6.85, and pH ≈6.85, for Chibuluma, TSF14 and TSF15A tailing samples respectively.

Table 3-5: Static ARD prediction of tailings samples

Sample	Sulphur Grade [%]	MPA [kg H2SO4/ton]	ANC [kg H2SO4/ton]	NAPP [kg H2SO4/ton]	NAG pH	ARD Classification
Chibuluma	0,0009	0,03	114 ± 26,4	-113,47	6,77 ± 0,04	Non-acid forming
TSF14	0,0023	0,07	229 ± 8,5	-229,23	6,85 ± 0,04	Non-acid forming
TSF15A	0,0665	2,03	411 ± 2,9	-408,97	6,85 ± 0,04	Non-acid forming

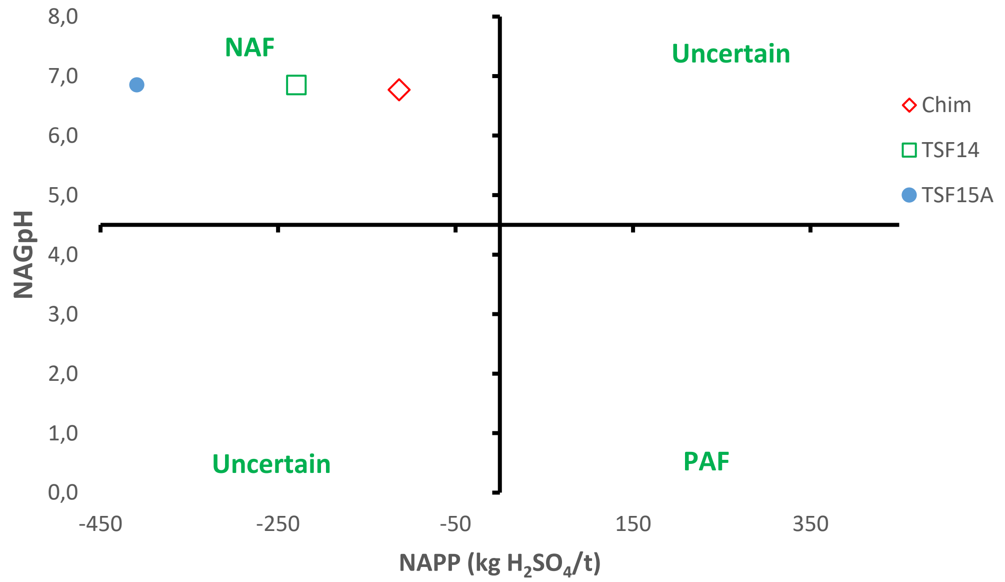


Figure 3-3: Classification of ARD potential for Chibuluma TSF, TSF14 and TSF15A copper-bearing tailings based on NAG and NAPP pH values

3.3.3. Biokinetic Tests

In order to evaluate the ARD generation potential under microbial conditions and generate data with respect to the relative rates of neutralization and acidification, the UCT biokinetic tests, also known as the biokinetic accelerated weathering tests, were carried out under non-pH controlled and pH-controlled conditions as outlined in Section 3.2.3. The leaching of the mixed tailings suspension was monitored over a 90-day period.

The pH and redox potential profiles under the non-pH controlled biokinetic test results are presented in Figure 3-4 and 3-5, and Supplementary Tables S3-1 to S3-6. From initial pH values of approximately pH 3.4, 5.4, and 5.5 following suspension of Chibuluma TSF, TSF14 and TSF15A copper tailing samples in media at pH 2.0, the pH of all samples increased to over pH 7.0 in the initial 10 days. The pH increases suggested dissolution of ANC in tailing samples. High ANC values (114 – 410 kg H_2SO_4/ton) were observed in the ABA statics. The pH of the samples on average remained above pH 7.0 throughout the experiment without pH control, confirming the NAPP-NAG classification. After the time period of 21 days, no substantial changes in pH of the tailing samples were observed. The redox potential was monitored as an indication of iron-oxidising microorganism's activities over time and is presented in Figure 3-5. The low redox potential (below 500 mV) obtained was indicative of the absence of notable microbial activities and the relative pre-eminence of ferrous iron over ferric iron and were maintained over the 90-day experiment. Low microbial activity is expected at neutral conditions as high pH values have a negative effect on microbial community used as an inoculum. The high pH and low redox potentials maintained was consistent with the classification of non-acid generation. The high neutralisation could be impeding the reaction of sulphide minerals.

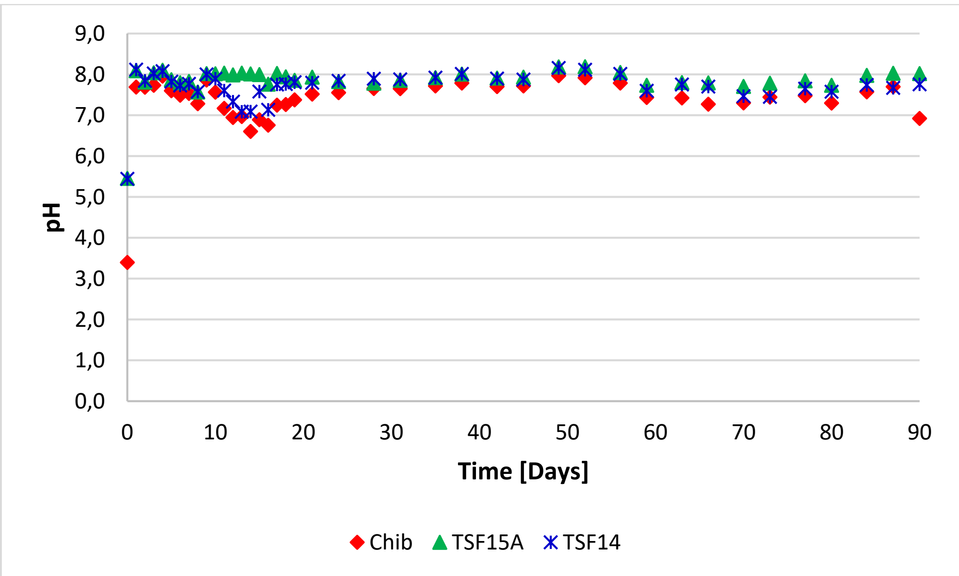


Figure 3-4: pH profiles for non-pH controlled and inoculated standard biokinetic tests performed on Chibuluma TSF, TSF14 and TSF15A copper tailings sample. n = 3

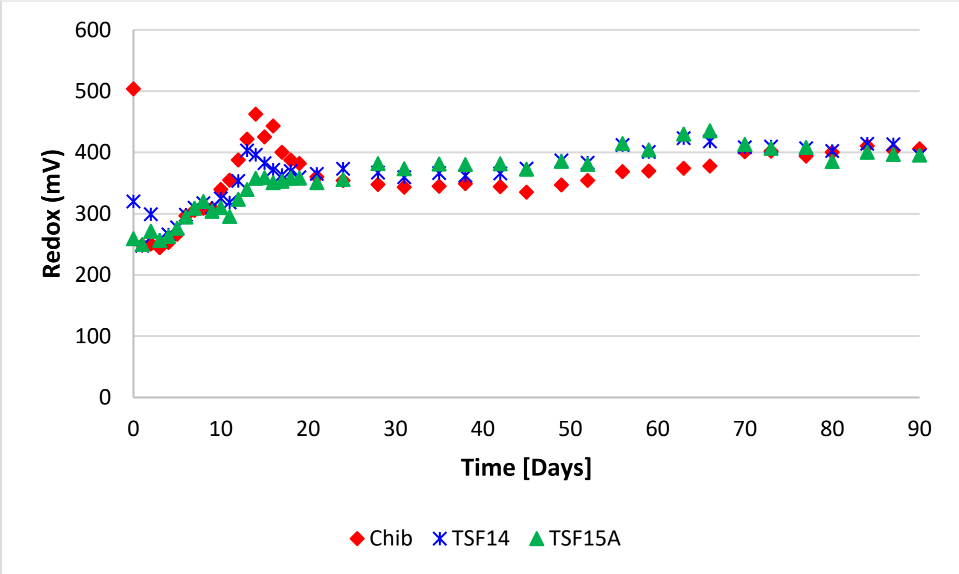


Figure 3-5: Redox potential (mV) profiles for non-pH controlled and inoculated standard biokinetic tests performed on Chibuluma TSF, TSF14 and TSF15A copper tailings sample. n = 3

The pH-controlled tests were run concomitantly with the non-pH-controlled. A lower pH environment was maintained through the addition of 0.5M sulphuric acid every day to return the pH to pH 2.0 to enable microbial community to facilitate the regeneration of ferric iron needed to leach the tailings material at a faster rate. While the pH-controlled flasks for Chibuluma and TSF14 had similar profiles for acid consumption, the flasks containing TSF15A consumed significantly more acid to control the pH (Figure 3-8). The high acid consumption in TSF15A flasks is expected due to the presence of significant ANC minerals in the sample. This corresponds with the ANC values described in Section 3.2.2

These tests were conducted under conditions for the Fe- and S-oxidising microbial consortium to thrive. The pH below 2.5 enables microbial regeneration of lixiviants (ferric iron and H^+) and minimises Fe^{3+} precipitation which accelerates the rate of ARD generation thus favouring the microbial community of iron and sulphur oxidisers. The pH increased rapidly over the initial 3 days of the experiment, owing to the dissolution of gangue minerals like carbonates. Notably, the observed initial high pH values are consistent with the reported high concentration of acid neutralising minerals and correlate with the ANC values. The pH in flasks containing Chibuluma and TSF14 increased each day for 3days, but less each day, while in flasks containing TSF15A samples, the pH increases were longer, for 8 days. Thereafter, no significant changes in pH of the tailing samples were observed, suggesting that the ANC minerals had been depleted. As an attestation of microbial activities in the experiments, the redox potential of the flasks, presented in Figure 3-7, was monitored throughout the experiment to give an indication of ferrous iron concentration to ferric iron. A steady rise in potential was observed after 3 days indicating oxidation of ferrous iron to ferric iron, reaching ≈660 mV as acid neutralisation potential was depleted. In the Chibuluma and TSF14 samples, the concentrations of ferrous iron were small and comparable ranging from 0 to 100 mg/L whereas the TSF15A sample reported the highest concentration (250 mg/L). Ferrous iron increased over the first 20 days suggesting that leaching was occurring with Ferrous iron converted to ferric iron. This is supported by an increase in total iron concentration the leachate. A decline in ferrous iron was observed after day 21, which suggests reduction in microbial oxidation. Consequently, no more increase in total iron after day 38 was observed, as ferric iron was converted to ferrous iron (Figure 3-9 and 3-10).

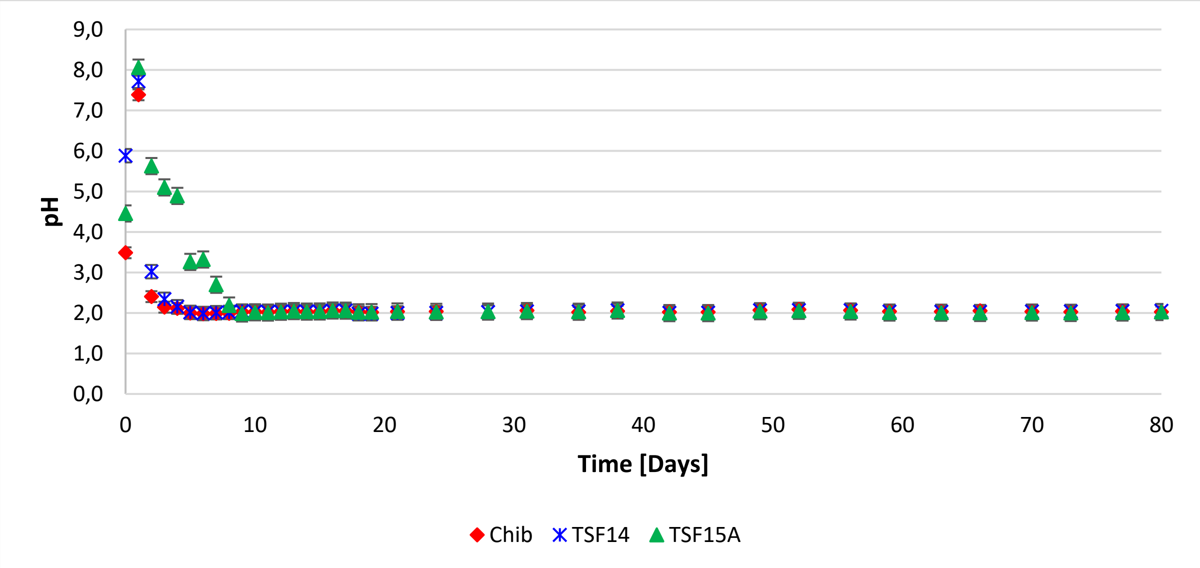


Figure 3-6: pH profiles for pH controlled and inoculated standard biokinetic tests performed on Chibuluma TSF, TSF14 and TSF15A copper tailings sample. n = 3. The pH was returned to pH 2 each day through the addition of 0.5M sulphuric acid

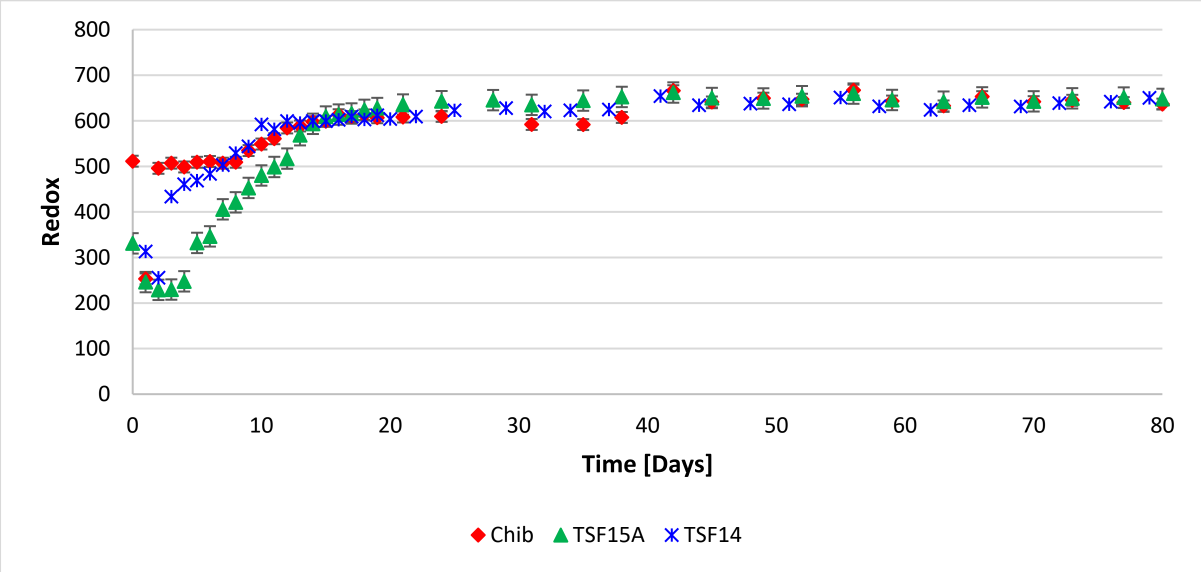


Figure 3-7: Redox potential profiles for pH controlled and inoculated standard biokinetic tests performed on Chibuluma TSF, TSF14 and TSF15A copper tailings sample. n = 3

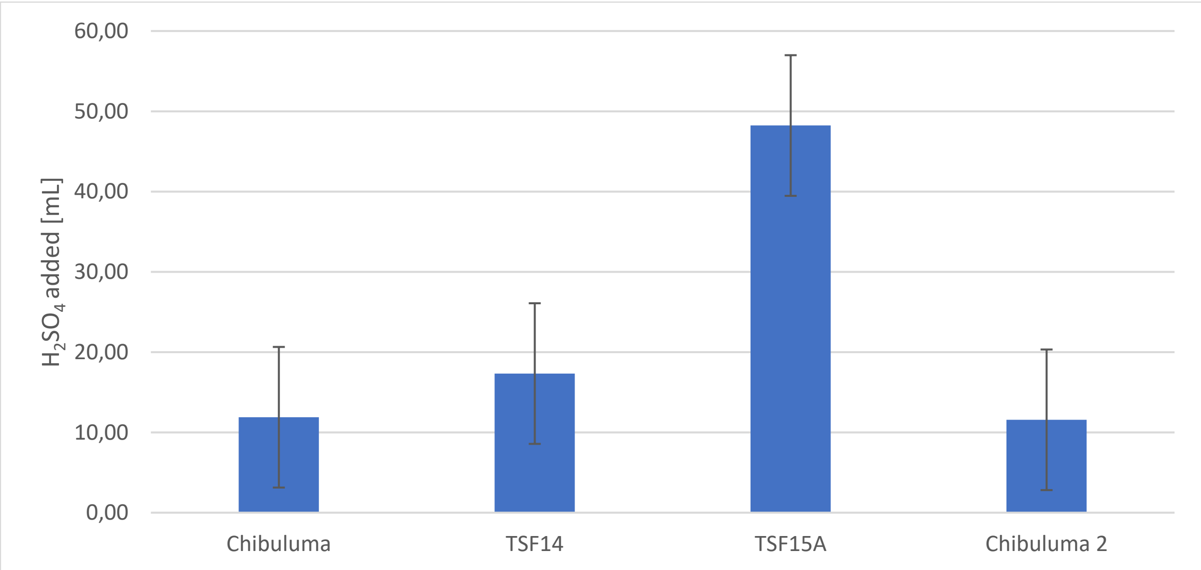


Figure 3-8: Volumes of 96-98 % H2SO4 used to adjust the pH to 2.0 for controlled biokinetic tests. n = 3

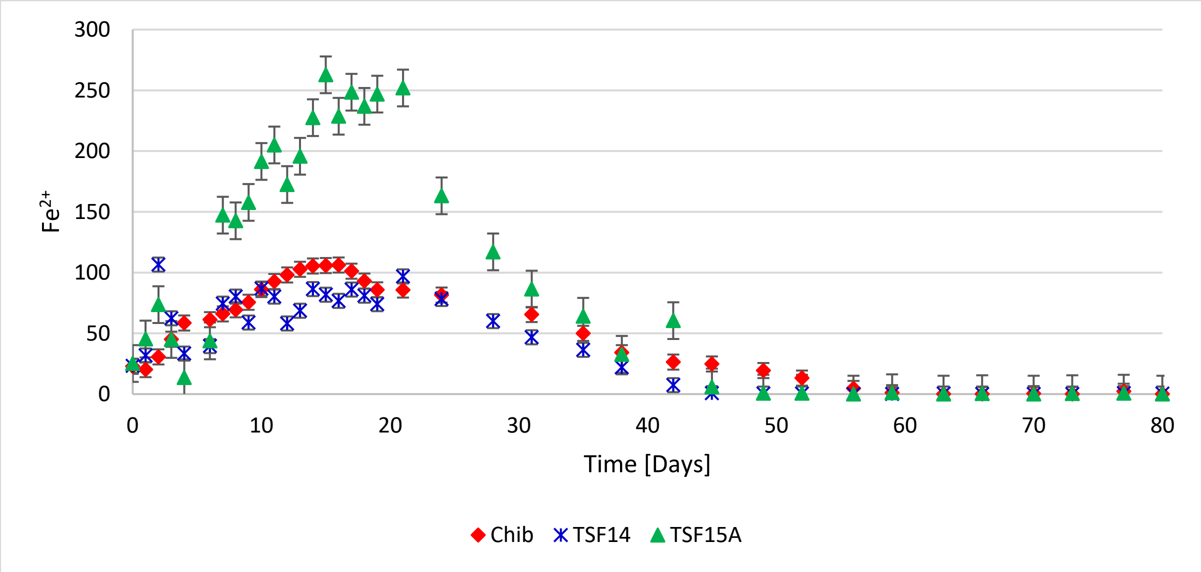


Figure 3-9: Cumulative ferrous iron concentration profiles for biokinetic experiments under pH-controlled conditions using Chibuluma, TSF14 and TSF15A tailings samples under the same durations. n = 3

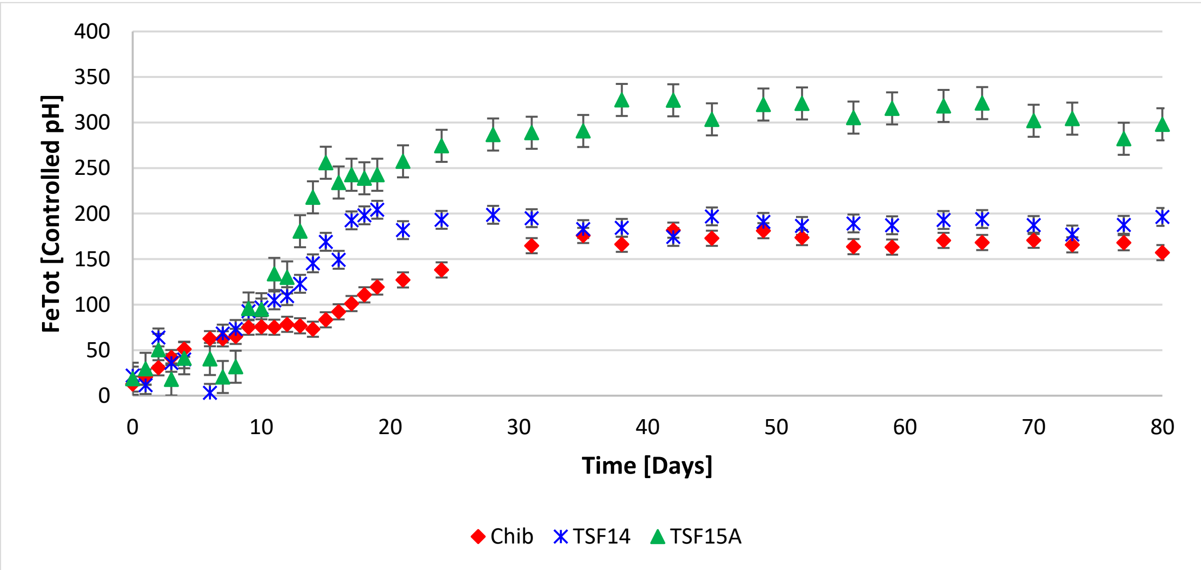


Figure 3-10: Cumulative total iron concentration profiles for biokinetic experiments under pH-controlled conditions using Chibuluma, TSF14 and TSF15A tailings samples under the same durations

A comparative analysis of the biokinetic and static test results for the tailing samples indicate that they had similar NAG pH values, while the NAPP values differed. However, they had a similar locality in the non-acid forming region in the amalgamated ABA/NAG classification plot (Figure 3-3). All the samples were classified as non-acid forming (NAF), with the potential for neutralisation greater than the capacity for acid formation. The biokinetic test results given in Figure 3-4, yielded results similar with static tests in terms of classification.

3.3.4. Metal Mobilization from Tailing Samples under Column Leach Test Conditions

The column leaching experiment was conducted for the tailing samples to simulate the open flow environment under TSF conditions. The inoculum was used to accelerate the generation of conditions similar to TSFs over time, and the feed flowrate of 40 mL/h, corresponding to a precipitation of 36 mm per day, was chosen. This amount of rainfall is common on the Copperbelt mining areas in Zambia (Beilfuss, 2012). Additionally, a high irrigation rate was applied to minimise the experimental period. Figures 3-11 – 3-13 present the release profiles of pH and redox potential.

The pH for column 1, irrigated with deionised H_2O, increased over the first 10 days while the redox potential decreased. The decrease in pH from initial pH 5.0 to pH 4.0 could be attributed to the acid agglomeration process. From Day 18 to 120, the pH settled around pH 7.0 i.e. it lay outside the optimum for bioleaching micro-organisms (Watling et al., 2015); this high pH suggests little microbial activity after the 10-day period. This initial high pH is expected due to the neutralising capacity of the tailing samples (Section 3.3.2) (Sracek et al., 2011).

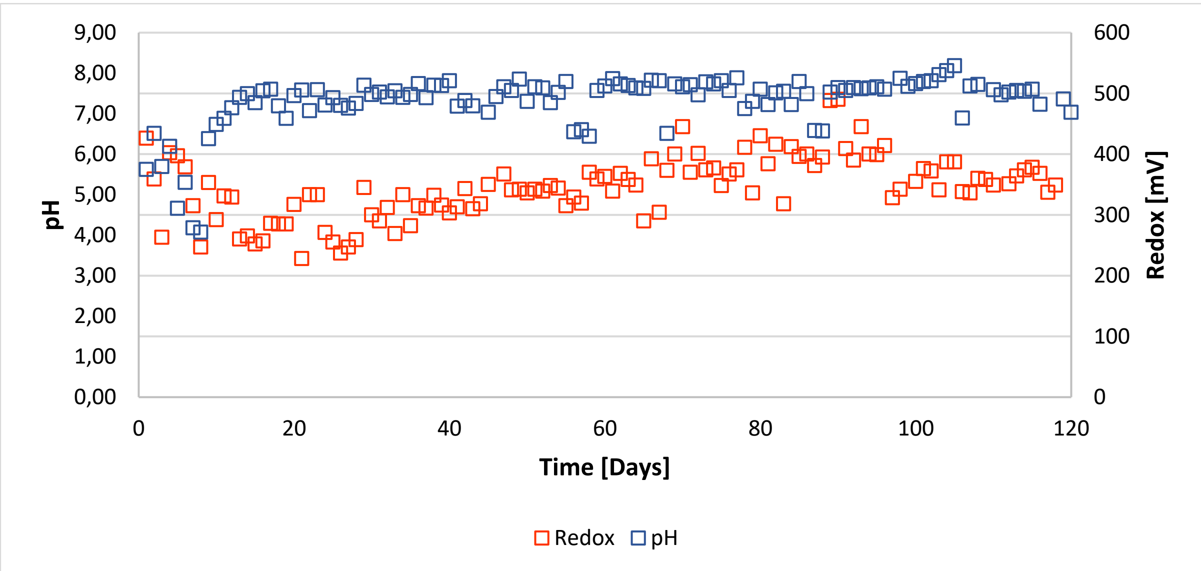


Figure 3-11: pH and redox potential profiles on continuous irrigation of agglomerated and inoculated tailings with deionised water media under oxygen limitation (column 1)

For the Chibuluma columns irrigated with acidified media (2, 3, 4), at pH 1.5. The initial pH of the leachate solution was approximately pH 5.0 for the first 5 days, and thereafter, a steady drop in pH was observed with a successive increase in the redox potential (Figure 3-12 and 3-13). The decrease in pH was most rapid for the inoculated column under continuous irrigation. After 13 days, the pH lay within the optimum for bioleaching micro-organisms (Watling, 2006), therefore, this stable pH suggests some microbial activity after the 13-day period and leaching of acid neutralising minerals from the samples. In Column 5 containing TSF14 and irrigated with acidified medium, the pH was maintained at pH 7.0 for some 7 days, thereafter, decreasing to pH 2.0 by day 25. In the case of Column 6, containing TSF15A and irrigated with acidified medium, a high pH of 7 was observed over a longer period compared to the other similar columns owing to the higher ANC of TSF15A (≈411 kg/t H_2SO_4). A sudden drop in pH was observed after 54 days to approximately pH 2.0, suggesting the depletion of rapidly soluble neutralising capacity; this was maintained until the end of the experiment (Figure 3-12).

The redox potential profile (Figure 3-12) shows that during the initial acid mineral leaching following agglomeration, the redox potential was low (250 – 400 mV). Large changes in redox potential were observed after day 14, reaching 620 mV on day 21 for columns 2 and 4, and 580 mV on day 60 for column 6; in all cases, at the point at which pH decrease was observed, indicating an oxidizing environment present in the column. The redox potential showed a sharp rise in inoculated and continuous irrigated columns 2 and 5 to a value above 600 mV, except for column 6. This difference in column 6 could be related to the high ANC values observed, however, after day 60, the column was microbially active. In column 3, gradual increase of redox was observed and stabilized after day 78 with redox ≈643 mV. The delay in changes was as a result of less irrigant, as more time was required to neutralise the ANC. Equally, the redox potential for column 4 delayed due to the fact that the column was not inoculated. The data suggests that indigenous microorganisms take longer to ramp up oxidation, since they are dominated by weaker iron oxidisers (Kumar and Gopal, 2015).

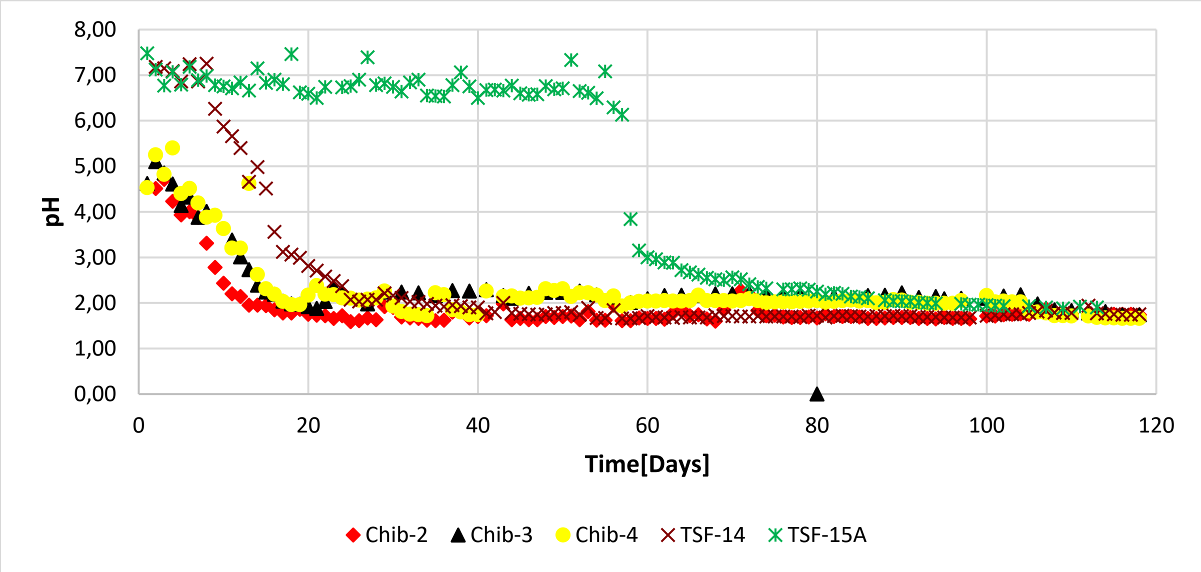


Figure 3-12: pH profiles on continuous irrigation of agglomerated and inoculated tailings with acidified media under oxygen limitation for columns 2, 3, 4, 5, and 6

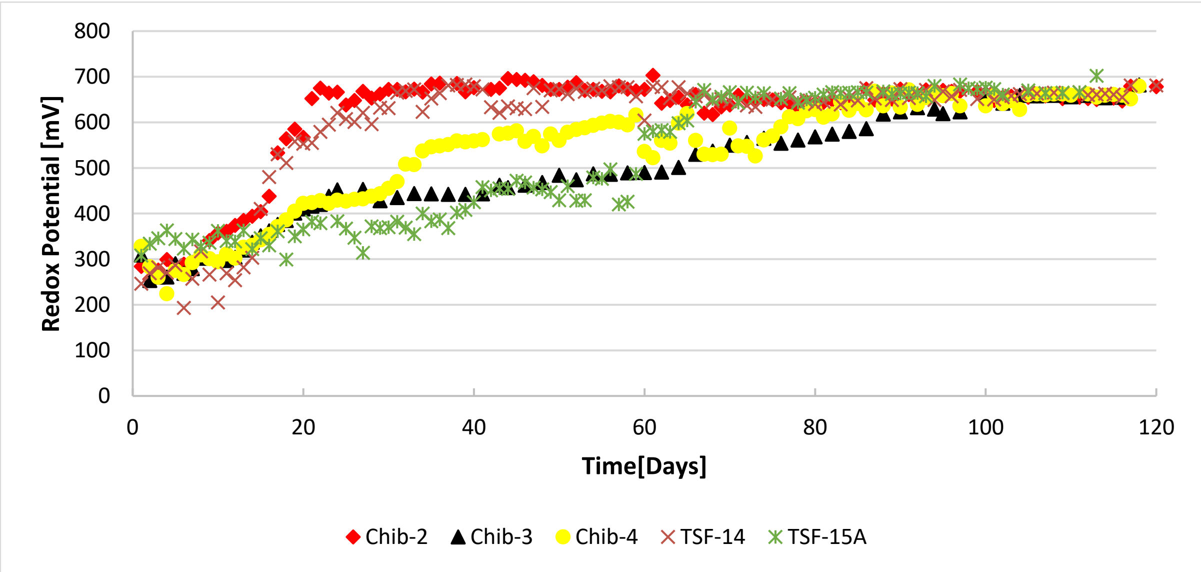


Figure 3-13: Redox potential profiles on continuous irrigation of agglomerated and inoculated tailings with acidified media under oxygen limitation for columns 2, 3, 4, 5 and ***6***

Based on changes observed in the pH and redox potential profiles, selected leachates were taken for analysis of elemental concentrations. Leachates collected on days 5, 20, 50, 80, 110, and 140 were selected for metal analysis. In column 1, the elements with the highest mobility under non-acidified column conditions were Ca and Fe (Figure 3-14) with approximately ≈82.3 ppm and ≈0.95 ppm deporting under non-acidic conditions respectively. Although solubilization of pyritic Fe is expected to occur under oxidative conditions, notable Fe precipitations under elevated pH conditions in column 1 were observed. It is probable that the Fe precipitates that formed at elevated pH conditions resulted in the removal of the elements of interest (Cu, Co, Mn, Ni and Pb) from the leachate conditions. Notably, mobilization of Mg (≈0.56 ppm) and Cu (≈0.2 ppm) was observed to be higher than Co, Ni, Pb and Mn (≈0.01 ppm). The low mobilization of elements in column 1 could be attributed to the low presence of sulphide bearing minerals and high neutralizing capacity.

Analysis of the Column 1 leachate samples suggested that high metal mobilization occurred during the first 20 days of the column leach experiments. The metal concentration in the leachate varied with leaching time (Figure 3-14 and 3-15). The observed fluctuations in metal elevation might be attributed to metal precipitation and hydrolysis at high pH conditions. The lowest leachate concentration of Ca, Cu, Mn, Co, and Zn was reached after 90 days, and then remained consistently low.

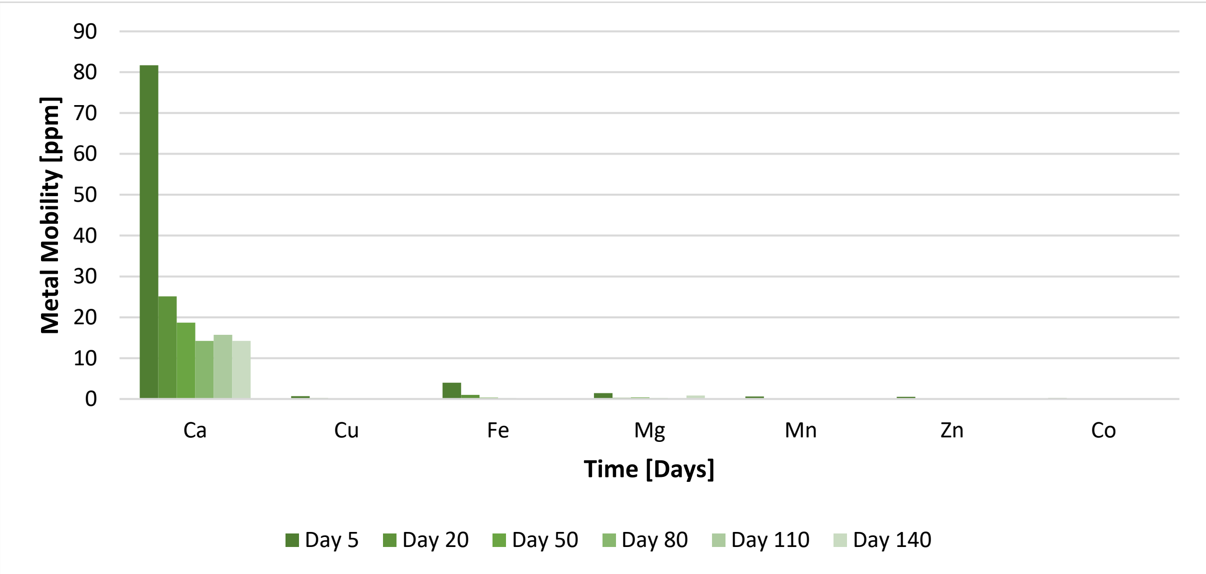


Figure 3-14: Mobilization of environmentally significant elements for tailing samples in column 1 under non-acid conditions

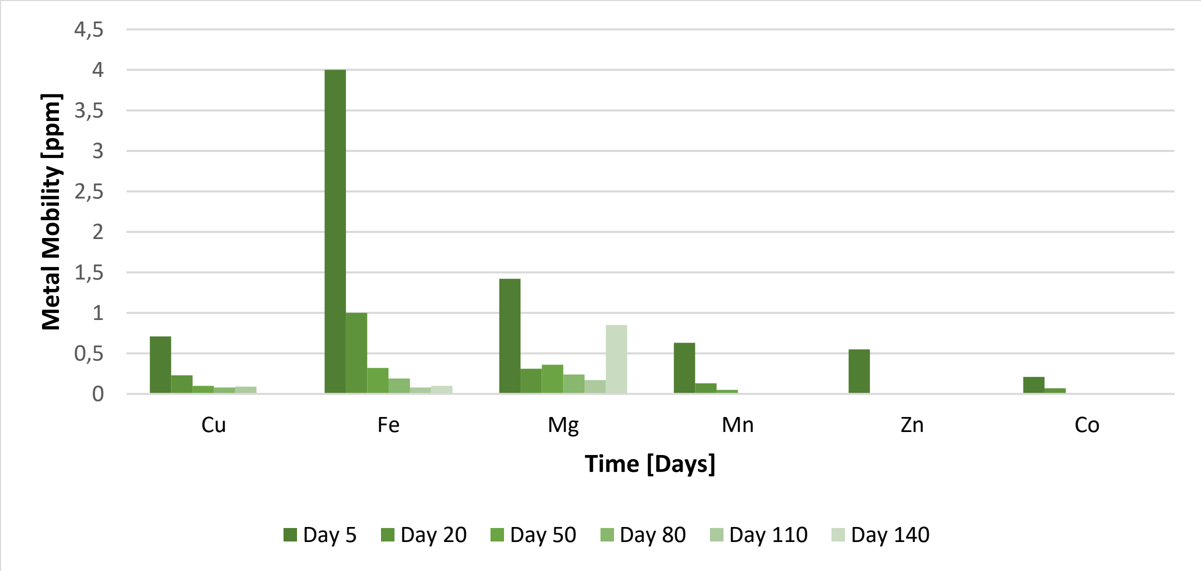


Figure 3-15: Mobilization of environmentally significant elements for tailing samples in column 1 under non-acid conditions (excluding Ca)

Metal mobilization from tailing samples under acidic conditions and non-acidic conditions is presented in Figure 3-16 to 3-18 and Table S3-7. Higher mobilities of Cu, Ca and Mn were observed in the first 5 days, with copper concentrations in column 2, 3 and 5, compared to the other columns. This may result from high elemental solubilities at lower pH than at elevated pH. At day 20 (Figure 3-17), significant increase in solubilization of Fe element was observed to be high compared to other metals. The increase in released iron suggests that more ferrous was being converted to ferric iron. Significant mobilization of the elements Cu, Ca, Al and Mg was equally noticed under acidic irrigation at day 20 (Figure 3-17), while low mobilization was observed under non-acidic conditions. Solubilization of elements Co, Mn, Ni, and Zn remained low under acidic conditions. The mobilization of iron remained high by day 110 (Figure 3-18), while a decline in other metals was observed.

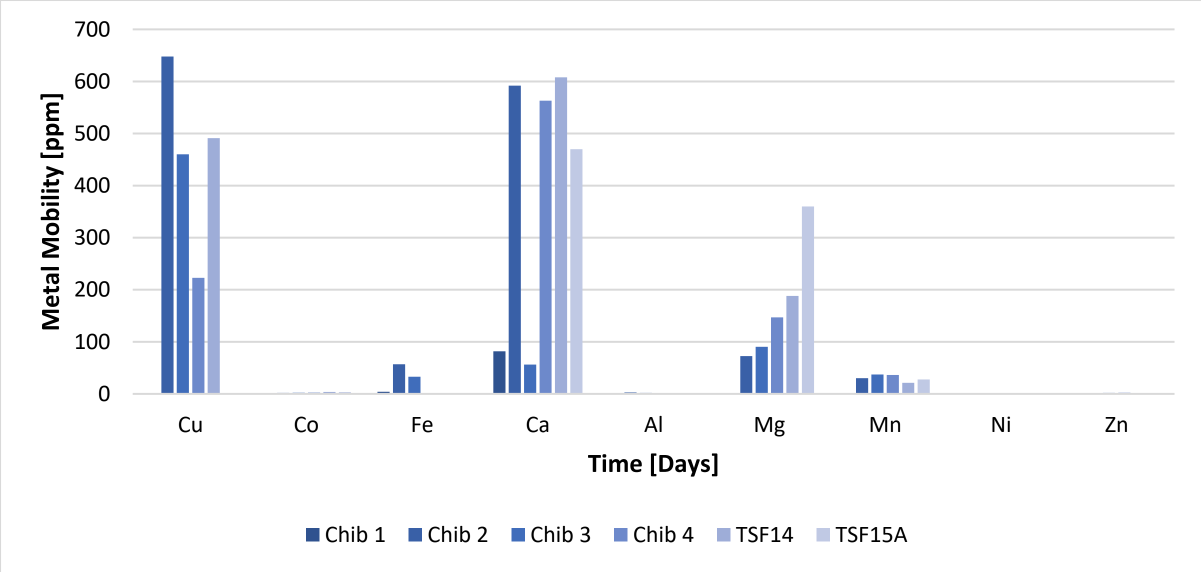


Figure 3-16: Mobilization of environmentally significant elements for Chibuluma TSF under acid conditions and non-acid conditions at day 5

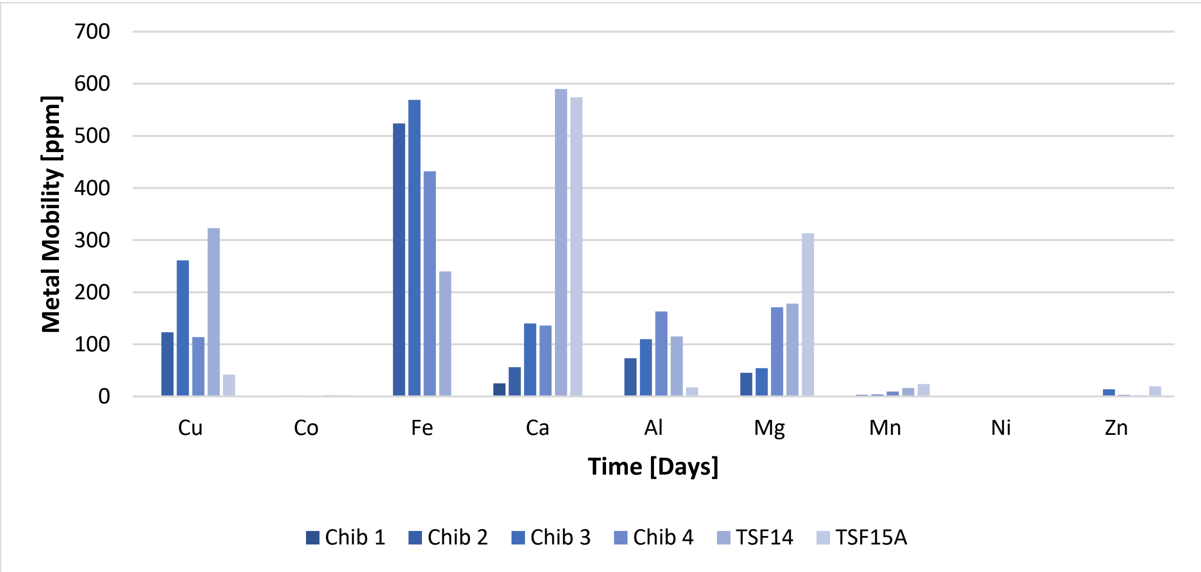


Figure 3-17: Mobilization of environmentally significant elements for Chibuluma TSF under acid conditions and non-acid conditions at day 20

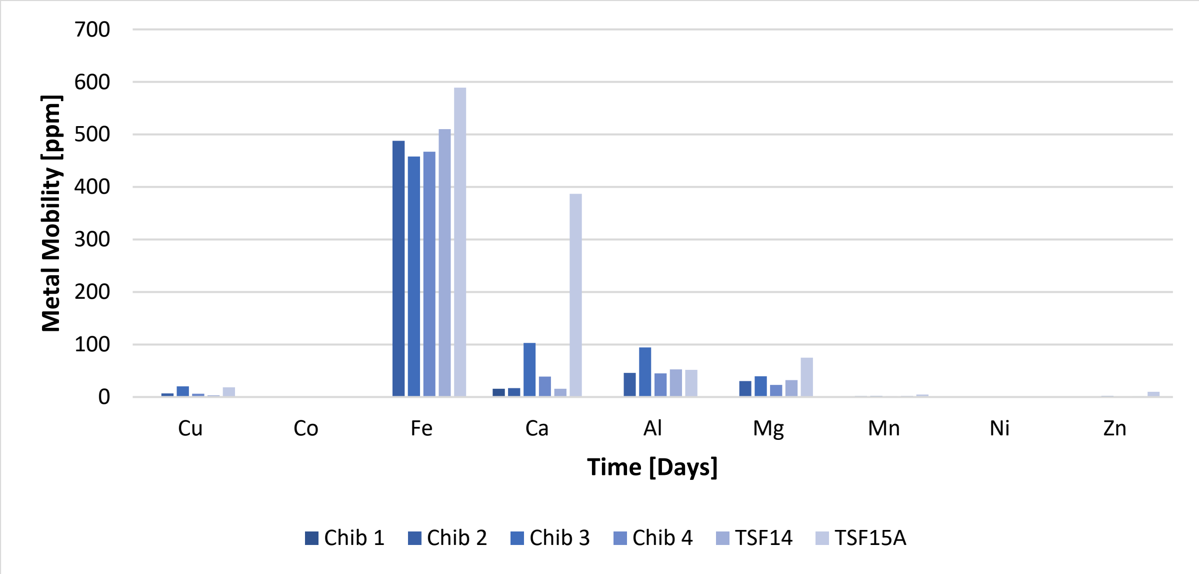


Figure 3-18: Mobilization of environmentally significant elements for Chibuluma TSF under acid conditions and non-acid conditions at day 110

The cumulative leaching of metals under acidified and inoculated conditions as a function of time were similarly observed to decrease to a steadily low value except for Ni and Co. Table S3-17 shows the amount of metals released from the copper tailing samples within the columns varied with time. The released amount for each metal showed a decreasing trend except Fe, Al and Ni. For instance, in column 2, for Cu, Ca, Mg, Mn, Co and Zn, the released amount changed from 648, 592, 72.6, 30.1, 1.96, 0.88 ppm at day 5 to 14.1, 43.3, 33.3, 2.43, 0.2 and 0.48 ppm at day 80, to 2.79, 14.6, 35.1, 1.8, 0.2 and 0.42 at 140 days, respectively. The released amounts of Fe had an increasing trend for the first 20 days. In column 2 the released amount of Fe increased from 56.9 ppm at Day 5 to 524 ppm at Day 20, then maintained stability of ≈500 ppm throughout the experiment to Day 140. No clear trends were observed for Al and Ni.

The leaching behaviour of all metals examined were observed to depend on pH and contact time. Release of metals was higher at low pH such as 2, while at neutral pH metal concentrations, except Ca, were typically < 1 ppm (Figure 3-14). Equally, the metal concentrations in leachates increased with increase in contact time, reaching maximum values by day 20 and then a steady decrease was observed except with Fe. Possibly the peak levels in metal concentrations represent the limited fractions of metals likely to easily dissolve in the copper tailings.

3.3.5. Potential Ecological Risk Associated with Metal Mobilization from Tailing Samples under Column Leach Conditions Based on Metal Content

Results from the estimated ecological risk assessment associated with metal deportment from the column tests are reported in Table 3-6 to 3-11. The soluble metal concentrations were used to assess the ecological risks according to the ranking scoring method developed by Broadhurst and Petrie (2010). These together with the natural background concentrations and water quality limits (Table S3-8) of each metal species, are used to calculate the risk potential factors and hazard potential factors. Thereafter, these factors were ranked to determine which metal species showed potential risk to the environment. IRMA standards were used for water quality limits for metal species, while background values from upstream sites were used for Mn.

The potential ecological risks associated with the six tailing samples showed significant differences. The potential mobility of elements Fe, Ca, Cu, Al, Mg, Mn, Zn, Co, and Ni showed significant risks for tailing material under different conditions in columns 2 – 6. Particularly, Fe and Cu exhibited high ecological risk under the column conditions. Although a high mobility of Ca, Al, Mg and Mn was observed, the concentration of these elements varied from low to moderate, resulting in a moderate ecological risk. Throughout all the column experiments, the observed significant dissolution of Ca, Al and Mg metal ions did not present a high ecological risk. Essentially, these elements are mostly concentrated within the gangue minerals that are present within the copper tailing samples (Table 3-2). As a result, even though the analysis of leachates designated potential for ecological degradation, these elements did not pose significant risks under disposal conditions. Low to negligible ecological risk profiles were found with elements of interest Pb, Co, Zn, and Ni for the tailing samples under acidic conditions. This could be attributed to the low metal concentration observed in the tailing samples (Table 3-3).

Following the non-acidic conditions in column 1, significant risk remained with the deportment of Fe and Cu, albeit in lower quantities relative to the acidic column bioleach conditions. The potential mobility of Ca, Co and Mn though low posed an environmental hazard for Chibuluma TSF sample, however, the potential risk related with deportment of these elements were decreased towards the last stage of the experiment. The other elements of interest Pb, Zn, Mg, and Ni exhibited negligible mobilization percentages. The lack of significant metal mobility in column 1 tailings, suggests that the liberation of metals of concern in the host minerals is more likely under sulphide concentration stream or oxidative environment. However, over time, the low mobilization of elements has potential to pose high ecological risks (McCarthy, 2011). Studies by Qu et al. (2010) have shown that long-term exposure to low metal contamination were reflected in the changes of macroinvertebrate community structures in the high mountain of Gangqu River in China. The consequences on macroinvertebrate diversity were significant, indicating the effects of chronic long-term exposure to low metal contamination.

Table 3-6: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 5

Environmental Significance		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Cu	Cu		Cu	
	B	100 - 1000		Mn<Fe	Fe<Mn	Mn<Fe<Cu	Fe	Mn<Fe
	C	10 - 100			Co	Co	Co<Mn	Co
Moderate		1,0 - 10	Cu<Fe	Co				
Low		0,1 -1	Mn<Co	Ca	Zn<Ni<Ca	Mg<Ca	Mg<Ca	Mg<Ca
Negligible		< 0,1	Mg<Pb<Zn<Ni<Ca	Pb<Al<Ni<Zn<Mg	Pb<Al<Mg	Al<Pb<Ni	Pb<Zn<Al	Cu<Al<Pb<Ni

Table 3-7: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 20

Environmental Significance		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Fe	Cu<Fe	Fe	Cu<Fe	
	B	100 - 1000		Cu		Cu		
	C	10 - 100				Mn	Cu<Mn	Fe<Cu<Mn
Moderate		1,0 - 10	Fe	Al<Mn<Co	Mn<Al<Zn<C	Co<Al	Al	Zn<Co
Low		0,1 -1	Co<Cu<Mn<Ca	Ni	Ni	Mg<Ni<Zn	Zn<Mg<Ca	Al<Mg<Ca
Negligible		< 0,1	Zn<Pb<Mg	Pb<Ca<Mg<Zn	Pb<Mg<Ca	Pb<Ca	Pb<Ni	Pb<Ni

Table 3-8: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 50

Environmental		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Fe	Fe	Fe	Fe	Fe
	B	100 - 1000						
	C	10 - 100			Al<Cu	Al		Mn<Cu
Moderate		1,0 - 10	Fe	Cu	Co<Ni<Mn	Mn<Cu	Cu<Co	Al<Co<Zn
Low		0,1 -1	Cu<Co	Cu<Al<Mn	Zn	Co<Zn<Ni	Zn<Al<Mn	Mg<Ca
Negligible		< 0,1	Mg<Zn<Mn<Pb<Ca	PB<Ca<Mg<Zn<Ni	Pb<Mg<Ca	Pb<Ca<Mg	Pb<Mg<Ca<Ni	Pb<Mg

Table 3-9: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 80

Environmental Significance		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Fe	Fe	Fe	Fe	Fe
	B	100 - 1000						
	C	10 - 100			Al<Cu	Al		
Moderate		1,0 - 10	Fe	Cu	Ni<Co	Cu<Mn		Cu<Zn<Mn<Cu
Low		0,1 -1	Cu	Co<Al<Mn	Zn<Ca<Mn	Mg<Ni<Co	Zn<Co<Cu<Mn<Al	Ca<Al
Negligible		< 0,1	Mg<Mn<Pb<Co<Ca	Pb<Ca<Zn<Mg<Ni	Pb<Mg	Pb<Ca<Zn	Pb<ca<Mg	Pb<Ni<Mg

Table 3-10: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 110

Environmental Significance		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Fe	Fe	Fe	Fe	Fe
	B	100 - 1000						
	C	10 - 100						
Moderate		1,0 - 10		Ni<Cu	Ni<Al<Cu			Zn<Mn<Cu
Low		0,1 -1	Fe	Co<Mn<Al	Zn<Co<Mn	Ni<Co<Mn<Al<Cu	Co<Cu<Mn<Al	Ca<Al<Co
Negligible		< 0,1	Mg<Ca<Zn<Co<Cu	Pb<Ca<Zn<Mg	Pb<Mg<Ca	Pb<Mg<Ca<Zn	Ca<Pb<Mg<Zn	Pb<Ni<Mg

Table 3-11: Ecological Risk Associated with Metal Mobilization for Tailing Samples from the Column Leach Processes Collected on Day 140

Environmental Significance		RPF/1000	Chib 1 Tails	Chib 2 Tails	Chib 3 Tails	Chib 4 Tails	TSF14 Tails	TSF15A Tails
High	A	1000 - 10000		Fe	Fe	F	Fe	Fe
	B	100 - 1000						
	C	10 - 100						
Moderate		1,0 - 10				Cu		Mn<Zn<Cu
Low		0,1 -1		Co<Cu<Mn<Al	Co<Mn<Al<Cu	Co<Mn<Al	Co<Cu<Mn<Al	Ca<Co<Al
Negligible		< 0,1	Mg<Co<Cu<Ca<Fe	Pb<Ca<Zn<Mg<Ni	Pb<Ni<Ca<Mg<Zn	Ca<Pb<Zn<Mg<Ni	Ca<Pb<Mg<Ni	Pb<Ni<Mg

3.4. Discussion

3.4.1. Characterising the ARD Generating Potential of Copper Tailing Waste using Static and Biokinetic Tests

Three copper tailing samples from Chibuluma TSF (active), TSF15A (active) and TSF14 (historical) were investigated to characterise their ARD generation and metal mobility potential. From the results, the metal profiles in the tailing samples were observed to be similar, with acid neutralising minerals dominating the samples. High quantities of gangue and acid consuming minerals such as quartz, muscovite, and feldspar dominated the tailing samples. Characteristic peaks for acid generating minerals were not reported from the XRD results, possibly this could be attributed to their relative low concentrate or interference and coverage with abundant Ca and Al or they were already oxidised during the mineral processing operation (Rodríguez et al., 2018). The elemental analysis that the tailing samples contained at least 10 different minerals, with high concentration of metals Fe, Cu, Mn, As and Co.

The ABA and NAG characterisation results performed on the copper tailing waste reported similar patterns with regards to ARD classification, in that the samples were non-acid forming. They occurred at a similar location in the non-acid forming section of the integrated ABA/NAG classification plot. Although the samples were all non-acid forming, the NAPP values differed. Particularly, high NAPP values were reported in TSF15A sample (-409 kg H_2SO_4/t), followed by TSF14 (-229 kg H_2SO_4/t). This could be attributed to the dominance of gangue and acid consuming minerals. These observations are similar to the findings by Sracek et al. (2012, 2011), which reported that the copper mine waste on the Zambian Copperbelt, has a high neutralising capacity system. The net acid consuming behaviour under high carbonate content of gangue minerals is expected.

While a suite of standard static ARD chemical tests was selected for this study, their impediments are understood. Partly, these have been overcome through the development of the biokinetic test, a useful tool in characterisation ARD in terms of providing information that both validates and enhances the static test results, through provision of information in a relative timescale of neutralisation and acidification (Harrison et al., 2010; Hesketh et al., 2010). The biokinetic tests were conducted as a function of time, the results yielded are congruous with the standard static test with regards to classification, i.e., all the copper tailing samples were non-acid generating under microbial activity in batch culture. Commensurate with Miller (2008), the NAG test may under or overestimate the potential for acid generation of samples, due to the influence of organic acid. Nevertheless, the samples classified as non-acid forming on the basis of the standard static tests were equally non-acid forming under microbial colonisation conditions over the duration of the biokinetic test. The results showed that the acid neutralising reactions in the biokinetic test occurred rapidly. More acid was required to consume the acid neutralising minerals in the TSF15A copper tailing sample than TSF14 and least for Chibuluma TSF. The dissimilarity in the time frame and consumption of acid neutralising minerals, and the quality of effluents likely from the copper TSFs has been demonstrated in the column leach studies.

3.4.2. Characterising Water Related Ecological Impacts Associated with Copper Tailing Waste: Potential Drainage Quality and Metal Deportment

The static and biokinetic ARD characterization tests primarily focus on the potential for ARD generation as indicator for ecological risk, with little attention placed on risks associated with metal mobilization under waste deposal conditions. To identify elements likely to pose ecological risks, leachates from column tests designed to mimic waste deposit conditions, reported in Section 3.3.4, were analysed. The probable ecological risks associated with metal mobilization were conducted through a risk evaluation analysis developed by Broadhurst and Petrie (2010) to identify elements likely to mobilize from mine waste based on common deleterious elements within each tailing sample. The concentrations measured from the collected column leachates were employed to calculate the risk assessment factors.

From the results, it was evident that the pH and redox potential for column 1 did not vary significantly between day 1 and day 150. The input of high neutralising elements and low sulphur content were observed to significantly impact the drainage quality of column 1. The high pH is likely to lead to a sequence of reactions where metals precipitate as hydroxides from the TSFs (Sracek et al., 2010). Transportation of contaminants in systems with high neutralisation capacity often occurs in suspension, as a result, suspended load may be more significant than contaminant transport in dissolved state (Cánovas et al., 2008). Nevertheless, metal concentration in suspended form was not analysed in this study. The collected and tested leachates from column 1 for days 5, 20, 50, 80, 110 and 150 under high pH showed relatively low concentrations of dissolved metal species. The reported low mobilization of dissolved metals indicates that ecological risk on the water resources is low. However, studies have shown that over time, the persistence of low metal release could result in significant ecological damage to receiving aquatic ecosystem (Ali et al., 2019; Qu et al., 2010). This is further explored in Chapters 4 and 5.

Owing to the potential for acidification following depletion of readily weathering acid neutralising potential, the metal mobility was also investigated under acid conditions, representing potential long-term conditions. Compared to column 1, the drainage quality from columns 2 to 5 showed much higher mobilities of metal species Ca, Fe, Cu, Al, Mg and Mn. The increase in metal concentration may be used as a proxy to indicate the influence of low pH in metal solubilization. The observed high metal release under low pH could ultimately result in the progressive degradation of the ambient aquatic ecosystem. From the analysis of leachates collected from the columns, the metal concentration variations were observed across tailing samples. Overall, significant mobilisation of Fe, Cu, Mn, Mg and Al was observed compared to the other metal species. Iron concentration after day 20 remained high throughout the experiments while a decline was observed in other metal species. It is plausible that abiotic reactions with Fe may influence or even dominate behaviour patterns of reducible pollutants, particularly in Fe bearing minerals. Elsner et al. (2004) observed that such reactions can be captivated by specific interactions of the oxidant.

The influence of pH in the TSFs is of great concern because there is a considerable high ecological risk associated with Fe and Cu, and moderate risk for Ca, Al, Mg and Mn under low pH conditions. Any activity within the TSFs likely to cause acidification may cause ambient water resources to shift to undesirable state. As revealed by the study on metal mobilisation from TSFs under column leach tests, most of the likely water quality impacts and related ecosystem degradation would be as a result of changes in pH levels triggered by reactions in the TSFs. Therefore, ARD and metal mobilisation impacts must be avoided through improved management and rehabilitation of TSFs to avoid depletion of neutralising capacity. In addition, increased monitoring of the drainage quality seeping from the TSFs is of importance, especially in TSFs with lower buffering capacity. The fact that relatively high and low concentrations of Ca, Fe and Cu were observed in both neutral and acidic drainage from the column leachate analysis, is of concern and highlights the need to properly manage the mine waste sites to avoid the TSFs impacting the aquatic ecosystem over time.

3.5. Conclusion

The analysis of the potential for ARD generation through the different approaches, including mineralogy analysis, amalgamation of NAG and NAPP analysis, and biokinetic tests, combined with the column leach analysis employed to assess potential for metal mobilization and its related ecological risks, was applied to tailing samples from copper mine wastelands. The core purposes were to provide an overall assessment of the tailing material in a limited time span. The ARD static testing carried out on the tailing samples from TSFs in the Kafue River catchment on the Zambian Copperbelt revealed a non-acid forming classification. This could be attributed to the lower sulphur content of the tailing samples coupled with a high neutralising ability as observed in the XRD results. The standard ARD classifications for potential acid generation for both tailing samples, together with the acid consuming and acid generating elements was verified using the biokinetic tests. The biokinetic tests also indicated the rapid depletion of neutralising capacity when exposed to acidic conditions. To aid in the understanding of potential ecological risks associated with the mobilization of metals within the TSFs, column bioleach tests were conducted with respect to conditions likely to cause metal mobilization. This was applied to evaluate potential risks related to mobilization of elements under conditions representative of TSF scenarios.

Although the tailing samples were classified as non-acid forming following the static and biokinetic ARD tests, ecological risk associated mobilization of elements Fe, Ca, Cu, Al, Mg, Mn, Zn, Co, Ni and Pb remained. Investigation of the leachate solutions that were collected from the column leach tests established Fe, Cu and Mn as elements worthy of note concerning ecological degradation for the TSFs under these conditions These results were particularly interesting considering the non-acid forming classification and emphasize the need to use a suite of pertinent tests to confirm the overall ecological risk. This knowledge is key to understanding and guiding more detailed risk assessment studies as well as designing relevant mitigation strategies. This research has demonstrated the importance of assessing both the acidity and overall ecological risks, including mobilization of metals across mine wastelands. Furthermore, investigations of potential for metal mobilization might help to understand or anticipate the likely impacts on the ecosystems, thus help to redesign, and repurpose these waste streams.

3.6. References

Alexander, K.B.O., 2013. An investigation into accelerated leaching for the purpose of ARD mitigation. Masters' thesis, University of Cape Town

Ali, H., Khan, E., Ilahi, I., 2019. Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation [WWW Document]. Journal of Chemistry. https://doi.org/10.1155/2019/6730305

Beilfuss, R., 2012. A Risky Climate for Southern African Hydro: assessing hydrological risks and consequences for Zambezi River Basin Dams. https://doi.org/10.13140/RG.2.2.30193.48486

Ben Ali, H.E., Neculita, C.M., Molson, J.W., Maqsoud, A., Zagury, G.J., 2019. Performance of passive systems for mine drainage treatment at low temperature and high salinity: A review. Minerals Engineering 134, 325–344. https://doi.org/10.1016/j.mineng.2019.02.010

Broadhurst, J.L., Petrie, J.G., 2010. Ranking and scoring potential environmental risks from solid mineral wastes. Minerals Engineering, Special issue: Sustainability, Resource Conservation and Recycling 23, 182–191. https://doi.org/10.1016/j.mineng.2009.09.008

Broughton, D.W., 2013. Geology and ore deposits of the Central African Copperbelt (Thesis). Colorado School of Mines.

Cánovas, C.R., Hubbard, C.G., Olías, M., Nieto, J.M., Black, S., Coleman, M.L., 2008. Hydrochemical variations and contaminant load in the Río Tinto (Spain) during flood events. Journal of Hydrology 350, 25–40. https://doi.org/10.1016/j.jhydrol.2007.11.022

Carvalho, P.C.S., Neiva, A.M.R., Silva, M.M.V.G., Antunes, I.M.H.R., 2014. Metal and metalloid leaching from tailings into streamwater and sediments in the old Ag–Pb–Zn Terramonte mine, northern Portugal. Environmental earth sciences 71, 2014. DOI 10.1007/s12665-013-2605-7

Chileshe, M.N., Syampungani, S., Festin, E.S., Tigabu, M., Daneshvar, A., Odén, P.C., 2020. Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. Journal of Forest Research 31, 1283–1293. https://doi.org/10.1007/s11676-019-00921-0

Christophoridis, C., Kosma, A., Evgenakis, E., Bourliva, A., Fytianos, K., 2019. Determination of heavy metals and health risk assessment of cheese products consumed in Greece. Journal of Food Composition and Analysis 82, 103238. https://doi.org/10.1016/j.jfca.2019.103238

Davey, J., Roberts, S., Wilkinson, J., 2020. Copper- and cobalt-rich, ultrapotassic bittern brines responsible for the formation of the Nkana-Mindola deposits, Zambian Copperbelt. Geology 49, 341-345. https://doi.org/10.1130/G48176.1

Fan, L., Zhou, X., Luo, H., Deng, J., Dai, L., Ju, Z., Zhu, Z., Zou, L., Ji, L., Li, B., Cheng, L., 2016. Release of Heavy Metals from the Pyrite Tailings of Huangjiagou Pyrite Mine: Batch Experiments. Sustainability 8, 96. https://doi.org/10.3390/su8010096Fernando, W.A.M., Ilankoon, I.M.S.K., Syed, T.H., Yellishetty, M., 2018. Challenges and opportunities in the removal of sulphate ions in contaminated mine water: A review. Minerals Engineering 117, 74–90. https://doi.org/10.1016/j.mineng.2017.12.004

Guo, Y., Huang, P., Zhang, W., Yuan, X., Fan, F., Wang, H., Liu, J., Wang, Z., 2013. Leaching of heavy metals from Dexing copper mine tailings pond. Transactions of Nonferrous Metals Society of China 23, 3068–3075. https://doi.org/10.1016/S1003-6326(13)62835-6

Harrison, S.T., Broadhurst, J.L., Van Hille, R., Oyekola, O., Bryan, C., Hesketh, A., Opitz, A., 2010. A systematic approach to sulphidic waste rock and tailings management to minimise acid rock drainage formation.

Heikkinen, P.M., Räisänen, M.L., 2009. Trace metal and As solid-phase speciation in sulphide mine tailings – Indicators of spatial distribution of sulphide oxidation in active tailings impoundments. Applied Geochemistry 24, 1224–1237. https://doi.org/10.1016/j.apgeochem.2009.03.007

Hesketh, A.H., Broadhurst, J.L., Bryan, C.G., van Hille, R.P., Harrison, S.T.L., 2010. Biokinetic test for the characterisation of AMD generation potential of sulfide mineral wastes. Hydrometallurgy, 18th International Biohydrometallurgy Symposium, IBS2009, Bariloche-Argentina, 13-17 September 2009 104, 459–464. https://doi.org/10.1016/j.hydromet.2010.01.015

Jamieson, H.E., Walker, S.R., Parsons, M.B., 2015. Mineralogical characterization of mine waste. Applied Geochemistry, Environmental Geochemistry of Modern Mining 57, 85–105. https://doi.org/10.1016/j.apgeochem.2014.12.014

Komadel, P. and Stucki, J.W. 1988. Quantitative assay of minerals for Fe (super 2+) and Fe (super 3+) using 1, 10-phenanthroline; III, A rapid photochemical method. *Clays and Clay Minerals*, *36*(4):379-381.

Korkmaz, C., Ay, Ö., Ersoysal, Y., Köroğlu, M.A., Erdem, C., 2019. Heavy metal levels in muscle tissues of some fish species caught from north-east Mediterranean: Evaluation of their effects on human health. Journal of Food Composition and Analysis 81, 1–9. https://doi.org/10.1016/j.jfca.2019.04.005

Kumar, B.L., Gopal, D.V.R.S., 2015. Effective role of indigenous microorganisms for sustainable environment. 3 Biotech 5, 867–876. https://doi.org/10.1007/s13205-015-0293-6

Larsson, M., Nosrati, A., Kaur, S., Wagner, J., Baus, U., Nydén, M., 2018. Copper removal from acid mine drainage-polluted water using glutaraldehyde-polyethyleneimine modified diatomaceous earth particles. Heliyon 4, e00520. https://doi.org/10.1016/j.heliyon.2018.e00520

Lovison, G., Gore, S.D., Patil, G.P., 1994. 4 Design and analysis of composite sampling procedures: A review, in: Handbook of Statistics, Environmental Statistics. Elsevier, pp. 103–166. https://doi.org/10.1016/S0169-7161(05)80006-9

Madzivire, G., Maleka, R.M., Tekere, M., Petrik, L.F., 2019. Cradle to cradle solution to problematic waste materials from mine and coal power station: Acid mine drainage, coal fly ash and carbon dioxide. Journal of Water Process Engineering, SI: Sust Water Processing 30, 100474. https://doi.org/10.1016/j.jwpe.2017.08.012

Mao, C., Song, Y., Chen, L., Ji, J., Li, J., Yuan, X., Yang, Z., Ayoko, G.A., Frost, R.L., Theiss, F., 2019. Human health risks of heavy metals in paddy rice based on transfer characteristics of heavy metals from soil to rice. Catena 175, 339–348. https://doi.org/10.1016/j.catena.2018.12.029

Masindi, V., Chatzisymeon, E., Kortidis, I., Foteinis, S., 2018. Assessing the sustainability of acid mine drainage (AMD) treatment in South Africa. Science of The Total Environment 635, 793–802. https://doi.org/10.1016/j.scitotenv.2018.04.108

Mbewe, G., Mutondo, M., Maseka, K., Sichilongo, K., 2016. Assessment of Heavy-Metal Pollution in Sediments and Tilapia Fish Species in Kafue River of Zambia. Archives of Environmental Contamination and Toxicology 71, 383–393. https://doi.org/10.1007/s00244-016-0295-3

McCarthy, T.S., 2011. The impact of acid mine drainage in South Africa. South African Journal of Science 107, 01–07. https://doi.org/10.4102/sajs.v107i5/6.712

Miller, S., 2008. Development of ARD assessment for coal process wastes (ACARP project C15034). Australia: Environmental Geochemistry International Pty Ltd.

Naidu, G., Ryu, S., Thiruvenkatachari, R., Choi, Y., Jeong, S., Vigneswaran, S., 2019. A critical review on remediation, reuse, and resource recovery from acid mine drainage. Environmental Pollution 247, 1110–1124. https://doi.org/10.1016/j.envpol.2019.01.085

Neculita, C.M., Rosa, E., 2019. A review of the implications and challenges of manganese removal from mine drainage. Chemosphere 214, 491–510. https://doi.org/10.1016/j.chemosphere.2018.09.106

Nordstrom, D.K., Blowes, D.W., Ptacek, C.J., 2015. Hydrogeochemistry and microbiology of mine drainage: An update. Applied Geochemistry, Environmental Geochemistry of Modern Mining 57, 3–16. https://doi.org/10.1016/j.apgeochem.2015.02.008

Opitz, A., 2013. An investigation into accelerated leaching for the purpose of ARD mitigation. Masters thesis, University of Cape Town, South Africa.

Opitz, A.K.B., Becker, M., Harrison, S.T.L., Broadhurst, J.L., 2016. Characterising Environmental Risks Associated with Sulfide-bearing Gold Wastes. Proceedings IMWA 2016, Freiberg/Germany

Patil, G.P., 1995. Editorial: Composite sampling. Environmental and Ecological Statistics 2, 169–179. https://doi.org/10.1007/BF00456662

Plante, B., Benzaazoua, M., Bussière, B., 2011a. Kinetic Testing and Sorption Studies by Modified Weathering Cells to Characterize the Potential to Generate Contaminated Neutral Drainage. Mine Water and the Environment 30, 22–37. https://doi.org/10.1007/s10230-010-0131-3

Plante, B., Benzaazoua, M., Bussière, B., 2011b. Predicting Geochemical Behaviour of Waste Rock with Low Acid Generating Potential Using Laboratory Kinetic Tests. Mine Water and the Environment 30, 2–21. https://doi.org/10.1007/s10230-010-0127-z

Qu, X., Wu, N., Tang, T., Cai, Q., Park, Y.-S., 2010. Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams. Ann. Limnol. - Int. J. Lim. 46, 291–302. https://doi.org/10.1051/limn/2010027

Rodríguez, L., Gómez, R., Sánchez, V., Villaseñor, J., Alonso-Azcárate, J., 2018. Performance of waste-based amendments to reduce metal release from mine tailings: One-year leaching behaviour.

Journal Environmental Management 209, 1–8. https://doi.org/10.1016/j.jenvman.2017.12.031

Singh, R., Mangat, N.S., 1996. Systematic Sampling, in: Singh, R., Mangat, N.S. (Eds.), Elements of Survey Sampling, Kluwer Texts in the Mathematical Sciences. Springer Netherlands, Dordrecht, pp. 145–164. https://doi.org/10.1007/978-94-017-1404-4_6

Smart, R., Skinner, W.M., Levay, G., Gerson, A.R., Thomas, J.E., Sobieraj, H., Schumann, R., Weisener, C.G., Weber, P.A., Miller, S.D. and Stewart, W.A. 2002. ARD test handbook: project P387A, prediction and kinetic control of acid mine drainage. *AMIRA, International Ltd, Ian Wark Research Institute, Melbourne, Australia*.Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Sracek, O., Filip, J., Mihaljevič, M., Kříbek, B., Majer, V., Veselovský, F., 2011. Attenuation of dissolved metals in neutral mine drainage in the Zambian Copperbelt. Environ Monit Assess 172, 287–299. https://doi.org/10.1007/s10661-010-1334-6

Sracek, O., Mihaljevič, M., Kříbek, B., Majer, V., Veselovský, F., 2010. Geochemistry and mineralogy of Cu and Co in mine tailings at the Copperbelt, Zambia. Journal of African Earth Sciences 57, 14–30. https://doi.org/10.1016/j.jafrearsci.2009.07.008

Stewart, W., Miller, S., Smart, R., 2006. Advances In Acid Rock Drainage (ARD) Characterisation of Mine Wastes 1. Journal of the American Society of Mining and Reclamation 2006, 2098-2119. DOI:10.21000/JASMR06022098

Torremans, K., Gauquie, J., Boyce, A.J., Barrie, C.D., Dewaele, S., Sikazwe, O., Muchez, Ph., 2013. Remobilisation features and structural control on ore grade distribution at the Konkola stratiform Cu–Co ore deposit, Zambia. Journal of African Earth Sciences 79, 10–23. https://doi.org/10.1016/j.jafrearsci.2012.10.005

Vriens, B., Peterson, H., Laurenzi, L., Smith, L., Aranda, C., Mayer, K.U., Beckie, R.D., 2019. Long-term monitoring of waste-rock weathering at the Antamina mine, Peru. Chemosphere 215, 858–869. https://doi.org/10.1016/j.chemosphere.2018.10.105

Wang, P., Sun, Z., Hu, Y., Cheng, H., 2019. Leaching of heavy metals from abandoned mine tailings brought by precipitation and the associated environmental impact. Science of The Total Environment 133893. https://doi.org/10.1016/j.scitotenv.2019.133893

Watling, H.R., Shiers, D.W., Collinson, D.M., 2015. Extremophiles in Mineral Sulphide Heaps: Some Bacterial Responses to Variable Temperature, Acidity and Solution Composition. Microorganisms 3, 364–390. https://doi.org/10.3390/microorganisms3030364

Weber, P., Hughes, J., Conner, L.B., Lindsay, P., St, R., Smart, C., 2006. Short-Term Acid Rock Drainage Characteristics Determined by Paste pH And Kinetic NAG Testing: Cypress Prospect, New Zealand 1. Journal of the American Society of Mining and Reclamation 2006, 2289-2310. DOI:10.21000/JASMR06022289

3.7. Supplementary Material

Table S3-1: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for non-controlled pH batch biokinetic test Chibuluma sample

Chibuluma Un-controlled pH										
Day	**pH**	**Stdev**	**Redox**	**Stdev**	**[Fe2+]**	**Stdev**	**[FeTot]**	**Stdev**	**Fe3+**	**Stdev**
0	3,39	0,03	504	6,02	26,4	19,4	20,9	14,9	-5,43	6,46
1	7,69	0,14	249	2,62	12	8,82	12,4	7,73	0,39	1,1
2	7,68	0,09	250	2,05	11,2	2,39	1,16	0,95	-10,1	3,05
3	7,73	0,25	244	2,62	12	4,28	6,98	4,14	-5,04	2,39
4	7,96	0,08	253	5,31	72,1	7,6	31	4,49	-41,1	7,96
5	7,6	0,13	266	3,68						
6	7,49	7,49	296	1,25	33,3	22	45,7	14,4	12,4	7,67
7	7,53	0,11	305	1,89	31	14	17,4	7,17	-13,6	9,12
8	7,28	0,18	309	2,36	29,8	3,59	29,8	9,51	0	5,93
9	7,86	0,03	308	6,65	6,2	8,77	26	2,9	19,8	11,6
10	7,56	0,07	340	7,41	45,1	4,78	21,3	2,9	-23,6	2,19
11	7,17	0,02	355	11,4	26,4	8,51	19	9,7	-7,36	2,19
12	6,94	0,18	388	9,43	38	48	43	51,8	5,04	4,28
13	6,97	0,05	422	5,19	26,4	5,23	21,3	6,03	-5,04	2,74
14	6,6	0,19	462	16,1	42,6	13,2	40,7	10,3	-1,94	2,9
15	6,89	1,01	425	5,44	20,5	5,8	20,2	2,9	-0,39	3,95
16	6,75	0,09	443	23	12,8	3,8	16,7	4,49	3,88	1,45
17	7,25	0,28	400	34,9	19,4	4,87	31,8	6,87	12,4	2,39
18	7,26	0,34	388	23,2	21,3	7,67	20,9	5,93	-0,39	1,98
19	7,37	0,31	382	23	22,9	8,61	20,2	6,18	-2,71	3,33
21	7,52	0,11	360	7,87	37,2	11	19,4	12,6	-17,8	2,39
24	7,55	0,07	354	8	10,9	7,19	19,8	6,23	8,91	1,98
28	7,65	0,04	348	4,64	27,1	2,9	26	6,18	-1,16	5,78
31	7,65	0,01	343	3,3	22,9	11,8	15,1	13,7	-7,75	3,59
35	7,72	0	345	2,49	5,04	2,39	19	5,4	14	7,77
38	7,79	0,02	349	6,38	8,14	5,29	3,88	1,45	-4,26	6,46
42	7,7	0,03	344	13,4	32,2	7,25	33,3	6,67	1,16	0,95
45	7,72	0,03	335	0,82	32,2	6,67	25,2	4,87	-6,98	10,8
49	7,97	0,07	347	19,1	28,3	7,91	29,1	7,42	0,78	1,45
52	7,92	0,1	354	6,6	29,1	6,23	27,1	3,95	-1,94	3,05
56	7,79	0,13	369	3,09	22,5	4,87	29,1	10,1	6,59	5,23
59	7,43	0,06	370	3,86	51,9	22,8	66,3	28,9	14,3	6,1
63	7,42	0,05	374	10,2	34,9	4,93	38	4,68	3,1	4,39
66	7,27	0,04	378	2,62	34,1	2,9	30,6	4,49	-3,49	1,64
70	7,3	0,14	401	5,72	13,2	5,72	12,4	3,84	-0,78	5,23
73	7,44	0,15	402	4,32	40,7	11	32,6	3,42	-8,14	8,28
77	7,48	0,02	394	8,98	22,9	5,72	22,1	11,9	-0,78	6,32
80	7,3	0,04	400	3,09	39,9	7,37	38	2,39	-1,94	5,23
84	7,57	0,09	410	2,05	48,1	4,28	45,7	3,95	-2,33	0,95
87	7,7	0,12	403	4,19	47,3	13,8	45,4	13,1	-1,94	1,45
90	6,92	0,14	406	7,48	5,04	3,95	8,91	2,9	3,88	2,19

Table S3-2: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for pH-controlled batch biokinetic test Chibuluma sample

Chibuluma pH-controlled (flasks 4-6)										
Day	**pH**	**Stdev**	**Redox**	**Stdev**	**[Fe2+]**	**Stdev**	**[FeTot]**	**Stdev**	**Fe3+**	**Stdev**
0	3,49	0,24	511	3,4	7,36	3,95	12,8	3,42	5,43	5,23
1	7,39	0,09	253	1,41	26,7	18	19,4	12,3	-7,36	6,1
2	2,4	0,22	496	20,3	36,4	14,7	36,4	15,4	-14,7	3,33
3	2,14	0,08	507	7,12	42,3	9,74	37,2	9,16	-5,04	1,45
4	2,11	0,02	499	3,4	43,8	39,7	40,7	3,42	-3,1	43,1
5	2	0	509	3,27						
6	1,98	0,01	511	3,3	9,3	3,29	20,9	6,23	11,6	6,65
7	1,99	0,01	507	3,56	48,8	5,29	43,4	3,84	-5,43	1,45
8	1,99	0,01	509	11,2	46,1	3,59	41,9	2,85	-4,26	2,19
9	2,03	0	535	22,9	35,7	4,78	64	2,85	28,3	1,98
10	2,03	0	549	33,3	55	1,98	25,2	3,95	-29,8	3,95
11	2,02	0	561	23,8	52,3	8,11	41,5	8,51	-10,9	5,56
12	2,03	0	584	18,8	132	140	130	140	-1,94	1,45
13	2,04	0,01	589	24,2	65,1	11,4	59,3	14,7	-5,81	3,42
14	2,03	0,02	598	13,1	76,7	30,6	70,9	26	-5,81	5,29
15	2,02	0,02	599	8,29	62,8	4,14	56,2	3,05	-6,59	4,49
16	2,06	0,01	613	1,89	46,1	11,5	45	2,19	-1,16	9,49
17	2,05	0,02	609	5,44	43,8	9,74	46,9	4,49	3,1	6,32
18	2,03	0,01	613	0,82	32,6	5,29	33,3	1,45	0,78	3,95
19	2,02	0,01	607	2,87	43	3,42	46,9	5,4	3,88	3,05
21	2,03	0	608	5,73	54,7	1,64	38	7,19	-16,7	6,32
24	2,04	0	610	3,4	37,2	4,35	43,4	1,45	6,2	5,8
28	2,05	0,01	611	2,16	45,4	2,51	40,7	1,64	-4,65	2,51
31	2,06	0,01	592	2,16	67,8	9,51	54,7	8,7	-13,2	4,87
35	2,02	0	592	5,44	41,1	5,8	24,8	5,56	-16,3	0,95
38	2,04	0	607	5,25	49,6	5,8	50	4,93	0,39	3,84
42	2,02	0,01	666	7,48	62,8	1,64	65,5	2,39	2,71	3,95
45	2,02	0,01	641	8,38	53,5	4,14	48,8	2,85	-4,65	1,9
49	2,07	0	650	9,53	68,2	6,67	68,2	5,23	0	2,51
52	2,08	0	647	9,46	60,9	4,78	66,7	18,7	5,81	15,3
56	2,06	0,01	667	11,1	43,8	5,23	39,5	2,51	-4,26	2,74
59	2,04	0,01	643	9,74	53,9	8,77	54,7	9,35	0,78	2,39
63	2,03	0	633	11,5	49,2	3,84	54,3	3,05	5,04	1,98
66	2,05	0	653	2,94	57,8	6,74	51,9	3,84	-5,81	8,44
70	2,03	0,01	642	6,68	60,5	10,3	61,2	10,5	0,78	3,05
73	2,03	0,02	645	3,74	73,6	7,37	71,3	8,82	-2,33	4,35
77	2,04	0,01	640	1,63	66,3	8,11	71,7	6,67	5,43	8,61
80	2,03	0,01	637	7,93	75,2	5,72	79,8	3,84	4,65	1,9
84	2,07	0,01	657	10	74,8	8,51	77,1	12	2,33	4,35
87	2,06	0,02	671	1,7	92,6	7,62	90,7	8,44	-1,94	6,46
90	2,07	0,03	665	4,64	61,6	9,64	57	10	-4,65	9,06

Table S3-3: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for non-controlled pH batch biokinetic test TSF14 sample

TSF 14 Un-controlled pH (flasks 1-3)										
Day	**pH**	**Stdev**	**Redox**	**Stdev**	**[Fe2+]**	**Stdev**	**[FeTot]**	**Stdev**	**Fe3+**	**Stdev**
0	5,44	0,15	320	18,2	12,8	9,64	10,5	6,58	-2,33	4,14
1	8,12	0,02	248	0,47	23,3	10,8	10,1	1,45	-13,2	12,2
2	7,84	0,05	299	4,55	18,6	6,58	10,1	7,13	-8,53	3,84
3	8,02	0,02	258	1,7	6,98	2,85	1,16	1,16	-5,81	2,51
4	8,08	0,01	266	3,27	83,3	12,2	12,4	7,91	-70,9	6,65
5	7,82	0,03	278	4,99						
6	7,73	0,04	298	2,05	50,8	2,9	61,2	6,32	10,47	4,75
7	7,77	0,02	310	1,7	15,5	8,82	14,3	3,84	-1,16	5,02
8	7,58	0,03	317	6,48	62	23,1	42,6	19,2	-19,4	13,8
9	8	0,01	310	3,3	41,1	7,13	44,2	8,54	3,1	1,45
10	7,89	0,05	325	5,1	27,1	3,84	35,3	1,45	8,14	2,51
11	7,6	0,18	318	5,31	34,5	26,7	31,8	16,2	-2,71	10,6
12	7,33	0,1	353	4,78	11,2	1,98	3,1	2,9	-8,14	1,64
13	7,08	0,06	403	4,9	20,2	2,9	15,5	2,19	-4,65	3,29
14	7,09	0,23	396	2,16	20,9	4,35	19	3,59	-1,94	1,1
15	7,58	0,08	382	4,78	38,8	8,82	20,9	1,9	-17,8	9,51
16	7,13	0,04	372	2,62	27,1	21,2	23,6	21,2	-3,49	0
17	7,75	0,04	363	4,9	56,6	41,3	62	35,4	7,25	7,25
18	7,76	0,03	370	2,87	24,8	16,1	22,9	13,6	-1,94	3,05
19	7,81	0,04	359	1,25	112	121	105	123	-6,98	5,93
21	7,79	0,04	365	5,89	38,4	3,42	42,6	18	4,26	20,1
24	7,84	0,02	373	14,1	5,43	3,84	11,6	2,85	6,2	3,05
28	7,89	0,02	367	4,97	38	8,61	31	4,28	-6,98	5,02
31	7,88	0,02	359	8,06	38,8	7	19,4	2,9	-19,4	4,68
35	7,92	0,02	366	6,94	11,2	3,05	19,4	2,39	8,14	3,8
38	8,01	0,02	362	7,26	10,1	7,91	20,5	10,1	10,5	2,51
42	7,9	0,02	366	2,62	24	2,39	21,3	1,98	-2,71	1,45
45	7,87	0,03	374	1,7	20,9	1,64	23,6	3,33	2,71	2,19
49	8,15	0,01	387	8,64	31,4	2,51	28,7	2,19	-2,71	4,28
52	8,11	0,04	383	1,7	20,9	21,4	25,6	26,4	4,65	5,02
56	8,01	0,02	412	3,74	20,2	15,4	20,2	11,5	0	4,14
59	7,6	0,05	401	1,7	17,4	9,64	15,5	8,51	-1,94	3,84
63	7,75	0,03	423	1,7	26,4	24,2	26,7	24,7	0,39	0,55
66	7,7	0,01	418	2,16	26,4	14,4	21,3	13,7	-5,04	1,1
70	7,47	0,05	408	1,7	14,3	7,19	14,7	5,4	0,39	1,98
73	7,45	0,05	410	1,25	45	13,2	40,3	6,87	-4,65	6,65
77	7,65	0,05	407	2,05	38,3	8,7	41,5	8,82	3,1	5,23
80	7,58	0,04	402	6,65	50,4	8,29	41,1	7,37	-9,3	0,95
84	7,74	0,11	414	5,35	44,2	10,3	48,1	12,9	3,88	3,84
87	7,67	0,04	413	2,87	23,7	7,77	40,3	6,87	0,78	5,8
90	7,75	0,06	395	5,72	13,6	6,46	14,3	4,87	0,78	6,18

Table S3-4: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for pH-controlled batch biokinetic test TSF14 sample

TSF 14 Controlled pH (flasks 4-6)										
Day	**pH**	**Stdev**	**Redox**	**Stdev**	**[Fe2+]**	**Stdev**	**[FeTot]**	**Stdev**	**Fe3+**	**Stdev**
0	5,88	0,04	313	5,91	23,3	7,42	22,1	24	-1,16	20,1
1	7,72	0,06	256	6,02	31,8	13,5	11,6	2,51	-20,2	11,3
2	3,02	0,16	434	13,1	107	15,8	64	16,5	-42,6	13,5
3	2,34	0,06	461	3,4	62,4	10,2	36,1	6,65	-26,4	4,78
4	2,15	0,05	469	5,44	33,3	9,51	39,9	14,2	6,59	21,1
5	2,01	0	484	8,38						
6	1,99	0,01	503	19	39,5	0,95	3,1	3,59	-36,4	3,84
7	2,01	0	529	39,5	74,4	8,7	68,2	3,05	-6,2	8,19
8	2,01	0	544	25,6	80,2	1,64	73,3	3,42	-6,98	4,14
9	2,05	0	592	17,1	58,9	4,78	92,6	3,33	33,7	1,64
10	2,03	0,02	581	15,2	86,8	2,9	62	3,33	-24,8	0,55
11	2,02	0,01	599	2,62	80,2	4,93	73,6	6,46	-6,59	1,98
12	2,03	0,01	595	5,25	58,1	1,9	55,4	2,9	-2,71	4,78
13	2,04	0,01	599	3,09	68,6	7,42	61,6	5,7	-6,98	3,42
14	2,03	0,01	599	10,2	86,4	2,39	72,9	3,59	-13,6	1,98
15	2,03	0,01	602	10,3	81,8	1,98	67,8	3,84	-14	2,85
16	2,06	0,01	609	6,34	76,7	3,42	71,7	3,33	-5,04	1,1
17	2,06	0,01	603	8,83	86,1	18,5	88,4	18,7	2,33	0,95
18	1,98	0	612	9,2	81	6,67	81,4	4,35	0,39	4,68
19	1,98	0	604	3,74	74	4,68	74,4	0,95	0,39	4,87
21	2	0	609	3,86	96,9	13,3	70,9	8,28	-26	17
24	2	0	623	3,27	83	5,23	82,6	3,42	-0,39	1,98
28	2,02	0	628	2,94	145	3,59	127	11,9	-17,8	9,7
31	2,04	0	620	3,3	154	43,5	136	45,7	-18,2	2,74
35	2,04	0,01	623	3,74	130	90,5	104	56,1	-26	9,02
38	2,06	0	625	6,34	123	36,9	98,8	3,42	-23,6	39,8
42	2	0	654	1,25	109	10,1	100	6,03	-8,53	6,46
45	2,01	0	634	3,68	86,4	7,13	80,6	1,45	-5,81	7,77
49	2,06	0	638	0,94	96,9	11,3	96,5	4,35	-0,39	9,02
52	2,07	0	636	0,47	86,8	10,5	85,7	12,9	-1,16	2,51
56	2,06	0	651	1,63	89,2	1,45	84,5	1,45	-4,65	0,95
59	2,03	0	632	0,94	88	6,46	87,2	6,65	-0,78	0,55
63	2,03	0,01	624	0,82	87,2	6,85	91,9	3,42	4,65	3,42
66	2,02	0,01	634	4,19	89,2	8,19	93	5,93	3,88	3,84
70	2,04	0,02	631	5,79	79,5	19,5	93,4	13,8	14	14
73	2,04	0,01	639	8,65	95,7	11,3	95,7	12,6	0	2,51
77	2,05	0,01	642	6,16	70,5	8,82	79,1	6,65	8,53	3,84
80	2,05	0,01	650	7,32	90,7	3,42	89,2	8,07	-1,55	4,78
84	2,08	0,01	662	4,78	87,2	6,23	84,9	6,65	-2,33	1,64
87	2,08	0,01	667	2,49	113	8,19	115	5,93	1,94	2,39
90	2,08	0,02	666	1,25	80,2	3,42	83	6,74	2,71	3,33

Table S3-5: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for non-controlled pH batch biokinetic test TSF15A sample

TSF 15A Un-controlled pH (flask 1-3)										
Day	**pH**	**stdev**	**Redox**	**stdev**	**Fe2+**	**Stdev**	**FeTot**	**Stdev**	**Fe3+**	**Stdev**
0	5,45	0,46	259	9,42	16,3	5,78	8,91	2,39	-7,36	5,8
1	8,09	0,03	250	1,25	65,5	26,7	15,9	7,91	-49,6	24,3
2	7,81	0,04	271	3,09	27,1	11,5	5,43	1,98	-21,7	9,74
3	8,04	0,01	256	0,47	11,2	7,67	8,91	5,4	-2,33	3,42
4	8,1	0,01	263	2,16	68,6	19,1	9,3	7,17	-59,3	16,9
5	7,87	0,02	276	9,93						
6	7,79	0,02	295	2,62	51,6	4,28	64	1,9	12,4	2,39
7	7,83	0,02	309	1,41	38	15,7	35,3	15,2	-2,71	0,55
8	7,57	0,06	320	4,64	53,1	17,5	48,1	23,3	-5,04	6,03
9	8,02	0,02	304	0,82	26,4	2,9	29,1	0,95	2,71	3,59
10	8,01	0,01	310	0,47	31	10,4	30,6	2,9	-0,39	7,96
11	8,03	0	295	0,47	30,6	3,59	19,8	0,95	-10,9	4,39
12	7,99	0,01	324	2,49	5,43	1,45	6,59	3,95	1,16	4,14
13	8,03	0,01	339	6,24	22,5	6,32	14	3,42	-8,53	3,05
14	8,01	0,01	358	2,62	43	16,8	33,3	20,5	-9,69	30,7
15	7,99	0,01	359	2,62	26,4	7,91	20,5	6,67	-5,81	1,9
16	7,76	0,06	351	7,59	3,1	1,98	5,81	1,9	2,71	0,55
17	8,02	0,01	353	2,16	5,04	1,98	4,65	2,51	-0,39	1,1
18	7,94	0,09	358	12	12,8	7,42	11,2	7	-1,55	1,98
19	7,85	0,26	358	11,1	23,3	13,1	20,5	9,12	-2,71	8,51
21	7,93	0,06	351	4,03	57,4	20	53,1	7,62	-4,26	17,1
24	7,82	0,15	356	9,84	21,3	8,07	31,8	15,7	10,5	14
28	7,78	0,15	382	3,09	28,7	7,25	22,1	6,85	-6,59	0,55
31	7,85	0,07	373	4,64	54,3	3,95	28,68	4,78	-25,6	5,02
35	7,92	0,04	381	2,94	17,1	7,19	6,98	4,14	-10,1	11,3
38	8,01	0,02	380	12,1	16,3	7,77	16,7	11,3	0,39	3,59
42	7,91	0,01	381	11,3	42,3	7,67	40,7	9,06	-1,55	3,84
45	7,93	0,02	373	0,47	42,3	18,3	36,4	12,4	-5,81	5,93
49	8,18	0,02	385	1,89	62,4	3,84	58,1	3,29	-4,26	2,39
52	8,19	0,01	380	0,94	6,2	5,56	5,43	4,39	-0,78	1,45
56	8,04	0,02	414	1,25	8,14	0,95	1,55	0,55	-6,59	0,55
59	7,73	0,02	404	2,16	1,16	0,95	1,94	0,55	0,78	1,45
63	7,8	0,02	430	3,56	5,43	3,59	11,6	3,8	6,2	4,87
66	7,79	0,03	435	5,91	21,3	5,23	5,23	9,69	-2,71	0,55
70	7,7	0,05	413	5,1	19,4	11,3	18,2	7,37	-1,16	4,14
73	7,78	0,05	407	2,49	20,9	10,4	18,6	7,6	-2,33	2,85
77	7,84	0,07	408	6,53	19,4	10,1	14,3	3,84	-5,04	8,77
80	7,73	0,03	385	8,83	38,8	18,9	39,2	17,6	0,39	2,19
84	7,97	0,11	400	1,25	17,8	5,4	12	7,13	-5,81	2,51
87	8,03	0,02	397	3,68	31,4	11,6	33,7	6,85	2,33	5,7
90	8,02	0,05	396	5,56	19,8	7,77	8,14	4,35	-11,6	8,44

Table S3-6: Measured pH, redox potential, ferrous iron, total iron, ferric iron, and their standard deviations for pH-controlled batch biokinetic test Chibuluma sample

TSF 15A Controlled pH (flask 4-6)										
Day	**pH**	**Stdev**	**Redox**	**Stdev**	**[Fe2+]**	**Stdev**	**[FeTot]**	**Stdev**	**Fe3+**	**Stdev**
0	4,46	0,47	331	45,3	25,2	10,1	18,6	7,54	-6,59	3,05
1	8,06	0,05	246	4,08	45,4	16,5	29,5	12	-15,9	6,18
2	5,63	0,16	229	10,7	73,6	20,4	50,4	17,1	-23,3	4,35
3	5,1	0,41	230	29,6	45	14,9	17,8	7,73	0,78	8,07
4	4,89	0,52	248	23,8	13,6	8,61	41,1	4,39	27,5	12,4
5	3,26	0,31	332	21,1						
6	3,32	0,36	346	6,85	43,8	22,7	40,3	8,61	-3,49	28,8
7	2,7	0,16	406	3,68	147	29,9	20,5	3,59	-127	32,1
8	2,18	0	421	6,68	143	17	31,8	2,39	-111	17,1
9	1,99	0,02	453	15,9	158	19,5	95,7	16,3	-62	35,6
10	2,02	0,04	480	50,2	192	27,2	95	40,1	-96,5	62,6
11	2,01	0,01	499	60,5	205	21,2	134	32,9	-71,3	47,7
12	2,03	0,01	517	50,5	173	44,9	130	17,1	-42,6	34,7
13	2,05	0,02	568	48,8	196	41	181	30	-15,1	16,5
14	2,03	0	593	40,4	228	13	218	7,91	-9,69	5,23
15	2,04	0	609	33	263	17,4	256	21,5	-6,98	6,23
16	2,06	0,01	614	29,2	229	8,82	234	2,9	5,43	11
17	2,06	0	616	26,3	249	3,59	243	6,1	-5,81	2,85
18	2,02	0	624	18,8	237	7,91	239	10,5	1,94	2,9
19	2,02	0,01	628	11,4	247	5,23	243	16,3	-4,26	11,1
21	2,04	0	636	5,79	273	12,2	258	11,3	-15,1	0,95
24	2,03	0	643	1,41	273	13,8	274	11,6	1,94	3,05
28	2,03	0	645	6,24	299	13,8	287	14	-11,6	9,64
31	2,05	0	635	0,82	300	17,8	289	16,2	-11,6	1,64
35	2,03	0,01	644	0,47	323	17	291	14,1	-32,2	4,39
38	2,06	0,01	652	6,24	317	19	325	20,5	8,14	13,7
42	2	0	662	2,45	337	31,4	324	23,9	-11,6	7,77
45	2	0	650	4,08	310	11,1	304	9,06	-6,2	3,05
49	2,04	0	649	0,82	323	3,42	320	4,35	-3,49	1,9
52	2,05	0	654	2,45	324	17,4	321	12,6	-3,49	4,93
56	2,04	0	660	1,7	314	4,35	305	9,12	-8,53	6,46
59	2,01	0	646	1,25	319	8,97	316	5,8	-3,49	3,29
63	2,01	0	642	4,32	319	8,82	318	6,32	-1,16	5,29
66	2	0,01	651	1,7	324	10,3	321	10,4	-3,1	2,9
70	2,01	0,02	643	5,25	301	10,7	302	9,51	1,16	1,9
73	2	0,02	649	4,5	308	23,6	304	23,8	-3,49	8,11
77	2,01	0,01	651	4,55	295	8,19	282	20,4	-12,4	12,4
80	2,02	0,03	648	5,1	301	11,4	298	17,5	-3,1	6,87
84	2,05	0,03	664	5,73	320	7	318	7,19	-1,94	0,55
87	2,04	0,04	670	5,91	309	16,3	303	14,2	-5,43	7,91
90	2,03	0,03	669	4,92	283	11,5	275	6,32	-7,36	5,8

Table S3-7: Total concentration of elements in mg/L, from leachates collected from days 5, 20, 50, 80, 110 and 140

	Cu						Co					
	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Day 5	0,71	648	460	223	491	0,12	0,21	1,96	2,34	2,53	3,77	3,48
Day 20	0,23	123	261	114	323	42,1	0,07	1,45	1,4	1,26	2,32	2,23
Day 50	0,1	12,3	30,6	22,1	9,07	45,7		0,3	0,91	0,51	0,45	1,15
Day 80	0,08	14,1	55,1	7,95	4,25	19	0,01	0,2	0,8	0,5	0,29	0,73
Day 110	0,09	7,02	20,3	6,37	3,32	18,4	0,01	0,19		0,23	0,23	0,61
Day 140	0,01	2,79	5,75	9,23	2,86	17,5		0,2	0,18	0,22	0,2	0,56
	Fe						Ca					
Day 5	4	56,9	33				81,7	592	56,2	563	608	470
Day 20	1	524	569	432	240	0,11	25,1	56,1	140	136	590	574
Day 50	0,32	515	355	360	554	313	18,7	22,5	75,5	126	58,8	511
Day 80	0,19	506	323	308	513	578	14,2	43,3	222	83,7	25,1	428
Day 110	0,08	488	458	467	510	589	15,7	16,9	103	38,8	15,8	387
Day 140	0,1	501	540	475	520	607	14,2	14,6	26,1	11,7	16,1	353
	Al						Mg					
Day 5		2,96	1,98	0,51			1,42	72,6	90,4	147	188	360
Day 20		73,5	110	163	115	17,3	0,31	45,4	54,3	171	178	313
Day 50		52,6	233	316	58,8	69,7	0,36	36	30,6	112	47	157
Day 80		47,8	482	333	60,5	52,2	0,24	33,3	154	133	44	93,6
Day 110		45,9	94,4	45,1	52,6	51,6	0,17	30,3	39,5	23,2	32,3	74,9
Day 140		52,3	45	47,5	55,7	63,4	0,85	35,1	28,5	34,3	39,9	71,9
	Mn						Ni					
Day 5	0,63	30,1	37,1	36,2	21,4	27,7		0,1	1,09	0,4	0,18	0,18
Day 20	0,13	3,11	3,64	9,39	16,3	23,9		0,29	1,27	0,7	0,38	0,35
Day 50	0,05	2,26	5,49	4,87	2,51	9,68		0,18	3,43	0,55	0,27	0,28
Day 80	0,01	2,43	6,23	5,03	2,39	5,67		0,2	3,48	0,65	0,25	0,26
Day 110		1,8	2,04	1,5	2,05	4,66		0,19		0,15	0,23	0,24
Day 140		1,8	1,53	1,67	2,01	4,26		0,21	0,19	0,19	0,24	0,23
	Zn											
Day 5	0,55	0,88	1,91	2,14	0,17	0,43						
Day 20	0,01	1,08	13,7	3,06	2,25	19,6						
Day 50	0,01	0,48	1,96	1,89	1,92	14,9						
Day 80		0,48	2,04	1,33	1,65	12,6						
Day 110	0,01	0,35	1,96	0,59	1,34	10,1						
Day 140		0,42	0,4	0,41	1,21	11,4						

Table S3-8: IRMA Surface Fresh Water Quality Criteria

Metals & Metalloids	Units	Criteria	Most Sensitive Use	Source
Aluminium	ppm	0,03	Aquaculture	AUS, WHO
Arsenic	ppm	0,01	Human Health - Drinking Water	USEPA, Health CA, AUS, WHO
Calcium	ppm	100		WHO
Cobalt	ppm	0,05	Agriculture - Irrigation	AUS, CCME, FAO, USEPA, SA
Copper	ppm	1,3	Aquatic Organisms Fresh Water	USEPA, WHO
Iron	ppm	0,01	Aquaculture	AU, WHO
Magnesium	ppm	30	Aquaculture	WHO
Manganese	ppm	0,2 (Background Value)	Aquaculture	AUS
Nickel	ppm	0,1	Aquatic Organisms Fresh Water	WHO
Lead	ppm	0,015	Aquatic Organisms Fresh Water	USEPA, WHO
Zinc	ppm	5	Aquatic Organisms Fresh Water	USEPA, FAO

CHAPTER 4: A WATERSHED APPROACH IN INVESTIGATING IMPACTS OF MINE WASTE: A CASE STUDY OF NSELAKI, MULULU AND FIKONDO STREAMS

Water is an essential commodity to life. One of the prerequisites of sustainable development is to reduce contamination of freshwater resources. There is growing concern about the conditions of freshwater on the Zambian Copperbelt. Anthropogenic activities such as mining affects freshwater through discharge of effluents and seepage from waste rock impoundments and tailings, thus water pollution from waste rock and tailings needs to be monitored. Impacts of mining may need to be managed for centuries, hence the need for improved monitoring and mitigation strategies. This study was set up to investigate water quality in water resources within perimeters of influence from copper tailing storage facilities (TSF), providing opportunity to better understand potential interventions to reduce impact of TSFs on water resources and associated land areas. Through increasing the intensity of water and sediment sampling within selected streams in the Kafue River catchment, it is envisaged that new insights with respect to pollution mitigation strategies will facilitate best practice to minimise environmental impact of mining and thereby improve quality of life. This study will help increase understanding of the principal sources of contamination in the catchment in relation to land-use activities.

4.1. Introduction

Globally, there is a concern with regard to the impact of metals and metalloids in the biosphere, as they impact on human health and the provisioning of the ecosystem services (Csavina *et al.*, 2012; Dutta *et al*., 2020). Mining activities generate significant amounts of environmental contaminants (Briffa *et al*., 2020; Cimboláková *et al*., 2019; Masindi and Muedi, 2018). A wide range of contaminants that may be derived from these activities include production of acid rock drainage (ARD), mobilisation of heavy metals, and release of mine effluent (Higueras *et al*., 2016; Stewart, 2020). One of the ecological footprints of mining activities is the transformation of landscape into mining generated wastelands such as waste rock, overburden and tailings materials (Festin *et al*., 2019; Venkateswarlu *et al.,* 2016). Elemental mobilization from metalliferous mine wastelands poses serious risk on ecosystem integrity through ground or surface water contamination, bioaccumulation in food chains, and uptake by vegetation (Adamczyk-Szabela *et al*., 2015).

In Zambia, active and historical mine wastelands such as tailings storage facilities (TSF) have been observed as the principal sources of metal contaminants affecting the aquatic ecosystems (Chileshe *et al*., 2020; Ikenaka *et al*., 2010; Kapungwe, 2013). Particularly, the Kafue River catchment in the Copperbelt Province has been described as one of the most contaminated catchments in Zambia because of the long-term presence of mining related activities (Kambole, 2003; Ntengwe, 2005). The water and sediment qualities in the Kafue River and its tributaries have also been observed to be significantly affected by a blend of contaminants from mining on different spatial and temporal scales (von der Heyden and New, 2004). This situation has raised various ecological concerns particularly with streams in close proximity to TSFs where high incidences of metal contamination and reduction of resident aquatic community structures (macroinvertebrates and fish) have occurred (Lindahl, 2014; Mundike, 2004). However, information on the effects of metal deportment from TSF on rivers and streams in a highly mining-impacted catchment is inadequate. Therefore, the Copperbelt region in which both active and historical TSFs exist, provides an opportunity for evaluating the differences in selected abiotic variables under similar potentially impacted conditions of such mine wastelands.

Regardless of the presence of voluminous wastelands generated by mining activities in Zambia (whereby for instance, over 10000 ha is projected to contain about 791 million tons of tailing material on the Copperbelt) (Festin *et al*., 2019); there is a dearth of information regarding the effects of metal deportment from TSF on rivers and streams that lie downstream of the TSF in a highly mining-impacted catchment. Owing to this, the current study was set up to evaluate the impact of metal mobilization from TSFs on the aquatic ecosystem through a multiple comparative approach of TSFs and adjacent surface water resources. The study focused on understanding the impact of metal deportment from active TSFs (Chibuluma TSF, and TSF15A), and historical TSF (TSF14) on surrounding water resources (the Nselaki Stream, Fikondo Stream, and Mululu Stream respectively).

4.2. Materials and Methods

4.2.1. Site Selection and Description

Nselaki Stream, Fikondo Stream, and Mululu Stream were chosen for inclusion in the study based on their similarities in land use patterns (Figure 4-1). These streams are potential hot spots for metal contamination (Auditor General, 2014), because of their close proximity to TSFs. Additionally, these streams are comparable as they share similar climatic, geographical, and geological characteristics (Riedel *et al*., 2010; Shimaponda-Mataa *et al*., 2017), although the scale of impacts may differ due to the differences in the nature of the surrounding TSF (active or historical). The study sites were strategically selected to enhance comparison of impacts between the TFSs on water quality. TSF14 (78 years old), which is potentially anticipated to be influencing the chemical and physical signatures in Mululu Stream is not active, while the other two streams are surrounded by active TSFs (Moka, 2016). TSF14 was decommissioned in 2001. Chibuluma TSF and TSF15A are 35 and 42 years old, respectively. Characteristics of study area are summarised in Table 4-1.

A number of sampling sites were selected across the catchment areas namely, Nselaki Stream catchment (16 sampling points), Mululu Stream catchment (19 sampling points) and Fikondo Stream catchment (8 sampling points). The sites were monitored during and post rainy season over a period of three years (2018 – 2020). During the rainy seasons, the total sampling times were 5 whilst post rainy seasons 6. Freshwater quality signatures reported at Nselaki Stream at the downstream sampling points were compared to those noted in Mululu Stream and Fikondo Stream.

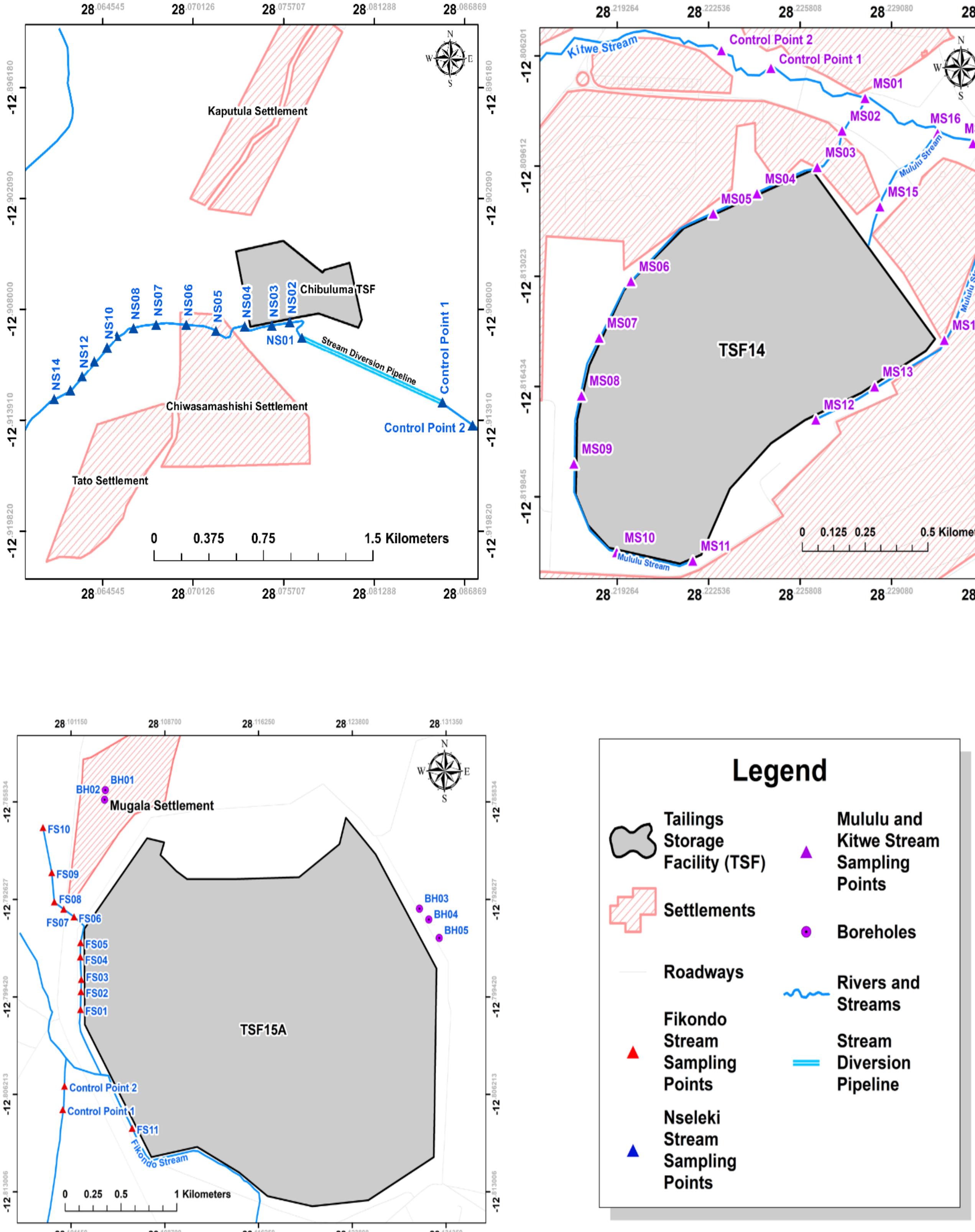


Figure 4-1: The map shows selected sampling points in Nselaki, Fikondo and Mululu streams, as well as TSFs in close proximity

Table 4-1: Summary of characteristics of study area in Kafue River catchment

Town		Kitwe	Kitwe	Kalulushi
Stream		**Fikondo**	**Mululu**	**Nselaki**
Tailing Storage Facilities - Characteristics	Active/Passive	TSF15A - Active	TSF14 - Decommissioned 2001	Chibuluma TSF
	Vegetation cover	Poor	Good	Poor
Major Upstream Activities		Agriculture	Residential Areas	Agriculture
Agriculture	Irrigation water and transportation mode	Irrigation furrows - water from Fikondo Stream	Mululu Stream	Irrigationfurrows - water from Nselaki Stream
	Type of irrigation	Furrows	Buckets, Plastic Containers	Furrows, Buckets, Plastic Containers, Flooding
	Type of soil	Clay loam, reddish brown	Clay, greyish brown	Sandy loam, greyish brown
	Type of crops	Rape, Cabbage, Chinese Cabbage, Green Beans, Tomatoes, Sweet Potatoes, Pumpkins, Amaranthus	Pumpkins, Cassava, Sweet Potatoes, Amaranthus, Rape, Egg Plants	Rape, Cabbage, Tomatoes, Chilli, Sweet Potatoes, Pumpkins, Maize, Amaranthus, Sugar Cane
	Area	26,4 Hectares	1.23 Hectares	10 Hectares
Industrial area			Garages	
Other			Veichle car wash	
Type of community setup	Formal settlement		Nkana East, Parklands	Chiwasamashishi, Tato, Kaputula villages
	Informal settlement	Mugala Compound		

4.2.2. Water and Sediment Collection

At each site, sediment samples were collected at 0 – 10 cm depth from the streams in triplicates, and in consonance with methods described by USEPA (2001). The sediment samples were stored in pre-cleaned plastic containers and taken to the laboratory for analysis

4.2.3. Water and Sediment Quality Analysis

Water quality variables, namely pH, temperature, DO, TDS, and turbidity were observed at each site using a HI98193 dissolved oxygen BOD/OUR/SOUR meter and HQ4200 portable multi-meter on site. At each site, water samples were collected in clean, pre-rinsed polyethylene sample container, filtered on site, and then stored in a cooler box before being taken to the laboratory for chemical analysis. At the laboratory, water samples were digested using concentrated nitric acid, in which 5 mL of acid was added to 50 mL of water sample. This was then heated in a beaker until it boiled, and the volume had reduced to about 20 ml. Another 5 mL of nitric acid was added to the mixture and heated further for 10 minutes, and then allowed to cool. The resultant solution was then poured into a 50 mL volumetric flask after which distilled water was added to make it up to the mark. The PinAAcle 900H Atomic Absorption Spectrometer was used to analyse metal concentration in the solutions.

The sediment samples were oven dried in the laboratory for 48 hours at 70 °C until constant weight was attained. Determination of granulometric composition of sediment samples was conducted by dry sieving for sand fractions, and hydrometer method for clay and silt. Samples were sieved to obtain a particle size of < 75 µm and to remove large pebbles, since metals are well known to stick to fine particles (Adamu *et al.*, 2015). The sediment samples were digested using concentrated nitric acid, where 1 g of dry sample was added in a beaker containing 30 ml of nitric acid. Thereafter, three drops of hydrofluoric acid (HF) were added to the mixture, and then the samples were heated with an electronic plate set at 120^{o}C for 20 minutes. The mixture was then allowed to cool after boiling, thereafter, filtered through into a 50 ml volumetric flask using filter paper, and made up to 50 ml with deionized water. The Atomic Absorption Spectrometer (AAS) was then used to determine metal concentration in the sediment samples.

The precision and reproducibility of analysis was monitored by analysing each sample in duplicate. These included two sample duplicates and one procedural blank sample. The relative percentage in differences between the duplicate samples were within 5%.

4.2.4. Statistical Analysis

The results from the streams were handled separately in order to determine the influence of the TSF on the streams. The Analysis of Variance (ANOVA) was used to test if there were significant differences in the reported metal concentrations in water and sediment samples. Significance was accepted at probability ≤ 0.05. Additionally, the datasets were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests (Mishra et al., 2019); to assess whether the data samples is within some tolerance. The homogeneity of variance was evaluated using Levene's and Brown-Forsythe's tests, this essentially ensures that distribution of outcomes for independent groups are comparable (James et al., 2013). The non-parametric or data that deviates from normality was transformed using box-Cox to meet ANOVA assumptions. Transformation helps to accommodate all observations into a distribution that is less skewed than original data (Vélez et al., 2015). Principal Component Analysis (PCA) was also conducted to evaluate the water quality difference between the streams within the catchment (Jolliffe, 2002). The results generated were expressed as an ordination disposition on a bidimensional base, where the placement of samples reflected the dissimilarities and similarities between sampling sites. Using this approach, anthropogenic activities responsible for impacts observed in the streams were determined.

4.3. Results

4.3.1. Water Quality – Physical Parameters

The spatial distribution of physical and chemical characteristics bound to the water samples at selected sampling sites in the three watersheds are presented in Tables S4-1 – S4-3 (see appendix). Further information on physical and chemical characteristics can be accessed on https://drive.google.com/drive/folders/1VkL0dmrP0Cc1M9UAZimtadsFgI44s1Eq?usp=sharing. The sampling points selected along Nselaki Stream had a relatively neutral pH throughout the sampling period. The pH and TDS values did not vary significantly among the sampling points (≈6.7 and 220-420 mg/l respectively). The lowest pH (≈5.1) was reported at site NS08, while sampling point NS06 was observed to have a higher TDS (≈613 mg/l). Similarly to Nselaki

Stream, concentrations in pH and TDS in Mululu Stream (≈6.8 and 271 mg/l respectively and Fikondo Stream (≈6.7 and 301 mg/l respectively) did not differ considerably While Mululu and Fikondo watersheds were equally dominated by a relatively neutral pH; incidences of low pH (≈5.5) were observed at sampling points MS10, MS08, FS06 and FS02 Overall. The water quality from Mululu Stream and Fikondo Stream had no significant difference from that of Nselaki Stream, particularly in respect to pH.

Based upon the comparable analysis of the water physical signature, our observations indicated that Fikondo Stream had higher turbidity concentrations (≈41.3 NTU) in comparison with Mululu (≈31.4 NTU) and Nselaki (≈32 NTU) streams. The highest turbidity value was reported at FS05 (178 NTU) during the rainy season. Higher levels of TDS were recorded at most of the sampling points of Nselaki Stream (≈ 495.4 mg/l) during the rainy season (Table S4-1). In Mululu Stream, turbidity and TDS concentrations varied significantly among sampling points. Lower concentrations of turbididty (≈19.1 NTU) and TDS (≈ 166 mg/l) were recorded at sampling points MS06 and MS03 respectively, during the rainy season. Post rainy season, sampling sites MS11 (≈10.5 NTU) and MS07 (and ≈ 218 mg/l) recorded the lowest turbididty and TDS respectively compared to other sites (≈18.5 NTU and ≈ 275 mg/l, respectively). The concentrations of DO in Mululu Stream (≈6.5 mg/l) varied significantly between sites, ranging from 3.49 mg/l to 9.4 mg/l. Similarly, siginificant variations of DO concentrations were reported in Nselaki Stream and Fikondo Stream. Sampling sites NS02 (3.16 mg/l) post rainy season and FS02 (2.49 mg/l) rainy season reported the lowest DO concentrations in Nselaki Stream and Fikondo Stream.

Multivariate analysis using the principal component analysis was conducted to evaluate the differences between the sampling sites. The colours and symbols represent datasets corresponding to particular sampling periods while the circles represented the major groupings in relation to similarities. The direction of the arrows represents the direction of increase of the corresponding principal component. The PCA analysis showed that most of the sampling sites within Nselaki Stream grouped together and were similar in water quality conditions, although sites NS06, NS08, and NS09 was noticed to be different from the other sites (Figure 4-2). The observed dissimilitude corresponds to a rise in concentrations of several contaminants. Notably, incidences of low pH values were reported at site NS06 (pH 5.2) and NS08 (pH 5.1) (Table S4-1), and which could be attributed to water from underground mining operations. Water discharged from underground was observed to be slightly acidic (≈ 5.6). Turbidity, and TDS were observed to significantly impact sites NS04, NS07, and NS08 (Table S4-1). The concentrations of TDS, turbidity and DO were observed to be high during the rainy season compared to post rainy season (Figure 4-3). The pattern was similar in Mululu and Fikondo streams (Figure 4-5 and 4-7).

Similarly, to Nselaki Stream, sites in Mululu Stream grouped together, although sites MS01, MS03, MS04, MS10, and MS16 (Figure 4-4), were observed to be different. Particularly sites, MS01, MS04 and MS10 were observed to have high turbidity values (≈70.2, ≈67.2, ≈95.2 NTU, respectively) (Table S4-2) during the rainy season. Water quality signatures reported at downstream sites in Mululu Stream, were comparable with those noted throughout Nselaki Stream. The pH and DO concentrations reported in Fikondo Stream throughout the study at selected sites did not vary significantly (≈6.71 and ≈6.23 mg/l). The water quality signature of Fikondo Stream was mostly dominated by low TDS and relatively high tubirdity compared to Nselaki and Mululu streams.

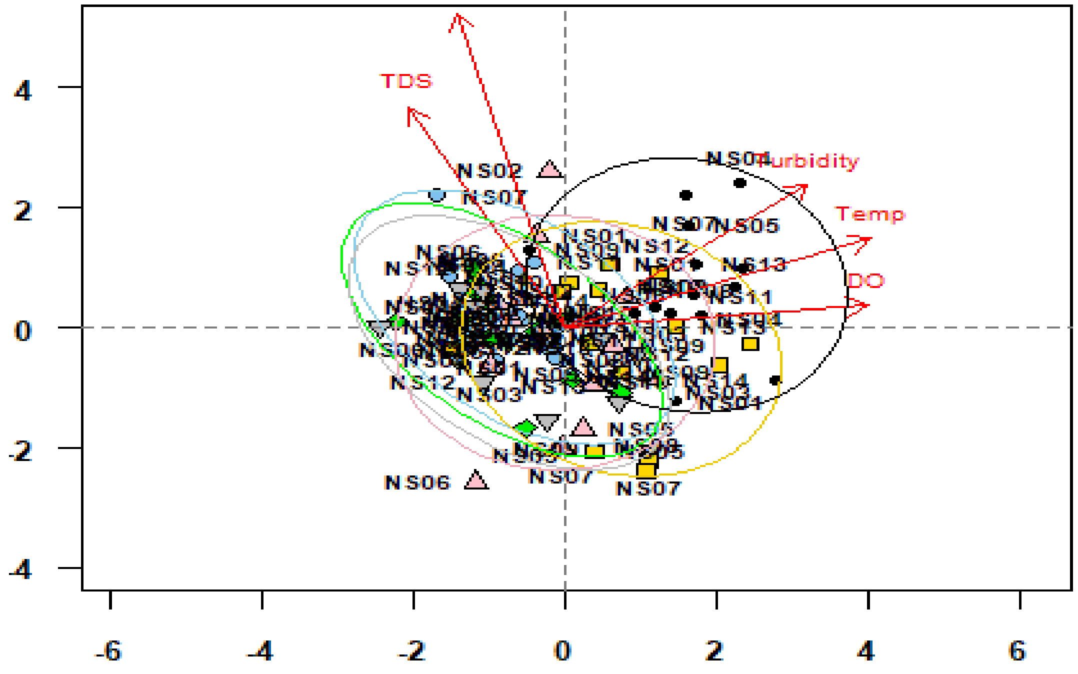


Figure 4-2: PCA biplots suggesting the similitude and differences of selected sampling sites in Nselaki Stream from the physical water quality parameters recorded at selected sites. PCA plot for Nselaki Stream accounts for 21.2% and 28% of the discrepancy observed on the 1st axis and 2nd axis. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

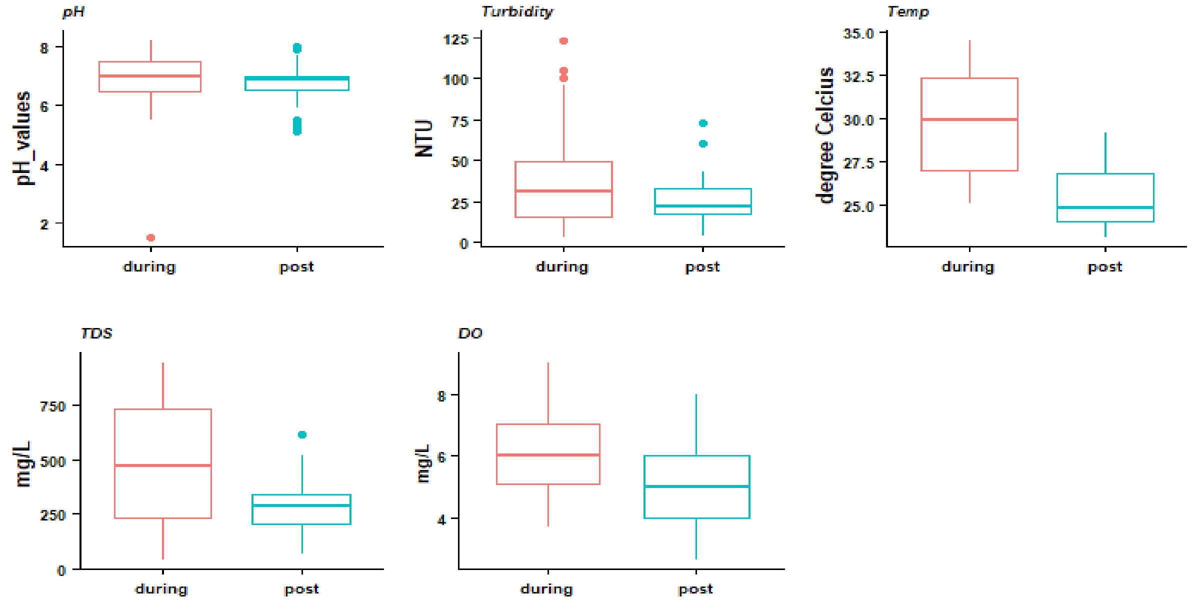


Figure 4-3: The boxplot shows the range and distribution of physical parameters pH, turbidity, temperature, TDS and DO in the water of selected sites in Nselaki Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes.

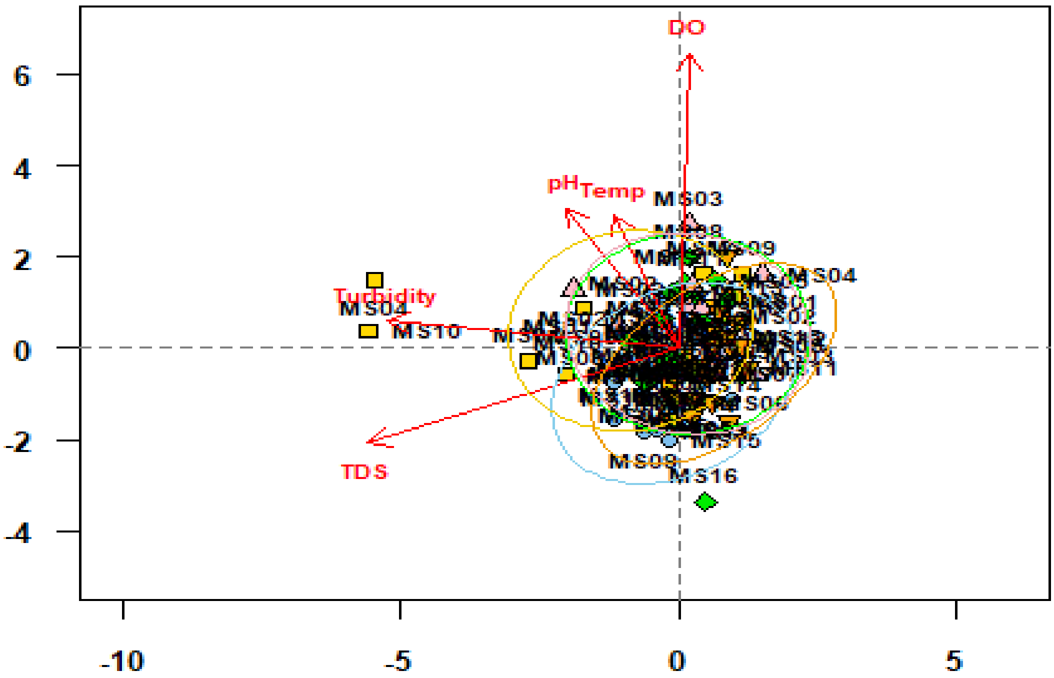


Figure 4-4: PCA biplots suggesting the similitude and differences of selected sampling sites in Mululu Stream from the physical water quality parameters recorded at selected sites. PCA plot for Mululu Stream accounts for 23.2% and 27.7% of the discrepancy observed on the 1st and

2nd axis. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

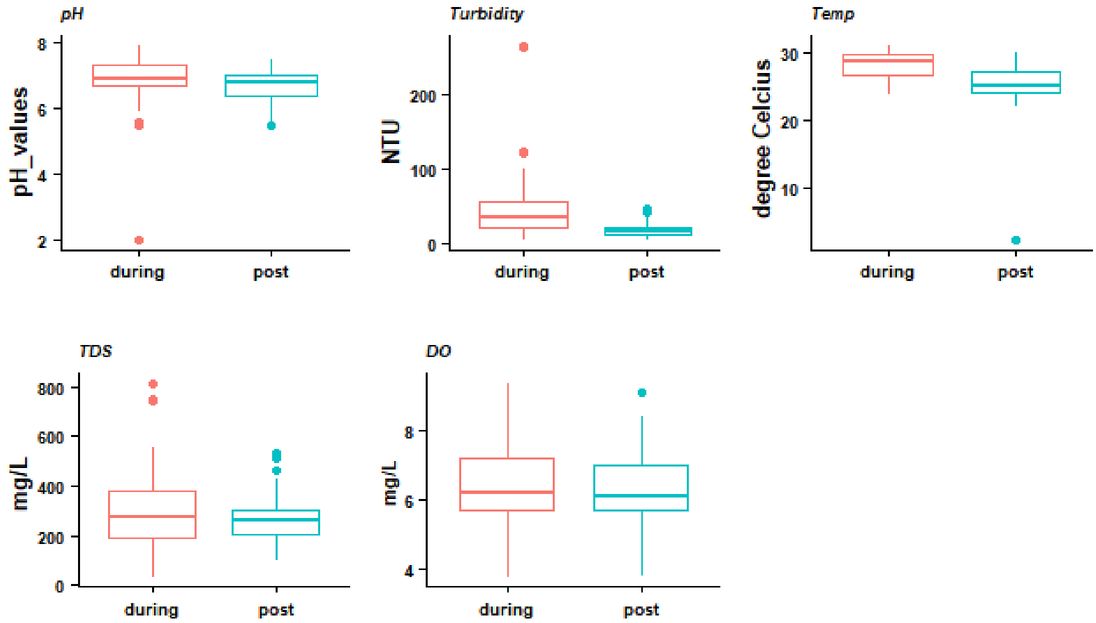


Figure 4-5: The boxplot shows the range and distribution of physical parameters pH, turbidity, temperature, TDS and DO in the water of selected sites in Mululu Streams. Significant differences during and post rainy season are indicated by the variability in the box plot size

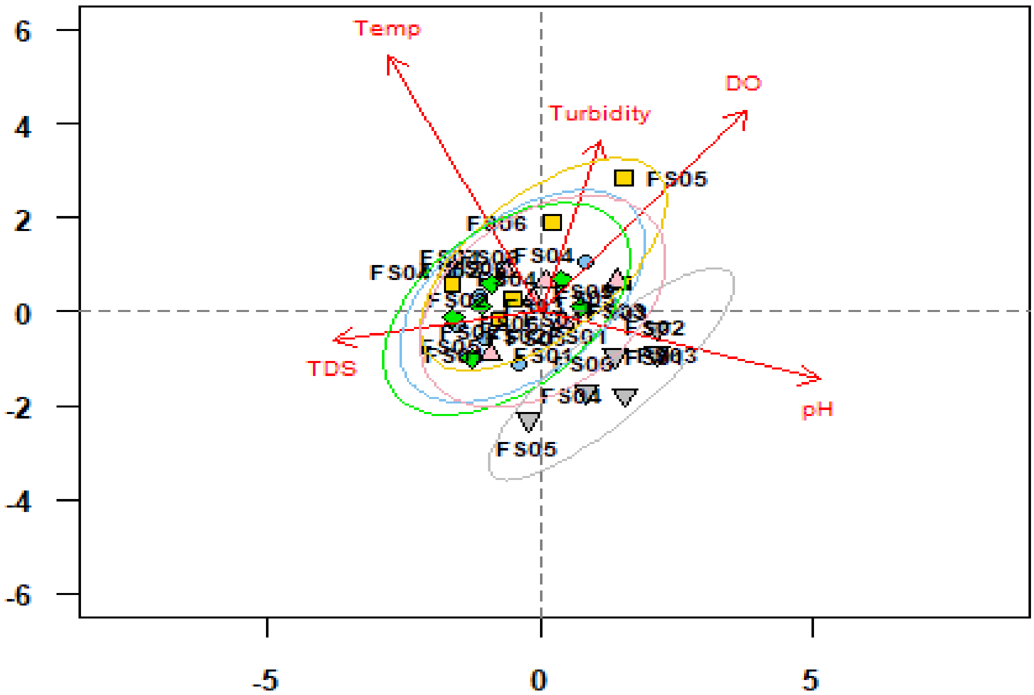


Figure 4-6: PCA biplots suggesting the similitude and differences of selected sampling sites in Fikondo Stream from the physical water quality parameters recorded at selected sites. PCA plot for Fikondo Stream accounts for 23.1% and 28% of the variation in the 1st and 2nd axis,

respectively. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

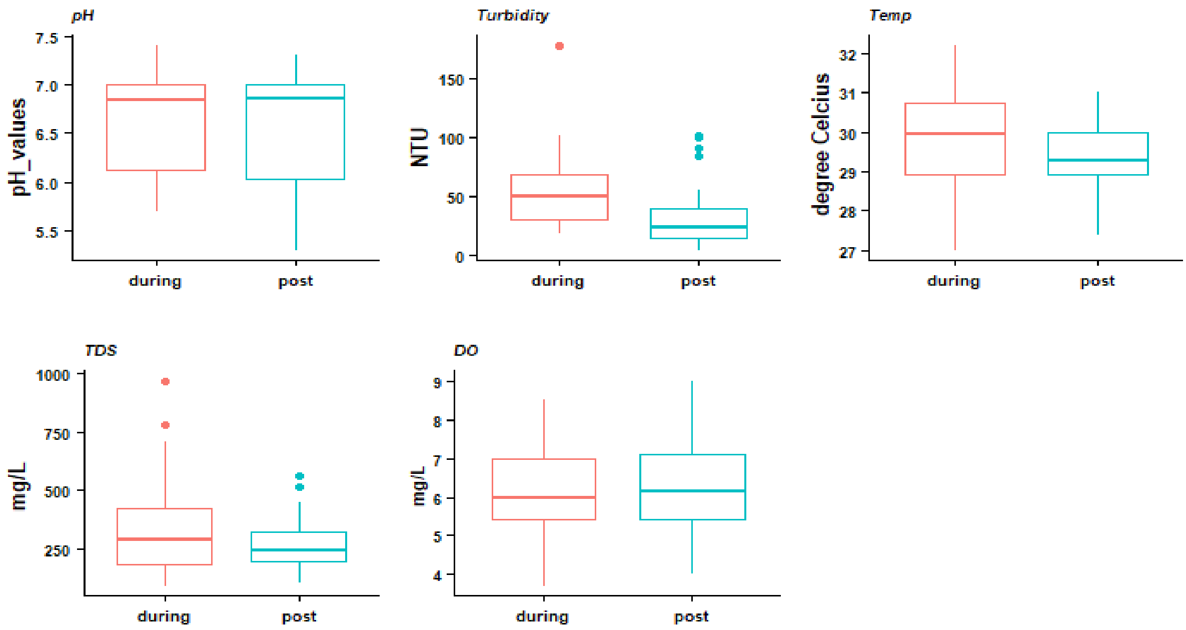


Figure 4-7: The boxplot shows the range and distribution of physical parameters pH, turbidity, temperature, TDS and DO in the water of selected sites in Fikondo Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes

4.3.2. Water Quality – Chemical Parameters

The spatial variation of selected metal concentrations evaluated within selected sites in Nselaki Stream, Mululu Stream, and Fikondo Stream are presented in Figures 4-8 to 4-10. Within Nselaki Stream, it was observed that there was a greater variability of Mn (≈0.11 ppm) and Cu (≈0.12 ppm) values compared to other elemental concentrations (Table S4-1). At the most downstream sites (NS08, NS10, NS11, NS12 and NS13), Cu concentrations were relatively high (≈ 0.14 ppm) post rainy season, throughout the sampling period. High Cu and Mn concentrations were measured at at NS08 (0.7 ppm and 0.4 ppm respectively) during the rainy season. Post rainy season, significant high Cu concentrations were observed at NS07 and NS04 (0.8 ppm and 0.7 ppm respectively) compared to other sites. A similar trend was reported regarding Mn concentrations at NS01 (≈0.17ppm), NS04 (≈0.23 ppm), NS06 (≈0.22 ppm), NS07 (≈0.2 ppm), and NS08 (≈0.19 ppm), which were higher in comparison to the other sampling points (≤0.15 ppm on average). Cobalt concentrations showed no significant variation although high Co concentrations (≈0.1 ppm) were reported at NS08 (during rainy season) and NS05, NS09 and NS13 (post rainy season) in comparison to the other sites (≤0.05). Higher Zn concentrations were observed at sampling points NS01 (post rainy season, ≈0.1 ppm) and NS09 (during rainy season, ≈0.11 ppm) when compared to the rest of the sampling points (<0.09 ppm). Lower concentrations of Pb (≈0.01 ppm) were observed at most of the sites.

The analysis of water samples from Mululu Stream and Fikondo Stream indicated that Zn, Co and Pb did not vary significantly among the selected sites (Figure 4-9). Copper concentrations were reported to be significantly higher at MS05 (≈0.17 ppm), MS08 (≈0.2 ppm), FS02 (≈0.18 ppm) and FS04 (≈0.3 ppm) compared to other sampling points (Table S4-2 and S4-3). Manganese concentrations showed variation when comparing the different sampling points in Mululu Stream, higher concentrations were observed at sites MS04 (≈0.21 ppm), MS07 (≈0.27 ppm), MS08 (≈0.3 ppm), MS09 (≈0.37 ppm) (Figure 4-9C) and MS03 (≈0.2 ppm) (Figure 4-9D) when compared to the rest of the sampling points (≈0.1 ppm). In Fikondo Stream, higher Mn concentrations were determined at sampling points FS02 (≈0.34 ppm), FS05 (≈0.42 ppm) and FS06 (≈0.38 ppm) in comparison to other sampling points (≈0.23 ppm) (Table S4-3).

Comparing all of the sites in Nselaki Stream to sites in Mululu and Fikondo Streams, higher Cu concentrations were observed at FS04 (≈0.3 ppm), NS07 (≈0.27 ppm) and NS04 (≈0.24 ppm) post rainy season compared to other sampling sites. The concentrations of Mn were generally observed to be similar, however, a significant increase was observed at sites MS07, MS08 MS09, FS02, FS05, and FS06 (≈0,35 ppm) in Mululu and Fikondo streams during the rainy season. On the other hand, the concentrations of Pb, Co, and Zn concentrations were observed to be similar between the three streams (Figure 4-11).

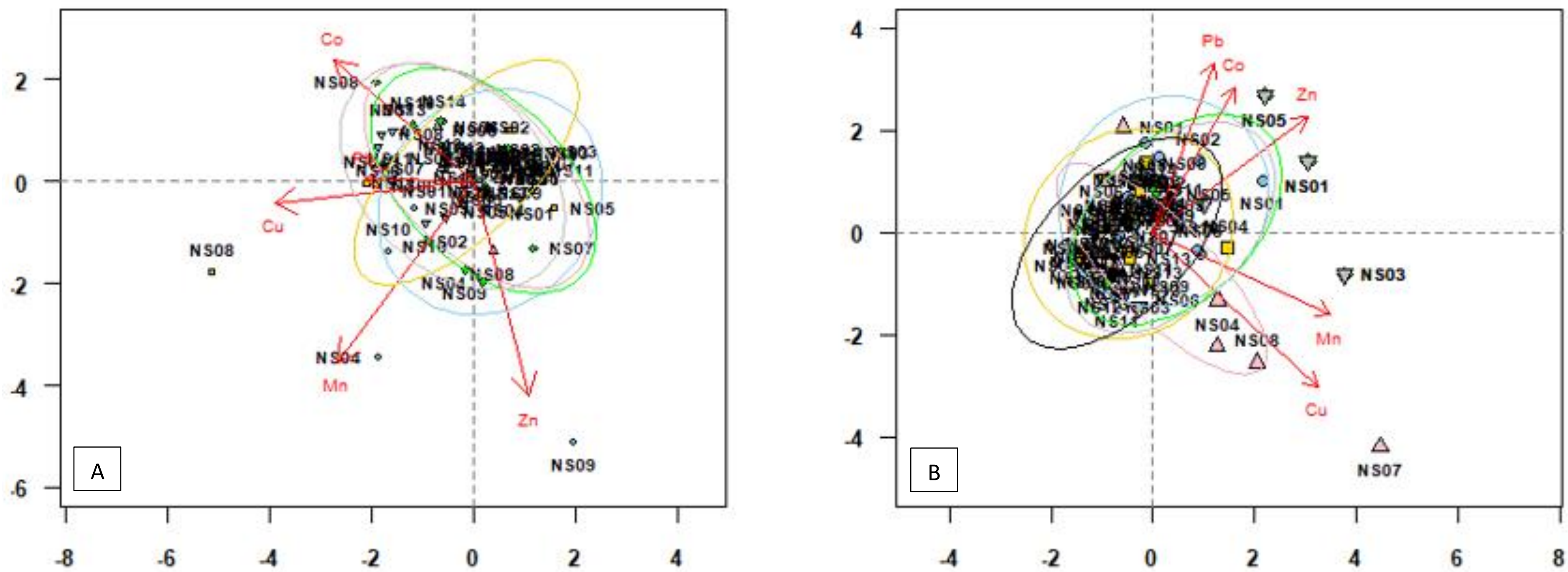


Figure 4-8: PCA biplots suggesting the similitude and differences of Nselaki Stream during (A) and post (B) rainy season derived from the selected measured chemical water quality parameters. PCA plot during season accounts for 27.7% and 22.5% of the variation in the 1st and 2nd axis. The PCA plot post rainy season accounts for 31% of the discrepancy on the 1st axis, while 2nd axis accounts for 22.6%. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

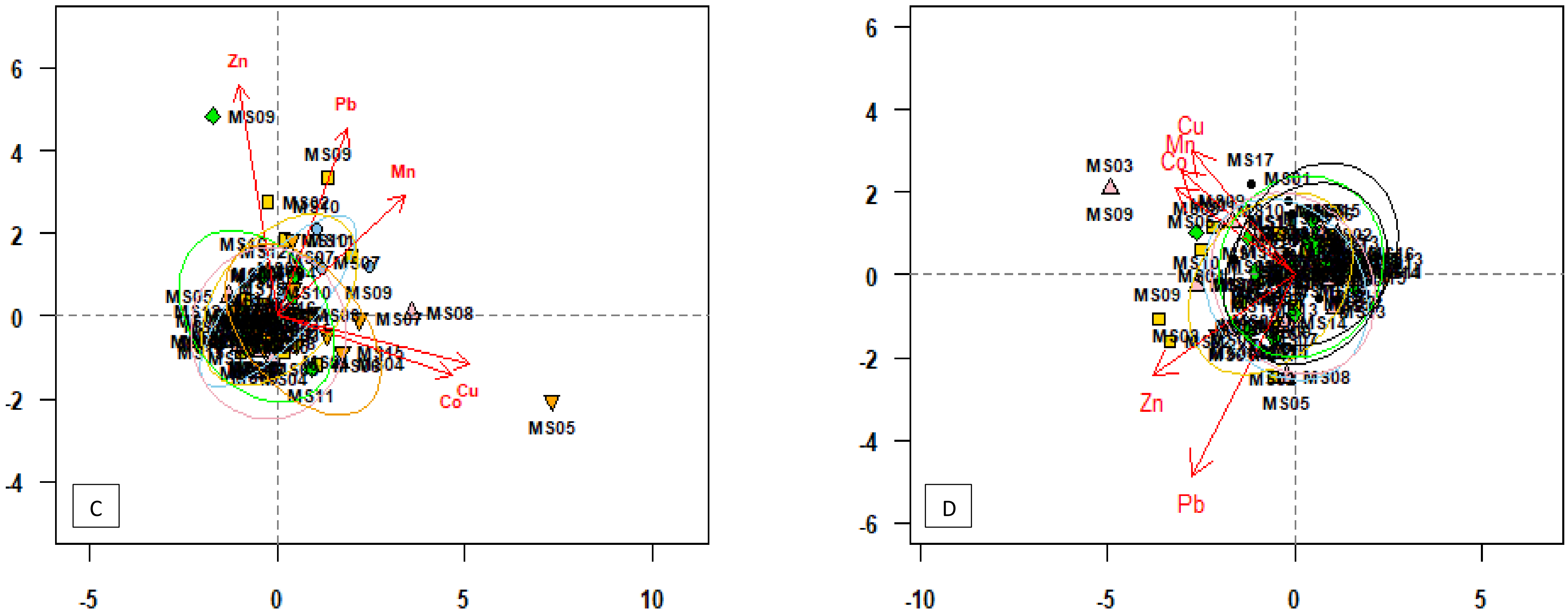


Figure 4-9: PCA biplots showing the similitude and differences of Mululu Stream during (C) and post (D) rainy season derived from selected measured chemical water quality parameters. PCA plot during season accounts for 30.3% and 22.6% of the variation in the 1st and 2nd axis. The PCA plot post rainy season accounts for 42.3% on the variation in the 1st axis, while the 2nd axis accounts for 20.9%. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

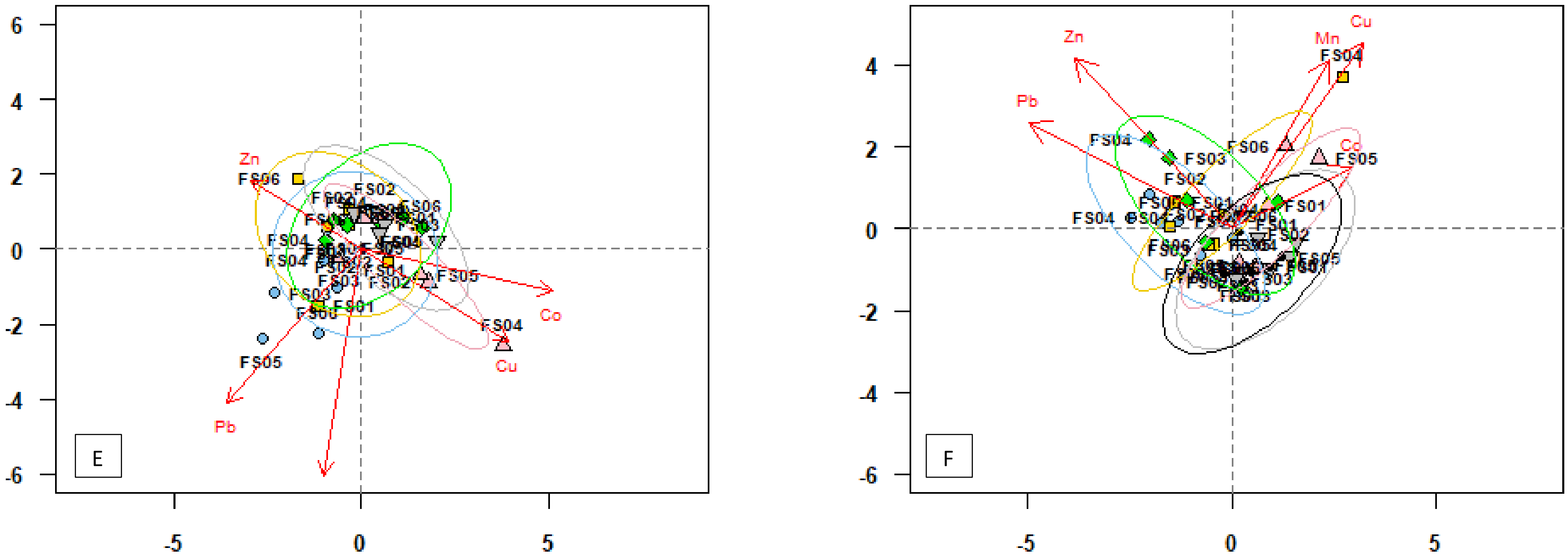


Figure 4-10: PCA biplots showing the similitude and differences of Fikondo Stream during (E) and post (F) rainy season derived from selected measured chemical water quality parameters. PCA plot during season accounts for 36.9% and 24.9% of the variation in the 1^{st} and 2^{nd} axis. The PCA plot post rainy season accounts for 30.5% on the variation in the 1^{st} axis while the 2^{nd} axis accounts for 26.4%. The symbols and colours represent different sampling periods *while major groupings are indicated by encirclements*

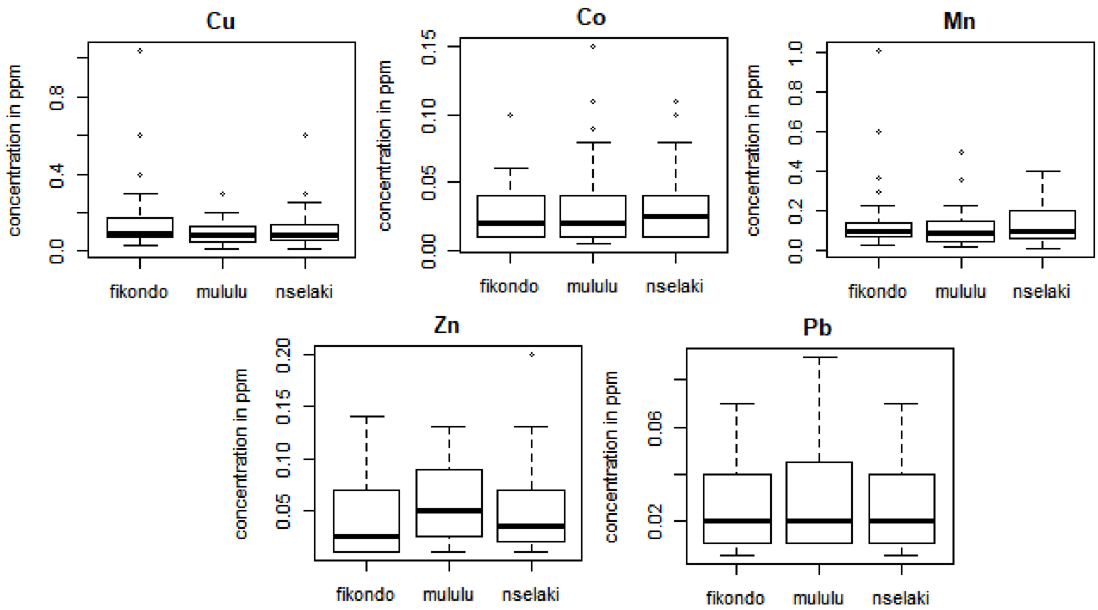


Figure 4-11: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese, (Zn) = Zinc, and (Pb) = Lead in the water of selected sites in Nselaki, Mululu and Fikondo Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes.

4.3.3. Sediment Quality

The spatial distribution of metals that are bonded with the sediment samples at the selected sampling points of Nselaki, Mululu and Fikondo streams is represented in Figure 4-12 to 4-14, Table 4-2 and Tables S4-4 to S4-6. From the results, the spatial distribution of Cu concentrations in Nselaki Stream during rainy season was high at NS01 (≈4000 ppm), NS02 (≈3118 ppm), NS03 (≈3968 ppm) and NS04 (≈3027 ppm) respectively, in comparison to the other sampling points. Copper concentration was observed to decrease with distance from pollution source (Table S4-4). However, no significant trend was observed post rainy season. Concentration of Mn was reported to be higher at NS01 (≈1707 ppm), NS10 (≈2176 ppm) and NS13 (≈2250 ppm) during the rainy season and NS01 (≈3083 ppm), NS04 (≈2439 ppm) and NS10 (≈2036) respectively, post rainy season. Cobalt concentrations were observed to vary at different sampling points with the highest concentration found at NS02 (≈1016 ppm) which showed significant difference with the concentrations reported at other sampling points. Elevated Zn concentrations were observed during the rainy season at NS10 (≈277 ppm) when compared to the rest of the sampling points (≈181 ppm), whilst lower Pb concentrations were recorded at NS04 (≈20.7 ppm) during rainy season and NS08 (≈19,3 ppm) post rainy season, compared to the rest of the sampling points (≈37.6 ppm).

Within the Mululu Stream, the spatial distribution of Co was observed to be vary significantly, high Co concentration was reported at MS12 and MS17 (≈620 ppm and ≈562 ppm, respectively) during the rainy season (Table S4-5). Low concentrations were observed in Zn (≈153.6 ppm) and Pb (≈ 47.7 ppm) compared to other metal species. The highest concentrations of Zn were recorded during rainy season at MS12 (≈256 ppm), MS113 (≈271 ppm) and MS17 (≈252 ppm), respectively; Pb at MS03 (≈70.7 ppm), MS06 (≈62.3 ppm), MS07 (≈64.3 ppm), and MS10 (≈85.7 ppm) respectively. Copper concentrations were observed to be higher during the rainy season (≈1983 ppm) compared to post rainy season (≈1522 ppm). Notably, concentration of Mn did not differ significantly across all sampling point throughout the sampling period. At Fikondo Stream, variations in Cu and Mn concentration were observed at the selected sampling points, with the apex concentration found at FS01 (≈2915 ppm, and ≈1795 ppm respectively) post rainy season. Equally, Cobalt concentration was observed to be significantly high at FS01 (≈379 ppm) post rainy season compared to the rest of the sampling points (≈241 ppm), whilst lower concentrations were recorded during the rainy season (≈185 ppm). When compared to the rainy season, Zn concentrations post rainy season were slightly higher (≈53.4 ppm and ≈102.4 ppm, respectively), while Pb variation across all sampling points was not significant.

When comparing the three stream systems it was found that Nselaki Stream had higher Cu, Co, Mn and Pb concentrations in the sediments compared to Mululu and Fikondo Streams (Table 4-2). Fikondo Stream had lower Zn concentrations at the selected sampling points, whilst Mululu and Nselaki Streams Zn concentrations were generally observed to be similar. Overall, changes in seasons had significant influence in metal concentrations, particularly in Nselaki Stream, during rainy season a significant increase was observed.

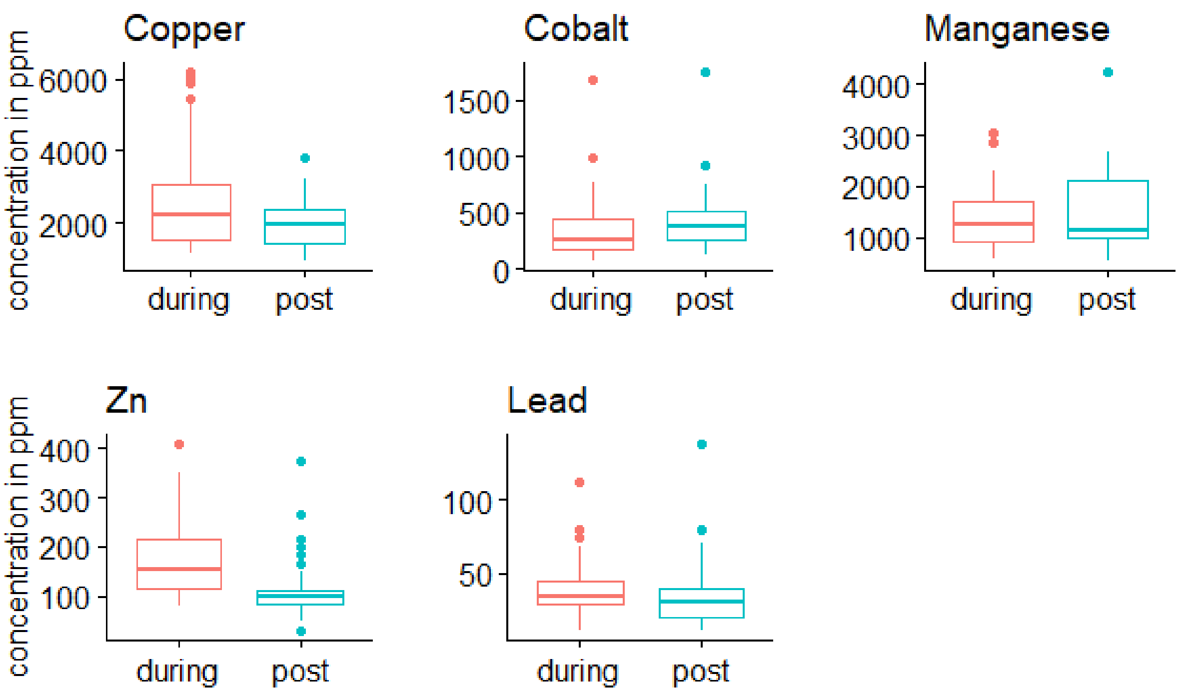


Figure 4-12: The boxplot shows the range and distribution of Cu, Co, Mn, Zn and concentrations in bottom sediments of selected sites in Nselaki Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes

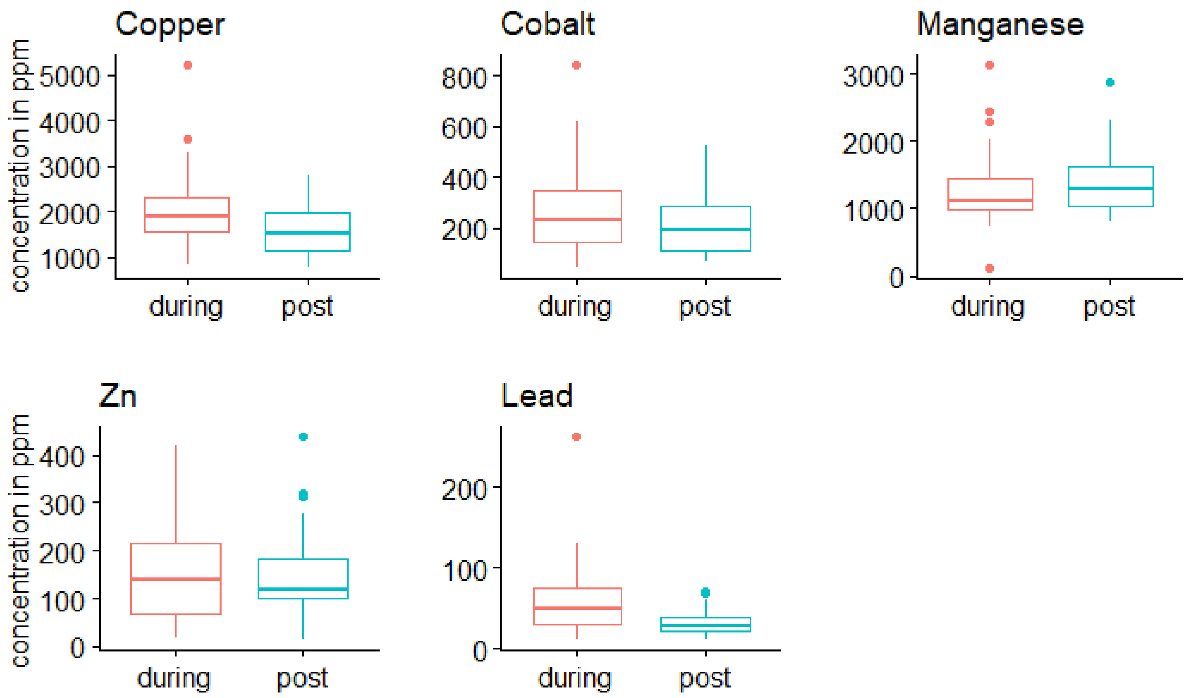


Figure 4-13: The boxplot shows the range and distribution of Cu, Co, Mn, Zn and Pb concentrations in bottom sediments of selected sites in Mululu Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes

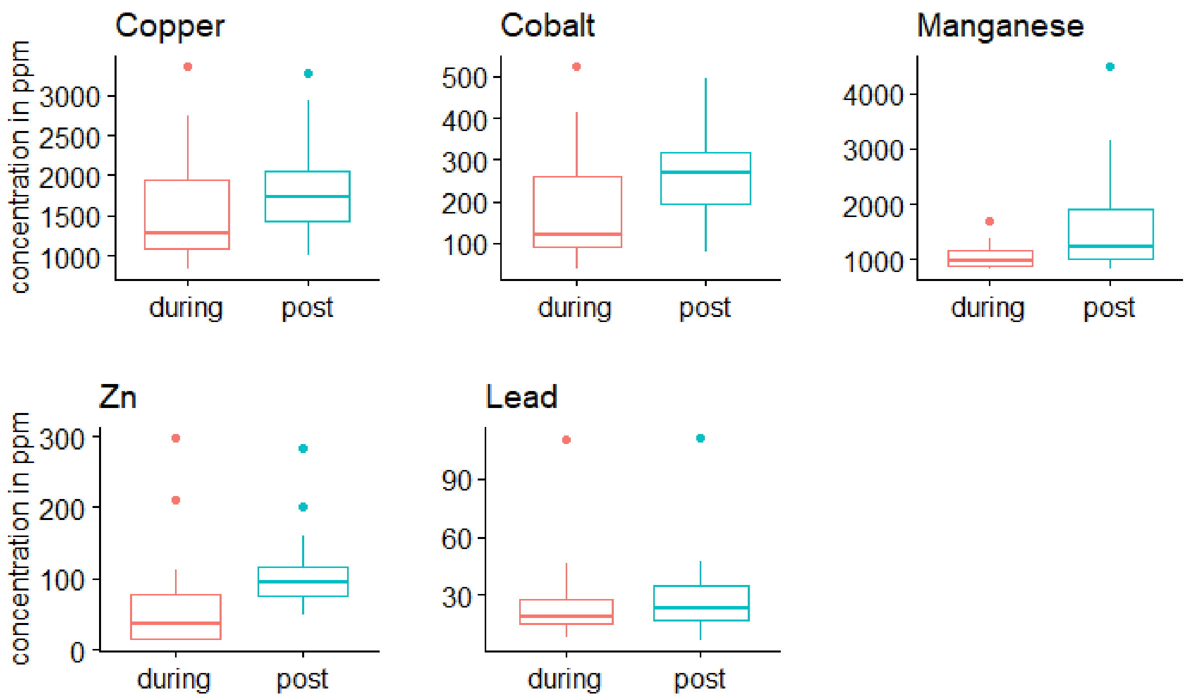


Figure 4-14: The boxplot shows the range and distribution of Cu, Co, Mn, Zn and Pb concentrations in bottom sediments of selected sites in Fikondo Streams. Significant differences during and post rainy season are indicated by the variability in the box plot sizes

Table 4-2: Mean concentration, background values, and multiple values within surface sediments of Nselaki Stream, Fikondo Stream and Mululu Stream (ppm). Background values were taken from upstream sampling points

	Cu	Co	Mn	Zn	Pb		Cu	Co	Mn	Zn	Pb
Nselaki Stream	**Rainy Season**						**Post Rainy Season**				
Mean Values	2628	348	1364	181	39		1971	425	1544	115	34
Background	372	70	172	58	19		592	79	271	19	11
Multiple	7	5	8	3	2		3	5	6	6	3
Fikondo Stream	**Rainy Season**						**Post Rainy Season**				
Mean Values	1570	239	1076	25	19		1824	264	1611	110	29
Background	316	108	291	22	14		563	90	340	78	17
Multiple	5	2	4	1	1		3	3	5	1	2
Mululu Stream	**Rainy season**						**Post Rainy Season**				
Mean Values	1983	262	1280	158	57		1586	216	1420	149	31
Background	467	89	316	96	26		585	135	458	87	19
Multiple	4	3	4	2	2		3	2	3	2	2

4.4. Discussion

4.4.1. Water and Sediment Quality

The observation from this study is that certain water and sediment parameters varied significantly between Nselaki Stream, Mululu stream, and Fikondo Stream. Among the selected parameters observed, turbidity varied significantly between the streams. A number of reasons may be attributed to the variation between streams. For example, the concentration of turbidity downstream in Nselaki Stream may be attributed to the tailing material from Chibuluma TSF and other associated land use activities that tend to loosen the soils during the rainy season. It is likely that the observed increase in turbidity was associated with runoff during the rainy season and re-suspension post rainy season (Mosley *et al.*, 2012). Notably, fine tailings sediments were dominant in the selected sampling points, suggesting significant migration of tailings material during the rainy season.

Changes were observed post rainy season in the concentrations of some metals and pH within sites NS06, NS07 and NS08. This could be attributed to the seepage from the toe drain of Chibuluma TSF and water return pond; this was possibly the source of slightly lower pH and high metal concentrations. The pH variations in a given system is likely to have significant effect on metal mobilization and distribution (Zhang *et al*., 2018). Studies by Guo *et al.*, (2013) have shown that pH is one of the major factors that affects the adsorption attributes of metals and regulates the insolubility/solubility of carbonates, phosphates, and hydroxides of metals, thus affecting metal hydrolysis in organic matter and sediments, and the formation of ion pairs. This was noticeable at sites NS06 to NS08 as the observation correlated with a rise in certain dissolved elements, plausible introduced by runoff from Chibuluma TSF and underground water. It was also observed from the spatial analysis that Nselaki Stream runs through the toe drain of Chibuluma TSF, making it susceptible to discharge from Chibuluma TSF. As a result, the contaminants migrating from Chibuluma TSF are likely to affect and change the water quality of Nselaki (Karlsson *et al*., 2018). For instance, higher concentrations of TDS, turbidity, phosphorous and nitrogen pollutants may be transported into the aquatic ecosystem thus leading to eutrophication (van den Berg *et al.*, 2020). The increase in metal contamination in the sediments and TDS downstream from NS02 to NS10 corresponds well with this. Agricultural practices are another major sources of TDS, turbidity, nitrogen, and phosphorous (Adonadaga *et al.*, 2020). The addition of higher concentrations of nutrients in the course of fertilizer application can result in gradual degradation of the integrity of the aquatic ecosystem (Dodds *et al.*, 2009); the observed agricultural activities near sites NS12, NS13, and NS14 is therefore a cause for concern.

The water quality in Fikondo Stream was noticeably not different compared to Nselaki Stream. Turbidity, TDS and pH can be utilized as a proxy to show the quality of water (García-

Ávila *et al*., 2018). The investigation of water samples executed at various sampling points within Fikondo Stream during and post rainy season, showed that TDS were within acceptable limits stipulated by the World Health Organization (WHO) and Initiative for Responsible Mining Assurance (IRMA). The highest TDS value of 785 mg/L at FS02 were below the maximum allowable limit (maximum limit 1000 mg/L). Notably, pH concentrations in Fikondo Stream did not vary significantly, however, incidences of lower pH values were observed at FS06, and FS02 (post rainy season). Overall, the pH was observed to be neutral, this could be attributed to the fact that the drainage quality from TSF15A impacting the stream is mainly neutral. As reported in the previous chapter (chapter 3, section 3.3.2), the tailing material were composed of high acid neutralising minerals. Turbidity values were observed to be above the acceptable limit (5 NTU) set by WHO.

The spatial distribution of metal concentrations in Fikondo Stream was not site specific, and the variation in metal concentration downstream was not significant across all sampling points. Higher Cu and Mn concentrations were found at sites FS01, FS02, and FS04. The common denominator that these sites share, is that they are entry points for seepage from TSF15A. Fovet *et al*. (2020), and Fritz *et al.* (2018) observed that the chemical and physical signature of a stream changes when there is connectivity between water resources and pollution sources. Equally other studies have shown changes in chemical and physical signature downstream based on intervening units, environmental settings, proximity, material disparity and relative size, of connectivity of upstream water resources (Fovet *et al*., 2020; Shogren *et al.*, 2019). The different water quality signatures observed in Fikondo Stream is in conformity with such observation. This may be the reason why a rise in metal concentration in the sediment and water samples was reported downstream.

A downstream comparative analysis between the increase in metals (Cu, Co, Mn, Zn and Pb) at various points FS01, FS02, FS04, FS05, and FS06, observed changes in metal concentrations within the sediments. The sediments were characterized by medium to fine grained material with no other large material similar to the material found in the toe drains of TSF15A. It may therefore be said that the fine material in the sediments of Fikondo Stream is linked to effluents discharge from TSF15A. Fine grained particles with their larger surface area are more likely to be anthropogenically enriched with associated metal(loid)s (Martín-Crespo et al., 2019). The conditional inference indicates a potential impact on chemical and physical signature in Fikondo Stream.

Based on what has been observed in Fikondo and Nselaki streams, and put into context of Mululu Stream, TDS and turbidity concentrations were observed to be lower in Mululu Stream. This could be attributed to reduction in discharge of effluents owing to the good vegetation cover observed at TSF14. Vegetation cover reduces eroding of particles in the top-soil into aquatic resources, packing of stream-beds/river-beds with sediments, and mobilization of metals (Karaca *et al.*, 2018). No substantial variation was observed in pH values among the sampling points in Mululu Stream. The pH values of all the sampling points were ranging between 6 and 7.6. There was no significant variation in pH between Mululu Stream, Nselaki Stream, and Fikondo Stream. Equally, Cu, Co, Mn, Zn and Pb concentrations in sediments were generally observed to be similar, although Cu concentrations were slightly lower (≈1785 ppm) compared to Nselaki Stream and Fikondo Stream (≈2300 ppm). On the other hand, the chemical and physical signature of Mululu Stream could be also impacted by effluents from industrial areas draining into the stream at sampling points MS04, MS05, and MS07. Industrial effluents are some of the principal contributors to water contamination problems (Edokpayi *et al*., 2017). Additionally, it was observed that a number of vehicle wash services were operating near Mululu Stream between MS10 and MS11, thus increasing the variety of contaminants affecting the stream. The untreated vehicle wash wastewater could increase turbidity, TDS, temperature, reduce available habitat, pH, and increase metal concentration from vehicle body parts, lubricants, and fuel (Rai *et al.*, 2020). Although no notable changes in the physical and chemical variables were reported in relation to the observed land use patterns, it is envisaged that the physical and chemical signatures of Mululu Stream is impacted by three blends of pollutants from TSF14, industrial areas, and vehicle wash wastewater. However, the fine-grained material similar to tailings material dominating the sediment of Mululu Stream suggests that TSF14 could be the major source of pollution in the stream.

4.4.2. Comparative Assessment

Based on the observations from Nselaki Stream, Fikondo Stream, and Mululu Stream, and when contextualized within the risks associated with TSFs in close proximity to the streams; the following consideration may be of value in the management of aquatic ecosystems on the

Zambian Copperbelt in the face of increased mining, industrial, and urbanization activities. The influence of TSFs on the physical and chemical signature will be of paramount concern, as it may cause changes in the water regime. As observed in the study of Nselaki Stream and Fikondo Stream, most of the contaminants and related deterioration of aquatic ecosystem were as a consequence of mobilization of suspended and dissolved metals from the active TSFs (Chibuluma TSF and TSF15A, respectively). Equally, TSF14 was observed to have significant influence on water quality in Mululu Stream, although the impacts were observed to be less compared to Nselaki and Fikondo streams. This could be attributed to the good vegetative cover observed on TSF14; vegetation can play a significant role in minimising migration of contaminants (Ernst, 1988). The study revealed that mainly the adulteration of water quality observed in Nselaki Stream, Fikondo Stream, and Mululu Stream, could be attributed to the changes in turbidity, pH, and TDS brought about by TSFs discharge. Turbidity was observed to be above the acceptable limits, whilst low incidences of slightly acidic pH (≈5.3) were reported. A synthesis of various studies indicates that a meld of physical and chemical combination of chemical and physical stressors generates a difficult environment and reduces aquatic ecosystem services (Dzwairo and Mujuru, 2017; Fernandes *et al*., 2016; Hatje *et al.*, 2017). Monitoring pH, turbidity and TDS concentration is therefore important because of the close proximity (<500 m) of TSFs to the selected streams. These contaminants are anticipated to continue filtering into the streams especially during the rainy season.

The sediment samples of Nselaki Stream, Fikondo Stream, and Mululu Stream reported significant concentration of metal elements like Cu, Co, Mn, Zn, and Pb, presenting a probable threat on the aquatic ecosystem and reuse of wastewater. In addition, the influence of agricultural practices within the stream bank of Nselaki Stream, and Fikondo Stream, and untreated vehicle wash wastewater in Mululu Stream could be contributing to metal congregation. These activities have the potential of causing serious environmental impacts and destabilize the aquatic ecosystem services through destruction of the natural settings of the streams (Ngole-Jeme and Fantke, 2017). The current study has shown the effect that TSFs have downstream with regard to increased metal contamination. Thus, drainage quality entering the streams should be monitored and managed to minimise a shift in the ecosystem, which might result in negative impacts on downstream end users. As has been noted in the study, the streams play a major role in supporting livelihood of unplanned settlements downstream, providing the much-needed water for irrigation and domestic use. The integrity of the streams must therefore be preserved against severe environmental decline induced by the mine wastelands and protect the ecological diversity present within the ecosystems.

4.5. Conclusion

From the results of Nselaki Stream, Fikondo Stream and Mululu Stream catchments, it was observed that these three streams were comparable. This was based on the underlying geology, geographical location, as well as land use practices that they were either at risk of or subjected to. Thus, providing us with unique insights into learning from the streams so that this knowledge could potentially be applied in other similar settings for improved water resource management. The various changes in water and sediment quality observed in the streams, suggest that pH, TDS, and turbidity were essential variables to control and monitor within the streams because of the domino effect they might have on metal mobilization among other things. The effect of TSFs on the ambient surface water resources was accentuated with regards to their influence on the quality of water and sediments. This supports the need for improved control and monitoring of TSFs. It is therefore important to find remedial measures that will reduce the impact of the streams in future, and this study has provided opportunity for understanding likely changes induced by similar activities in another similar impacted catchment. This is true concerning certain metal concentrations like Cu and Mn being present in higher concentrations downstream from pollution source.

4.6. References

Adamczyk-Szabela, D., Markiewicz, J., Wolf, W.M., 2015. Heavy Metal Uptake by Herbs. IV. Influence of Soil pH on the Content of Heavy Metals in Valeriana officinalis L. Water, Air and Soil Pollution 226. https://doi.org/10.1007/s11270-015-2360-3

Adamu, C.I., Nganje, T.N., Edet, A., 2015. Major and trace elements pollution of sediments associated with Abandoned Barite Mines in parts of Oban Massif and Mamfe Embayment, SE Nigeria. Journal of Geochemical Exploration 151, 17–33. https://doi.org/10.1016/j.gexplo.2014.12.010

Adonadaga, M.-G., Frimpong, C., Ampadu, B., Alare, R., Owusu, G., 2020. Assessment of agricultural practices on water quality of the Tono Dam in the Upper East Region of Ghana. African Journal of Environmental Science and Technology 14, 114-122. Doi: 10.5897/AJEST2019.2798

Auditor General Report, 2014. *Management of Environmental Degradation Caused by Mining Activities in Zambia,* Lusaka: s.n.Becker, M., Dyantyi, N., Broadhurst, J.L., Harrison, S.T.L., Franzidis, J.-P., 2015. A mineralogical approach to evaluating laboratory scale acid rock drainage characterisation tests. Minerals Engineering 80, 33–36. https://doi.org/10.1016/j.mineng.2015.06.015

Bhateria, R., Jain, D., 2016. Water quality assessment of lake water: a review. Sustain. Water Resources Management 2, 161–173. https://doi.org/10.1007/s40899-015-0014-7

Briffa, J., Sinagra, E., Blundell, R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6, e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Brown, S., Mahoney, M., Sprenger, M., 2014. A comparison of the efficacy and ecosystem impact of residual-based and topsoil-based amendments for restoring historic mine tailings in the Tri-State mining district. Science of The Total Environment 485–486, 624–632. https://doi.org/10.1016/j.scitotenv.2014.03.029

Cappuyns, V., Rudy, S., 2008. The Use of Leaching Tests to Study the Potential Mobilization of Heavy Metals from Soils and Sediments: A Comparison. Water, Air, and Soil Pollution 191, 95–111. https://doi.org/10.1007/s11270-007-9609-4

Chiarenzelli, J.R., Skeels, M.C., 2014. End-member River water composition in the acidified Adirondack Region, Northern New York, USA. Journal of Hydrology 2, 97–118. https://doi.org/10.1016/j.ejrh.2014.08.003

Chileshe, M.N., Syampungani, S., Festin, E.S., Tigabu, M., Daneshvar, A., Odén, P.C., 2020. Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. Journal of Forestry Research 31, 1283–1293. https://doi.org/10.1007/s11676-019-00921-0

Cimboláková, I., Uher, I., Laktičová, K.V., Vargová, M., Kimáková, T., Papajová, I., 2019. Heavy Metals and the Environment. Environmental Factors Affecting Human Health. https://doi.org/10.5772/intechopen.86876

Csavina, J., Field, J., Taylor, M.P., Gao, S., Landázuri, A., Betterton, E.A., Sáez, A.E., 2012. A Review on the Importance of Metals and Metalloids in Atmospheric Dust and Aerosol from Mining Operations. Science of the Total Environment 433C, 58–73. https://doi.org/10.1016/j.scitotenv.2012.06.013

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environmental Science and Technology 43, 12–19. https://doi.org/10.1021/es801217q

Donkor, A.K., Bonzongo, J.-C.J., Nartey, V.K., Adotey, D.K., 2005. Heavy Metals in Sediments of the Gold Mining Impacted Pra River Basin, Ghana, West Africa. Soil and Sediment Contamination 14, 479–503. https://doi.org/10.1080/15320380500263675

Dutta, S., Datta, A., Zaid, A., Bhat, J.A., 2020. Metalloids and Their Impact on the Environment, in: Metalloids in Plants. John Wiley & Sons, Ltd, pp. 19–26. https://doi.org/10.1002/9781119487210.ch2

Dzwairo, B., Mujuru, M., 2017. Assessment of Impacts of Acid Mine Drainage on Surface Water Quality of Tweelopiespruit Micro-Catchment, Limpopo Basin. Water Quality 338-359. https://doi.org/10.5772/65810

Edokpayi, J.N., Odiyo, J.O., Durowoju, O.S., 2017. Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. Water Quality 2017, 401-416. https://doi.org/10.5772/66561

Ernst, W.H.O., 1988. Response of Plants and Vegetation to Mine Tailings and Dredged Materials, in: Salomons, W., Förstner, U. (Eds.), Chemistry and Biology of Solid Waste: Dredged Material and Mine Tailings. Springer, Berlin, Heidelberg, pp. 54–69. https://doi.org/10.1007/978-3-642-72924-9_3

Fernandes, G.W., Goulart, F.F., Ranieri, B.D., Coelho, M.S., Dales, K., Boesche, N., Bustamante, M., Carvalho, F.A., Carvalho, D.C., Dirzo, R., Fernandes, S., Galetti, P.M., Millan, V.E.G., Mielke, C., Ramirez, J.L., Neves, A., Rogass, C., Ribeiro, S.P., Scariot, A., Soares-Filho, B., 2016. Deep into the mud: ecological and socio-economic impacts of the dam breach in Mariana, Brazil. Natureza and Conservação 14, 35–45. https://doi.org/10.1016/j.ncon.2016.10.003

Festin, E.S., Tigabu, M., Chileshe, M.N., Syampungani, S., Odén, P.C., 2019. Progresses in restoration of post-mining landscape in Africa. Journal of Forestry Research 30, 381–396. https://doi.org/10.1007/s11676-018-0621-x

Fovet, O., Ndom, M., Crave, A., Pannard, A., 2020. Influence of dams on river water-quality signatures at event and seasonal scales: The Sélune River (France) case study. River Research and Applications 36, 1267–1278. https://doi.org/10.1002/rra.3618

García-Ávila, F., Ramos- Fernández, L., Pauta, D., Quezada, D., 2018. Evaluation of water quality and stability in the drinking water distribution network in the Azogues city, Ecuador. Data in Brief 18, 111–123. https://doi.org/10.1016/j.dib.2018.03.007

Gonzalo, C., Camargo, J.A., 2013. The impact of an industrial effluent on the water quality, submersed macrophytes and benthic macroinvertebrates in a dammed river of Central Spain. Chemosphere 93, 1117–1124. https://doi.org/10.1016/j.chemosphere.2013.06.032

Guo, Y., Huang, P., Zhang, W., Yuan, X., Fan, F., Wang, H., Liu, J., Wang, Z., 2013. Leaching of heavy metals from Dexing copper mine tailings pond. Transactions of Nonferrous Metals Society of China 23, 3068–3075. https://doi.org/10.1016/S1003-6326(13)62835-6

Hatje, V., Pedreira, R.M.A., de Rezende, C.E., Schettini, C.A.F., de Souza, G.C., Marin, D.C., Hackspacher, P.C., 2017. The environmental impacts of one of the largest tailing dam failures worldwide. Scientific Reports 7. https://doi.org/10.1038/s41598-017-11143-x

Higueras, P.L., Sáez-Martínez, F.J., Reyes-Bozo, L., 2016. Characterization and remediation of contamination: the influences of mining and other human activities. Environmental Science and Pollution Research 23, 5997–6001. https://doi.org/10.1007/s11356-016-6388-8

Hogsden, K.L., Harding, J.S., 2011. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science 31, 108–120. https://doi.org/10.1899/11-091.1

Huiyan, Y., Zhongfang,. Chen, Yuelong,. Qian, R., Guo, Chi,. Zhu, 2005. A study of the effect of soil pH on chemical species of cadmium by simulated experiments. Earth science frontiers 12, 252–260.

Ikenaka, Y., Nakayama, S., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy Metal Contamination of Soil and Sediment in Zambia. African Journal of Environmental Science and Technology 4, 729–739. https://doi.org/10.4314/ajest.v4i11.71339

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. Introduction, in: James, G., Witten, D., Hastie, T., Tibshirani, R. (Eds.), An Introduction to Statistical Learning: With Applications in R, Springer Texts in Statistics. Springer, New York, NY, pp. 1–14. https://doi.org/10.1007/978-1-4614-7138-7_1

Jolliffe, I.T., 2002. Principal Component Analysis, 2nd ed, Springer Series in Statistics. Springer-Verlag, New York. https://doi.org/10.1007/b98835

Kambole, M.S., 2003. Managing the water quality of the Kafue River. Physics and Chemistry of the Earth, Parts A/B/C 28, 1105–1109. https://doi.org/10.1016/j.pce.2003.08.031

Kapungwe, E.M., 2013. Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. Journal of Geography and Geology 5, 55-72. https://doi.org/10.5539/jgg.v5n2p55

Karaca, O., Cameselle, C., Reddy, K.R., 2018. Mine tailing disposal sites: contamination problems, remedial options and phytocaps for sustainable remediation. Reviews in Environmental Science and Biotechnology 17, 205–228. https://doi.org/10.1007/s11157-017-9453-y

Karlsson, T., Räisänen, M.L., Lehtonen, M., Alakangas, L., 2018. Comparison of static and mineralogical ARD prediction methods in the Nordic environment. Environmental Monitoring and Assessment 190, 719. https://doi.org/10.1007/s10661-018-7096-2

Lehtiniemi, M., Engström-Öst, J., Viitasalo, M., 2005. Turbidity decreases anti-predator behaviour in pike larvae, Esox lucius. Environmental Biology of Fishes 73, 1–8. https://doi.org/10.1007/s10641-004-5568-4

Li, M.S., 2006. Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: A review of research and practice. Science of The Total Environment 357, 38–53. https://doi.org/10.1016/j.scitotenv.2005.05.003

Lindahl, J., 2014. Environmental Impact of Mining in Zambia: Towards Better EnvironmentalManagement and Sustainable Exploitation of Mineral Resources, s.l.: s.n.

Martín-Crespo, T., Gómez-Ortiz, D., Martín-Velázquez, S., 2019. Geoenvironmental Characterization of Sulfide Mine Tailings. Applied Geochemistry with Case Studies on Geological Formations, Exploration Techniques and Environmental Issues Geochemistry 1-25. https://doi.org/10.5772/intechopen.84795

Masindi, V., Muedi, K.L., 2018. Environmental Contamination by Heavy Metals. Heavy Metals. Intechopen 2018, 115-133 https://doi.org/10.5772/intechopen.76082

Mbonambi, Z., 2016. An investigation into the environmental impacts of informal settlements on water: a case of Kennedy Road informal settlement in Durban, KwaZulu-Natal. [WWW Document]. undefined. URL /Thesis/An-investigation-into-the-environmental-impacts-of-Mbonambi/6924c9fe6ff87b17716eac10d557f48d8d0469d7 (accessed 10.19.20).

McGrane, S.J., 2016. Impacts of urbanisation on hydrological and water quality dynamics, and urban water management: a review. Hydrological Sciences Journal 61, 2295–2311. https://doi.org/10.1080/02626667.2015.1128084

Mendez, M.O., Maier, R.M., 2008. Phytostabilization of Mine Tailings in Arid and Semiarid Environments—An Emerging Remediation Technology. Environmental Health Perspectives 116, 278–283. https://doi.org/10.1289/ehp.10608

Mishra, P., Pandey, C.M., Singh, U., Gupta, A., Sahu, C., Keshri, A., 2019. Descriptive Statistics and Normality Tests for Statistical Data. Ann Card Anaesth 22, 67–72. https://doi.org/10.4103/aca.ACA_157_18

Moka Environmental and Geotechnical Consultants, 2016. Musi Tailing Dump, Chibuluma TSF and TSF15A Statutory Inspection, Independent Competent Persons Report

Mosley, L.M., Zammit, B., Leyden, E., Heneker, T.M., Hipsey, M.R., Skinner, D., Aldridge, K.T., 2012. The Impact of Extreme Low Flows on the Water Quality of the Lower Murray River and Lakes (South Australia). Water Resources Management 26, 3923–3946. https://doi.org/10.1007/s11269-012-0113-2

Mundike, J., 2004. Bwana Mkubwa Mine; Water Pollution Report, Ndola, Zambia.

Ngole-Jeme, V.M., Fantke, P., 2017. Ecological and human health risks associated with abandoned gold mine tailings contaminated soil. PLoS One 12. https://doi.org/10.1371/journal.pone.0172517

Ntengwe, F.W., 2005. An overview of industrial wastewater treatment and analysis as means of preventing pollution of surface and underground water bodies—the case of Nkana Mine in Zambia. Physics and Chemistry of the Earth, Parts A/B/C, Integrated Water Resources Management (IWRM) and the Millennium Development Goals: Managing Water for Peace and Prosperity 30, 726–734. https://doi.org/10.1016/j.pce.2005.08.014

Oberholster, P.J., Botha, A.-M., Cloete, T.E., 2008. Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution 156, 184–192. https://doi.org/10.1016/j.envpol.2007.12.028

Oyewumi, O., Feldman, J., Gourley, J.R., 2017. Evaluating stream sediment chemistry within an agricultural catchment of Lebanon, Northeastern USA. Environmental Monitoring and Assessment 189, 141. https://doi.org/10.1007/s10661-017-5856-z

Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: Environmental implications and human health risk. Harmful Algae 61, 31–45. https://doi.org/10.1016/j.hal.2016.11.006

Rahmanian, N., Ali, S.H.B., Homayoonfard, M., Ali, N.J., Rehan, M., Sadef, Y., Nizami, A.S., 2015. Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia [WWW Document]. Journal of Chemistry. https://doi.org/10.1155/2015/716125

Rai, R., Sharma, S., Gurung, D.B., Sitaula, B.K., Shah, R.D.T., 2020. Assessing the impacts of vehicle wash wastewater on surface water quality through physico-chemical and benthic macroinvertebrates analyses. Water Science 0, 1–11. https://doi.org/10.1080/11104929.2020.1731136

Riba, I., Delvalls, T.Á., Forja, J.M., Gómez-Parra, A., 2004. The influence of pH and salinity on the toxicity of heavy metals in sediment to the estuarine clam Ruditapes philippinarum. Environmental Toxicology and Chemistry 23, 1100–1107. https://doi.org/10.1897/023-601

Riedel, N., Vounatsou, P., Miller, J.M., Gosoniu, L., Chizema-Kawesha, E., Mukonka, V., Steketee, R.W., 2010. Geographical patterns and predictors of malaria risk in Zambia: Bayesian geostatistical modelling of the 2006 Zambia national malaria indicator survey (ZMIS). Malaria Journal 9, 37. https://doi.org/10.1186/1475-2875-9-37

Sarma, K., Barik, S.K., 2011. Coal mining impact on vegetation of the Nokrek Biosphere Reserve, Meghalaya, India. Biodiversity 12, 154–164. https://doi.org/10.1080/14888386.2011.629779

Shimaponda-Mataa, N.M., Tembo-Mwase, E., Gebreslasie, M., Achia, T.N.O., Mukaratirwa, S., 2017. Modelling the influence of temperature and rainfall on malaria incidence in four endemic provinces of Zambia using semiparametric Poisson regression. Acta Tropica. 166, 81–91. https://doi.org/10.1016/j.actatropica.2016.11.007

Shogren, A.J., Zarnetske, J.P., Abbott, B.W., Iannucci, F., Frei, R.J., Griffin, N.A., Bowden, W.B., 2019. Revealing biogeochemical signatures of Arctic landscapes with river chemistry. Scientific Reports 9, 12894. https://doi.org/10.1038/s41598-019-49296-6

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. Introduction, in: James, G., Witten, D., Hastie, T., Tibshirani, R. (Eds.), An Introduction to Statistical Learning: With Applications in R, Springer Texts in Statistics. Springer, New York, NY, pp. 1–14. https://doi.org/10.1007/978-1-4614-7138-7_1

Stewart, A.G., 2020. Mining is bad for health: a voyage of discovery. Environmental Geochemistry and Health 42, 1153–1165. https://doi.org/10.1007/s10653-019-00367-7

Tutu, H., McCarthy, T.S., Cukrowska, E., 2008. The chemical characteristics of acid mine drainage with particular reference to sources, distribution and remediation: The Witwatersrand Basin, South Africa as a case study. Applied Geochemistry 23, 3666–3684. https://doi.org/10.1016/j.apgeochem.2008.09.002

USEPA, 2001. Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual. Report No. EPA-823-F-01-023. United States Environmental Protection Agency, Washington, DC, USA.

van den Berg, M.F., Botha, A.M., Bierman, A., Oberholster, P., 2020. Assessing Domestic Wastewater Effluent with a Battery of Bioassays after Treatment with a Specific Consortium of Microalgae and Different Flocculation Methods. Water, Air and Soil Pollution 231, 257. https://doi.org/10.1007/s11270-020-04627-6

Vélez, J.I., Correa, J.C., Marmolejo-Ramos, F., 2015. A new approach to the Box–Cox transformation. Frontiers in Applied Mathematics and Statistics 1

Venkateswarlu, K., Nirola, R., Kuppusamy, S., Thavamani, P., Naidu, R., Megharaj, M., 2016. Abandoned metalliferous mines: ecological impacts and potential approaches for reclamation. Reviews in Environmental Science and Biotechnology 15, 327–354. https://doi.org/10.1007/s11157-016-9398-6

von der Heyden, C.J., New, M.G., 2004. Sediment chemistry: a history of mine contaminant remediation and an assessment of processes and pollution potential. Journal of Geochemical Exploration 82, 35–57. https://doi.org/10.1016/j.gexplo.2003.11.001

Wang, X., Liu, Y., Zeng, G., Chai, L., Xiao, X., Song, X., Min, Z., 2008. Pedological characteristics of Mn mine tailings and metal accumulation by native plants. Chemosphere 72, 1260–1266. https://doi.org/10.1016/j.chemosphere.2008.05.001

Wuana, R.A., Okieimen, F.E., 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation [WWW Document]. ISRN Ecology 2011. https://doi.org/10.5402/2011/402647

Zeilhofer, P., Topanotti, V.P., 2008. GIS and ordination techniques for evaluation of environmental impacts in informal settlements: A case study from Cuiabá, central Brazil. Applied Geography 28, 1–15. https://doi.org/10.1016/j.apgeog.2007.07.009

Zhang, Y., Zhang, H., Zhang, Z., Liu, C., Sun, C., Zhang, W., Marhaba, T., 2018. pH Effect on Heavy Metal Release from a Polluted Sediment [WWW Document]. Journal of Chemistry. https://doi.org/10.1155/2018/7597640

4.7. Supplementary Material

Table S4-1: Range, mean and standard deviation of chemical and physical parameters of water samples within Nselaki Stream.

Sample Description		Cu	Co	Mn	Zn	Pb	pH	Turbidity	TDS	DO
Rainy Season										
NS01	Range	0,01 - 0,09	0,01 - 0,03	0,01 - 0,16	0,01 - 0,06	0,01 - 0,05	6,1 - 7,7	7 - 100	530 - 780	09-May
	Mean±SD	0,04 ± 0,03	0,02 ± 0,01	0,05 ± 0,06	0,03 ± 0,02	0,02 ± 0,02	7,24 ± 0,61	48,8 ± 38,5	687 ± 86,9	7,02 ± 1,33
NS02	Range	0,01 - 0,2	0,01 - 0,03	0,02 - 0,24	0,01 - 0,02	0,01 - 0,03	5,9 - 7,7	5 - 123	471 - 777	6,8 - 8,6
	Mean±SD	0,1 ± 0,07	0,02 ± 0,01	0,09 ± 0,08	0,01 ± 0,00	0,01 ± 0,01	7,2 ± 0,66	60,2 ± 42,1	677 ± 108	7,9 ± 0,59
NS03	Range	0,01 - 0,08	0,01 - 0,01	0,01 - 0,08	0,01 - 0,04	0,01 - 0,02	6,6 - 7,6	7 - 44	601 - 779	3,7 - 7,9
	Mean±SD	0,04 ± 0,03	0,01 ± 0,00	0,04 ± 0,03	0,02 ± 0,01	0,01 ± 0,00	7,34 ± 0,38	17,2 ± 13,5	703 ± 64	6,08 ± 1,44
NS04	Range	0,03 - 0,5	0,01 - 0,02	0,06 - 0,74	0,01 - 0,1	0,01 - 0,02	6,1 - 8,1	9 - 61	61 - 940	4,1 - 6
	Mean±SD	0,14 ± 0,18	0,01 ± 0,00	0,26 ± 0,25	0,03 ± 0,04	0,01 ± 0,00	7 ± 0,83	31,6 ± 19,3	447 ± 370	5,38 ± 0,72
NS05	Range	0,01 - 0,06	0,01 - 0,01	0,01 - 0,11	0,01 - 0,08	0,01 - 0,03	6,2 - 8,2	9 - 39	71 - 936	4,36 - 8,1
	Mean±SD	0,03 ± 0,02	0,01 ± 0,00	0,06 ± 0,04	0,04 ± 0,02	0,01 ± 0,01	7,18 ± 0,68	17,4 ± 11,2	533 ± 336	6,11 ± 1,36
NS06	Range	0,01 - 0,23	0,01 - 0,06	0,01 - 0,17	0,01 - 0,04	0,01 - 0,04	5,6 - 8,1	12 - 76	42 - 928	5,19 - 7,2
	Mean±SD	0,12 ± 0,07	0,03 ± 0,02	0,09 ± 0,05	0,02 ± 0,01	0,02 ± 0,01	6,9 ± 0,79	33 ± 24,3	561 ± 293	6,26 ± 0,74
NS07	Range	0,01 - 0,17	0,01 - 0,05	0,01 - 0,1	0,01 - 0,12	0,01 - 0,03	5,6 - 7,9	23 - 60	123 - 793	4,7 - 6,6
	Mean±SD	0,1 ± 0,04	0,02 ± 0,01	0,05 ± 0,04	0,03 ± 0,04	0,02 ± 0,01	6,86 ± 0,80	36,2 ± 15,7	450 ± 282	5,6 ± 0,77
NS08	Range	0,08 - 0,7	0,01 - 0,11	0,01 - 0,4	0,01 - 0,07	0,01 - 0,03	5,5 - 7,7	4 - 96	340 - 798	5 - 8,2
	Mean±SD	0,26 ± 0,24	0,04 ± 0,04	0,15 ± 0,14	0,03 ± 0,02	0,02 ± 0,01	6,88 ± 0,79	44 ± 32,3	547 ± 189	6,28 ± 1,24
NS09	Range	0,04 - 0,21	0,01 - 0,02	0,06 - 0,14	0,03 - 0,11	0,01 - 0,04	6 - 7,4	3 - 105	165 - 806	4,39 - 7
	Mean±SD	0,10 ± 0,06	0,01 ± 0,00	0,11 ± 0,03	0,11 ± 0,11	0,02 ± 0,01	6,72 ± 0,53	42 ± 33,7	590 ± 231	5,72 ± 1,06
NS10	Range	0,07 - 0,27	0,01 - 0,06	0,05 - 0,3	0,01 - 0,07	<0,01 - 0,03	6,1 - 7,1	19 - 60	191 - 346	4,3 - 6,8
	Mean±SD	0,15 ± 0,08	0,03 ± 0,02	0,14 ± 0,09	0,03 ± 0,03	0,02 ± 0,01	6,8 ± 0,36	39,2 ± 17,1	246 ± 57,6	5,54 ± 0,97
NS11	Range	0,02 - 0,41	0,01 - 0,05	0,03 - 0,09	0,02 - 0,04	<0,01 - 0,02	6,8 - 7,5	14 - 71	175 - 551	3,92 - 6,1
	Mean±SD	0,15 ± 0,14	0,02 ± 0,01	0,06 ± 0,02	0,03 ± 0,01	0,02 ± 0,01	7,08 ± 0,23	37 ± 19,6	317 ± 134	5,56 ± 0,83
NS12	Range	0,06 - 0,19	0,01 - 0,03	0,02 - 0,1	0,01 - 0,03	0,01 - 0,01	6,5 - 7,2	17 - 49	252 - 474	3,76 - 5,7
	Mean±SD	0,12 ± 0,05	0,02 ± 0,01	0,06 ± 0,03	0,02 ± 0,01	0,01 ± 0,00	6,94 ± 0,24	33,6 ± 12,5	349 ± 77,2	4,93 ± 0,66
NS13	Range	0,02 - 0,09	0,01 - 0,05	0,02 - 0,09	0,01 - 0,03	<0,01 - 0,04	5,8 - 6,9	10 - 70	118 - 320	5,61 - 8
	Mean±SD	0,06 ± 0,03	0,03 ± 0,01	0,05 ± 0,03	0,02 ± 0,01	0,03 ± 0,01	6,22 ± 0,39	31 ± 20,7	205 ± 70	6,68 ± 0,87
NS14	Range	0,05 -0,08	0,02 0,06	0,04 - 0,1	0,01 - 0,03	0,01 - 0,02	6,2 - 6,9	36 - 55	208 - 436	5,11 - 7,52
	Mean±SD	0,07 ± 0,01	0,04 ± 0,02	0,06 ± 0,03	0,02 ± 0,01	0,01 ± 0,00	6,57 ± 0,29	44,7 ± 7,85	352 ± 102	6,28 ± 0,99
PostRainy Season										
Sample Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**TDS**	**DO**
NS01	Range	0,01 - 0,25	0,01 - 0,05	0,05 - 0,4	0,01 - 0,2	0,01 - 0,09	6 - 7,6	15 - 60	196 - 425	4,12 - 6
	Mean±SD	0,1 ± 0,11	0,03 ± 0,02	0,17 ± 0,12	0,1 ± 0,08	0,04 ± 0,03	6,99 ± 0,54	31,5 ± 16,2	279 ± 85,4	4,57 ± 0,66
NS02	Range	0,05 - 0,14	0,01 - 0,02	0,02 - 0,2	0,01 - 0,1	0,01 - 0,05	6,7 - 8	4,04 - 73	211 - 304	3,16 - 6,1
	Mean±SD	0,08 ± 0,03	0,01 ± 0,00	0,14 ± 0,06	0,05 ± 0,03	0,03 ± 0,02	7,07 ± 0,44	38 ± 22,1	247 ± 30,5	4,29 ± 1,12
NS03	Range	0,07 - 0,6	0,01 - 0,09	0,04 - 0,3	0,02 - 0,12	<0,01 - 0,04	6,3 - 7,1	11 - 42	154 - 342	3,21 - 8
	Mean±SD	0,3 ± 0,23	0,04 ± 0,03	0,13 ± 0,12	0,08 ± 0,04	0,02 ± 0,01	6,81 ± 0,31	19,5 ± 10,7	282 ± 71,4	4,9 ± 1,6
NS04	Range	0,04 - 0,7	0,01 - 0,03	0,01 - 0,4	0,01 - 0,07	0,02 - 0,05	6,7 - 7,7	11 - 80	98 - 421	4,01 - 7,8
	Mean±SD	0,19 ± 0,24	0,02 ± 0,01	0,23 ± 0,17	0,03 ± 0,02	0,04 ± 0,01	6,99 ± 0,32	33,7 ± 24,2	279 ± 105,4	5,62 ± 1,31
NS05	Range	0,03 - 0,21	0,01 - 0,1	0,01 - 0,19	0,01 - 0,08	0,01 - 0,07	6,2 - 7,2	6 - 33	109 - 521	3,5 - 7
	Mean±SD	0,13 ± 0,09	0,05 ± 0,04	0,08 ± 0,08	0,05 ± 0,03	0,05 ± 0,02	6,73 ± 0,45	18,8 ± 9,3	289 ± 142	5,35 ± 1,46
NS06	Range	0,01 - 0,1	0,01 - 0,03	0,09 - 0,6	0,02 - 0,09	<0,01 - 0,03	5,2 - 7,1	10-32	260 - 613	2,67 - 7,5
	Mean±SD	0,05 ± 0,03	0,02 ± 0,01	0,22 ± 0,19	0,05 ± 0,03	0,01 ± 0,01	6,47 ± 0,71	15 ± 3,32	421 ± 118	4,7 ± 1,48
NS07	Range	0,03 - 0,8	0,01 - 0,03	0,02 - 0,71	0,01 - 0,03	0,01 - 0,02	5,3 - 7,9	17 - 37	145 - 555	3,7 - 8,6
	Mean±SD	0,2 ± 0,27	0,02 ± 0,01	0,2 ± 0,23	0,01 ± 0,01	0,02 ± 0,01	6,55 ± 0,93	23,8 ± 6,62	341 ± 135	4,95 ± 1,73
NS08	Range	0,06 - 0,4	0,01 - 0,05	0,01 - 0,5	0,01 - 0,04	0,01 - 0,02	5,1 - 7	25 - 38	65 - 516	3,5 - 6
	Mean±SD	0,15 ± 0,12	0,02 ± 0,01	0,19 ± 0,16	0,02 ± 0,01	0,01 ± 0,00	6,4 ± 0,79	33,3 ± 4,46	228 ± 150	4,55 ± 0,87
NS09	Range	0,02 - 0,21	0,01 - 0,1	0,01 - 0,23	0,01 - 0,03	0,01 - 0,07	6,4 - 7	9,0 - 26	94 - 496	3,96 - 7,9
	Mean±SD	0,09 ± 0,06	0,05 ± 0,04	0,13 ± 0,08	0,02 ± 0,01	0,02 ± 0,02	6,67 ± 0,22	17,5 ± 5,35	222 ± 135	5,31 ± 1,22
NS10	Range	0,08 - 0,17	0,01 - 0,05	0,07 - 0,3	0,01 - 0,05	<0,01 - 0,03	6,0 - 7,0	19 - 43	122 - 420	5 - 6,1
	Mean±SD	0,11 ± 0,03	0,03 ± 0,01	0,13 ± 0,08	0,03 ± 0,02	0,02 ± 0,01	6,58 ± 0,35	31,5 ± 8,50	277 ± 93,3	5,83 ± 0,40
NS11	Range	0,08 - 0,2	0,01 - 0,04	0,03 - 0,09	0,01 - 0,05	<0,01 - 0,04	6,6 - 7,3	21 - 64	154 - 426	5,34 - 8
	Mean±SD	0,14 ± 0,04	0,02 ± 0,01	0,07 ± 0,02	0,02 ± 0,02	0,02 ± 0,01	6,9 ± 0,26	32,3 ± 15,1	282 ± 89,5	6,28 ± 0,94
NS12	Range	0,07 - 0,3	0,01 - 0,04	0,03 - 0,1	0,01 - 0,06	<0,01 - 0,02	6,1 - 7,4	16 - 41	98 - 450	4,78 - 7,3
	Mean±SD	0,14 ± 0,09	0,02 ± 0,01	0,08 ± 0,03	0,03 ± 0,02	0,02 ± 0,01	6,82 ± 0,46	24,3 ± 9,30	262 ± 109	5,86 ± 1,06
NS13	Range	0,05 - 0,2	0,01 - 0,11	0,05 - 0,09	0,01 - 0,03	<0,01 - 0,01	6 - 7,2	18 - 36	191 - 400	5,2 - 8,4
	Mean±SD	0,14 ± 0,06	0,04 ± 0,03	0,08 ± 0,02	0,02 ± 0,01	0,01 ± 0,00	6,78 ± 0,39	26,2 ± 7,31	276 ± 70,1	6,75 ± 0,99
NS14	Range	0,05 - 0,11	0,02 - 0,08	0,02 - 0,1	0,02 - 0,05	<0,01 - 0,04	5,8 - 7,2	23 - 55	128 - 417	3,97 - 7
	Mean±SD	0,08 ± 0,02	0,04 ± 0,03	0,06 ± 0,03	0,03 ± 0,01	0,02 ± 0,01	6,52 ± 0,51	34,2 ± 10,1	262 ± 90,2	5,27 ± 1,00

Table S4-2: Range, mean and standard deviation of chemical and physical parameters of water samples within Mululu Stream.

Rainy Season										
Sample Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**TDS**	**DO**
MS01	**Range**	**0,01 - 0,41**	**<0,01 - 0,01**	**0,01 - 0,31**	**0,01 - 0,02**	**0,01 - 0,03**	**6,2 -7,7**	**10 - 100**	**138 - 741**	**6 - 8,3**
	Mean±SD	0,12± 0,15	0,01 ± 0,00	0,11 ± 0,11	0,012 ± 0,00	0,02 ± 0,01	7,18 ± 0,56	70,2 ± 31,6	316 ± 218	7,14 ± 0,95
MS02	**Range**	**0,01 - 0,14**	**0,01 - 0,02**	**0,02 - 0,09**	**0,01 - 0,13**	**0,01 - 0,04**	**6,4 - 6,83**	**12,1 - 123**	**192 - 378**	**5,9 - 8**
	Mean±SD	0,07 ± 0,05	0,015 ± 0,01	0,08 ± 0,04	0,04 ± 0,05	0,02 ± 0,01	7,05 ± 0,48	56,2 ± 39,1	289 ± 80,9	6,96 ± 0,68
MS03	**Range**	**0,05 - 0,27**	**0,01 - 0,04**	**0,01 - 0,22**	**0,01 - 0,03**	**0,01 - 0,03**	**6,6 - 7,6**	**7 - 100**	**32,5 - 223**	**5,8 - 9**
	Mean±SD	0,10 ± 0,08	0,02 ± 0,01	0,09 ± 0,08	0,02 ± 0,01	0,02 ± 0,01	7,16 ± 0,36	36,6 ± 34,1	166 ± 69,9	7,12 ± 1,04
MS04	**Range**	**0,02 - 0,5**	**<0,01 - 0,02**	**0,06 - 0,74**	**0,01 - 0,02**	**<0,01 - 0,01**	**6,5 - 7,6**	**9 - 263**	**72 - 749**	**4,75 - 8,1**
	Mean±SD	0,19 ± 0,17	0,02 ± 0,01	0,21 ± 0,26	0,01 ± 0,01	0,01 ± 0	7,12 ± 0,38	67,2 ± 98,1	362 ± 220	6,79 ± 1,27
MS05	**Range**	**0,01 - 0,84**	**<0,01 - 0,16**	**0,01 - 0,42**	**0,01 - 0,08**	**0,01 - 0,03**	**6,2 - 7,5**	**9 - 34**	**270 - 552**	**5 - 8,1**
	Mean±SD	0,23 ± 0,31	0,05 ± 0,07	0,15 ± 0,16	0,03 ± 0,03	0,02 ± 0,01	6,92 ± 0,44	21,8 ± 9,95	333 ± 114	6,22 ± 1,17
MS06	**Range**	**0,01 - 0,18**	**<0,01 - 0,06**	**0,01 - 0,72**	**0,01 - 0,02**	**0,01 - 0,04**	**5,6 - 7,9**	**3 - 49**	**128 - 815**	**5,19 - 6**
	Mean±SD	0,09 ± 0,07	0,03 ± 0,02	0,2 ± 0,26	0,01 ± 0,00	0,02 ± 0,01	6,96 ± 0,77	19,1 ± 16,5	339 ± 257	5,66 ± 0,32
MS07	**Range**	**0,03 - 0,23**	**0,01 - 0,12**	**0,01 - 0,9**	**0,01 - 0,05**	**0,01 - 0,05**	**5,6 - 7,9**	**19 - 120**	**98 - 417**	**4,7 - 7,8**
	Mean±SD	0,1 ± 0,08	0,04 ± 0,04	0,27 ± 0,35	0,02 ± 0,02	0,02 ± 0,02	6,64 ± 0,74	52,4 ± 36,8	269 ± 134	6,24 ± 0,99
MS08	**Range**	**0,01 - 0,7**	**0,01 - 0,02**	**0,01 - 1**	**0,01 - 0,04**	**0,01 - 0,03**	**5,5 - 7,7**	**11,2 - 96**	**140 - 749**	**5,9 - 8,2**
	Mean±SD	0,23 ± 0,25	0,01 ± 0,00	0,3 ± 0,38	0,02 ± 0,01	0,02 ± 0,01	6,82 ± 0,78	64,6 ± 33,3	398 ± 219	7,12 ± 0,85
MS09	**Range**	**0,02 - 0,31**	**0,01 - 0,06**	**0,06 - 0,79**	**0,02 - 0,33**	**0,01 - 0,06**	**6,7 - 7,6**	**7 - 65**	**30,4 - 434**	**4,7 - 7,4**
	Mean±SD	0,1 ± 0,11	0,02 ± 0,02	0,37 ± 0,33	0,09 ± 0,12	0,02 ± 0,02	7,06 ± 0,3	36 ± 18,8	195 ± 137	6,28 ± 1,22
MS10	**Range**	**0,01 - 0,2**	**0,01 - 0,03**	**0,01 - 0,6**	**0,01 - 0,14**	**0,01 - 0,05**	**6,8 - 7,7**	**5 - 266**	**311 - 750**	**5 - 6,3**
	Mean±SD	0,08 ± 0,07	0,03 ± 0,01	0,2 ± 0,24	0,05 ± 0,05	0,02 ± 0,02	7,18 ± 0,34	95,2 ± 91,4	452 ± 162	5,58 ± 0,49
MS11	**Range**	**0,01 - 0,15**	**0,01 - 0,09**	**0,01 - 0,5**	**0,01 - 0,08**	**0,01 - 0,05**	**7 - 7,7**	**11 - 51**	**76 - 522**	**5 -7,3**
	Mean±SD	0,07 ± 0,05	0,04 ± 0,03	0,18 ± 0,19	0,04 ± 0,03	0,02 ± 0,02	7,3 ± 0.29	23,8 ± 15,2	262 ± 145	6,42 ± 0,91
MS12	**Range**	**0,04 - 0,14**	**0,01 - 0,03**	**0,02 - 0,2**	**0,03 - 0,08**	**<0,01 - 0,02**	**5,6 - 7**	**19 - 64**	**117 - 509**	**6,1 - 9,4**
	Mean±SD	0,08 ± 0,03	0,02 ± 0,01	0,08 ± 0,07	0,06 ± 0,02	0,01 ± 0,00	6,32 ± 0,48	40,8 ± 17,1	266 ± 141	7,24 ± 1,24
MS13	**Range**	**0,04 - 0,09**	**0,01 - 0,04**	**0,03 - 0,12**	**0,01 - 0,06**	**<0,01 - 0,03**	**6,1 - 7**	**19 - 70**	**100 - 320**	**4,6 - 8,1**
	Mean±SD	0,07 ± 0,02	0,02 ± 0,01	0,09 ± 0,03	0,03 ± 0,02	0,02 ± 0,01	6,64 ± 0,37	44 ± 20,7	243 ± 89,3	5,85 ± 1,56
MS14	**Range**	**0,01 - 0,07**	**0,01 - 0,03**	**0,01 - 0,08**	**0,01 - 0,05**	**0,01 - 0,01**	**5,9 - 7**	**29 - 51**	**272 - 376**	**6 - 7,7**
	Mean±SD	0,04 ± 0,02	0,02 ± 0,01	0,03 ± 0,02	0,02 ± 0,02	0,01 ± 0,00	6,5 ± 0,49	38,4 ± 7,26	299 ± 39,8	5,92 ± 0,92
MS15	**Range**	**0,03 - 0,2**	**0,01 - 0,06**	**0,01 - 0,41**	**0,01 - 0,04**	**<0,01 - 0,02**	**6,7 - 7,2**	**18 - 40**	**231 - 456**	**4,38 - 7,3**
	Mean±SD	0,09 ± 0,06	0,03 ± 0,02	0,12 ± 0,15	0,02 ± 0,01	0,01 ± 0,01	6,9 ± 0,19	27,6 ± 8,36	323 ± 85,3	5,91 ± 1,21
MS16	**Range**	**0,01 - 0,15**	**0,01 - 0,05**	**0,02 - 0,11**	**0,01 - 0,07**	**0,01 - 0,04**	**5,6 - 7,2**	**17 - 55**	**166 - 510**	**5,27 - 7,3**
	Mean±SD	0,06 ± 0,05	0,02 ± 0,01	0,06 ± 0,03	0,04 ± 0,02	0,02 ± 0,01	6,54 ± 0,6	35,6 ± 13,8	300 ± 124	5,85 ± 1,1
MS17	**Range**	**0,03 - 0,1**	**0,01 - 0,04**	**0,03 - 0,06**	**0,01 - 0,05**	**<0,01 - 0,02**	**6,8 - 7,2**	**11 - 73**	**93 - 401**	**6,1 - 8**
	Mean±SD	0,07 ± 0,03	0,02 ± 0,01	0,05 ± 0,01	0,03 ± 0,02	0,02 ± 0,01	7 ± 0,17	32 ± 21,3	241 ± 100	6,66 ± 0,76
PostRainy Season										
Sample Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**TDS**	**DO**
MS01	**Range**	**0,05 - 0,3**	**0,01 - 0,08**	**0,05 - 0,13**	**0,01 - 0,13**	**0,01 - 0,04**	**6 - 7,1**	**8,0 - 20**	**192 - 321**	**4,9 - 7,2**
	Mean±SD	0,15 ± 0,08	0,05 ± 0,03	0,08 ± 0,03	0,05 ± 0,05	0,02 ± 0,01	6,57 ± 0,41	15 ± 4,57	260 ± 48,9	6,13 ± 0,8
MS02	**Range**	**0,08 - 0,18**	**0,01 - 0,04**	**0,05 - 0,36**	**0,01 - 0,12**	**0,01 - 0,08**	**6,5 - 7,1**	**4,04 - 23**	**186 - 434**	**5,02 - 7,1**
	Mean±SD	0,1 ± 0,03	0,02 ± 0,01	0,13 ± 0,1	0,06 ± 0,04	0,04 ± 0,03	6,9 ± 0,2	13,2 ± 6,14	258 ± 87,1	6,2 ± 0,64
MS03	**Range**	**0,08 - 0,3**	**0,01 - 0,11**	**0,04 - 0,5**	**0,01 - 0,07**	**0,01 - 0,04**	**6,2 - 7,2**	**10,9 - 30**	**185 - 301**	**3,79 - 7,8**
	Mean±SD	0,13 ± 0,08	0,04 ± 0,03	0,2 ± 0,16	0,05 ± 0,03	0,02 ± 0,01	6,68 ± 0,31	20 ± 5,63	249 ± 43,5	5,86 ± 1,16
MS04	**Range**	**0,03 - 0,12**	**0,01 - 0,15**	**0,04 - 0,14**	**0,01 - 0,12**	**0,01 - 0,06**	**5,8 - 7,3**	**5,43 - 26**	**179 - 307**	**4,25 - 7**
	Mean±SD	0,05 ± 0,03	0,07 ± 0,05	0,16 ± 0,16	0,05 ± 0,04	0,03 ± 0,02	6,64 ± 0,55	12,2 ± 7,13	238 ± 49,5	6,09 ± 0,97
MS05	**Range**	**0,03 - 0,17**	**0,01 - 0,05**	**0,04 - 0,5**	**0,02 - 0,08**	**0,01 - 0,09**	**6,7 - 7,1**	**6,0 -19**	**165 - 566**	**4,3 - 7,7**
	Mean±SD	0,1 ± 0,05	0,03 ± 0,01	0,18 ± 0,17	0,05 ± 0,02	0,04 ± 0,03	6,96 ± 0,12	11,2 ± 3,98	295 ± 130	6,38 ± 1,06
MS06	**Range**	**0,08 - 0,27**	**0,01 - 0,04**	**0,01 - 0,23**	**0,01 - 0,08**	**0,01 - 0,06**	**5,9 - 7,3**	**4,7 - 40**	**125 - 311**	**3,97 - 7**
	Mean±SD	0,13 ± 0,06	0,02 ± 0,01	0,11 ± 0,09	0,04 ± 0,02	0,03 ± 0,02	6,77 ± 0,44	21,1 ± 15,1	256 ± 71,9	5,79 ± 1,03
MS07	**Range**	**0,04 - 0,1**	**0,01 - 0,04**	**0,05 - 0,14**	**0,03 - 0,1**	**0,01 - 0,04**	**6,2 - 7**	**5,43 - 32**	**172 - 265**	**4,67 - 6,5**
	Mean±SD	0,07 ± 0,02	0,03 ± 0,01	0,09 ± 0,03	0,06 ± 0,03	0,03 ± 0,01	6,76 ± 0,31	16,9 ± 9,21	218 ± 41	5,72 ± 0,66
MS08	**Range**	**0,03 - 0,3**	**0,01 - 0,1**	**0,04 - 0,1**	**0,05 - 0,11**	**6,3 - 6,95**	**6,0 - 6,8**	**23,5 - 29,8**	**201 - 349**	**5,02 - 7,5**
	Mean±SD	0,16 ± 0,08	0,05 ± 0,03	0,08 ± 0,02	0,08 ± 0,02	0,05 ± 0,02	6,66 ± 0,22	16 ± 7,33	283 ± 53,4	6,5 ± 0,9
MS09	**Range**	**0,09 - 0,3**	**0,01 - 0,11**	**0,09 - 0,5**	**0,01 - 0,1**	**0,01 - 0,08**	**6,1 - 7,2**	**4,7 - 30**	**179 - 300**	**3,9 - 7**
	Mean±SD	0,15 ± 0,08	0,07 ± 0,03	0,25 ± 0,15	0,07 ± 0,03	0,04 ± 0,03	6,73 ± 0,39	18,3 ± 8,01	223 ± 60,9	5,91 ± 1,02
MS10	**Range**	**0,04 - 0,23**	**0,01 - 0,15**	**0,09 - 0,17**	**0,02 - 0,12**	**0,01 - 0,04**	**5,5 - 7,1**	**5,43 - 26**	**145 - 291**	**4,25 - 6,49**
	Mean±SD	0,1 ± 0,07	0,05 ± 0,05	0,12 ± 0,03	0,06 ± 0,03	0,02 ± 0,01	6,76 ± 0,59	15,7 ± 7,62	239 ± 49,1	5,67 ± 0,75
MS11	**Range**	**0,04 - 0,19**	**0,01 - 0,09**	**0,04 - 0,5**	**0,01 - 0,08**	**0,01 - 0,06**	**6,4 - 7**	**6,0 - 14**	**225 - 350**	**3,49 - 7**
	Mean±SD	0,08 ± 0,05	0,03 ± 0,03	0,17 ± 0,16	0,06 ± 0,02	0,02 ± 0,02	6,88 ± 0,22	10,5 ± 2,75	303 ± 38,5	5,66 ± 1,20
MS12	**Range**	**0,03 - 0,17**	**0,01 - 0,05**	**0,05 - 0,09**	**0,01 - 0,03**	**<0,01 - 0,01**	**5,8 - 6,8**	**19 - 50**	**188 - 421**	**6 - 8,1**
	Mean±SD	0,09 ± 0,04	0,02 ± 0,01	0,08 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	6,28 ± 0,37	33,8 ± 12,1	302 ± 88,9	7,27 ± 0,65
MS13	**Range**	**0,02 - 0,07**	**0,01 - 0,02**	**0,01 - 0,06**	**0,01 - 0,05**	**<0,01 - 0,02**	**6,1 - 7,2**	**9 - 44**	**113 - 425**	**5,7 - 8,4**
	Mean±SD	0,05 ± 0,02	0,01 ± 0,00	0,05 ± 0,02	0,02 ± 0,01	0,01 ± 0,00	6,8 ± 0,36	21,8 ± 10,8	286 ± 93,4	7,33 ± 0,96
MS14	**Range**	**0,02 - 0,2**	**0,01 - 0,03**	**0,04 - 0,09**	**0,01 - 0,05**	**0,01 - 0,05**	**5,8 - 7,4**	**11,0 - 21**	**163 - 511**	**4,47 - 9,1**
	Mean±SD	0,08 ± 0,06	0,02 ± 0,01	0,07 ± 0,02	0,02 ± 0,01	0,02 ± 0,01	6,8 ± 0,56	17,5 ± 3,30	293 ± 130	6,47 ± 1,53
MS15	**Range**	**0,01 - 0,12**	**0,01 - 0,06**	**0,02 - 0,13**	**0,01 - 0,09**	**<0,01 - 0,02**	**6,1 - 7,2**	**31-Oct**	**190 - 404**	**5,29 - 7,3**
	Mean±SD	0,07 ± 0,04	0,03 ± 0,02	0,05 ± 0,04	0,03 ± 0,03	0,02 ± 0,01	6,8 ± 0,40	19,3 ± 8,36	290 ± 81,3	6,21 ± 0,69
MS16	**Range**	**0,01 - 0,16**	**<0,01 - 0,04**	**0,02 - 0,08**	**0,01 - 0,06**	**<0,01 - 0,03**	**6 - 7,5**	**14 - 35**	**143 - 535**	**4,5 - 7,1**
	Mean±SD	0,09 ± 0,07	0,02 ± 0,01	0,05 ± 0,02	0,03 ± 0,02	0,01 ± 0,01	6,88 ± 0,45	22 ± 7,72	340 ± 168	5,85 ± 0,78
MS17	**Range**	**0,02 - 0,3**	**0,01 - 0,09**	**0,04 - 0,1**	**0,01 - 0,09**	**<0,01 - 0,04**	**5,6 - 6,9**	**10,0 - 30**	**100 - 540**	**5,68 - 8,4**
	Mean±SD	0,1 ± 0,10	0,03 ± 0,03	0,08 ± 0,02	0,04 ± 0,03	0,02 ± 0,01	6,45 ± 0,47	21,3 ± 7,25	277 ± 147	6,61 ± 0,99

Table S4-3: Range, mean and standard deviation of chemical and physical parameters of water samples within Fikondo Stream.

Rainy Season										
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**TDS**	**DO**
FS01	**Range**	**0,01 - 0,31**	**0,01 - 0,03**	**0,01 - 1,2**	**0,01 - 0,03**	**0,01 - 0,04**	**6,07 - 7,1**	**45 - 86**	**203 - 621**	**3,7 - 8,5**
	Mean±SD	0,09 ± 0,11	0,02 ± 0,01	0,38 ± 0,43	0,02 ± 0,01	0,02 ± 0,01	6,73 ± 0,36	59,4 ± 15,1	362 ± 145	5,67 ± 1,67
FS02	**Range**	**0,01 - 1,05**	**0,01 - 0,03**	**0,06 - 1,36**	**0,01 - 0,04**	**0,01 - 0,02**	**5,9 - 7,4**	**30 - 70**	**126 - 969**	**2,49 - 7,1**
	Mean±SD	0,27 ± 0,39	0,02 ± 0,01	0,54 ± 0,06	0,02 ± 0,04	0,02 ± 0,00	6,74 ± 0,59	51,6 ± 15,2	505 ± 317	6,18 ± 0,93
FS03	**Range**	**0,03 - 0,1**	**0,01 - 0,02**	**0,09 - 0,36**	**0,01 - 0,03**	**0,01 - 0,07**	**6,1 - 7,1**	**25 - 101**	**173 - 512**	**5,3 - 8**
	Mean±SD	0,08 ± 0,02	0,01 ± 0,00	0,25 ± 0,12	0,02 ± 0,01	0,03 ± 0,02	6,62 ± 0,56	66,6 ± 33,3	262 ± 126	6,36 ± 1,01
FS04	**Range**	**0,01 - 1,03**	**0,01 - 0,06**	**0,01 - 1,2**	**0,01 - 0,02**	**0,01 - 0,04**	**6,2 - 7,3**	**31 - 63**	**165 - 514**	**5,7 - 7,6**
	Mean±SD	0,28 ± 0,40	0,03 ± 0,02	0,34 ± 0,44	0,01 ± 0,00	0,02 ± 0,01	6,52 ± 0,51	43,6 ± 13,5	311 ± 156	6,28 ± 0,67
FS05	**Range**	**0,02 - 0,14**	**0,01 - 0,04**	**0,08 - 1,75**	**<0,01 - 0,04**	**<0,01 - 0,06**	**5,7 - 7**	**26 - 178**	**130 - 710**	**4,1 - 7,6**
	Mean±SD	0,08 ± 0,05	0,02 ± 0,01	0,58 ± 0,67	0,02 ± 0,01	0,02 ± 0,02	6,68 ± 0,49	59,2 ± 59,5	360 ± 211	6,52 ± 1,27
FS06	**Range**	**0,01 - 0,23**	**0,01 - 0,04**	**0,09 - 2,46**	**0,01 - 0,07**	**<0,01 - 0,02**	**07-Jun**	**18 - 97**	**89 - 425**	**5,39 - 8**
	Mean±SD	0,09 ± 0,08	0,02 ± 0,01	0,59 ± 0,94	0,03 ± 0,02	0,02 ± 0,01	6,44 ± 0,39	57,6 ± 30,7	232 ± 122	6,04 ± 1,04
Post Rainy Season										
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**TDS**	**DO**
FS01	**Range**	**0,05 - 0,6**	**0,01 - 0,1**	**0,06 - 0,23**	**0,01 - 0,1**	**0,01 - 0,05**	**5,9 - 7,2**	**4,04 - 101**	**122 - 378**	**5,21 - 7,14**
	Mean±SD	0,18 ± 0,19	0,03 ± 0,03	0,12 ± 0,05	0,05 ± 0,04	0,03 ± 0,01	6,82 ± 0,44	31 ± 32,86	238 ± 77,1	6,38 ± 0,66
FS02	**Range**	**0,03 - 0,18**	**0,01 - 0,04**	**0,06 - 0,2**	**0,01 - 0,09**	**<0,01 - 0,04**	**5,5 - 7,4**	**16 - 91**	**147 - 315**	**5 - 8,5**
	Mean±SD	0,09 ± 0,05	0,03 ± 0,01	0,13 ± 0,06	0,05 ± 0,03	0,02 ± 0,01	6,68 ± 0,66	41,5 ± 25,8	226 ± 65,9	6,60 ± 1,15
FS03	**Range**	**0,04 - 0,15**	**0,01 - 0,05**	**0,05 - 0,3**	**0,01 - 0,1**	**<0,01 - 0,07**	**5,9 - 7,2**	**5,43 - 37**	**101 - 335**	**4,21 - 9**
	Mean±SD	0,08 ± 0,04	0,03 ± 0,01	0,13 ± 0,08	0,04 ± 0,03	0,04 ± 0,02	6,73 ± 0,42	23,6 ± 10,4	213 ± 84,3	6,37 ± 1,45
FS04	**Range**	**0,03 - 1,04**	**0,01 - 0,06**	**0,04 - 0,37**	**0,01 - 0,14**	**0,01 - 0,06**	**6,5 - 7,1**	**6 - 43**	**198 - 517**	**4,01 - 6,6**
	Mean±SD	0,3i ± 0,34	0,03 ± 0,02	0,13 ± 0,11	0,05 ± 0,05	0,03 ± 0,02	6,93 ± 0,20	22,8 ± 14,5	348 ± 109	5,63 ± 0,82
FS05	**Range**	**0,1 - 0,23**	**0,01 - 0,06**	**0,08 - 1,01**	**0,01 - 0,07**	**<0,01 - 0,04**	**5,8 - 7,3**	**7,3 - 84**	**98 - 309**	**5,3 - 7,6**
	Mean±SD	0,15 ± 0,05	0,03 ± 0,02	0,25 ± 0,34	0,03 ± 0,02	0,02 ± 0,01	6,79 ± 0,53	33,1 ± 25,4	203 ± 81	6,35 ± 1,02
FS06	**Range**	**0,03 - 0,4**	**0,01 - 0,03**	**0,05 - 0,6**	**0,01 - 0,05**	**0,01 - 0,06**	**5,3 - 69**	**19 - 100**	**123 - 563**	**4,17 - 8,4**
	Mean±SD	0,16 ± 0,13	0,02 ± 0,01	0,16 ± 0,2	0,03 ± 0,02	0,03 ± 0,02	16,6 ± 23,5	34,2 ± 29,7	359 ± 151	6,39 ± 1,55

Table S4-4: Range, mean and standard deviation of metal concentration (ppm) of sediment samples within Nselaki Stream

Site Description		Cu	Co	Mn	Zn	Pb
Rainy Season						
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**
NS01	**Range**	**2589 - 6010**	**80 - 385**	**1115 - 2240**	**104 - 140**	**10 - 40**
	Mean±SD	4000 ± 1460	253 ± 128	1707 ± 461	118 ± 15,9	29,3 ± 13,7
NS02	**Range**	**1193 - 5860**	**220 - 1675**	**910 - 1760**	**88 - 350**	**32 - 68**
	Mean±SD	3118 ± 1991	805 ± 627	1257 ± 364	219 ± 107	52,7 ± 15,2
NS03	**Range**	**1540 - 6165**	**182 - 380**	**800 - 1560**	**100 - 247**	**25 - 42**
	Mean±SD	3968 ± 1895	275 ± 81,2	1243 ± 323	169 ± 60,4	35,7 ± 7,59
NS04	**Range**	**1925 - 4905**	**150 - 440**	**865 - 1270**	**102 - 185**	**11 - 32**
	Mean±SD	3027 ± 1335	327 ± 127	1018 ± 179	133 ± 37,2	20,7 ± 8,65
NS05	**Range**	**1430 - 5460**	**98 - 770**	**782 - 1340**	**135 - 155**	**15 - 40**
	Mean±SD	3067 ± 1730	323 ± 316	1037 ± 230	145 ± 8,18	30 ± 10,8
NS06	**Range**	**1240 - 3185**	**126 - 275**	**1001 - 1125**	**125 - 233**	**22 - 53**
	Mean±SD	2517 ± 783	217 ± 56,4	1047 ± 48,2	176 ± 38,4	35,7 ± 11,2
NS07	**Range**	**1200 - 5390**	**410 - 990**	**589 - 725**	**79 - 185**	**11 - 78**
	Mean±SD	2719 ± 1641	670 ± 208	665 ± 49	148 ± 42,5	33 ± 16,7
NS08	**Range**	**1400 - 2200**	**115 - 295**	**787 - 1490**	**120 - 289**	**28 - 36**
	Mean±SD	1832 ± 286	215 ± 64,8	1096 ± 254	185 ± 64,5	32 ± 2,83
NS09	**Range**	**1230 - 2348**	**101 - 185**	**900 - 1620**	**135 - 349**	**29 -34**
	Mean±SD	1619 ± 447	151 ± 31,2	1144 ± 291	209 ± 86	30,7 ± 2,04
NS10	**Range**	**1700 - 2990**	**101 - 603**	**1587 - 2841**	**109 - 410**	**43 - 61**
	Mean±SD	2440 ± 471	374 ± 180	2176 ± 446	277 ± 109	51,7 ± 6,38
NS11	**Range**	**1360 - 3100**	**240 - 545**	**786 - 2309**	**112 - 190**	**29 - 35**
	Mean±SD	2103 ± 418	415 ± 135	1573 ± 322	150 ± 17,4	31,3 ± 5,1
NS12	**Range**	**1799 - 2800**	**70 - 320**	**1200 - 1918**	**200 - 289**	**19 - 80**
	Mean±SD	2251 ± 359	197 ± 88,4	1480 ± 272	230 ± 36,1	53 ± 22
NS13	**Range**	**1335 - 3100**	**215 - 660**	**1700 - 3040**	**138 - 344**	**19 - 112**
	Mean±SD	2145 ± 630	463 ± 161	2250 ± 496	225 ± 75,3	57,7 ± 34,2
NS14	**Range**	**1400 - 2871**	**163 - 209**	**800 - 2100**	**89 - 277**	**27 - 74**
	Mean±SD	1990 ± 550	181 ± 17,2	1396 ± 464	156 ± 74,1	48,7 ± 16,8
Post Rainy Season						
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**
NS01	**Range**	**1623 - 3260**	**324 - 400**	**812 - 4218**	**86 - 90**	**16 -56**
	Mean±SD	2714 ± 772	374 ± 35,8	3083 ± 1606	88,7 ± 1,89	29,3 ± 18,9
NS02	**Range**	**2350 - 2500**	**654 - 1741**	**797 - 1097**	**26,9 - 62**	**11 - 138**
	Mean±SD	2450 ± 70,7	1016 ± 512	997 ± 141	50,3 ± 16,6	53,3 ± 59,9
NS03	**Range**	**1542 - 2541**	**275 - 925**	**915 - 2669**	**74 - 374**	**20 -49**
	Mean±SD	1875 ± 471	492 ± 306	1500 ± 827	174 ± 141	33 ±4,24
NS04	**Range**	**2334 - 3834**	**151 - 650**	**2098 - 2610**	**102 - 200**	**19 - 25**
	Mean±SD	2834 ± 707	317 ± 235	2439 ± 241	135 ± 46,2	24,3 ± 0,94
NS05	**Range**	**1992 - 2299**	**112 - 376**	**845 - 1399**	**82 - 105**	**12 - 52**
	Mean±SD	2094 ± 145	288 ± 125	1030 ± 261	97,3 ± 10,8	25,3 ± 18,9
NS06	**Range**	**1070 - 2379**	**240 - 742**	**546 - 2091**	**89 - 149**	**31 -33**
	Mean±SD	1506 ± 617	407 ± 237	1576 ± 728	109 ± 28,3	31,7 ± 0,94
NS07	**Range**	**997 - 1414**	**516 -540**	**1001 - 1694**	**112 - 165**	**19 -22**
	Mean±SD	1136 ± 170	527 ± 13,9	1232 ± 283	130 ± 21,6	21 ± 1,22
NS08	**Range**	**1200 - 2425**	**428 - 554**	**616 - 925**	**49 -52**	**13 -32**
	Mean±SD	1615 ± 508	470 ± 51,4	822 ± 126	50 ± 1,22	19,3 ± 1,76
NS09	**Range**	**1198 - 2118**	**184 - 190**	**1122 - 1223**	**77 - 112**	**15 - 36**
	Mean±SD	1811 ± 376	188 ± 2,45	1189 ± 41,2	88,7 ± 14,3	29 ± 8,57
NS10	**Range**	**1978 - 3019**	**289 - 620**	**1675 - 2300**	**100 - 121**	**25 - 80**
	Mean±SD	2667 ± 422	421 ± 124	2036 ± 229	110 ± 7,45	44,7 ± 21,7
NS11	**Range**	**2018 - 2235**	**312 - 421**	**1237 - 2310**	**88 - 101**	**21 - 65**
	Mean±SD	2104 ± 81,7	349 ± 44,3	1776 ± 379	95,7 ± 4,81	46 ± 16
NS12	**Range**	**1040 - 2670**	**219 - 465**	**992 - 1033**	**99 - 264**	**28 - 70**
	Mean±SD	1751 ± 590	312 ± 94,3	1011 ± 14,6	176 ± 58,8	44 ± 16,1
NS13	**Range**	**973 - 1981**	**198 - 345**	**1089 - 2417**	**102 - 215**	**34 - 52**
	Mean±SD	1610 ± 392	280 ± 53,1	1836 ± 480	142 ± 45	42 ± 6,48
NS14	**Range**	**1083 - 1685**	**400 - 602**	**980 - 1124**	**89 - 186**	**11 - 49**
	Mean±SD	1429 ± 220	506 ± 71,7	1089 ± 68,2	164 ± 47,2	33,7 ± 14,2

Table S4-5: Range, mean and standard deviation of metal concentration (ppm) of sediment samples within Mululu Stream

Rainy Season						
Site Description		Cu	Co	Mn	Zn	Pb
MS01	Range	**1066 - 3600**	**41 - 382**	**1182 - 1260**	**64 - 215**	**19 - 74**
	Mean±SD	2348 ± 1035	191 ± 142	1214 ± 33,4	114 ± 71,2	38,3 ± 25,3
MS02	Range	**1572 - 1952**	**154 - 160**	**800 - 1090**	**18 - 134**	**20 - 56**
	Mean±SD	1766 ± 155	156 ± 2,63	910 ± 128	65,3 ± 49,7	37,3 ± 14,7
MS03	Range	**1282 - 3180**	**89 - 525**	**989 - 1732**	**51 - 200**	**54 - 112**
	Mean±SD	2115 ± 792	330 ± 181	1276 ± 326	147 ± 68,2	70,7 ± 29,4
MS04	Range	**1596 - 2820**	**139 - 382**	**905 - 2450**	**92 - 406**	**18 - 86**
	Mean±SD	2048 ± 549	235 ± 105	1562 ± 651	224 ± 133	42 ± 31,2
MS05	Range	**992 - 2382**	**109 - 255**	**921 - 2292**	**70 - 185**	**15 - 112**
	Mean±SD	1773 ± 580	166 ± 63,9	1411 ± 624	127 ± 47	55 ± 41,4
MS06	Range	**825 - 2300**	**100 - 200**	**929 - 1389**	**49 - 133**	**14 - 110**
	Mean±SD	1556 ± 602	137 ± 45	1106 ± 202	96 ± 35	62,3 ± 39,2
MS07	Range	**1400 - 2310**	**141 - 234**	**1150 - 2295**	**53 - 144**	**14 - 125**
	Mean±SD	1937 ± 389	195 ± 39,4	1618 ± 490	112 ± 41,8	64,3 ± 45,9
MS08	Range	**1204 - 2210**	**208 - 256**	**930 - 1758**	**37 - 108**	**20 - 81**
	Mean±SD	1695 ± 411	238 ± 21,4	1222 ± 380	63 ± 32	51,7 ± 25
MS09	Range	**1905 - 2400**	**310 - 385**	**1107 - 1300**	**140 - 420**	**32 - 54**
	Mean±SD	2138 ± 203	338 ± 33,3	1226 ± 84,8	242 ± 126	41,3 ± 9,29
MS10	Range	**1460 - 3300**	**177 - 841**	**990 - 1610**	**30 - 220**	**58 - 129**
	Mean±SD	2207 ± 790	411 ± 304	1230 ± 272	116 ± 78,5	85,7 ± 31
MS11	Range	**998 - 2500**	**114 - 380**	**800 - 1001**	**111 - 160**	**42 - 86**
	Mean±SD	1894 ± 647	213 ± 119	933 ± 93,8	130 ± 21,6	56,7 ± 20,7
MS12	Range	**1250 - 2171**	**196 - 620**	**891 - 1650**	**200 - 314**	**26 - 79**
	Mean±SD	1835 ± 415	405 ± 173	1177 ± 337	256 ± 46,6	45 ± 24,1
MS13	Range	**989 - 1967**	**235 - 302**	**1006 - 2039**	**239 - 300**	**19 - 82**
	Mean±SD	1549 ± 412	258 ± 31,1	1421 ± 445	271 ± 25	50 ± 25,7
MS14	Range	**1116 - 1821**	**288 - 412**	**1100 - 3121**	**55 - 181**	**11 - 60**
	Mean±SD	1390 ± 308	340 ± 42,4	2015 ± 836	133 ± 55,5	40,3 ± 21,1
MS15	Range	**2012 - 3100**	**215 - 371**	**875 - 1379**	**89 - 298**	**44 - 49**
	Mean±SD	2375 ± 513	302 ± 65	1112 ± 207	172 ± 90,7	47 ± 2,16
MS16	Range	**1745 - 2700**	**96 - 485**	**1020 - 1541**	**31 - 264**	**61 - 261**
	Mean±SD	2262 ± 394	228 ± 182	1345 ± 232	170 ± 101	126 ± 95,7
MS17	Range	**1564 - 5200**	**109 - 562**	**740 - 1932**	**44 - 401**	**29 - 83**
	Mean±SD	2826 ± 1680	312 ± 188	1319 ± 487	252 ± 152	62 ± 23,6
Post rainy Season						
Site Description		Cu	Co	Mn	Zn	Pb
MS01	Range	**1521 - 2122**	**68,5 - 143**	**1024 - 2102**	**12,2 - 170**	**33,1 - 51**
	Mean±SD	1774 ± 311	104 ± 37,4	1564 ± 539	86,4 ± 79,3	41,4 ± 9,02
MS02	Range	**998 - 2012**	**95,8 - 230**	**998 - 2031**	**26,9 - 208**	**10 - 34,1**
	Mean±SD	1560 ± 516	178 ± 72,1	1427 ± 538	128 ± 92,4	23,4 ± 12,3
MS03	Range	**1421 - 1968**	**99 - 250**	**805 - 1207**	**101 - 182**	**25 - 46,7**
	Mean±SD	1613 ± 307	152 ± 84,7	973 ± 209	128 ± 46,5	34,9 ± 11
MS04	Range	**834 - 1296**	**167 - 310**	**1084 - 2872**	**79 - 109**	**18 - 28,8**
	Mean±SD	1072 ± 232	238 ± 71,7	1793 ± 950	92 ± 15,4	21,9 ± 5,97
MS05	Range	**1304 - 1606**	**165 - 516**	**1193 - 2109**	**99 - 128**	**13,3 - 32**
	Mean±SD	523 ± 185	307 ± 185	1638 ± 459	115 ± 14,7	24,1 ± 9,68
MS06	Range	**1060 - 2001**	**80 - 424**	**1041 - 1682**	**100 - 211**	**25 - 43**
	Mean±SD	1678 ± 535	250 ± 172	1336 ± 326	143 ± 59,4	33 ± 9,17
MS07	Range	**1014 - 1950**	**99 - 189**	**1382 - 2000**	**105 - 275**	**16 - 36**
	Mean±SD	1551 ± 483	148 ± 45,5	1626 ± 329	182 ± 86	24,7 ± 10,3
MS08	Range	**1166 - 2310**	**115 - 225**	**903 - 2116**	**84 - 105**	**21 - 30**
	Mean±SD	1576 ± 637	183 ± 59,7	1547 ± 610	94,7 ± 10,5	24,7 ± 4,73
MS09	Range	**739 - 1669**	**101 - 128**	**993 - 1047**	**90 - 181**	**13 - 56**
	Mean±SD	1125 ± 485	116 ± 13,8	1026 ± 29,1	127 ± 47,7	29 ± 23,5
MS10	Range	**1039 - 1251**	**90 - 217**	**824 - 1004**	**100 - 103**	**25 -38**
	Mean±SD	1180 ± 122	136 ± 70,4	914 ± 90	101 ± 1,53	32,7 ± 6,81
MS11	Range	**1125 - 2150**	**99 -177**	**1107 - 1682**	**77 - 100**	**22 - 31**
	Mean±SD	1594 ± 518	147 ± 42	1303 ± 328	86 ± 12,3	25,3 ± 4,93
MS12	Range	**1792 - 2811**	**314 - 528**	**1561 - 2027**	**214 - 311**	**29 - 69**
	Mean±SD	2461 ± 580	405 ± 111	1720 ± 266	255 ± 50,1	46 ± 20,7
MS13	Range	**1023 - 2564**	**226 - 291**	**1590 - 2035**	**95 - 319**	**25 - 59**
	Mean±SD	1746 ± 775	266 ± 34,8	1849 ± 231	207 ± 112	42,3 ± 17
MS14	Range	**996 - 1923**	**163 - 311**	**1115 - 1268**	**118 - 201**	**17 - 71**
	Mean±SD	1458 ± 464	222 ± 78,4	1205 ± 79,8	163 ± 419	42 ± 27,2
MS15	Range	**1027 - 1594**	**105 - 417**	**1001 - 1200**	**116 - 255**	**24 - 60**
	Mean±SD	1247 ± 304	292 - 165	1106 ± 100	176 ± 71,6	39 ± 18,7
MS16	Range	**928 - 1879**	**101 - 419**	**1310 - 1602**	**182 - 437**	**17 - 29**
	Mean±SD	1545 ± 535	247 ± 161	1497 ± 162	311 ± 128	21,7 ± 6,43
MS17	Range	**2050 - 2340**	**183 - 341**	**989 - 2302**	**99 - 182**	**15 - 39**
	Mean±SD	2168 ± 152	281 ± 85,8	1624 ± 658	142 ± 41,6	25,3 ± 12,3

Table S4-6: Range, mean and standard deviation of metal concentration (ppm) of sediment samples within Fikondo Stream

Rainy Season						
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**
FS01	**Range**	**1066 - 2018**	**36 - 150**	**1022 - 1260**	**14 - 210**	**19-Aug**
	Mean±SD	1438 ± 416	101 ± 48	1131 ± 98,3	85,7 ± 88,3	13 ± 4,55
FS02	**Range**	**952 - 1500**	**89 - 412**	**830 - 1190**	**14 - 80**	**15 - 44**
	Mean±SD	1192 ± 229	218 ± 140	970 ± 158	46,3 ± 27	26,3 ± 12,7
FS03	**Range**	**979 - 1282**	**65 - 375**	**810 - 1289**	**11 - 112**	**23 - 110**
	Mean±SD	1080 ± 143	236 ± 129	1000 ± 208	58 ± 41,5	59,7 ± 36,8
FS04	**Range**	**1728 - 3349**	**51 - 523**	**832 - 1678**	**12 - 89**	**14 - 38**
	Mean±SD	2392 ± 693	220 ± 215	1297 ± 350	41,7 ± 33,8	23,3 ± 10,5
FS05	**Range**	**1271 - 2729**	**109 - 310**	**890 - 1091**	**12 - 297**	**15 - 29**
	Mean±SD	2028 ± 597	182 ± 91	967 ± 88,4	113 ± 131	21,7 ± 5,74
FS06	**Range**	**825 - 1513**	**92 - 249**	**929 - 1001**	**12 - 69**	**14 - 19**
	Mean±SD	1173 ± 281	150 ± 70,2	962 ± 29,8	41 ± 23,3	16,7 ± 2,06
Post Rainy Season						
Site Description		**Cu**	**Co**	**Mn**	**Zn**	**Pb**
FS01	**Range**	**2556 - 3266**	**358 - 401**	**1111 - 2304**	**85 - 283**	**30-Dec**
	Mean±SD	2915 ± 290	379 ± 17,6	1795 ± 503	153 ± 91,7	21 ± 7,35
FS02	**Range**	1688 - 2419	228 - 299	1355 - 2002	74 - 118	36 - 47
	Mean±SD	2072 ± 300	263 ± 29	1700 ± 266	101 ± 19,5	41,7 ± 4,5
FS03	**Range**	1265 - 1766	185 - 305	995 - 1017	65 - 160	22-Jun
	Mean±SD	1536 ± 207	235 ± 50,9	1010 ± 10,4	111 ± 38,8	13 ± 6,68
FS04	**Range**	1530 - 1895	189 - 313	982 - 3144	71 - 99	19 - 111
	Mean±SD	1669 ± 161	259 ± 51,8	1784 ± 967	84 ± 11,5	52,7 ± 41,4
FS05	**Range**	999 - 1408	121 - 496	998 - 2476	49 - 201	16 - 36
	Mean±SD	1142 ± 188	312 ± 153	1563 ± 652	101 ± 71	23,7 ± 8,81
FS06	**Range**	1192 - 1870	80 - 218	823 - 1574	86 - 149	17 - 31
	Mean±SD	1607 ± 297	136 ± 59,4	1146 ± 316	112 26,9	24,3 ± 5,74

CHAPTER 5: A COMPARATIVE ANALYSIS OF MACROINVERTEBRATE COMMUNITY STRUCTURES FROM NSELAKI, MULULU AND FIKONDO STREAMS ON THE ZAMBIAN COPPERBELT USING MULTIFARIOUS LINES OF ATTESTATION

Biomonitoring can be defined as "the systematic use of living organisms in order to evaluate the changes or conditions of the environment". Impacts of external factors on aquatic ecosystems and differences between location can be monitored over time to assess anthropogenic impacts and their consequences. In this study, the use of benthic macroinvertebrates to categorize and understand the influence of metal mobilization from copper tailings was investigated. The functional approach (based on behavioural and morphological characteristics) and taxonomic approach (measurement of invertebrate diversity or richness) was used to characterise the conditions of aquatic ecosystems. This approach is valuable in assessing certain abiotic variables on water resources, whilst providing a integrated summary of biodiversity changes associated with variation in water quality. Biomonitoring can be useful as an early warning system to detect sudden alterations in water quality occasioned by anthropogenic related activities such as mining. This is important in providing timely mitigation measures that would lessen societal and environmental impacts.

5.1. Introduction

Rivers and streams are among the most threatened ecosystems in the world (Krajenbrink et al., 2019; Vörösmarty et al., 2010), due to increase in anthropogenic activities on a global scale. Their health is influenced by a multitude of factors; practically, it is not possible to measure in detail every single factor. However, their ecological health can be evaluated using selected ecological indicator groups (abiotic or biotic) that are representative of the ecosystem (de Klerk et al., 2012; Gutiérrez-Cánovas et al., 2019; Klerk and Wepener, 2013) in totality. The assessment of the ecological integrity of a river or stream may be used to define the combined effects of all the activities that drain into the aquatic ecosystem (Desrosiers et al., 2019; Everall et al., 2017; Li et al., 2010). Studies have shown that the ecological integrity of an aquatic ecosystem can be determined by assessing the composition of aquatic organisms of a biotic community (Agboola et al., 2019; Guareschi and Wood, 2019; Gutiérrez-Cánovas et al., 2019) to indicate the health status of an ecosystem (Laini et al., 2018; Li et al., 2010). Physical and chemical monitoring of the aquatic ecosystem can be enhanced using biological assessments. Biological assessments integrate different factors affecting the rivers and provides a direct assessment of the ecosystem's health (Mathers et al., 2016; Smith et al., 2019). The physical and chemical signature of water samples may serve only as a snapshot of the health of the ecosystem, due to variability in concentrations, dependent on the timing of precipitation events, discharges and water flow patterns (Karaouzas et al., 2019; von Schiller et al., 2017).

Macroinvertebrates in particular are an important group of aquatic species that can be used in the integrated assessment and monitoring of water quality (Alemneh et al., 2017; Chellaiah and Yule, 2018; Martinez-Haro et al., 2015; Novotny et al., 2005), because of the sensitivity of selected species to environmental stressors. Additionally, their relative abundance in aquatic environments and constant exposure to surrounding water resources (Chellaiah and Yule, 2018; Masese et al., 2014), together with vulnerability to changes in water quality make them suitable species for monitoring water quality.

Land use activities, such as mining, can affect the integrity of an aquatic ecosystem (Keovilignavong, 2019; Northey et al., 2019; Werner et al., 2019). On the other hand, mining is of strategic importance in countries like Zambia and as such proactive monitoring is required to permit sustainable use of water resources. The Kafue River catchment in the

Copperbelt Province of Zambia is among the most polluted regions in Southern Africa (Kapungwe, 2013; Ntengwe and Maseka, 2006; Sracek et al., 2012, Sracek, 2015; von der Heyden and New, 2004). The catchment has been subjected to copper mining for more than 80 years (Sikamo et al., 2016), thus understanding the impact of mining related activities such as mine waste on the selected aquatic ecosystems and associated spatial and temporal changes in aquatic community assemblages is important. This may help to gain sufficient ecological information relating to aquatic community changes subject to copper mining impacts. Such information is lacking in Zambia, thus creating challenges in proper management of aquatic systems in the face of potential growth of mining industry in the near future (Sikamo et al., 2016). The Kafue River catchment is one such area at the centre of historical and increased current mining developments (Mwaanga et al., 2019). Whilst copper deposits in the old mines within the region are slowly nearing depletion, exploration activities to exploit other copper deposits within the area are increasing. These are anticipated to occur mainly within the Kafue catchment, placing the water resources at increased risk of mine pollution related activities. The catchment is characterised by high rainfall which is critical in instigating metal mobilization from the mine wastelands (Colombani et al., 2020; Lim et al., 2009; Shimaponda-Mataa et al., 2017). Thus, the impact of mining activities in the catchment remains a source of concern.

The current study made use of this rare opportunity to undertake a comparative study of macroinvertebrate community assemblages along the Nselaki Stream, Fikondo Stream, and Mululu Stream, to better understand the impacts of mining activities and assist with management of water resources. A range of endpoints to be used as early warning signs of adverse impacts on biotic community integrity were evaluated in this study. These may be used to monitor and assess the impact of mining.

5.2. Materials and Methods

5.2.1. Study Areas and Study Design

Within the Mululu Stream, eleven sites were selected, in Nselaki Stream nine sites were selected whilst in Fikondo Stream six sites were selected (Figure 5-1) as representative of the stream system. Site selection was based on accessibility and land use activities. Samples from each stream were collected seasonally for three consecutive years (2018 – 2020) in order to collect data that account for differences in hydrological extremes and seasonal variations for a number of chemical parameters. At each of the selected sites, *in situ* parameters for water quality were measured, water samples were collected and taken to the laboratory for analysis of a suite of variables (The same results reported in chapter 4 are used in this chapter). Changes in fauna composition of these streams were assessed by focussing on diversity of macroinvertebrate assemblages. A biotic index score was calculated based on site-specific habitat assessments. The biotic index score was derived by compiling a list of potential macroinvertebrate assemblages from literature and then assessing each site for the availability of these assemblages (Aazami et al., 2015; Du et al., 2017; Gonçalves and Menezes, 2011; Shah and Lloyd, 2009). Assessment from the sites included scoring ecological health status using the biotic index score (on a scale from 1.0 = poor to 3.6 = excellent) adopted from other studies (Gonçalves and Menezes, 2011; Water-Monitoring, 2007). The index scores (Table 5-1) represent graded scores which permit comparison between streams or rivers. For the sites in the Mululu Stream, site names were abbreviated as MS (i.e., MS01), NS was used to abbreviate sites in Nselaki Stream (i.e., NS01), whilst for Fikondo Stream the abbreviation FS was used (i.e., FS01). The results were compared with similar studies conducted within the Copperbelt region (Mundike, 2004; Mudenda, 2018).

The species were grouped according to their sensitivity indicator (Table 5-1), in order to derive the overall indication of stream condition based on community populations (Water-Monitoring, 2010). Biotic index values were based on sensitivity score assigned to certain taxa by Resh and Jackson, (1993), Rosenberg and Resh, (1993) and Bis and Kosmala, (2008).

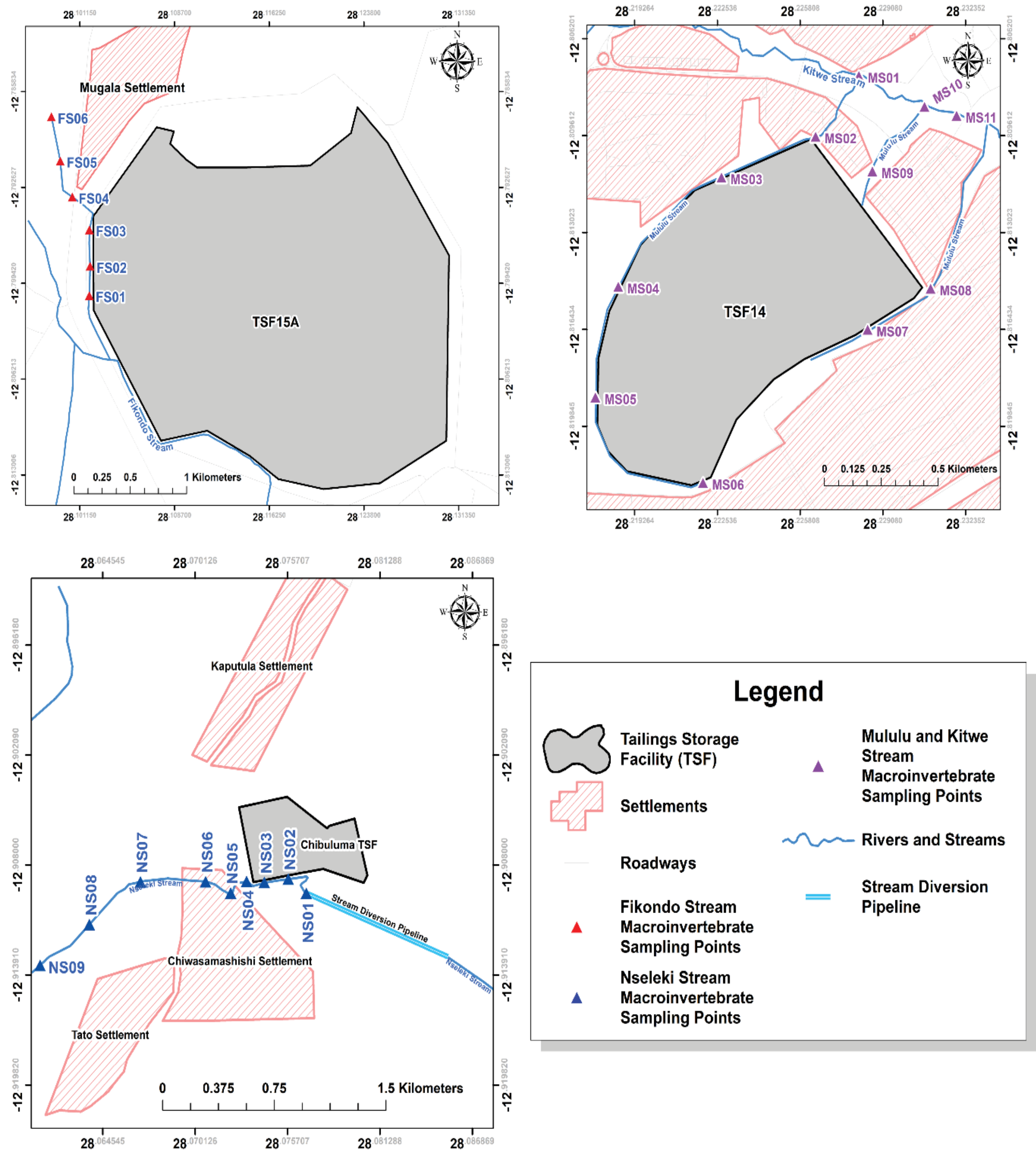


Figure 5-1: The study area, Fikondo Stream (upper left) reflecting the six study sites (FS01 - FS06), Nselaki Stream (lower left) reflecting nine study sites (NS01 – NS09) and Mululu Stream (right) reflecting eleven study sites (MS01 – MS11).

5.2.2. Assessment of Integrity of Aquatic Ecosystem

5.2.2.1. *Water Quality*

In situ water quality parameters such as pH, temperature, DO, total dissolved solids (TDS) and turbidity were measured using a HI98193 dissolved oxygen BOD/OUR/SOUR meter and HQ4200 portable multi-meter. Standard procedures (APHA, AWWA, WEF, 2012) were used to analyse a suite of different parameters from the collected water samples at each site. Quality assurance and quality control protocols throughout the analysis were followed. This included measuring the reproducibility by duplicating the first sample of each batch. Water samples upstream were analysed for comparative purposes. This water quality analysis has been presented in detail in Chapter 4 and is integrated here to better interpret the macroinvertebrate results. The variables introduced in Chapter 4 (see chapter 4, section 4-7; Tables 4-1 to 4-6) and used here include pH, DO, TDS, turbidity, Cu, Co, Pb, Mn and Zn.

5.2.2.2. *Macroinvertebrates*

The biotic index, a rapid assessment tool based on assemblages of the aquatic macroinvertebrates which assess the ecological integrity of a stream or river (Bhatt and Pandit, 2010; Sirisinthuwanich et al., 2016; Zakaria and Mohamed, 2019), was used in this study. The biotic index is designed for low, moderate and high flow hydrology that is typically encountered during the rainy seasons. Using this method, sampling of macroinvertebrates can be done efficiently across different seasons without being restricted to time as is the case with South African Scoring System Version 5 (SASS5) (Dickens and Graham, 2002). Equally, the number of species found is not important; rather, the variety of species and how they are categorized tells us the biotic index score. The biotic index score was calculated as follows.

The total number of organisms in each category was multiplied by the relevant group value, given below:

- Group 1 – The value is 4
- Group 2 – The value is 3
- Group 3 – The value is 2
- Group 4 – The value is 1

The products were recorded and totalled to give the total value (b). The numbers of organisms from each group were also totalled to give the total number of organisms (a). The biotic index score was then calculated as the quotient of (b) and (a).

Biotic Index Score = b/a

This value was used to verify health of the stream (Table 5-1) (Water-Monitoring, 2007).

Table 5-1: Score patterns of biotic index

Group	Pollution Response	Index Score	Ecological Status
1	Sensitive	3.5^{+}	Excellent
2	Semi-sensitive	2.6 - 3.5	Good
3	Semi-tolerant	2.1 - 2.5	Average
4	Tolerant	1.0 -2.0	Poor

The study made use of D-frame nets to sample aquatic macroinvertebrates at the identified biotopes. The sampled macroinvertebrates were placed on a white dish pan for subsequent identification using the benthic macroinvertebrate key (Birmingham et al., 2005). Various attributes such as shell, legs etc., were considered when identifying the macroinvertebrates.

There are two approaches when using macroinvertebrates in assessing ecological integrity, namely a functional approach based on behavioural and morphological characteristics, or a taxonomic approach based on diversity and richness of macroinvertebrates. Both can be used in characterising aquatic ecosystem conditions (Cummins et al., 2005; Leslie and Lamp, 2017). In the functional assessment, feeding groups developed by Merritt et al. (2002) and Cummins et al. (2005) are adopted. The macroinvertebrates identified at each sampling site are classified under the following classes: predators, scrapers, shredders, gathering collectors, filtering collectors and unknown (Merritt and Cummins, 2008; Yoshimura et al., 2006). In the case of taxonomic assessment, macroinvertebrates sampled are identified using Southern Africa freshwater guides (Arimoro, 2009; Gaigher, 2010; Harrison, 2009) and using the benthic macroinvertebrate key (Birmingham et al., 2005), thereafter the biotic index score was calculated. In this study, the taxonomic approach was utilised more.

5.2.2.3. *Macroinvertebrate Sensitivity Indication and Reference Set*

The anticipated lists of macroinvertebrate communities expected to occur at each sampling site were assembled using various datasets. Although no databases have been compiled for macroinvertebrates assemblages for the Copperbelt province in Zambia, reference lists from South Africa eco-regions were borrowed due to climatic similarities with Zambia (Bere and

Nyamupingidza, 2013; Gerber et al., 2002). The list of adapted species was compiled by aggregating species sampled physically at each site, in conjunction with species anticipated to occur there, based on historical data and presence of suitable habitat.

5.2.3. Statistical Analysis

A concatenation of statistical analysis was applied in order to illuminate the spatial changes in the macroinvertebrate community structures in the streams. Univariate statistical analyses incorporated the Shannon diversity index (H') and Simpson's index (D) of diversity, to assess the diversity of macroinvertebrates at each sampling site. The higher the values of H', the higher the diversity of reported species in the selected community. The lower the value of H', the lower the diversity. A value of H' = 0 indicates a community that has only one species. Owing to the difficulties in interpreting H' score due to the integration of different variables, the Simpson's index of diversity was included to complement the scores obtained from Shannon diversity index (Chiarucci et al., 2011). The value of the Simpson's index ranges between 0 and 1, the greater the value, the greater the sample diversity. This was calculated according to Equation 5-1 and 5-2 obtained from Shannon (H') (1948) and Simpson (D) (1949):

$$\mathrm{H}' = -\sum_{i=1}^{s} P_{\mathrm{i}} \ln P_{\mathrm{i}} \qquad (5\text{-}1)$$

$$\mathrm{D} = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) \qquad (5\text{-}2)$$

Where in the Shannon index, Σ is the sum of calculations, s is the number of species, P_i is the proportion (n/N) of number of individuals of a particular (n) divided by the total number of individual organisms found (N) and ln is the natural log.

In the calculation of Simpson index, n is the number of individuals of a particular species, N is the total number of individual organisms found and Σ is sum of individuals of each species (n) found.

Bivariate statistical analysis, using Pearson correlation coefficient was carried out using two separate variables in order to evaluate the linear correlation between the different variables. Multivariate statistical analyses were also used to evaluate the relationships between the identified water quality variables and macroinvertebrate community structures using a multitude of decision trees (Random Forest analysis). For this purpose, redundancy analysis (RDA) was used as a method to extract and summarise variation in a set of response variables (macroinvertebrate species data) to be explained by a set of explanatory variables (environmental variables) (Legendre and Anderson, 1999). The RDA summarizes the linear relations between multiple dependent variables and multiple independent variables. The values used were the best fit data estimated from multiple regressions between each response variable and a second matrix of environmental data. This permitted us to determine the relationship between macroinvertebrate community structures and environmental data. The Analysis of Variance (ANOVA) in combination with Fisher's LSD post-hoc test, was used to determine significance. The normality of the data was assessed by the Shapiro-Wilk W test, Kolmogorov-Smirnov test, and Lilliefors test, while the homogeneity of variance was evaluated by Levene's and Browm, and Forsythe's tests. The probability value less than or equal to 0.05 was considered significant (Wild and Seber, 1999).

5.3. Results

The spatial distribution of macroinvertebrates collected in Nselaki, Fikondo and Mululu streams are presented in Table 5-2 and Tables S5-1 to S5-6, respectively. Colour coding is used in Tables S5-1 to S5-6 to represent sensitivity categories. Particularly, blue represents sensitive macroinvertebrates, green semi-sensitive macroinvertebrates, brown semi-tolerant macroinvertebrates and red tolerant macroinvertebrates. During the study, a total of 562 macroinvertebrate species were collected in Mululu Stream, 450 in Nselaki Stream and 318 in Fikondo Stream. At each site, macroinvertebrates were collected, physical and chemical parameters were equally measured. Thereafter, the biotic index score for each site was calculated. Cumulatively, the biotic index scores from each site were used to rate the stream habitat conditions. This permitted comparison among the streams in relation to the major land use activities (mine waste) in the catchment.

5.3.1. Sensitivity Indications and Habitat Availability

In Nselaki Stream, a significant decrease in sensitivity of the macroinvertebrates at sites NS02 (Index score = 1.7) post rainy season and NS06 (Index score = 1.9) during rainy season, was observed compared to other sampling sites (Index score > 2) even though there was similarity in habitat condition to the rest of the sites (Table 5-3). The spatial distribution of macroinvertebrate community structures indicated that Nselaki Stream was mainly composed of semi-sensitive, semi-tolerant and tolerant species. Cumulatively, ≈46% of macroinvertebrate structure in Nselaki Stream were semi-sensitive species, ≈28% tolerant, ≈24.2% semi-tolerant and ≈1.8% sensitive. The macroinvertebrate functional evaluation highlighted a significant increase in semi-sensitive species and decrease to tolerant species at sites NS08 and NS09, compared to the rest of the sites. Based on the comparative analysis of macroinvertebrate assemblages, our observations suggested that there was no significant difference in habitat condition during the rainy and post rainy season (Table 5-3). *Talitridae* (Amphipod) and *Gnathobdellidae* (Leech) were observed to be the most dominant species in the stream (Table S5-1 and S5-2). Contrastingly, sensitive macroinvertebrates were reported in upstream sampling sites upstream, indicating good habitat conditions compared to downstream sites (Table 5-3).

Within Fikondo Stream, low sensitivity macroinvertebrate populations were observed within the sampling sites downstream (Table 5-3). The functional assessment of the macroinvertebrate in the stream showed that the community assemblages at various sites consisted mainly of semi-sensitive (≈44.4%), semi-tolerant (≈26.7%) and tolerant (≈28%) respectively (Table 5-2). A shift in macroinvertebrate community structure was observed at site FS06, through a decrease in the proportion of semi-sensitive species compared to the general trend in Nselaki Stream of increase in semi-sensitive species with distance from pollution source (Table 5-3). Other than the site peculiar changes observed above, the overall degree of sensitivity and habitat availability of macroinvertebrate assemblages were similar in Nselaki and Fikondo streams. All biotic index scores in Fikondo Stream lie below ≤ 2.5 (Table 5-3).

The spatial distribution of macroinvertebrate community structures in Mululu Stream did not vary significantly across all sampling sites. Over 50% of the macroinvertebrate populations observed within the stream comprised of semi-sensitive species (288 out 562 species collected) (Table 5-2). There was an increase in availability of sensitive macroinvertebrates from *Megaloptera* (Alderfly larva and Dobsonfly larva) and *Plecoptera* (Stonefly larva) families in Mululu Stream compared to Nselaki and Fikondo streams. The presence of sensitive macroinvertebrates is a useful indicator of good water quality conditions. The analysis of biotic indices indicated good water quality (Biotic index score > 2.5) post rainy season at sampling sites MS01 and MS04, when compared to the rest of the sites (Biotic index score = ≈2.3) (Table 5-3).

When comparing all the sites in Mululu Stream to the sites in Nselaki Stream and Fikondo Stream, species diversity was observed to be similar. The macroinvertebrate functional assessment in the streams indicated that the community structure at various sites mainly consisted of predators, gathering collectors and scrappers (Table 5-4, S5-1 to S5-6) that are semi-sensitive, semi-tolerant and tolerant species to pollution. The observed macroinvertebrate community structures suggest similarities habitat conditions conducive for non-sensitive species. Overall, the biotic index score for Mululu Stream (≈2.4) was higher compared to Nselaki Stream and Fikondo Stream (≈2.2). Biotic index scores showed water quality reductions from upstream to downstream except for Fikondo Stream. The biotic indices at the upstream reference sites on the Mululu Stream (≈2.7) and Nselaki Stream (≈2.6) are indicative of good water quality. Index scores indicated significant differences between upstream and downstream sites.

Table 5-2: Density and diversity of macroinvertebrates found in Mululu Stream, Nselaki Stream and Fikondo Stream

Stream	Macroinvertebrate Species	Sensitivity Group	Rainy season	Post rainy season	Tolerance to pollution	Individual Biotic Index Score
Mululu Stream	Alderfly larva	1	1	2	Sensitive	4
	Dobsonfly larva	1	2	0	Sensitive	4
	Stonefly larva	1	4	9	Sensitive	4
	Carddisfly Larva	2	6	6	Sem-sensitive	3
	Crane Fly Larva	2	5	4	Sem-sensitive	3
	Damselfly Larva	2	34	42	Sem-sensitive	3
	Dragonfly Larva	2	35	38	Sem-sensitive	3
	Mayfly Larva	2	30	32	Sem-sensitive	3
	Riffle Beetle	2	4	8	Sem-sensitive	3
	Water Penny	2	20	24	Sem-sensitive	3
	Amphipod	3	37	35	Semi-tolerant	2
	Black Fly Larva	3	28	25	Semi-tolerant	2
	Midge Larva	3	1	0	Semi-tolerant	2
	Isopod	4	24	25	Tolerant	1
	Leech	4	29	27	Tolerant	1
	Snails	4	9	14	Tolerant	1
	Tubifex Worm	4	2	0	Tolerant	1
	Total	42	271	291		

Stream	Macroinvertebrate Species	Sensitivity Group	Rainy season	Post rainy season	Tolerance to pollution	Individual Biotic Index Score
Nselaki Stream	Alderfly larva	1	0	2	Sensitive	4
	Stonefly larva	1	3	3	Sensitive	4
	Carddisfly Larva	2	4	4	Sem-sensitive	3
	Crane Fly Larva	2	1	4	Sem-sensitive	3
	Damselfly Larva	2	25	28	Sem-sensitive	3
	Dragonfly Larva	2	26	27	Sem-sensitive	3
	Mayfly Larva	2	17	16	Sem-sensitive	3
	Riffle Beetle	2	8	4	Sem-sensitive	3
	Water Penny	2	22	21	Sem-sensitive	3
	Amphipod	3	30	34	Semi-tolerant	2
	Black Fly Larva	3	20	25	Semi-tolerant	2
	Isopod	4	20	24	Tolerant	1
	Leech	4	30	27	Tolerant	1
	Snails	4	12	12	Tolerant	1
	Tubifex Worm	4	1	0	Tolerant	1
	Total	38	219	231		

Stream	Macroinvertebrate Species	Sensitivity Group	Rainy season	Post rainy season	Tolerance to pollution	Individual Biotic Index Score
Fikondo Stream	Stonefly larva	1	1	2	Sensitive	4
	Carddisfly Larva	2	0	2	Sem-sensitive	3
	Crane Fly Larva	2	1	0	Sem-sensitive	3
	Damselfly Larva	2	17	21	Sem-sensitive	3
	Dragonfly Larva	2	21	21	Sem-sensitive	3
	Mayfly Larva	2	16	15	Sem-sensitive	3
	Riffle Beetle	2	4	5	Sem-sensitive	3
	Water Penny	2	11	7	Sem-sensitive	3
	Amphipod	3	25	23	Semi-tolerant	2
	Black Fly Larva	3	20	17	Semi-tolerant	2
	Midge Larva	3	0	0	Semi-tolerant	2
	Isopod	4	19	17	Tolerant	1
	Leech	4	18	21	Tolerant	1
	Snails	4	6	8	Tolerant	1
	Total	36	159	159		

Table 5-3: A summary of the macroinvertebrate functional groups, the results for the habitat assessments for the macroinvertebrate community structures, as well as the total sensitivity scores obtained for the macro invertebrate assemblage in respect to stream composition

Stream	Sites	Macroinvertebrate functional traits rainy season						Macroinvertebrate functional traits post rainy season					
		Sensitive	Semi senstive	Semi tolerant	Tolerant	Index score	Stream condition	Sensitive	Semi senstive	Semi tolerant	Tolerant	Index score	Stream condition
Mululu Stream	MS01	1	10	5	3	2.3	Fair	1	13	4	3	2.7	Good
	MS02	0	9	6	5	2.2	Fair	0	14	5	3	2.5	Fair
	MS03	0	7	8	4	2.1	Fair	0	13	4	7	2.4	Fair
	MS04	0	10	3	6	2.5	Fair	1	11	5	4	2.6	Good
	MS05	0	9	5	2	2.4	Fair	1	10	6	5	2.4	Fair
	MS06	1	8	6	7	2.0	Poor	1	11	6	2	2.5	Fair
	MS07	1	12	5	3	2.5	Fair	0	11	7	5	2.3	Fair
	MS08	0	12	3	7	2.3	Fair	1	10	5	6	2.2	Fair
	MS09	1	8	7	5	2.3	Fair	0	16	6	7	2.4	Fair
	MS10	2	11	4	5	2.4	Fair	0	11	4	6	2.2	Fair
	MS11	0	14	6	5	2.4	Fair	1	13	3	7	2.4	Fair
	Control 1	3	15	4	4	2.8	Good	2	15	3	4	2.7	Good
	Control 2	2	13	6	6	2.6	Good	3	13	3	6	2.7	Good
Nselaki Stream	NS01	0	9	4	7	2.1	Fair	0	9	6	6	2.1	Fair
	NS02	0	7	2	9	2	Poor	0	5	4	8	1.7	Poor
	NS03	1	9	5	7	2.1	Fair	0	9	6	8	2	Poor
	NS04	0	7	7	7	2	Poor	1	7	7	6	2.1	Fair
	NS05	1	9	5	9	2	Poor	0	9	5	3	2.4	Fair
	NS06	0	7	6	7	1.9	Poor	1	7	5	7	2	Poor
	NS07	0	9	5	4	2.5	Fair	0	12	8	4	2.4	Fair
	NS08	0	9	5	4	2.3	Fair	0	10	4	7	2.2	Fair
	NS09	1	11	3	5	2.4	Fair	0	12	4	4	2.6	Good
	Control 1	1	15	6	4	2.6	Good	1	16	5	6	2.7	Good
	Control 2	0	14	4	3	2.7	Good	2	13	5	4	2.7	Good
Fikondo Stream	FS01	0	11	5	4	2.4	Fair	0	8	7	6	2.1	Fair
	FS02	0	8	5	3	2.2	Fair	0	7	5	4	2	Poor
	FS03	0	10	7	6	2.2	Fair	0	11	6	6	2.2	Fair
	FS04	0	7	5	4	2.1	Fair	0	11	6	7	2.2	Fair
	FS05	0	9	5	5	2.2	Fair	1	11	4	7	2.3	Fair
	FS06	0	8	6	6	2.1	Fair	1	8	3	9	2.0	Poor
	Control 1	1	10	5	7	2.4	Fair	0	9	4	3	2.2	Fair
	Control 2	0	10	5	7	2.2	Fair	0	10	4	5	2.2	Fair

Table 5-4: Classification of macroinvertebrate species based on morphological and behaviour characteristics (Merritt and Cummins, 2008)

Family	Species	Functional Feeding Group
Megaloptera	Alderfly larva	Predators
Megaloptera	Dobsonfly larva	Predators
Plecoptera	Stonefly larva	Collectors/Gatherers
Trichoptera	Carddisfly Larva	Shredders/
Tipulidae	Crane Fly Larva	shredders
Odanata	Damselfly Larva	Predators
Odanata	Dragonfly Larva	Predators
Ephemeroptera	Mayfly Larva	Scrapers
Coleoptera	Riffle Beetle	Predators
Psephenidae	Water Penny	Scrapers
Talitridae	Amphipod	Feeders/Collectors
Simuliidae	Black Fly Larva	Collectors/Gatherers
Chironomidae	Midge Larva	Collectors/Gatherers
Asellidae	Isopod	shreders
Gnathobdellidae	Leech	Predators
Gastropoda	Snails	Scrapers
Tubificidae	Tubifex Worm	Scrapers/Collectors

5.3.2. Univariate Analysis on Macroinvertebrate Richness and Diversity Variation

The distribution of habitat condition according to response to environmental stressors in the selected streams are presented in Table 5-2. Macroinvertebrate structures observed in Nselaki Stream at downstream sites were compared to Fikondo Stream and Mululu Stream. The comparative analysis of the macroinvertebrate community structures in the streams showed that *Odanata* (Damselfly larva and Dragonfly larva*), Simuliidae* (Blackfly larva)*, Talitridae* (Amphipod)*, Gnathobdellidae* (Leech), and *Asellidae* (Isopod) families are the most adaptive species to the aquatic environment in the streams (Figure 5-2) (Table S5-1 to S5-6). These were observed to be more dominant at each sampling site. There were no significant variations observed in community structures downstream in all the sites, except for NS09 in Nselaki Stream, MS09 and MS10 in Mululu Stream, where a slight increase in sensitive species was observed (Table 5-3).

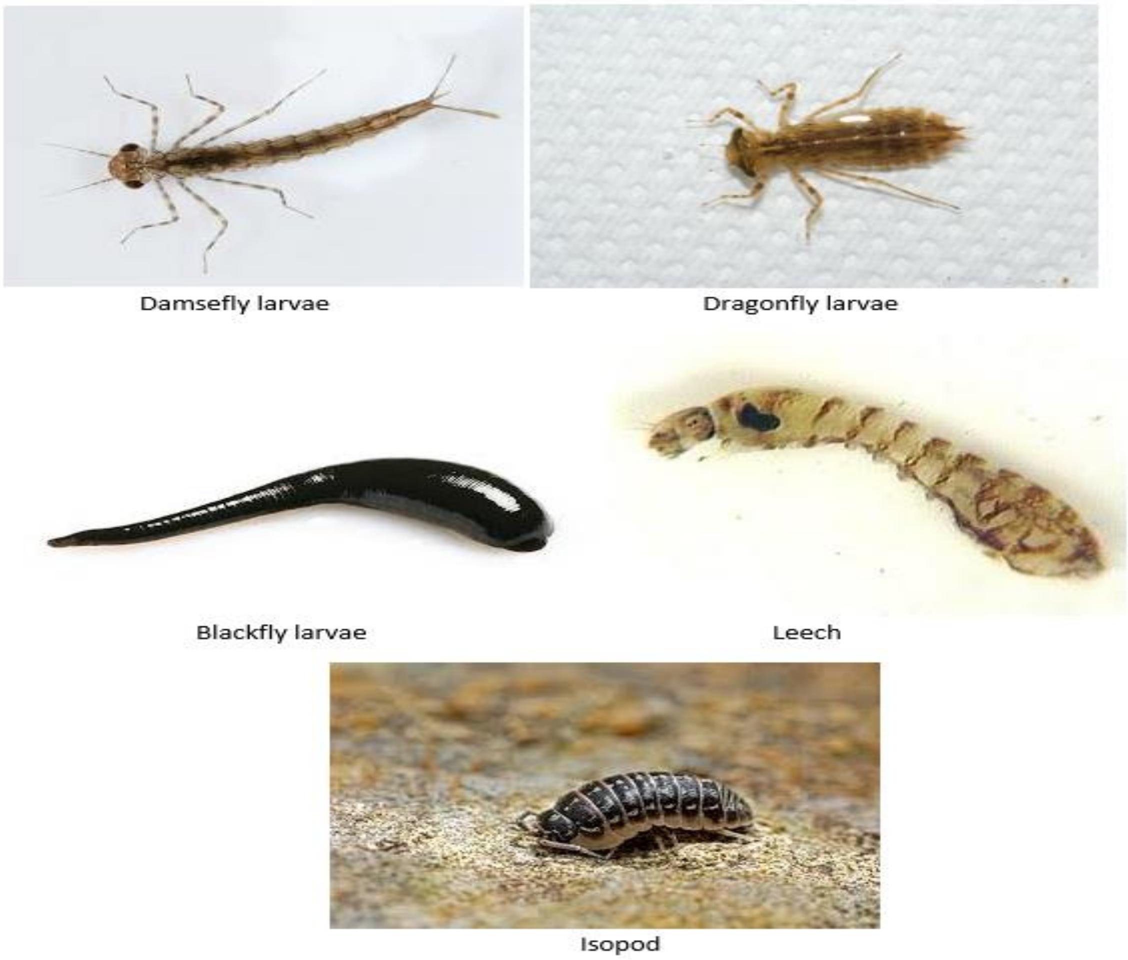


Figure 5-2: The most dominant macroinvertebrate species reported in Nselaki, Fikondo and Mululu streams

No significant trends were observed with regards to diversity and richness of macroinvertebrates community structures at the different sites in Nselaki Stream and Fikondo Streams, given in terms of the Shannon diversity index (H') and Simpson's index (D) of diversity. The highest diversity index in Nselaki Stream was observed at sampling sites NS06, NS08 and NS09 (H' = ≈ 2.45 and D = ≈0.7, respectively) (Figure 5-3), whilst in Fikondo Stream at sampling sites FS04 and FS06 (H' = ≈2.3 and D = ≈0.68, respectively) (Figure 5-4). The richness and diversity trend of macroinvertebrate communities in Mululu Stream generally followed a slight increase downstream from MS04 to MS11. Sites MS09 and MS11 were found to have the highest macroinvertebrate diversity and richness (H' = ≈2.57 and D = ≈0.75 respectively) (Figure 5-5), with the lowest values recorded at MS04 and MS05 (H' = ≈2.18 and D = ≈0.9). Based on the comparative analysis of the diversity and richness values, Mululu Stream had relatively higher values than those in Nselaki Stream and Fikondo Stream. This trend was similar to the biotic index score observed in Table 5-2. Overall, the diversity and richness values seen in the streams were high (≈0.65), with relatively less species that are sensitive to pollution.

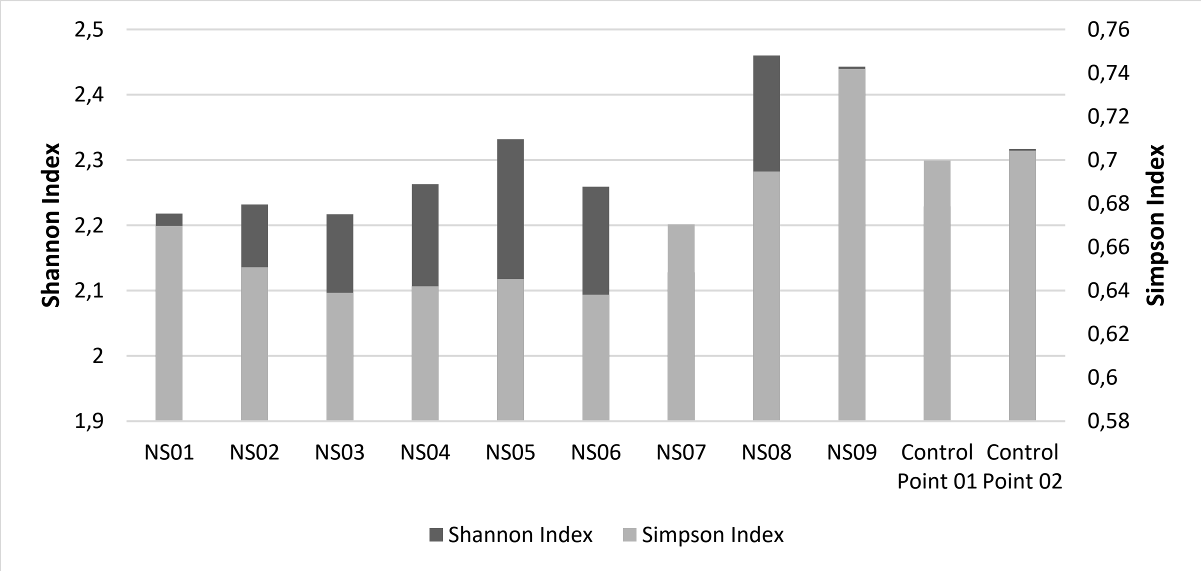


Figure 5-3: The Shannon diversity (H') and Simpson index of diversity (D) results for macroinvertebrates in Nselaki Stream

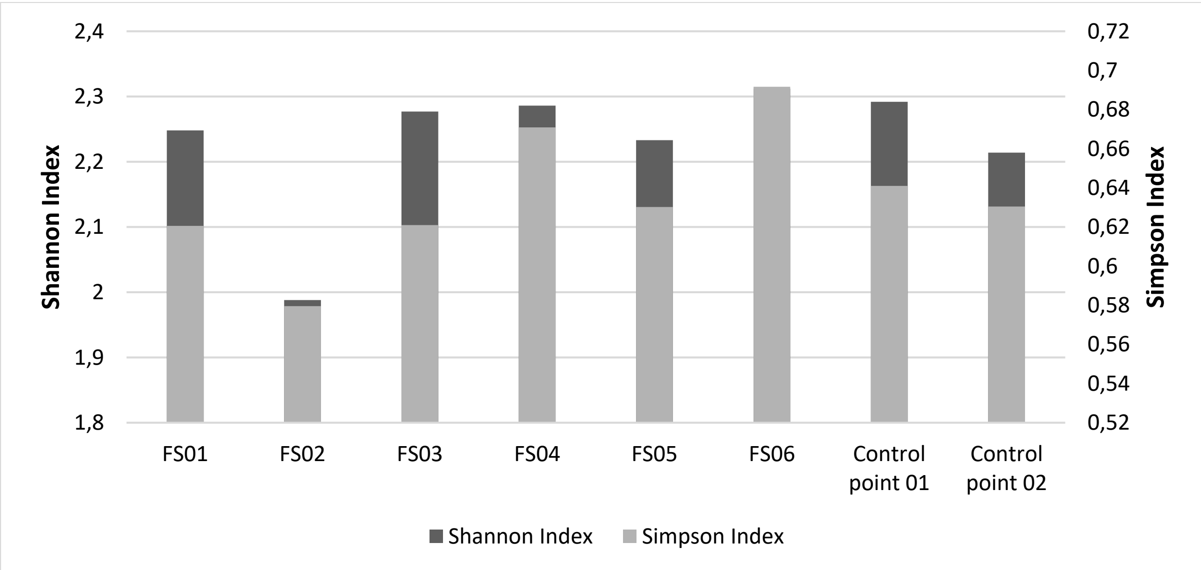


Figure 5-4: The Shannon diversity (H') and Simpson index of diversity (D) results for macroinvertebrates in Fikondo Stream

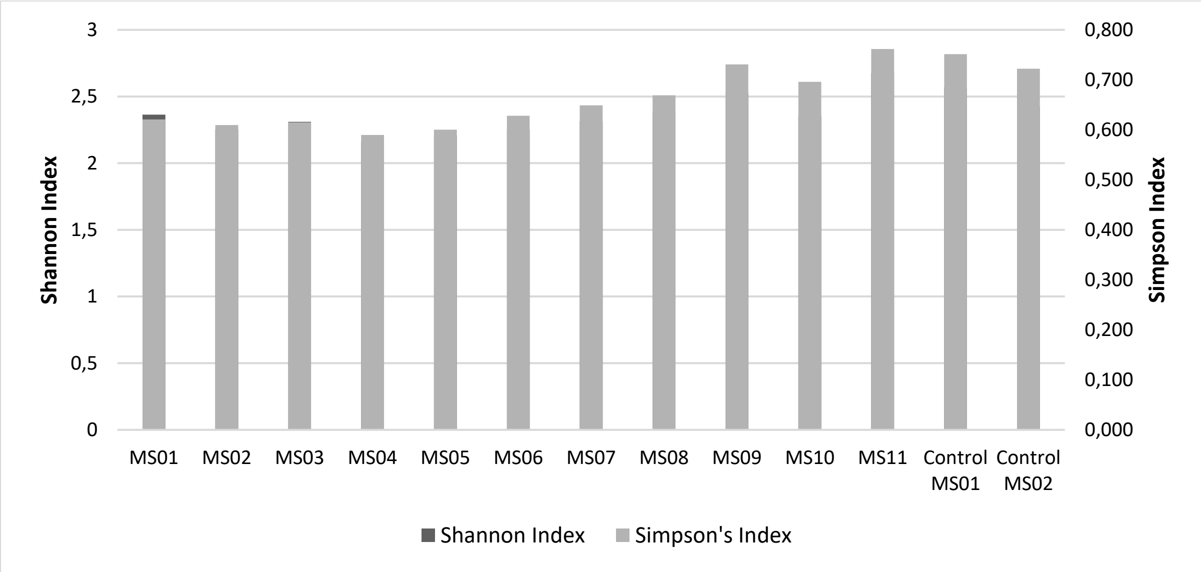


Figure 5-5: The Shannon diversity (H') and Simpson index of diversity (D) results for macroinvertebrates in Nselaki Stream

5.3.3. Bivariate Analysis

5.3.3.1. *Water Quality*

Most of the significant correlations in Nselaki Stream were either with pH or DO, as each of these variables showed notable correlation with four of the ten other tested variables (Figure 5-6 and Table 5-5). The physical and chemical characteristics are drawn from chapter 4. pH had a strong positive correlation with DO (r = 0.72) and Pb (r = 0.51,) and negative correlation with Co (r = -0.50) and temperature (r = -0.56). A strong positive correlation was also observed between DO, Pb and turbidity (r > ≈ 0.53), while a negative between DO and Cu (r = -0.61). Copper concentration was observed to be negatively correlated with Zn and Pb (r > ≈-0.62), while Co and turbidity were observed to be negatively correlated (r = -0.53). The relationship between Mn and TDS was equally observed to be negatively correlated (r = -0.52).

In Fikondo Stream, turbidity and Cu (Figure 5-7 and Table 5-6) were observed to be the most significant correlated variables, as both of these variables had significant correlation with four of the ten other variables tested. The pH had a weak correlation with most of the variables, although a moderately positive correlation was observed with Mn (r = 0.42). Very strong positive correlation was observed between Cu and Co (r = 0.92), Cu and TDS (r = 0.70) and turbidity and DO (r = 0.70). Cobalt, Pb, DO and TDS correlated at least with two other variables, respectively.

Temperature, Mn, and Zn were observed to be the pivotal variables in Mululu Stream as they showed significant correlation with other variables tested, each of these variables had significant correlation with less than three of the ten tested variables (Figure 5-7 and Table 5-8). Most of the significant correlation were observed to be positive except for correlation between turbidity and temperature which was negative (r = -0.55).

5.3.3.2. *Macroinvertebrate Diversity*

Macroinvertebrate community diversity (H') in Nselaki Stream (Figure 5-6 and Table 5-5), was observed to have a moderate to strong positive correlation with Pb (r = 0.50) and Zn (r = 0.62) respectively, whilst a strong negative correlation was observed with Cu (r = -0.58). pH and TDS were found to be weakly correlated with H' (r = 0.06 and -0.02, respectively).

In Fikondo Stream, strong significant positive correlations were found with Pb (r = 0.63) and temperature (r = 0.59), while correlation with TDS (r = -0.71) was strongly negatively correlated (Figure 5-7 and Table 5-6). Relatively moderate positive and negative correlation with pH (r = 0.38), DO (r = -0.39), Co (r = -0.33) and Zn (r = -0.38), respectively.

The macroinvertebrate community (H') in Mululu Stream (Figure 5-8 and Table 5-7) had relatively moderate positive correlations with pH (r = 0.36) and DO (r = 0.42). On the other hand, positive and negative significant correlation of a weak nature were observed with temperature (r = 0.18), Zn (r = 0.17), Cu (r = -0.24), Co (r = -0.15), Mn (r = -0.05), Pb (r = -0.17) and albeit non-significant correlation with TDS (r = 0.00).

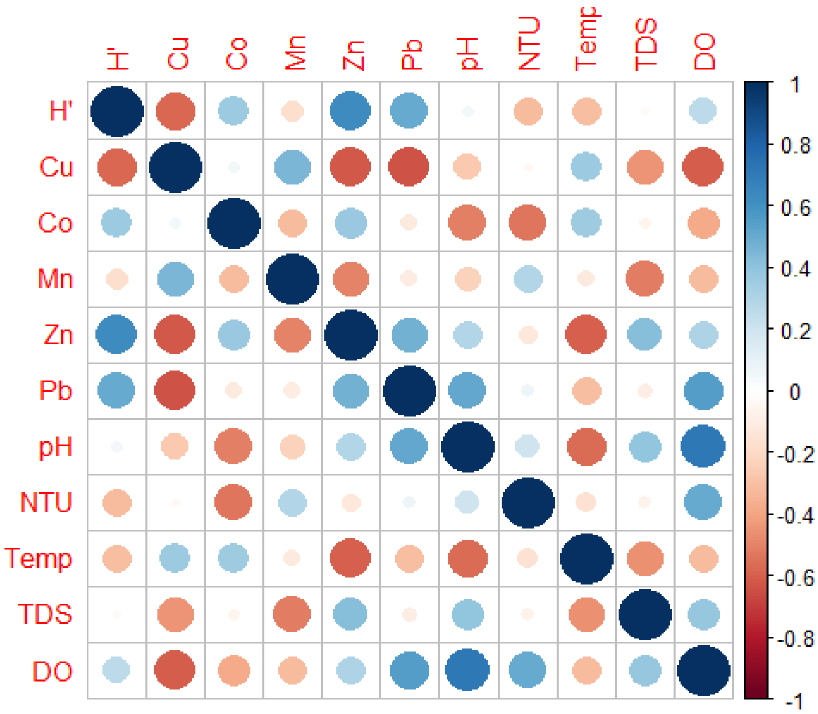


Figure 5-6: The correlations between the macroinvertebrate communities (using H' values) with the environmental variables selected in Nselaki Stream. The significant correlations are indicated by large circles, while the colour shows the differences in the type of correlation

Table 5-5: Correlation of macroinvertebrate community structure using H' with the selected environmental variables in Nselaki Stream

	H'	**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Turbidity**	**Temp**	**TDS**	**DO**
H'	1	-0.58	0.36	-0.18	0.62	0.50	0.06	-0.32	-0.31	-0.02	0.26
Cu	-0.58	1	0.06	0.46	-0.62	-0.63	-0.26	-0.03	0.36	-0.45	-0.61
Co	0.36	0.06	1	-0.31	0.37	-0.11	-0.50	-0.53	0.35	-0.06	-0.38
Mn	-0.18	0.46	-0.31	1	-0.49	-0.11	-0.23	0.30	-0.11	-0.52	-0.32
Zn	0.62	-0.62	0.37	-0.49	1	0.47	0.29	-0.13	-0.59	0.42	0.31
Pb	0.50	-0.63	-0.11	-0.11	0.47	1	0.51	0.07	-0.30	-0.10	0.55
pH	0.06	-0.26	-0.50	-0.23	0.29	0.51	1	0.21	-0.56	0.39	0.72
Turbidity	-0.32	-0.03	-0.53	0.30	-0.13	0.07	0.21	1	-0.16	-0.07	0.50
Temp	-0.31	0.36	0.35	-0.11	-0.59	-0.30	-0.56	-0.16	1	-0.4	-0.32
TDS	-0.02	-0.45	-0.06	-0.52	0.42	-0.10	0.39	-0.07	-0.45	1	0.39
DO	0.26	-0.61	-0.38	-0.32	0.31	0.56	0.72	0.50	-0.32	0.39	1

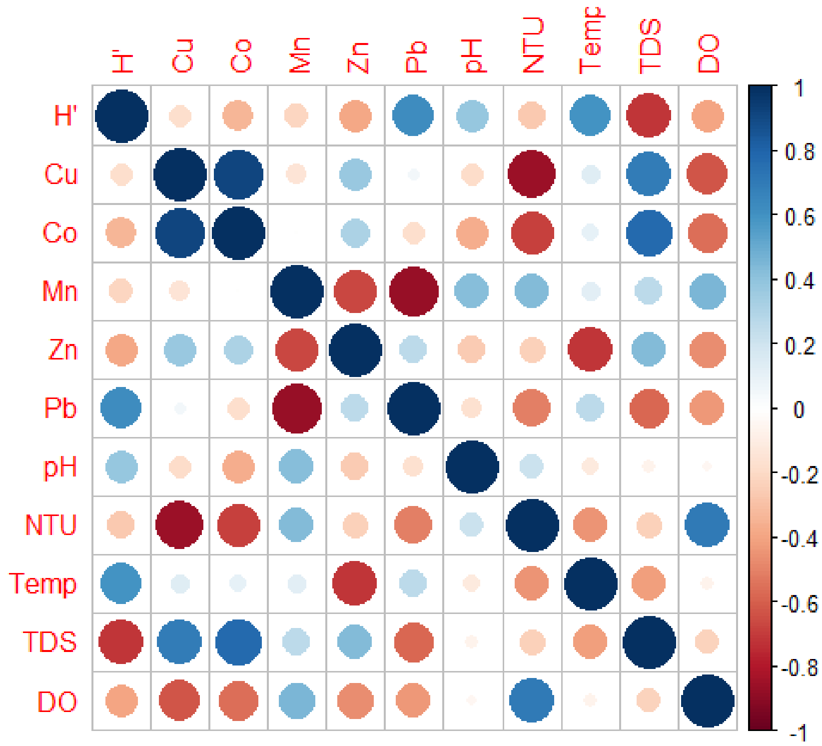


Figure 5-7: The correlations between the macroinvertebrate communities (using H' values) with the environmental variables selected in Fikondo Stream. The significant correlations are indicated by large circles, while the colour shows the differences in type of correlation

Table 5-6: Correlation of macroinvertebrate community structure using H' with the selected environmental variables in Fikondo Stream

	H'	Cu	Co	Mn	Zn	Pb	pH	Turbidity	Temp	TDS	DO
H'	1	-0.18	-0.33	-0.21	-0.38	0.63	0.38	-0.26	0.59	-0.71	-0.39
Cu	-0.18	1	0.92	-0.15	0.37	0.05	-0.19	-0.86	0.14	0.70	-0.63
Co	-0.33	0.92	1	-0.00	0.32	-0.18	-0.37	-0.69	0.11	0.78	-0.56
Mn	-0.21	-0.15	-0.00	1	-0.66	-0.87	0.42	0.43	0.13	0.27	0.46
Zn	-0.38	0.37	0.32	-0.66	1	0.26	-0.26	-0.24	-0.71	0.44	-0.47
Pb	0.63	0.05	-0.18	-0.87	0.26	1	-0.16	-0.51	0.27	-0.57	-0.45
pH	0.38	-0.19	-0.37	0.42	-0.26	-0.16	1	0.21	-0.11	-0.07	-0.05
Turbidity	-0.26	-0.86	-0.69	0.43	-0.24	-0.51	0.21	1	-0.44	-0.24	0.70
Temp	0.59	0.14	0.11	0.13	-0.71	0.27	-0.11	-0.44	1	-0.41	-0.07
TDS	-0.71	0.70	0.78	0.27	0.44	-0.57	-0.07	-0.24	-0.41	1	-0.22
DO	-0.39	-0.63	-0.56	0.46	-0.47	-0.44	-0.05	0.70	-0.07	-0.22	1

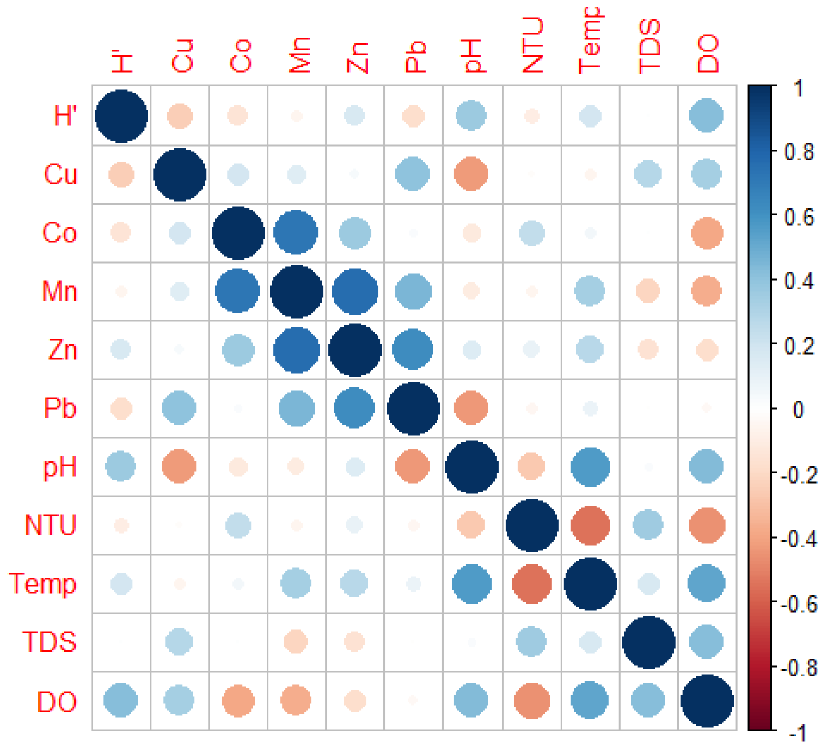


Figure 5-8: The correlations between the macroinvertebrate communities (using H' values) with the environmental variables selected in Mululu Stream. The significant correlations are indicated by large circles, while the colour shows the differences in type of correlation

Table 5-7: Correlation of macroinvertebrate community structure using H' with the selected environmental variables in Mululu Stream

	H'	Cu	Co	Mn	Zn	Pb	pH	NTU	Temp	TDS	DO
H'	1	-0.24	-0.15	-0.05	0.17	-0.17	0.36	-0.09	0.18	0.00	0.42
Cu	-0.24	1	0.19	0.14	0.04	0.41	-0.43	-0.01	-0.05	0.29	0.33
Co	-0.15	0.19	1	0.73	0.37	0.03	-0.12	0.25	0.05	0.00	-0.38
Mn	-0.05	0.14	0.73	1	0.76	0.45	-0.11	-0.06	0.33	-0.22	-0.36
Zn	0.17	0.04	0.37	0.76	1	0.63	0.14	0.10	0.27	-0.16	-0.17
Pb	-0.17	0.41	0.03	0.45	0.63	1	-0.43	-0.05	0.09	-0.01	-0.03
pH	0.36	-0.43	-0.12	-0.11	0.14	-0.43	1	-0.27	0.57	0.03	0.43
Turbidity	-0.09	-0.01	0.25	-0.06	0.10	-0.05	-0.27	1	-0.55	0.36	-0.45
Temp	0.18	-0.05	0.05	0.33	0.27	0.09	0.57	-0.55	1	0.17	0.53
TDS	0.00	0.29	0.00	-0.22	-0.16	-0.01	0.03	0.36	0.17	1	0.43
DO	0.42	0.33	-0.38	-0.36	-0.17	-0.03	0.43	-0.45	0.53	0.43	1

5.3.4. Multivariate Analysis

The redundancy analysis (RDA) was used as a method to extract and summarise the relationship between the macroinvertebrate community structures and water quality variables (physical properties and chemical properties in the sediments i.e., metal concentrations). The blue arrows represent the abiotic variables in relation to sampling points and macroinvertebrate species. This was helpful in evaluating particular abiotic variables influencing macroinvertebrates species at selected sites.

Within Nselaki Stream, it was observed that sites NS03, NS05 and NS009 were similar based on the taxonomic assemblage of the macroinvertebrates and physiochemical signature of water quality variables observed at these sites (Figure 5-9 and Table 5-3). The RDA analysis showed that pH, TDS, Cu, Zn, Pb and Co influenced the water and sediment quality signature at these sites. Observably, sensitive macroinvertebrates such as Stonefly Larva (*Plecoptera*) and Alderfly Larva (*Megaloptera*) were found on these sites. Contrastingly, site NS02 was observed to be different from other sites, with turbidity and DO significantly influencing the site. Equally a high elevation of Cu (≈3113 ppm), Zn (≈202 ppm) and Pb (≈46.8 ppm) was observed in the sediments. Macroinvertebrates tolerant to pollution such as *Gastropoda* (pouch snail), *Gnathobdellidae* (leech), *Asellidae* (isopod or aquatic sowbug) and *Talitridae* (amphipod) characterised the site. The RDA showed that the rest of the sites in Nselaki Stream grouped together.

The RDA analysis for Fikondo Stream grouped sites FS01, FS03, FS04 and FS05 together (Figure 5-10) based on the overlying water quality conditions and composition of the macroinvertebrate community structures. The sites were mainly dominated by macroinvertebrate species semi-tolerant and semi-sensitive to pollution such as *Talitridae* (amphipod or scud), *Simuliidae* (blackfly larva) and *Odanata* (damselfly larva and dragonfly larva). Site FS02 and FS06 were dissimilar from the other sites with FS02 having a relative low diversity of macroinvertebrates (Figure 5-7) and poor water quality conditions (Table 5-3) compared to other sites. The site was also characterised by less sensitive macroinvertebrates *Talitridae* (amphipod) and *Simuliidae* (blackfly larva).

In Mululu Stream, the RDA analysis reported a similar grouping based on macroinvertebrate assemblages and overlying water quality, during (d) and post (p) rainy season (Figure 5-11). It was observed that sites MS03, MS08 and MS10 were found to be dissimilar from the other sites, with MS08 substantially influenced by DO and TDS increase. The macroinvertebrate species found dominant at these sites include *Simuliidae* (blackfly larva), Odanata (damselfly Larva and dragonfly Larva) and *Gnathobdellidae* (leech). The most diversity of macroinvertebrate community structures was observed with MS01, MS08 and MS11, while

MS04 reported the lowest diversity (Figure 5-8). Site MS04 is one of the localities where effluents from industrial activities was noted.

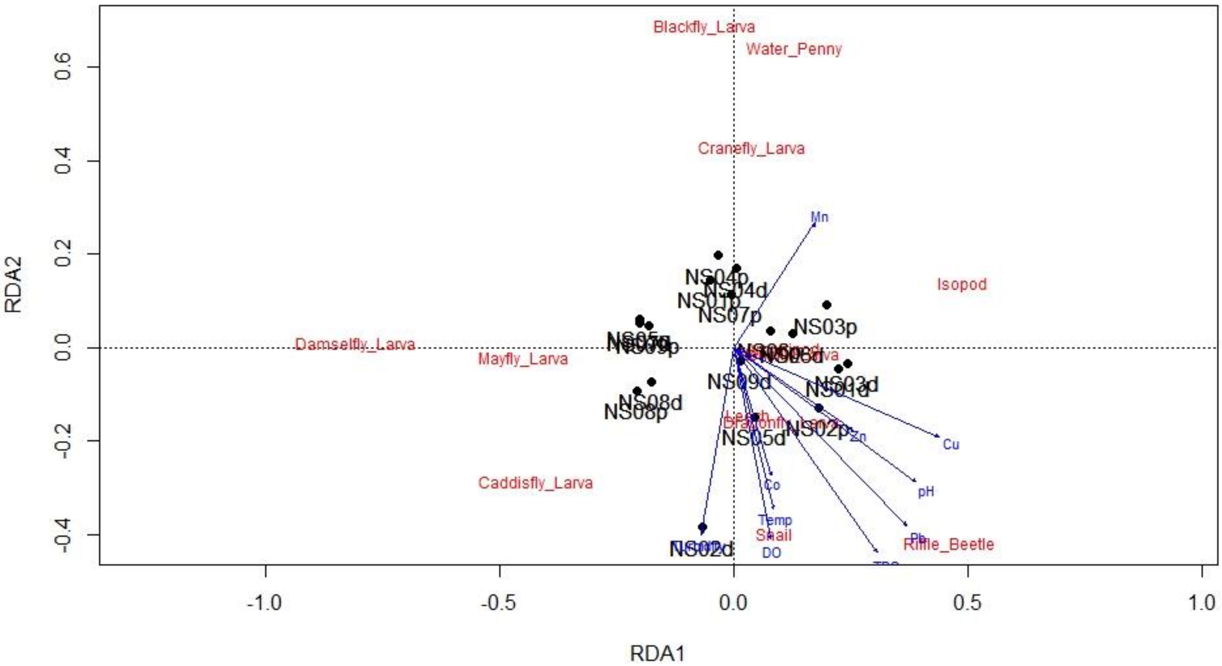


Figure 5-9: The RDA analysis for Nselaki Stream using macroinvertebrates species as the response variable, whilst overlain with selected water quality variables as explanatory variables. The blue arrows represent abiotic variables and red labels represent macroinvertebrate species.

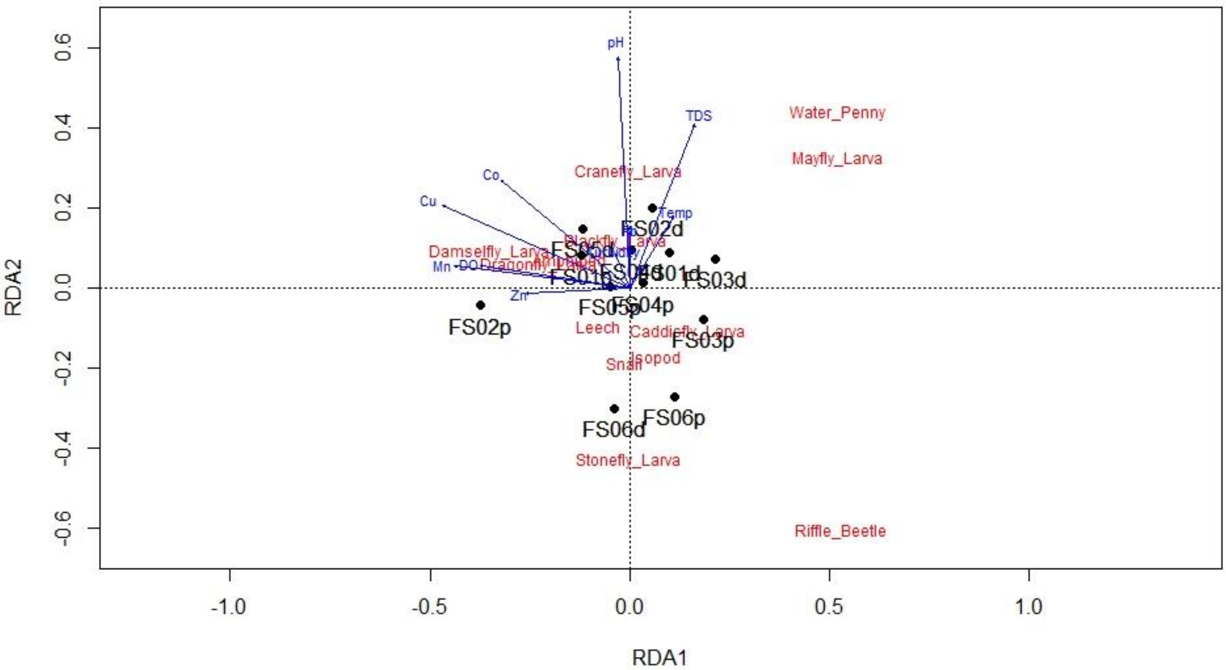


Figure 5-10: The RDA analysis for Fikondo Stream using macroinvertebrates species as the response variable, whilst overlain with selected water quality variables as explanatory

variables. The blue arrows represent abiotic variables and red labels represent macroinvertebrate species.

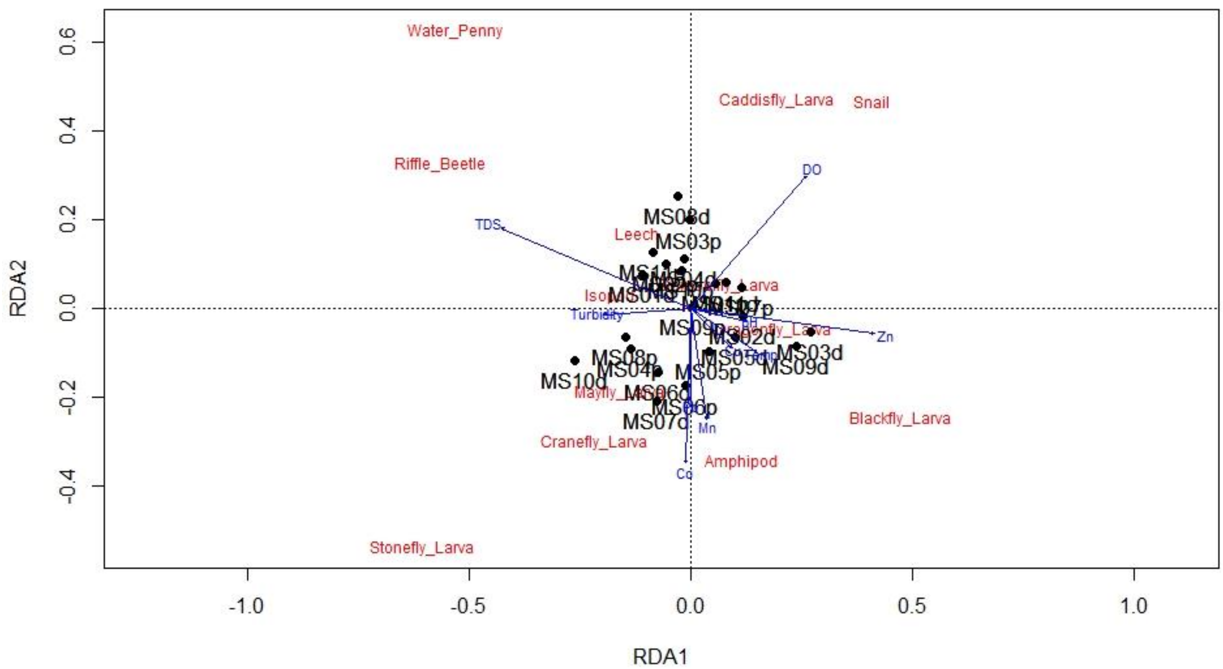


Figure 5-11: The RDA analysis for Mululu Stream using macroinvertebrates species as the response variable, whilst overlain with selected water quality variables as explanatory variables. The blue arrows represent abiotic variables and red labels represent macroinvertebrate species.

5.4. Discussion

5.4.1. Sensitivity Indicators and Habitat Availability

The analyses of macroinvertebrate community structures at selected sampling points across Nselaki Stream, Fikondo Stream and Mululu Stream indicated that there were no significant differences during and post rainy season between the sampling sites. A similar pattern of macroinvertebrate taxon was observed to dominate the sites. Significant differences were observed between the streams, with a high diversity of macroinvertebrate reported in Mululu Stream compared to the other streams. This could be attributed to the relative influence of TSFs on the streams. As noted in chapter 4, TSF14 near Mululu Stream was observed to have a good vegetation cover, which could be contributing to reduced contamination of Mululu Stream. On the other hand, the TSFs near Nselaki and Fikondo streams did not have a good vegetation cover, as a result, metal migration from TSFs is more likely, resulting in increased turbidity, TDS, metal concentration and sediment resuspension (Gleekia, 2016; Valipour et al., 2017). The relative low diversity of macroinvertebrate community structures observed in these streams could be attributed to this.

5.4.2. Nselaki Stream

The macroinvertebrate community structures in Nselaki Stream were observed to consist of semi-sensitive, semi-tolerant and tolerant species, particularly species from *Talitridae* (amphipod) and *Gnathobdellidae* (leech) families were the most dominant. These assemblages suggest changes in macroinvertebrate diversity associated with habitat conditions. The biotic index score showed that upstream, the water quality conditions were good, this supported by the presence of sensitive macroinvertebrates (Table 5-3). Downstream water quality had been compromised.

Significant correlations observed between the physical and chemical properties such as pH, DO, turbidity, Cu, Co, Mn, Zn and Pb, highlight their important role in Nselaki Stream. The observed decline in sensitive macroinvertebrate taxa diversity at selected sites like NS02 could be attributed to this. Notably, wastewater from underground operations and emergency tailing ponds is discharged into Nselaki Stream at this site. Consequently, water quality variables such as turbidity and DO were observed to be high at site NS02. It is widely acknowledged that DO availability influences the assemblages of aquatic communities as it essentially affects the species abundance and distribution (Amusan *et al*., 2018; Croijmans *et al*., 2021). Although aquatic macroinvertebrates have different response patterns to oxygen tolerance and requirements, some invertebrates such as *Ephemeroptera* (mayflies) are sensitive to low DO (Verberk et al., 2016). Similarly, the observed high levels of turbidity and TDS might result in a reduction in sensitive macroinvertebrate assemblage, thus lead to pre-eminence of tolerant taxa (Olson and Hawkins, 2017; Van de Meutter et al., 2006). Therefore, the changes in macroinvertebrate community structures suggests being related mostly to water quality conditions.

Although a relatively high richness / diversity was reported across the sampling sites in Nselaki Stream, these macroinvertebrate communities might be still affected by TSF in the catchment. The high metal concentrations in the stream sediments (chapter 4) downstream suggests continued presence of contaminants in the stream system. Studies by Jhariya et al. (2016) and Karaca et al. (2018) have shown that metal mobilization from mine waste remains a source of concern. Similarly, results from this study suggests that metal migration from the TSF has significant influence on downstream sampling sites in Nselaki Stream. Even though mobilization of metals from the TSF could be low, over time, prolonged exposure to low metal contamination could result in changes in composition of aquatic community structures (Qu et al., 2010). Either way, the impacts observed at NS02 and NS08 through the increase in metal concentration (Cu, Zn and Pb) in water and sediments could result in changes in macroinvertebrate diversity and appear to be correlated, which is an indication of the impact of TSF. Studies by Beghelli et al. (2016) and Marques et al. (2003) have reported a de-structuring of the community taxa in water resources impacted by Cu, Zn and Pb contamination, the evolution of these disturbances resulted in the decline in sensitive macroinvertebrate taxa such as *Athericidae, Baetidae, Ephemridae* and *Leptophlebiidae* and increase in tolerance taxa. Although no historical data was present to compare changes in macroinvertebrate assemblages downstream, the presence of sensitive species upstream and their absence downstream, coupled with dominance of tolerant species, suggests that metal migration from TSFs could be reason for this change in assemblages.

5.4.3. Fikondo Stream

Similarly, to Nselaki Stream, Cu and turbidity were observed to be significantly correlated with other physical and chemical variables in Fikondo Stream. Particularly, strong positive correlation existed between Cu and TDS, Cu and Co, and turbidity and DO. This may be an indication of anthropogenic impacts of copper mining in the stream. The decline in the number of macroinvertebrate species reported at site FS02, together with the high TDS, Cu and Mn, accentuate this association, however, no clear trends regarding changes in community structures along the stream could be observed. An increase in dominance of macroinvertebrate taxa which is known to tolerant to poor water quality, may result in changes in type of macroinvertebrate species (Kahlon and Julka, 2018). This is because the first changes observed in macroinvertebrate community assemblages mainly relate to species replacing one another other than reduction in diversity richness (Baumgartner and Robinson, 2017; Clemente et al., 2010). It is noteworthy that elevated TDS seldom occur independent of other environmental stressors in streams. Possible TDS covariates in streams may include, low pH, high metal concentration, sedimentation, degradation of stream and riparian habitat, modification of hydrology etc. Studies have shown that elevated TDS are toxic to macroinvertebrates (Olson and Hawkins, 2017; Timpano et al., 2010).

Comparatively, it was observed that the macroinvertebrate population sensitivities in Nselaki Stream and Fikondo Stream were similar, however, Nselaki Stream did have higher macroinvertebrate diversities than Fikondo Stream. Equally, the diversity in the upstream sampling points of Fikondo Stream was not different with the downstream trends, suggesting impacts from other land use activities. Particularly, the observed agriculture activities through the application of nutrients (fertilizers) and addition of pesticides on the fields, could be influencing the macroinvertebrate community structures and may result in impacts that may only be detected in indistinct benign stress pathways on a persistent scale instead of acute scale.

5.4.4. Mululu Stream

In contrast to Nselaki Stream and Fikondo Stream, Mululu Stream was characterised by relatively higher richness and diversity. The Shannon Diversity index (*H'*) indicated considerable taxonomic richness. Equally the biotic index score showed that on average, water quality in the stream was conducive for sensitive macroinvertebrates to survive, as it was less polluted. *Odanata* (Dragonfly larva and Damselfly larva) and *Ephemeroptera* (mayfly larva) were the most dominant macroinvertebrate families in Mululu Stream. Studies by Jomoc et al., (2013) have shown that macroinvertebrate species like Odonata are more likely to survive in sites with less disturbed waters. The presence of other sensitive species like *Ephemeroptera* (mayflies), *Trichoptera* (caddisflies) and *Plecoptera* (stoneflies) supports the narrative of less disturbed waters (Villantes, 2015). An increasing trend in diversity following a downstream gradient was observed, although a decline at MS09 and MS10 was noted. This may be linked to vehicle car wash operations taking place near these sites which may affect macroinvertebrate assemblages.

Generally, the sensitivity of macroinvertebrate taxa in Mululu Stream was considerably higher than the sensitivity of species populations in Nselaki Stream and Fikondo Stream. The macroinvertebrate fauna appears to be less affected by habitat conditions downstream than in Nselaki Stream and Fikondo Stream. This could be attributed to the good vegetation observed on TSF14. Vegetation growth plays a significant role in reduction of metal mobilization and erosion, thus reducing impacts on the surrounding water resources and land (Peco et al., 2021). It is noteworthy that although a high diversity of sensitive macroinvertebrates was observed in Mululu Stream, metal concentration in sediments was relatively high and comparable to Nselaki and Fikondo streams. This could be linked to other current land use activities other than TSF14 associated with the upper reaches compared to lower Mululu Stream reaches. Particularly, the presence of agriculture and vehicle car wash activities near the banks of the streams is another source of possible source of water contamination. One of the common effects of agriculture activities in stream ecosystems is the change and reduction of macroinvertebrate assemblages less tolerant to water quality modifications and increase in more tolerant taxa (Mamert et al., 2021; Meza-Salazar et al., 2020). This is an important consideration in the management of Nselaki Stream and Fikondo Stream. However, arguably, the macroinvertebrate community structures, and composition were more influenced by mine wastelands than any other land use activity and seasonal variations.

5.5. Conclusion

Ecological monitoring of surface water resources has culminated into an increase in understanding of the impacts of land use activities on the aquatic ecosystem. In this study, several techniques and statistical methods were exploited to characterize macroinvertebrate community structures in Nselaki, Fikondo and Mululu streams. Consequently, several lines and levels of evidence were produced for comparative assessment of the catchments and enhanced understanding of copper mine wasteland impacts on the biological integrity. The information generated might help in understanding the current health conditions of the streams as well as understanding which particular endpoints to monitor and designing suitable mitigation measures. The macroinvertebrate community structures in the streams were more diversified in Mululu Stream (H' = ≈2.4) than in Nselaki and Fikondo streams (H' = ≈2.2). Overall, the streams were dominated by macroinvertebrate taxa that is tolerant to pollution. Some of the selected physiochemical parameters such as TDS, turbidity, Cu, Pb, and Zn showed significant correlation with macroinvertebrate community structures. It is quite evident that from these results that influence of mine wastelands and associated pollutants in the streams was significant. As a result, the contamination of the streams by the mine wastelands needs to be minimised, particularly the mobilization of metals, in order to preserve the biotic integrity. Observably, Nselaki and Fikondo streams are likely to be more susceptible than Mululu Stream owing to a low vegetation cover on the mine wastelands. This may result in alteration of aquatic community structures, such as increase in tolerant species already observed at NS04, NS06 and FS02 downstream sampling points. On the other hand, good vegetation cover on the TSFs may help to reduce migration of contaminants on to the ambient environment.

The combined use of biotic and abiotic monitoring attested the value of providing more information with regards to environmental impacts that could be useful in subsequent mitigation measures. It is therefore recommended to include biomonitoring using macroinvertebrate community approach in monitoring habitat conditions and stream health. Owing to the uniqueness of this study, given that these streams face similar environmental challenges, the results generated in this study may contribute to current and future management practices of water resources threatened by copper mine wastelands. Consequently, the biotic and ecological integrity of surface water resources in upcoming copper mining regions in Zambia may be conserved, lest they share the same fate as Nselaki, Fikondo and Mululu streams.

5.6. References

Aazami, J., Esmaili-Sari, A., Abdoli, A., Sohrabi, H., Van den Brink, P.J., 2015. Monitoring and assessment of water health quality in the Tajan River, Iran using physicochemical, fish and macroinvertebrates indices. Journal of Environmental Health Science and Engineering 13, 29. https://doi.org/10.1186/s40201-015-0186-y

Agboola, O.A., Downs, C.T., O'Brien, G., 2019. Macroinvertebrates as indicators of ecological conditions in the rivers of KwaZulu-Natal, South Africa. Ecological Indicators 106, 105465. https://doi.org/10.1016/j.ecolind.2019.105465

Alemneh, T., Ambelu, A., Bahrndorff, S., Mereta, S.T., Pertoldi, C., Zaitchik, B.F., 2017. Modeling the impact of highland settlements on ecological disturbance of streams in Choke Mountain Catchment: Macroinvertebrate assemblages and water quality. Ecological Indicators 73, 452–459. https://doi.org/10.1016/j.ecolind.2016.10.019

Amusan, B., Idowu, M.A., Ogbogu, S., 2018. Comparative study of the macroinvertebrate community composition and water quality of ona and opa rivers, Southwestern Nigeria. West African Journal of Applied Ecology 26, 33–48.

APHA, AWWA, WEF. (2012). *Standard Methods for Examination of Water and Wastewater. 22nd Edition*. American Public Health Association, American Water Work Association, and Water Environment Federation, Washington, DC, USA.

Arimoro, F.O., 2009. Guides to the Freshwater Invertebrates of Southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata and Plecoptera. African Journal of Aquatic Science 34, 201–202. https://doi.org/10.2989/AJAS.2009.34.2.12.899

Baumgartner, S.D., Robinson, C.T., 2017. Changes in macroinvertebrate trophic structure along a land-use gradient within a lowland stream network. Aquatic Sciences 79, 407–418. https://doi.org/10.1007/s00027-016-0506-z

Beghelli, F., Pompêo, M., Rosa, A., Moschini-Carlos, V., 2016. Effects of copper in sediments on benthic macroinvertebrate communities in tropical reservoirs. Limnetica 35, 103–116.

Bere, T., Nyamupingidza, B., 2013. Use of biological monitoring tools beyond their country of origin: A case study of the South African Scoring System Version 5 (SASS5). Hydrobiologia 722. https://doi.org/10.1007/s10750-013-1702-7

Bhatt, J.P., Pandit, M.K., 2010. A macro-invertebrate based new biotic index to monitor river water quality. Current Science 99, 196–203.

Bis, B., Kosmala, G., 2008. Freshwater Macroinvertebrates Identification Guide. ISBN: ISBN:978-960-367-027-8

Birmingham, M., Heimdal, D., Hubbard, T., Krier, K., Leopold, R., Luzier, J., & Wilton, T. (2005). Benthic Macroinvertebrate Key. Available from: https:// greenspaceamdsb.pbworks.com/f/BMIKey2Edvmay05.pdf [accessed Jan. 2 2020].

Chellaiah, D., Yule, C.M., 2018. Riparian buffers mitigate impacts of oil palm plantations on aquatic macroinvertebrate community structure in tropical streams of Borneo. Ecological Indicators 95, 53–62. https://doi.org/10.1016/j.ecolind.2018.07.025

Chiarucci, A., Bacaro, G., Scheiner, S.M., 2011. Old and new challenges in using species diversity for assessing biodiversity. Philosophical Transactions of the Royal Society B: Biological Sciences 366, 2426–2437. https://doi.org/10.1098/rstb.2011.0065

Clemente, R., Hartley, W., Riby, P., Dickinson, N.M., Lepp, N.W., 2010. Trace element mobility in a contaminated soil two years after field-amendment with a greenwaste compost mulch. Environmental Pollution 158, 1644–1651. https://doi.org/10.1016/j.envpol.2009.12.006

Colombani, N., Gervasio, M.P., Castaldelli, G., Mastrocicco, M., 2020. Soil conditioners effects on hydraulic properties, leaching processes and denitrification on a silty-clay soil. Science of The Total Environment 733, 139342. https://doi.org/10.1016/j.scitotenv.2020.139342

Croijmans, L., Jong, J.F.D., Prins, H.H.T., 2021. Oxygen is a better predictor of macroinvertebrate richness than temperature—a systematic review. Environmental Research Letters 16, 023002. https://doi.org/10.1088/1748-9326/ab9b42

Cummins, K.W., Merritt, R.W., Andrade, P.C., 2005. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. Studies on Neotropical Fauna and Environment 40, 69–89. https://doi.org/10.1080/01650520400025720

de Klerk, A.R., de Klerk, L.P., Chamier, J., Wepener, V., 2012. Seasonal variations of water and sediment quality parameters in endorheic reed pans on the Mpumalanga Highveld. Water SA 38, 663–672.

Desrosiers, M., Usseglio-Polatera, P., Archaimbault, V., Larras, F., Méthot, G., Pinel-Alloul, B., 2019. Assessing anthropogenic pressure in the St. Lawrence River using traits of benthic macroinvertebrates. Science of The Total Environment 649, 233–246. https://doi.org/10.1016/j.scitotenv.2018.08.267

Dickens, C.W., Graham, P.M., 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27, 1–10. https://doi.org/10.2989/16085914.2002.9626569

Du, L.-N., Jiang, Y.-E., Chen, X.-Y., Yang, J.-X., Aldridge, D., 2017. A family-level macroinvertebrate biotic index for ecological assessment of lakes in Yunnan, China. Water Resources 44, 864–874. https://doi.org/10.1134/S0097807817090020

Everall, N.C., Johnson, M.F., Wood, P., Farmer, A., Wilby, R.L., Measham, N., 2017. Comparability of macroinvertebrate biomonitoring indices of river health derived from semi-quantitative and quantitative methodologies. Ecological Indicators 78, 437–448. https://doi.org/10.1016/j.ecolind.2017.03.040

Gaigher, I., 2010. Guides to the Freshwater Invertebrates of Southern Africa. Volume 5: Non-Arthropods. The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinea. African Journal of Aquatic Science 35, 107–108. https://doi.org/10.2989/16085914.2010.466645

Gerber, A., Gabriel, M.J.M., South Africa. Department of Water Affairs and Forestry. Resource Quality Services., 2002. Aquatic invertebrates of South African rivers: field guide. Department of Water Affairs and Forestry, Resource Quality Services, Pretoria.

Gleekia, A., 2016. Impacts of Iron Ore Mining on Water Quality and the Environment in Liberia. Conference paper 6th ASIAN MINING CONGRESS, 23-26

Gonçalves, F.B., Menezes, M.S. de, 2011. A comparative analysis of biotic indices that use macroinvertebrates to assess water quality in a coastal river of Paraná state, southern Brazil. Biota Neotropica 11, 27–36. https://doi.org/10.1590/S1676-06032011000400002

Guareschi, S., Wood, P.J., 2019. Taxonomic changes and non-native species: An overview of constraints and new challenges for macroinvertebrate-based indices calculation in river ecosystems. Science of The Total Environment 660, 40–46. https://doi.org/10.1016/j.scitotenv.2019.01.008

Gutiérrez-Cánovas, C., Arribas, P., Naselli-Flores, L., Bennas, N., Finocchiaro, M., Millán, A., Velasco, J., 2019. Evaluating anthropogenic impacts on naturally stressed ecosystems: Revisiting River classifications and biomonitoring metrics along salinity gradients. Science of The Total Environment 658, 912–921. https://doi.org/10.1016/j.scitotenv.2018.12.253

Harrison, J. du G., 2009. Guides to the Freshwater Invertebrates of Southern Africa. Volume 10: Coleoptera. African Journal of Aquatic Science 34, 205–206. https://doi.org/10.2989/AJAS.2009.34.2.14.901

Jhariya, D., Khan, R., Thakur, G.S., 2016. Impact of Mining Activity on Water Resource: An Overview study. Conference: National Seminar on Recent Practices & Innovations in Mining Industry (RPIMI 2016)

Jomoc, D.J.G., Flores, R.R.C., Nuneza, O., Villanueva, R.J., 2013. Species richness of Odonata in selected wetland areas of Cagayan de Oro and Bukidnon, Philippines. AACL Bioflux 6, 560–570.

Kahlon, S., Julka, J., 2018. Impact of Temperature and Dissolved Oxygen on the Macroinvertebrate Density of a Hill Stream in Western Himalaya. International Journal of Biology, Pharmacy and Allied Sciences 7, 18-22

Kambole, M.S., 2003a. Managing the water quality of the Kafue River. Physics and Chemistry of the Earth, Parts A/B/C 28, 1105–1109. https://doi.org/10.1016/j.pce.2003.08.031

Kapungwe, E.M., 2013. Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. Journal of Geography and Geology 5, 55. https://doi.org/10.5539/jgg.v5n2p55

Karaca, O., Cameselle, C., Reddy, K.R., 2018. Mine tailing disposal sites: contamination problems, remedial options and phytocaps for sustainable remediation. Reviews in Environmental Science and Biotechnology 17, 205–228. https://doi.org/10.1007/s11157-017-9453-y

Karaouzas, I., Smeti, E., Kalogianni, E., Skoulikidis, N.Th., 2019. Ecological status monitoring and assessment in Greek rivers: Do macroinvertebrate and diatom indices indicate same responses to anthropogenic pressures? Ecological Indicators 101, 126–132. https://doi.org/10.1016/j.ecolind.2019.01.011

Keovilignavong, O., 2019. Mining governance dilemma and impacts: A case of gold mining in Phu-Hae, Lao PDR. Resources Policy 61, 141–150. https://doi.org/10.1016/j.resourpol.2019.02.002

Klerk, A.R. de, Wepener, V., 2013. Macroinvertebrate Assemblage Changes as an Indicator of Water Quality of Perennial Endorheic Reed Pans on the Mpumalanga Highveld, South Africa. Journal of Environmental Protection 4, 10–21. https://doi.org/10.4236/jep.2013.47A002

Krajenbrink, H.J., Acreman, M., Dunbar, M.J., Hannah, D.M., Laizé, C.L.R., Wood, P.J., 2019. Macroinvertebrate community responses to river impoundment at multiple spatial scales.

Science of The Total Environment 650, 2648–2656. https://doi.org/10.1016/j.scitotenv.2018.09.264

Laini, A., Bolpagni, R., Cancellario, T., Guareschi, S., Racchetti, E., Viaroli, P., 2018. Testing the response of macroinvertebrate communities and biomonitoring indices under multiple stressors in a lowland regulated river. Ecological Indicators 90, 47–53. https://doi.org/10.1016/j.ecolind.2018.02.051

Legendre, P., Anderson, M.J., 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecological Monographs 69, 1–24. https://doi.org/10.2307/2657192

Leslie, A.W., Lamp, W.O., 2017. Taxonomic and functional group composition of macroinvertebrate assemblages in agricultural drainage ditches. Hydrobiologia 787, 99–110. https://doi.org/10.1007/s10750-016-2947-8

Li, L., Zheng, B., Liu, L., 2010. Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends. Procedia Environmental Sciences, International Conference on Ecological Informatics and Ecosystem Conservation (ISEIS 2010) 2, 1510–1524. https://doi.org/10.1016/j.proenv.2010.10.164

Lim, M., Han, G.-C., Ahn, J.-W., You, K.-S., Kim, H.-S., 2009. Leachability of Arsenic and Heavy Metals from Mine Tailings of Abandoned Metal Mines. International Journal of Environmental Research and Public Health 6, 2865–2879. https://doi.org/10.3390/ijerph6112865

Mamert, O., Tamsa Arfao, A., Tchakonté, S., Koji, E., Nyamsi, N.L., Tchatcho, N., Yannick, N., Nfongmo, Hubet, S., Serge, Z.T., 2021. Effects of Industrial Agriculture and Urbanization on Structure and Functional Organization of Macroinvertebrate of Coastal Streams in Cameroon. Journal of Water Resource and Protection 13. https://doi.org/10.4236/jwarp.2021.132009

Marques, M.J., Martínez-Conde, E., Rovira, J.V., 2003. Effects of Zinc and Lead Mining on the Benthic Macroinvertebrates of a Fluvial Ecosystem. Water, Air and Soil Pollution 148, 363–388. https://doi.org/10.1023/A:1025411932330

Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., Moreira-Santos, M., Reis-Henriques, A.M., Ribeiro, R., Santos, M.M., Marques, J.C., 2015. A review on the ecological quality status assessment in aquatic systems using community-based indicators and ecotoxicological tools: what might be the added value of their combination? Ecological Indicators 48, 8–16. https://doi.org/10.1016/j.ecolind.2014.07.024

Masese, F.O., Kitaka, N., Kipkemboi, J., Gettel, G.M., Irvine, K., McClain, M.E., 2014. Litter processing and shredder distribution as indicators of riparian and catchment influences on ecological health of tropical streams. Ecological Indicators 46, 23–37. https://doi.org/10.1016/j.ecolind.2014.05.032

Mathers, K.L., Chadd, R.P., Extence, C.A., Rice, S.P., Wood, P.J., 2016. The implications of an invasive species on the reliability of macroinvertebrate biomonitoring tools used in freshwater ecological assessments. Ecological Indicators 63, 23–28. https://doi.org/10.1016/j.ecolind.2015.11.051

Merritt, R.W., Cummins, K.W., 2008. An introduction to the Aquatic insects of North America, 4th ed. ed. Kendall/Hunt Pub. Co., Dubuque, Iowa.

Merritt, R. W., Cummins, K. W., Berg, M. B., Novak, J. A., Higgins, M. J., Wessel, K. J., and Lessard, J. L. (2002). Development and application of a macroinvertebrate functional-group approach in the bioassessment of remnant river oxbows in southwest Florida. *Journal of the North American Benthological Society*, *21*(2), 290–310.

Meza-Salazar, A.M., Guevara, G., Gomes-Dias, L., Cultid-Medina, C.A., 2020. Density and diversity of macroinvertebrates in Colombian Andean streams impacted by mining, agriculture and cattle production. PeerJ 8, e9619. https://doi.org/10.7717/peerj.9619

Mudenda, L., 2018. Assessment of Water Pollution Arising from Copper Mining in Zambia: A Case Study of Munkulungwe Stream in Ndola, Copperbelt Province. Master’s thesis, University of Cape Town, South Africa

Mundike, J., 2004. *Bwana Mkubwa Mine; Water Pollution Report,* Ndola, Zambia.

Mwaanga, P., Silondwa, M., Kasali, G., Banda, P.M., 2019. Preliminary review of mine air pollution in Zambia. Heliyon 5. https://doi.org/10.1016/j.heliyon.2019.e02485Namayandeh, A., Beresford, D.V., Somers, K.M., Dillon, P.J., 2018. Difference in benthic invertebrate communities of headwater streams can be detected using a short elevation gradient. International Aquatic Research 10, 153–164. https://doi.org/10.1007/s40071-018-0197-2

Northey, S.A., Mudd, G.M., Werner, T.T., Haque, N., Yellishetty, M., 2019. Sustainable water management and improved corporate reporting in mining. Water Resources and Industry 21, 100104. https://doi.org/10.1016/j.wri.2018.100104

Novotny, V., Bartošová, A., O'Reilly, N., Ehlinger, T., 2005. Unlocking the relationship of biotic integrity of impaired waters to anthropogenic stresses. Water Research 39, 184–198. https://doi.org/10.1016/j.watres.2004.09.002

Ntengwe, F.W., 2005a. An overview of industrial wastewater treatment and analysis as means of preventing pollution of surface and underground water bodies—the case of Nkana Mine in Zambia. Physics and Chemistry of the Earth, Parts A/B/C, Integrated Water Resources Management (IWRM) and the Millennium Development Goals: Managing Water for Peace and Prosperity 30, 726–734. https://doi.org/10.1016/j.pce.2005.08.014

Ntengwe, F.W., Maseka, K.K., 2006. The impact of effluents containing zinc and nickel metals on stream and river water bodies: The case of Chambishi and Mwambashi streams in Zambia. Physics and Chemistry of the Earth, Parts A/B/C, Water for Sustainable Socio-Economic Development, Good Health for All and Gender Equity 31, 814–820. https://doi.org/10.1016/j.pce.2006.08.027

Olson, J., Hawkins, C., 2017. Effects of total dissolved solids on growth and mortality predict distributions of stream macroinvertebrates. Freshwater Biology 62. https://doi.org/10.1111/fwb.12901

Peco, J.D., Higueras, P., Campos, J.A., Esbrí, J.M., Moreno, M.M., Battaglia-Brunet, F., Sandalio, L.M., 2021. Abandoned Mine Lands Reclamation by Plant Remediation Technologies. Sustainability 13, 6555. https://doi.org/10.3390/su13126555

Qu, X., Wu, N., Tang, T., Cai, Q., Park, Y.-S., 2010. Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams. Annales de Limnologie - International Journal of Liminology 46, 291–302. https://doi.org/10.1051/limn/2010027

Resh, V., Jackson, J., 1993. Rapid Assessment Approaches to Biomonitoring Using Benthic Macroinvertebrates. Freshwater Biomonitoring and Benthic Macroinvertebrates pp. 195–233.

Rosenberg, D.M., Resh, V.H. (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Springer US.

Shah, S.R., Lloyd, B., 2009. Biotic Index Methods Used for Water Quality Assessment. ISH Journal of Hydraulic Engineering 15, 108–120. https://doi.org/10.1080/09715010.2009.10514935

Shannon, C. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*(3, 4), 379–423, 623–656.

Shimaponda-Mataa, N.M., Tembo-Mwase, E., Gebreslasie, M., Achia, T.N.O., Mukaratirwa, S., 2017. Modelling the influence of temperature and rainfall on malaria incidence in four endemic provinces of Zambia using semiparametric Poisson regression. Acta Tropica 166, 81–91. https://doi.org/10.1016/j.actatropica.2016.11.007

Sikamo, J., Mwanza, A., Mweemba, C., 2016. Copper mining in Zambia - history and future. Journal of the Southern African Institute of Mining and Metallurgy 116, 491–496. https://doi.org/10.17159/2411-9717/2016/v116n6a1

Simpson, E.H. (1949) Measurement of diversity. Nature, 163, 688. doi:10.1038/163688a0

Sirisinthuwanich, K., Sangpradub, N., Hanjavanit, C., 2016. Development of biotic index to assess the phong and cheon rivers' healths based on benthic macroinvertebrates in Northeastern Thailand. AES Bioflux 9, 680–694.

Smith, A.J., Baldigo, B.P., Duffy, B.T., George, S.D., Dresser, B., 2019. Resilience of benthic macroinvertebrates to extreme floods in a Catskill Mountain River, New York, USA: Implications for water quality monitoring and assessment. Ecological Indicators 104, 107–115. https://doi.org/10.1016/j.ecolind.2019.04.057

Sracek, O., 2015. Formation of secondary hematite and its role in attenuation of contaminants at mine tailings: review and comparison of sites in Zambia and Namibia. Frontiers in Environmental Science 2. https://doi.org/10.3389/fenvs.2014.00064

Sracek, O., Choquette, M., Gélinas, P., Lefebvre, R., Nicholson, R.V., 2004. Geochemical characterization of acid mine drainage from a waste rock pile, Mine Doyon, Québec, Canada. Journal of Contaminant Hydrology 69, 45–71. https://doi.org/10.1016/S0169-7722(03)00150-5

Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Timpano, A., Schoenholtz, S., Zipper, C., Soucek, D., 2010. Isolating Effects of Total Dissolved Solids on Aquatic Life in Central Appalachian Coalfield Streams. Journal American Society of Mining and Reclamation 2010, 1284–1302. https://doi.org/10.21000/JASMR10011284

Valipour, R., Boegman, L., Bouffard, D., Rao, Y.R., 2017. Sediment resuspension mechanisms and their contributions to high-turbidity events in a large lake. Limnology and Oceanography 62, 1045–1065. https://doi.org/10.1002/lno.10485

Van de Meutter, F., Stoks, R., De Meester, L., 2006. The effect of turbidity state and microhabitat on macroinvertebrate assemblages: A pilot study of six shallow lakes, in: Hydrobiologia. pp. 379–390. https://doi.org/10.1007/1-4020-4111-X_32

Verberk, W.C.E.P., Durance, I., Vaughan, I.P., Ormerod, S.J., 2016. Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. Global Change Biology 22, 1769–1778. https://doi.org/10.1111/gcb.13240

Villantes, Y., 2015. Macroinvertebrates as bioindicators of water quality in labo and clarin rivers, misamis occidental, Philippines. International Journal of Biosciences 6, 62-73. https://doi.org/10.12692/ijb/6.9.62-73

von der Heyden, C.J., New, M.G., 2004. Groundwater pollution on the Zambian Copperbelt: deciphering the source and the risk. Science of The Total Environment 327, 17–30. https://doi.org/10.1016/j.scitotenv.2003.08.028

von Schiller, D., Acuña, V., Aristi, I., Arroita, M., Basaguren, A., Bellin, A., Boyero, L., Butturini, A., Ginebreda, A., Kalogianni, E., Larrañaga, A., Majone, B., Martínez, A., Monroy, S., Muñoz, I., Paunović, M., Pereda, O., Petrovic, M., Pozo, J., Rodríguez-Mozaz, S., Rivas, D., Sabater, S., Sabater, F., Skoulikidis, N., Solagaistua, L., Vardakas, L., Elosegi, A., 2017. River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors. Science of The Total Environment 596–597, 465–480. https://doi.org/10.1016/j.scitotenv.2017.04.081Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467, 555–561. https://doi.org/10.1038/nature09440

Vörösmarty, C., McIntyre, P., Gessner, M. *et al.* Global threats to human water security and river biodiversity. *Nature* **467**, 555–561 (2010). https://doi.org/10.1038/nature09440

Water-Monitoring, 2007. *Water Action Volunteers; University of Wisconism,* s.l.: Water Monitoring. University of Wisconsin, United States of America.

Werner, T.T., Bebbington, A., Gregory, G., 2019. Assessing impacts of mining: Recent contributions from GIS and remote sensing. The Extractive Industries and Society 6, 993-1012. https://doi.org/10.1016/j.exis.2019.06.011

Wild, C. J., and Seber, G. A. F. (1999). Chance Encounters: A First Course in Data Analysis and Inference. John Wiley & Sons, New York, USA.
Yoshimura, C., Tockner, K., Omura, T., Moog, O., 2006. Species diversity and functional assessment of macroinvertebrate communities in Austrian rivers. Limnology 7, 63–74. https://doi.org/10.1007/s10201-006-0170-4
Zakaria, M., Mohamed, M., 2019. Comparative analysis of Biotic Indices in water quality assessment: Case study at Sg. Bantang, Johor. IOP Conference Series: Earth and Environmental Science 269, 012047. https://doi.org/10.1088/1755-1315/269/1/012047

5.7. Supplementary Material

Table S5-1: Diversity of macroinvertebrate reported in Nselaki Stream during the rainy season

Nselaki Stream - Rainy Season			
Site	**Macsroinvertebrates**	**Index Score**	**Stream Condition**
NS01	Riffle Beetle, Water Penny, Amphipod, Snail, Leech, Snail	1,8	Poor
NS02	Mayfly Larva, Damesfly Larva, Snail, Leech	2,2	Fair
NS03	Dragonfly Larva, Water Penny, Amphipod, Leech, Isopod	2	Poor
NS04	Damselfly Larva, Mayfly Larva, Water Penny, Blackfly Larva, Isopod, Leech	2,2	Fair
NS05	Dragonfly Larva, Damselfly Larva, Amphipod, Blackfly Larva, Leech, Isopod, Snail	1,9	Poor
NS06	Amphipod, Black Fly Larva, Isopod, Leech	1,5	Poor
NS07	Dragonfly Larva, Water Penny, Amphipod, Leech	2,3	Fair
NS08	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
NS09	Mayfly Larva, Riffle Beetle, Snail, Leech	2,2	Fair
Control NS01	Damselfly Larva, Dragonfly Larva, Water Penny, Riffle Beetle, Amphipod, Leech	2,4	Fair
Control NS02	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Blackfly Larva, Isopod, Leech	2,7	Good
NS01	Water Penny, Riffle Beetle, Blackfly Larva, Leech, Isopod	2	Poor
NS02	Amphipod, Tubifex Worm, Leech	1,3	Poor
NS03	Mayfly Larva, Dragonfly Larva, Cranefly Larva, Amphipod, Blackfly Larva, Leech, Isopod	2,1	Fair
NS04	Dragonfly Larva, Water Penny, Amphipod, Blackfly Larva, Leech	2,2	Fair
NS05	Damselfly Larva, Dragonfly Larva, Riffle Beetle, Snail, Isopod, Leech	2	Poor
NS06	Damsefly Larva, Water Penny, Caddishfly Larva, Blackfly Larva, Amphipod	2,6	Good
NS07	Mayfly Larva, Damselfly Lara, Amphipod, Leech, Snails	2,5	Fair
NS08	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
NS09	Stonefly Larva, Damselfly Larva, Dragonfly Larva, Water Penny, Amphipod, Leech, Isopod	2,4	Fair
Control NS01	Caddisfly Larva, Damselfly Larva, Dragonfly Larva, Amphipod, Blackfly Larva	2,6	Good
Control NS02	Damelfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails	2,9	Good
NS01	Dragonfly Larva, Water Penny, Amphipod, Leech	2,3	Fair
NS02	Dragonfly Larva, Damselfly Lara, Amphipod, Leech, Snails	2,5	Fair
NS03	Riffle Beetle, Water Penny, Amphipod, Snail, Leech, Snail	1,8	Poor
NS04	Amphipod, Blackfly Larva, Snail, Isopod, Leech	1,4	Poor
NS05	Stonefly Larva, Dameselfly Larva, Mayfly Larva, Dragonfly Larva, Amphipod, Isopod, Leech	2,4	Fair
NS06	Water Penny, Dragonfly Larva, Damselfly Larva,Riffle Beetle, Amphipod, Leech, Isopod	2,3	Fair
NS07	Damselfly Larva, Dragonfly Larva, Water Penny, Amphipod, Blackfly Larva	2,6	Good
NS08	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,4	Fair
NS09	Damselfly Larva, Cranefly Larva, Water Penny, Leech	2,5	Fair
Control NS01	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Blackfly Larva, Isopod, Leech	2,7	Good
Control NS02	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod	2,8	Good
NS01	Water Penny, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,4	Fair
NS02	Caddisfly Larva, Dragonfly Larva, Riffle Beetle, Snail, Isopod, Leech	2	Poor
NS03	Dragonfly Larva, Water penny, Amphipod, Isopod	2,3	Fair
NS04	Water Penny, Mayfly Larva, Black Fly Larva, Isopod	2	Poor
NS05	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
NS06	Amphipod, Leech, Isopod, Snail	1,3	Poor
NS07	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
NS08	Mayfly Larva, Damesfly Larva, Snail, Leech	2,2	Fair
NS09	Damsefly Larva, Riffle Beetle, Water Penny, Amphipod, Black Fly Larva	2,6	Good
Control NS01	Stonefly Larva, Damselfly Larva, Dragonfly Larva, Water Penny, Amphipod, Blackfly Larva, Isopod	2,6	Good
Control NS02	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair

Table S5-2: Diversity of macroinvertebrate reported in Nselaki Stream post rainy season

Nselaki Stream - Post Rainy Season			
Site	**Macsroinvertebrates**	**Index Score**	**Stream Condition**
NS01	Water Penny, Dragonfly Larva, Amphipod, Leech	2,3	Fair
NS02	Dragonfly Larva, Riffle Beetle, Snail, Isopod, Leech	1,8	Poor
NS03	Dragonfly Larva, Amphipod, Isopod	2	Poor
NS04	Damselfly Larva, Mayfly Larva, Black Fly Larva, Isopod	2	Poor
NS05	Caddisfly Larva, Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	2,2	Fair
NS06	Blackfly Larva, Amphipod, Leech, Isopod, Snail	1,4	Poor
NS07	Mayfly Larva, Damselfly Larva, Riffle Beetle, Amphipod, Black Fly Larva	2,6	Good
NS08	Mayfly Larva, Damesfly Larva, Amphipod, Snail, Leech	2	Poor
NS09	Damsefly Larva, Water Penny, Amphipod, Black Fly Larva	2,5	Fair
Control NS01	Stonefly Larva, Caddisfly Larva, Damselfly Larva, Dragonfly Larva, Water Penny, Amphipod, Blackfly Larva, Isopod	2,6	Good
Control NS02	Alderfly Larva, Stonefly Larva, Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	3	Good
NS01	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva, Leeh, Snail	2	Poor
NS02	Damselfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
NS03	Water Penny, Dragonfly Larva, Damselfly Larva,Riffle Beetle, Amphipod, Leech, Isopod	2,3	Fair
NS04	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
NS05	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
NS06	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
NS07	Riffle Beetle, Mayfly Larva, Water Penny, Amphipod, Black Fly Larva, Snail, Leech	2,3	Fair
NS08	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,4	Fair
NS09	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
Control NS01	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Isopod, Leech	2,7	Good
Control NS02	Dragonfly Larva, Damselfly Larva,Mayfly Larva, Black Fly Larva, Isopod	2,4	Fair
NS01	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
NS02	Amphipod, Leech, Isopod, Snail	1,3	Poor
NS03	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
NS04	Alderfly, Amphipod, Blackfly Larva, Snail, Leach	2	Poor
NS05	Damselfly Larva, Cranefly Larva, Water Penny, Leech	2,5	Fair
NS06	Dragonfly Larva, Water penny, Amphipod, Isopod	2,3	Fair
NS07	Water Penny, Mayfly Larva, Damselfly Larva, Blackfly Larva, Amphipod	2,6	Good
NS08	Dragonfly Larva, Damselfly Larva, Amphipod, Blackfly Larva, Leech, Isopod, Snail	1,9	Poor
NS09	Dragonfly Larva, Damselfly Larva, Water Penny, Leech	2,5	Fair
Control NS01	Damsefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva	2,6	Good
Control NS02	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	2,7	Good
NS01	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
NS02	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
NS03	Water penny, Amphipod, Blackfly Larva, Leech, Isopod, Snail	1,7	Poor
NS04	Damselfly Larva, Water Penny, Amphipod, Blackfly Larva, Leech	2,2	Fair
NS05	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
NS06	Stonefly Larva, Dameselfly Larva, Mayfly Larva, Dragonfly Larva, Amphipod, Isopod, Leech	2,4	Fair
NS07	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
NS08	Caddisfly Larva, Damselfly Larva, Water Penny, Leech	2,5	Fair
NS09	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	2,7	Good
Control NS01	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	3	Good
Control NS02	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good

Table S5-3: Diversity of macroinvertebrate reported in Fikondo Stream during the rainy season

Fikondo Stream - Rainy Season			
Site	**Macsroinvertebrates**	**Index Score**	**Stream Condition**
FS01	Dragonfly Larva, Mayfly Larva, Water Penny, Leech	2,5	Fair
FS02	Dragonfly Larva, Damselfly Larva, Amphipod, Isopod	2,2	Fair
FS03	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
FS04	Mayfly Larva, Water Penny, Snails, Isopod, Leech	1,8	Poor
FS05	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
FS06	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
Control FS01	Dragonfly Larva, Mayfly Larva, Amphipod, Blackfly Larva	2,5	Fair
Control FS02	Damselfly Larva, Dragonfly Larva, Amphipod, Snails, Isopod, Leech	1,8	Poor
FS01	Damselfly, Cranefly, Rittle Beetle, Amphipod, Isopod	2,4	Fair
FS02	Dragonfly Larva, Amphipod, Black Fly Larva	2,3	Fair
FS03	Riffle Beetle, Water penny, Amphipod, Blackfly Larva, Isopod, Leach	2	Poor
FS04	Dragonfly Larva, Mayfly Larva, Amphipod, Blackfly Larva	2,5	Fair
FS05	Dragonfly, Damsefly Larva, Blackfly, Snail	2,3	Fair
FS06	Damselfly Larva, Dragonfly Larva, Riffle Beetle, Snail, Isopod, Leech	2	Poor
Control FS01	Stonefly Larva, Dameselfly Larva, Mayfly Larva, Dragonfly Larva, Amphipod, Isopod, Leech	2,4	Fair
Control FS02	Water Penny, Dragonfly Larva, Damselfly Larva,Riffle Beetle, Amphipod, Leech, Isopod	2,3	Fair
FS01	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
FS02	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Leech	2,4	Fair
FS03	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Isopod, Leech	2,2	Fair
FS04	Dragonfly Larva, Damselfly Larva, Amphipod, Isopod	2,2	Fair
FS05	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
FS06	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
Control FS01	Water Penny, Riffle Beetle, Amphipod, Blackfly Larva, Leech, Isopod, Snail	1,7	Poor
Control FS02	Damselfly Larva, Water Penny, Amphipod, Blackfly Larva, Leech	2,2	Fair
FS01	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
FS02	Water Penny, Mayfly Larva, Black Fly Larva, Isopod	2	Poor
FS03	Mayfly Larva, Water Penny, Amphipod, Black Fly Larva	2,5	Fair
FS04	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
FS05	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
FS06	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
Control FS01	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
Control FS02	Dragonfly, Damsefly Larva, Blackfly, Snail	2,3	Fair

Table S5-4: Diversity of macroinvertebrate reported in Nselaki Stream post rainy season

Fikondo Stream - Post Rainy Season			
Site	**Macsroinvertebrates**	**Index Score**	**Stream Condition**
FS01	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva, Leech, Snail	2	Poor
FS02	Damselfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
FS03	Water Penny, Dragonfly Larva, Damselfly Larva,Riffle Beetle, Amphipod, Leech, Isopod	2,3	Fair
FS04	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
FS05	Dragonfly Larva, Damselfly Larva, Mayfly Larva, Black Fly Larva, Leech, Isopod, Snail	2	Poor
FS06	Stonefly Larva, Dragonfly Larva, Riffle Beetle, Amphipod, Black Fly Larva, Leech, Isopod	2,3	Fair
Control FS01	Caddisfly Larva, Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,5	Fair
Control FS02	Damselfly Larva, Mayfly Larva, Water Penny, Leech	2,5	Fair
FS01	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
FS02	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
FS03	Riffle Beetle, Mayfly Larva, Water Penny, Amphipod, Black Fly Larva, Snail, Leech	2,3	Fair
FS04	Dragonfly Larva, Damselfly Larva, Riffle Beetle, Leech, Snail, Isopod	2	Poor
FS05	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,4	Fair
FS06	Dragonfly Larva, Damselfly Larva, Amphipod, Isopod	2,2	Fair
Control FS01	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
Control FS02	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
FS01	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
FS02	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
FS03	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
FS04	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
FS05	Stonefly Larva, Dameselfly Larva, Mayfly Larva, Dragonfly Larva, Amphipod, Isopod, Leech	2,4	Fair
FS06	Mayfly Larva, Water Penny, Snails, Isopod, Leech	1,8	Poor
Control FS01	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
Control FS02	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
FS01	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
FS02	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
FS03	Caddisfly Larva, Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	2,2	Fair
FS04	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
FS05	Water Penny, Dragonfly Larva, Amphipod, Leech	2,3	Fair
FS06	Dragonfly Larva, Riffle Beetle, Snail, Isopod, Leech	1,8	Poor
Control FS01	Dragonfly Larva, Amphipod, Isopod	2	Poor
Control FS02	Damselfly Larva, Mayfly Larva, Black Fly Larva, Isopod	2	Poor

Table S5-5: Diversity of macroinvertebrate reported in Mululu Stream during the rainy season

Mululu Stream - Rainy Season			
Site	Macsroinvertebrates	Index Score	Stream Condition
MS01	Water Penny, Mayfly Larva, Black Fly Larva, Isopod	2	Poor
MS02	Mayfly Larva, Water Penny, Amphipod, Black Fly Larva	2,5	Fair
MS03	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
MS04	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
MS05	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
MS06	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
MS07	Stonefly Larva, Dragonfly Larva, Mayfly Larva, Black Fly Larva	2,4	Fair
MS08	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	2,7	Good
MS09	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
MS10	Water Penny, Mayfly Larva, Black Fly Larva, Isopod	2	Poor
MS11	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
Control 01	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
Control 02	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Leech	2,4	Fair
MS01	Dragonfly Larva, Mayfly Larva, Water Penny, Leech	2,5	Fair
MS02	Dragonfly Larva, Damselfly Larva, Amphipod, Isopod	2,2	Fair
MS03	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
MS04	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	3,2	Good
MS05	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
MS06	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
MS07	Crane Fly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Blackfly Larva	2,6	Good
MS08	Riffle Beetle, Water penny, Amphipod, Blackfly Larva, Isopod, Leach	2	Poor
MS09	Dragonfly, Mayfly Larva, Damsefly Larva, Blackfly, Snail	2,6	Good
MS10	Stonefly Larva, Damisfly, Cranefly, Rittle Beetle, Amphipod, Isopod	2,7	Good
MS11	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
Control 01	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	3	Good
Control 02	Crane Fly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Blackfly Larva	2,6	Good
MS01	Dobsonfly, Dragonfly Larva, Damselfly Larva, Amphipod, Blackfly Larva, Midge Larva, Leech	2,5	Fair
MS02	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Isopod, Leach	2,2	Fair
MS03	Amphipod, Blackfly Larva, Snail	1,7	Poor
MS04	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
MS05	Dragonfly Larva, Damselfly Larva, Water Penny, Leech	2,5	Fair
MS06	Stonefly Larva, Cranefly Larva, Damselfly Larva, Amphipod, Isopod, Tubifex	2,3	Fair
MS07	Cranefly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Isopod, Leech	2,7	Good
MS08	Caddisfly Larva, Damselfly Larva, Water Penny, Leech	2,5	Fair
MS09	Alderfly, Amphipod, Blackfly Larva, Snail, Leach	2	Poor
MS10	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Leech	2,4	Fair
MS11	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Blackfly Larva, Isopod, Leech	2,7	Good
Control 01	Damelfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails	2,9	Good
Control 02	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Blackfly Larva, Isopod, Leech	2,3	Fair
MS01	Damselfly Larva, Water Penny, Riffle Beetle, Amphipod, Leech, Tubifex Worm	2,2	Fair
MS02	Damselfly Larva, Dragonfly Larva, Amphipod, Blackfly Larva, Leech, Snail	2	Poor
MS03	Caddisfly Larva, Damselfly Larva, Dragonfly Larva, Amphipod, Blackfly Larva	2,6	Good
MS04	Dragonfly Larva, Water penny, Amphipod, Isopod	2,3	Fair
MS05	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
MS06	Amphipod, Leech, Isopod	1,3	Poor
MS07	Dragonfly Larva, Damselfly Larva, Amphipod, Leech	2,2	Fair
MS08	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
MS09	Mayfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
MS10	Stonefly Larva, Dameselfly Larva, Mayfly Larva, Dragonfly Larva, Amphipod, Isopod, Leech	2,4	Fair
MS11	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Blackfly Larva, Isopod, Leech	2,7	Good
Control 01	Water penny, Riffle Beetle, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
Control 02	Dobsonfly Larva, Damselfly Larva, Dragonfly Larva, Water Penny, Amphipod, Leech, Isopod	2,4	Fair

Table S5-6: Diversity of macroinvertebrate reported in Mululu Stream during the rainy season

Mululu Stream - Post Rainy Season			
Site	**Macsroinvertebrates**	**Index Score**	**Stream Condition**
MS01	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
MS02	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
MS03	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	2,7	Good
MS04	Stonefly Larva, Dragonfly Larva, Water Penny, Amphipod	3	Good
MS05	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,4	Fair
MS06	Dragonfly Larva, Damselfly Larva, Amphipod, Isopod	2,2	Fair
MS07	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva	2,5	Fair
MS08	Damelfly Larva, Mayfly Larva, Black Fly Larva, Leech	2	Poor
MS09	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
MS10	Dragonfly Larva, Black Fly Larva, Amphipod, Isopod	1,8	Poor
MS11	Mayfly Larva, Damesfly Larva, Snail, Leech	2,2	Fair
Control 01	Damelfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails	2,9	Good
Control 02	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
MS01	Damselfly Larva, Cranefly Larva, Water Penny, Leech	2,5	Fair
MS02	Dragonfly Larva, Damselfly Larva, Water Penny, Leech	2,5	Fair
MS03	Riffle Beetle, Mayfly Larva, Water Penny, Amphipod, Black Fly Larva, Isopod, Leech	2,3	Fair
MS04	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Riffle Beetle, Amphipod, Leech, Isopod	2,2	Fair
MS05	Stonefly Larva, Dragonfly Larva, Mayfly Larva, Black Fly Larva	3	Good
MS06	Water Penny, Mayfly Larva, Damselfly, Blackfly Larva, Amphipod	2,6	Good
MS07	Cranefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,1	Fair
MS08	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Leech	2,4	Fair
MS09	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Isopod, Leech	2,7	Good
MS10	Dragonfly Larva, Damselfly Larva, Riffle Beetle, Amphipod, Isopod	2,4	Fair
MS11	Damselfly Larva, Mayfly Larva, Water Penny, Amphipod, Black Fly Larva	2,6	Good
Control 01	Stonefly Larva, Damsefly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva, Leech, Isopod	2,4	Fair
Control 02	Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Snails, Isopod, Leech	2,7	Good
MS01	Stonefly Larva, Dragonfly Larva, Damselfly Larva, Water penny, Blackfly Larva	3	Good
MS02	Water Penny, Riffle Beetle, Mayfly Larva, Damselfly Larva, Black Fly Larva, Amphipod, Isopod	2,4	Fair
MS03	Dragonfly Larva, Damselfly Larva, Amphipod, Blackfly Larva	2,5	Fair
MS04	Damselfly Larva, Dragonfly Larva, Black Fly Larva, Amphipod, Isopod, Leech	2,2	Fair
MS05	Dragonfly Larva, Damselfly Larva, Amphipod, Black Fly Larva, Leech, Snail	2	Poor
MS06	Damselfly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva	2,6	Good
MS07	Caddisfly Larva, Dragonfly Larva, Water Penny, Amphipod, Black Fly Larva	2,6	Good
MS08	Damsefly Larva, Water Penny, Mayfly Larva, Amphipod, Black Fly Larva, Pouch Snail, Isopod	2,1	Fair
MS09	Cranefly Larva, Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Black Fly Larva, Leech, Isopod	2,3	Fair
MS10	Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Blackfly Larva, Leech, Isopod, Snail	2	Poor
MS11	Stonefly Larva, Carddisfly Larva, Damselfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Isopod, Leech	2,6	Good
Control 01	Dragonfly Larva, Damselfly Larva, Riffle Beetle, Amphipod, Isopod	2,4	Fair
Control 02	Stonefly Larva, Damselfly Larva, Mayfly Larva, Black Fly Larva	3	Good
MS01	Caddisfly Larva, Alderfly Larva, Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech, Snail	2,6	Good
MS02	Caddisfly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Amphipod, Leech	2,5	Fair
MS03	Dragonfly Larva, Damselfly Larva, Riffle Beetle, Isopod, Snail	2,2	Fair
MS04	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod	2,8	Good
MS05	Cranefly Larva, Damselfly Larva, Water Penny, Amphipod, Black Fly Larva, Snail, Isopod	2,3	Fair
MS06	Stonefly Larva, Dragonfly Larva, Damselfly Larva,Mayfly Larva, Black Fly Larva, Leech	2,7	Good
MS07	Dragonfly Larva, Damselfly Larva, Mayfly Larva, Black Fly Larva, Leech, Isopod, Snail	2	Poor
MS08	Stonefly Larva, Dragonfly Larva, Riffle Beetle, Amphipod, Black Fly Larva, Leech, Isopod	2,3	Fair
MS09	Caddisfly Larva, Damsefly Larva, Dragonfly Larva, Mayfly Larva, Amphipod, Leech	2,5	Fair
MS10	Damselfly Larva, Mayfly Larva, Water Penny, Leech	2,5	Fair
MS11	Dragonfly Larva, Damselfly Larva, Riffle Beetle, Leech, Snail, Isopod	2	Poor
Control 01	Damsefly Larva, Dragonfly Larva, Mayfly Larva, Water Penny, Caddisfly Larva	3	Good
Control 02	Stonefly Larva, Alderfly Larva, Caddisfly Larva, Damselfly Larva, Mayfly Larva, Isopod, Leech, Snail	2,5	Fair

CHAPTER 6: HEAVY METAL CONCENTRATION IN SOIL AND VEGETABLES NEAR MINE WASTELAND AREA IN THE COPPERBELT PROVINCE OF ZAMBIA

The environmental impact of copper mining and its waste disposal strategies implemented on the Zambian Copperbelt cause significant reduction in water quality and other aquatic ecosystem services. Further, the potential of this contamination to cause food crop contamination has been postulated. Here, the environmental impact of irrigating food crops with water resources within perimeters that are susceptible to contamination by released metal species from the mine waste was investigated. The nemerow integrated pollution index (NIPI), bioaccumulation factor (BAF) and contamination load index (CLI) were used to highlight metal contamination on soil and selected food crops. The trends in water quality, food crops and mineral footprint along catchments located in the Zambian Copperbelt copper mining region was evaluated. The results provide a warning to stakeholders on the growing deterioration of water quality and the ecological risks posed by copper mine waste under current conditions for handling mine waste, particularly where there is no reduction in contamination sources. In particular, metal accumulation in agricultural crops is assessed to determine potential health risks. The findings highlight key recommendations for good practice to minimise environmental impact and the associated health impact.

6.1. Introduction

In many developing countries, metal contamination of arable land and food crops owing to industrial development and population growth has become a serious environmental and food safety concern (Ali et al., 2019; Anyanwu et al., 2018; Wuana and Okieimen, 2011). Studies have reported high accumulation of metals such as As, Pb, Cu, Zn, Ni and cadmium Cd in water, soils, vegetables, and dust across mining regions (Masindi and Muedi, 2018; Nwankwo and Elinder, 1979; Obasi and Akudinobi, 2020; Rai et al., 2019). At a global level, the emerging and diverse matters of food scarcity has become a concern owing to their inextricable link to the health and wellbeing of mankind (Rai et al., 2018). Metal contamination in arable land has a negative impact on crop production and safety (Nakayama et al., 2010), and food crops is one of the main pathways of metal uptake by humans. Particularly, vegetables are prone to heavy metal contamination owing to aerial burden and crop irrigation in mining areas. Accumulation of metals in the edible parts of vegetables in high quantities might likewise result in health complications to both humans and animals (Jolly et al., 2013).

Studies on agricultural lands irrigated by contaminated water have identified various challenges associated with contaminated water on crop farming. These challenges include insufficient knowledge on the temporal spatial variations in metal uptake of soils and food crops irrigated by contaminated water (Gola et al., 2016; Lu et al., 2015). The influence of the impact of irrigation with contaminated water, especially long-term application of irrigation with contaminated waters is well known (Nolde, 2005). Despite the risks affiliated with using contaminated or wastewater to irrigate food crops, it is a means of securing the necessities of life for a host of informal communities in several underdeveloped nations (Kookana et al., 2020; Saldías et al., 2017). In Africa, it is a conventional practice to grow food crops along the banks of water resources traversing industrial and mining areas (Edogbo et al., 2020; Emmanuel et al., 2018; Kapungwe, 2013; Kapwata et al., 2020; Ochieng et al., 2010; Suruchi and Khanna, 2011). Often such water resources have been observed to be contaminated by metals (Attiogbe and Nkansah, 2017; Jhariya et al., 2016; Mcintyre et al., 2018; Mudenda, 2018). There is a paucity of knowledge on the impacts of using metal contaminated water for crop irrigation in developing nations (Hamilton et al., 2007; Shakir et al., 2017). The present study evaluates the impact of mine wastelands (TSFs) on water quality, by means of analysing food crops irrigated by water resources within perimeters that are susceptible to TSF impacts in the Kafue River catchment. The objective of the contemporary survey was to compare the spatial variation of metal loads in crops and soils irrigated by water resources potentially contaminated by mine wasteland. This approach intended to contribute to the risk assessment of metal mobilization from mine wastelands in terms of the suitability of affected water for irrigation and ecological impacts resulting from metal migration.

6.2. Materials and Methods

6.2.1. Site Selection and Description

This study was undertaken near mine wastelands in the district of Kitwe and Lufwanyama, on the Copperbelt Province of Zambia (Figure 6-2). The study sites are located between latitude 12° 48' 3'' South and longitude 28°12' 23'' East (Kitwe Site), and latitude 12° 54' 34'' and longitude 28° 4' 48'' East (Chibuluma Site). In total, eleven sampling sites were selected at three study areas for the purposes of collecting soil and vegetables irrigated by water resources potentially contaminated by metal mobilization from mine wastelands to assess the extent and levels of selected metals in arable land and vegetables. The selected sites are in close proximity to Nselaki Stream (Chibuluma), Fikondo Stream (Kitwe), and Mululu Stream (Kitwe) (Figure 6-2) where crop cultivators irrigate with water affected by effluents from TSFs. These are illustrated in Figure 6-1.

Figure 6-1: Photos of agriculture sites irrigated by water from Fikondo Stream

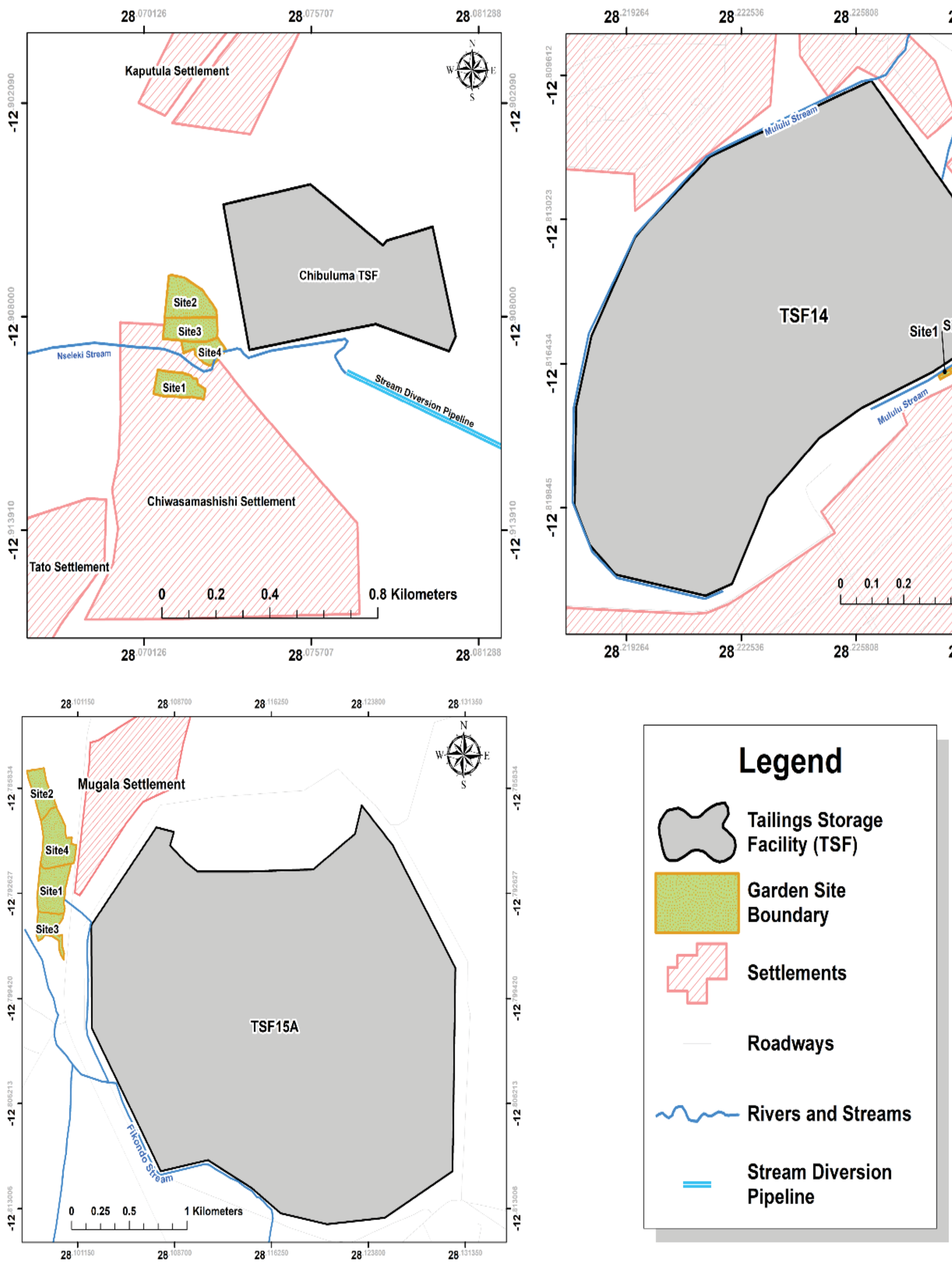


Figure 6-2: Sampling sites of vegetables irrigated by streams near TSF15A, Chibuluma TSF, and TSF14.

6.2.2. Research Design

Sampling of soils and vegetables was consented by the crop cultivators and seasonal sampling was conducted from October 2018 to May 2020. Soil and vegetable samples were collected downstream, while upstream only soil samples were collected because different types of crops were grown upstream, hence direct comparison was not possible. Although a few samples of pumpkin leaves were collected upstream of TSF15A. Background values for Mn used in this study were measure from these samples. Vegetable selection was influenced by the similarities in vegetables grown by the crop cultivators at the three sites downstream. This was beneficial in comparing variations in metal loads from the sites. A total of seventy-five samples each of soil and vegetables were collected at site near Chibuluma TSF, forty-five samples each of soil and vegetables were collected at site near TSF14, whilst a total of seventy-four samples each of soil and crops were collected at site near TSF15A. The number of samples collected was influenced by the intensity of agriculture activities in each location.

6.2.3. Determination of Metal Loads in Soils and Vegetables

Composite soil samples were collected at each plot at a 0-20 cm depth. They were then deposited in pre-cleaned plastic containers and transported to the analytical laboratory at the Copperbelt University for metal analysis. The soil samples were oven dried at 70 °C for 48 hours until constant weight; and thereafter, the representative samples were obtained from the composite sample using a 10-Dickie and Stockler rotary splitter. Sample sieving was conducted using a 75 µm sieve to remove large particles and sand. The soil samples were digested using concentrated nitric acid, where 1 g of the dried sample was added to a beaker containing 30 ml of nitric acid. Three drops of hydrofluoric acid (HF) were added to the suspension which was heated on an electronic plate set at 120^{o}C for 20 minutes. The suspension was allowed to cool after boiling; and thereafter filtered through into a 50 ml volumetric flask, using filter paper and the volume made up using deionized water. Perkin Elmer's PinAAcle 900T Atomic Absorption Spectrometer (AAS) was used to determine metal concentration in the soil samples.

Leaf samples of edible parts of vegetables (sweet potato leaves, pumpkin leaves, Amaranthus leaves and cabbage leaves) were randomly collected from the same sites where soil samples were collected and taken for laboratory analysis. The plant tissue samples were thoroughly washed using tap water in order to remove attached soil particles. Thereafter, the samples were rinsed with distilled water and oven dried for 24 hours at 70^0 C before being ground using a mortar to fine powder and sieved through a 0.18 µm sieve. From the fine ground samples, 2 g of each vegetable sample was added in a separate well-cleaned and dry 250 cm^3 conical flasks and 30 ml 0.5 M nitric acid added. The solution was heated for 30 minutes using an electronic plate set at *120*°C; afterwards, 10 ml of Perchloric acid ($HCLO_4$) was added and the solution transferred into 100 ml volumetric flask and diluted with deionized water. The total concentrations of selected metals (Cu, Co, Zn, Mn, and Pb) in the filtered digestates were analysed using the AAS.

6.2.4. Determination of Contamination Load in Soils and Crops

The contamination load of heavy metals in the soil samples was evaluated using the pollution index (Pi) and Nemerow integrated pollution index (NIPI). The Pi and NIPI are useful in highlighting the contaminant effects on soil quality (Lee et al., 2006; Nemerow, 1991) where Pi considers the contamination by individual metals relative to a background level and NIPI provides a composite measurement across all metals. Equation (1) and (2) were used in determining Pi and NIPI in the soil samples respectively:

$$Pi = \frac{C_m Sample}{B_n} \tag{1}$$

In which C_m (mg/kg) represents measured concentration of individual metals while B_n represents the background level for individual metals (mg/kg). The WHO/FAO contamination limits were adopted as B_n values because of the high metal concentration observed in metal species like Cu in upstream control points, except for Mn. There are no contamination limits for Mn, as a result, background values upstream were used. Thus, the metal load in upstream and downstream samples were benchmarked using this approach and variations compared between the sites. From this, the degree of contamination for individual metals was estimated (Hooda, 2010).

$$NIPI = \sqrt{\left[\left(\frac{C_m sample}{B_n}\right)_{aver}^2 + \left(\frac{C_m sample}{B_n}\right)_{max}^2\right]} \tag{2}$$

Where NIPI is the comprehensive contamination index of the soil contaminant, $(C_m/B_n)_{aver}$ represents the mean value for the single pollution index P_i, while $(C_m/B_n)_{max}$ represents the highest value for the single pollution index P_i.

For vegetable samples, the Bioaccumulation Factor (BAF) and Contamination Load Index (CLI) of Oti (2015) were employed to evaluate metal contamination levels with reference to regulations and standards stipulated by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) (Ezeofor et al., 2019; Oti, 2015). The BAF is calculated as follows:

$$\boldsymbol{BAF = \frac{Cplant\left(\mu g g^{-1}(edible\ parts)\right)}{Csoil\ (\mu g g^{-1})\ Concentration}} \qquad (3)$$

In which C_{plant} serves as the concentration of individual metals in the edible parts of the vegetable plant, while C_{soil} represents metal concentration in the host soil.

CLI is calculated as follows:

$$\boldsymbol{CLI = \frac{C_{crop}}{MPC}} \qquad (4)$$

Where C_{crop} represents the concentration of individual metals in the edible parts of the vegetable plants and MPC represents the maximum permissible limits of individual metal concentration in vegetables (Table S6-2). Values of CLI < 1 indicate that the soil or plant material is not or low contaminated whereas CLI >1 indicates that crop is contaminated (Laniyan and Adewumi, 2020).

6.2.5. Analysis of Data

The data for metal concentration in soils and crops collected were assessed for homogeneity of variance using Levene's test to ensure that distribution of outcomes for independent groups are comparable, whilst the normality of the datasets was tested using Shapiro- Wilk and Kolmogorov-Smirnov (K–S) tests at 95% confidence level (Mishra et al., 2019; James et al., 2013) to assess whether the data samples is within some tolerance. The means and standard deviations were calculated for heavy metal content in soils and crops and are presented in the tables 6-1 and 6-3. An inferential statistical t-test, the independent t-test, was conducted to identify statistically significant variations between the control and test sites. Furthermore, heavy metal concentration levels in soils and vegetable samples were contrasted with the maximum permissible limits by WHO/ FAO (Table S6-2), background values and results by other researchers from previous studies conducted in Zambia. In Zambia, there are no guidelines for permissible limits of metal concentration in soils; hence, by default, the European Union (Papapreponis et al., 2006) and United Kingdom (UK guidelines, 1989) guidelines were adopted as acceptable limits for soil.

6.3. Results

6.3.1. Metal Concentration in Selected Soil Samples

The spatial distribution of metal concentration in soil samples at selected sampling sites are shown in Tables S6-5, S6-7, and S6-9 (see supplementary material). The mean, range and pollution index (NIPI) are reported in Tables 6-1 and 6-2. The results indicated that metal concentration and pH in the soils were similar and exhibited the following trend: Cu > Mn > Co > Zn > Pb (Table 6-1). The soil samples downstream of Chibuluma TSF, TSF15A, and TSF14 showed elevated levels of all metals when compared to upstream (Table S6-3). Concentration of metals Cu and Mn in soils were observed to be considerably higher (≈1482.7 ppm and ≈960.6 ppm, respectively), compared to Co, Zn and Pb (≈210.7 ppm, ≈117.8 ppm and ≈40.5 ppm, respectively). Lead concentration was found to be lower at soils near Chibuluma TSF and TSF15A (≈32 ppm), compared to soils from control sites (≈39.8 ppm). There was no significant variation observed in metal concentration throughout the sampling period with regards to Cu and Mn at selected sites; although lower concentrations of Cu (≈623 ppm) were recorded at sites near TSF14, while for Mn, low concentrations (≈322 ppm) were recorded near Chibuluma TSF (Table 6-1).

The comparison of pollution index (Pi) showed relatively high Cu contamination across all sites including control sites, compared to the rest of the metals (Table 6-2). The soils near Chibuluma TSF and TSF15A were observed to be considerably contaminated by Mn, while moderate Zn and Pb contamination was found across all sampling sites. In the control sites, contamination of Co and Pb was observed to be moderate while Mn and Zn contamination was low. The pH range in soil samples was from pH 5.1 to 7.9 (slightly acid to mildly alkaline). The levels of Cu (≈1609 ppm), Co (≈224 ppm), Mn (≈650 ppm) and Zn (≈213 ppm) in agriculture soils near Chibuluma TSF were above WHO/FAO acceptable limits given in Table S6-2. A similar pattern emerged for samples from TSF15A and TSF14 (Table 6-1; Figure 6-3).

The NIPI showed severe soil contamination by Cu, Co, Mn, and Zn, indicated by NIPI values above 3 (NIPI ≥ 3) across all sampling sites downstream of the TSFs (Table 6-2). Notably, the NIPI reported moderate to severe contamination of Cu and Co across all control sampling sites. No significant contamination was observed in control soil samples for metals Mn, Zn and Pb, illustrated by the low reported NIPI values (NIPI ≤ 2).

Table 6-1: Concentration of metals (ppm) in selected soil samples

Sampling Sites	Number of samples	Values	Cu	Co	Mn	Zn	Pb	pH
Gardens near Chibuluma TSF								
Garden soil: Sweet Potatoes	20	Range	998 - 2201	92 - 301	397 - 1002	95 - 236	24 - 112	5,3 - 7,5
		Mean ± SD	1464 ± 371	179 ± 66,5	610 ± 188	138 ± 38,3	54,8 ± 23,7	6,63 ± 0,7
Garden soil: Pumpkins	20	Range	998 - 2300	99 - 400	322 - 900	99,3 - 260	15 - 57	5,9 - 7,9
		Mean ± SD	1654 ± 389	213 ± 78,3	614 ± 154	170 ± 53	32,2 ± 10,4	6,8 ± 0,6
Garden soil: Amaranthus	20	Range	990 - 2072	111 - 401	387 - 1013	90 - 397	26 - 61	5,5 - 7,5
		Mean ± SD	1510 ± 363	236 ± 74,1	666 ± 172	233 ± 92,4	41,6 ± 8,9	6,5 ± 0,6
Garden soil: Cabbage	15	Range	998 - 2567	163 - 415	411 – 1265	187 - 489	19 - 42	5,1 - 7,2
		Mean ± SD	1806 ± 376	267 ± 70,9	710 ± 227	311 ± 87,2	32,6 ± 6,7	6,4 ± 0,6
Gardens near TSF14								
Garden soil: Sweet Potatoes	14	Range	1009 - 2382	119 - 255	678 - 1292	132 - 317	32 - 112	5,9 - 7,9
		Mean ± SD	1627 ± 376	198 ± 37,3	1056 ± 213	203 ± 62	59 ± 19,5	6,8 ± 0,6
Garden soil: Pumpkins	12	Range	623 - 1800	112 – 532	470 - 1148	63 - 217	19 - 68	5,6 - 7,2
		Mean ± SD	1182 ± 337	292 ± 122	830 ± 182	121 ± 37,2	40,5 ± 14,9	6,5 ± 0,7
Garden soil: Amaranthus	19	Range	846 - 2162	85 - 305	417 - 1000	86 - 431	31 - 64	5,5 - 7,7
		Mean ± SD	1240 ± 418	200 ± 82,2	674 ± 157	190 ± 86,2	47,5 ± 9,2	6,7 ± 0,6
Gardens near TSF15A								
Garden soil: Sweet Potatoes	20	Range	891 - 2130	80 - 217	603 - 1724	86 - 247	22 - 77	5,5 - 7,4
		Mean ± SD	1519 ± 352	122 ± 36,4	1009 ± 239	150 ± 45	47,6 ± 16,1	6,6 ± 0,6
Garden soil: Pumpkins	20	Range	860 - 1677	94 - 307	704 - 1621	90 - 416	26 - 82	5,8 - 7,5
		Mean ± SD	1221 ± 274	197 ± 67,3	958 ± 222	202 ± 88	46,3 ± 14,8	6,6 ± 0,5
Garden soil: Amaranthus	20	Range	754 - 2014	83 - 318	756 - 1553	100 - 302	19 - 60	5,5 - 7,8
		Mean ± SD	1305 ± 350	197 ± 79,6	1147 ± 263	200 ± 70,8	31,7 ± 11,3	6,5 ± 0,6
Garden soil: Cabbage	14	Range	868 - 2365	86 - 308	641 - 1287	96,2 - 331	21 - 44	5,6 - 8
		Mean ± SD	1442 ± 477	172 ± 76,8	1060 ± 307	204 ± 81	32,5 ± 6,7	6,8 ± 0,7
WHO/FAO		Limit	100	40		70	60	5,5 – 7,5

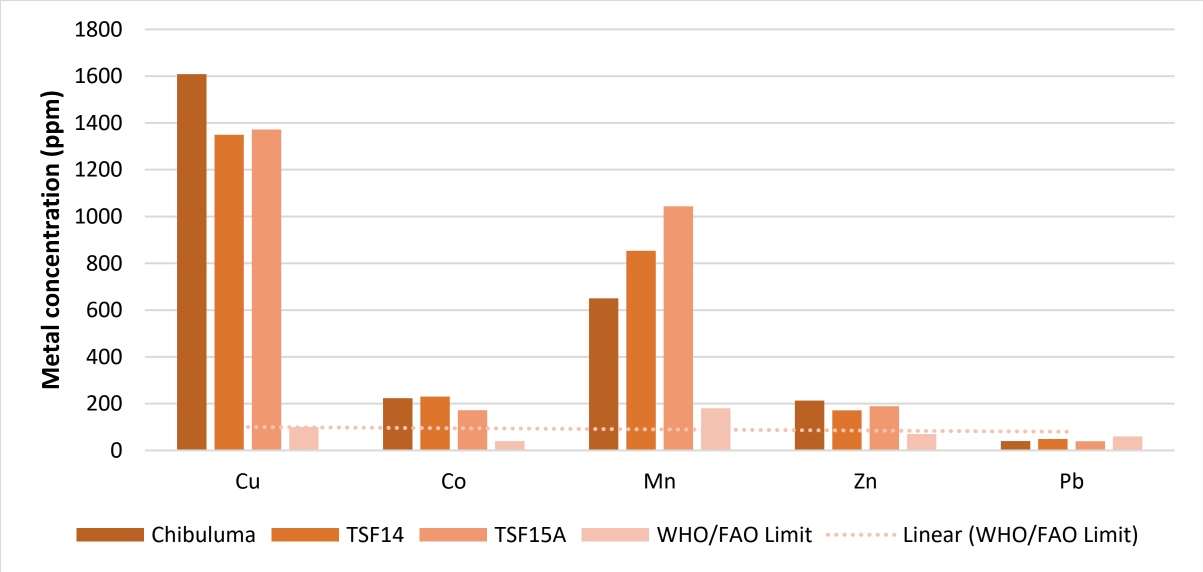


Figure 6-3: Metal concentration in soil samples near Chibuluma TSF, TSF14, TSF15A and WHO/FAO acceptable limits

Table 6-2: Heavy metal concentration (ppm), soil pollution index (Pi) and NIPI for agricultural soils near Chibuluma TSF, TSF14, TSF15A, and control sites. The colours denote the degree of contamination

Sampling site	Heavy Metals	Background Values (ppm)	Concentration (ppm)			Pollution Index (Pi)			NIPI/Contamination Level	
			Min	Mean	Max	Min	Mean	Max		
Chib TSF	Cu	100	998	1 608	2567	9,98	16,08	25,7	30,29	Severe
	Co	40	92	224	415	2,30	5,60	10,4	11,79	Severe
	Mn	200	322	379	1265	1,61	1,89	6,32	6,59	Severe
	Zn	70	90	213	489	1,29	3,04	6,99	7,62	Severe
	Pb	60	15	40,3	61	0,25	0,67	1,02	1,22	Alert
Control Sites	Cu	100	109	170	287	1,09	1,70	2,87	3,33	Severe
	Co	40	49,6	78,5	86	1,24	1,96	2,15	2,91	Moderate
	Mn	200	102	132	159	0,51	0,66	0,79	1,03	Light
	Zn	70	25	45	70	0,36	0,64	1,00	1,19	Light
	Pb	60	11	34,3	62	0,18	0,57	1,03	1,18	Light
TSF14	Cu	100	1009	1 350	2382	10,1	13,5	23,8	27,4	Severe
	Co	40	85	230	532	2,13	5,75	13,3	14,5	Severe
	Mn	180	417	853	1292	2,32	4,74	7,18	8,60	Severe
	Zn	70	63	171	431	0,9	2,44	6,16	6,62	Severe
	Pb	60	19	49	112	0,32	0,82	1,87	2,04	Moderate
Control Sites	Cu	100	91	157	210	0,91	1,57	2,10	2,62	Moderate
	Co	40	16	43	68	0,4	1,08	1,70	2,01	Moderate
	Mn	180	47	159	313	0,26	0,88	1,74	1,95	Light
	Zn	70	14	25	34	0,2	0,36	0,49	0,60	Safe
	Pb	60	18	21,7	26	0,30	0,36	0,43	0,56	Safe
TSF15A	Cu	100	754	1 372	2365	7,54	13,7	23,7	27,3	Severe
	Co	40	80	172	318	2	4,30	7,95	9,04	Severe
	Mn	159	603	1044	1724	3,79	6,57	10,8	12,68	Severe
	Zn	70	86	189	416	1,23	2,70	5,94	6,53	Severe
	Pb	60	19	39,5	82	0,32	0,66	1,37	1,52	Alert
Control Sites	Cu	100	243	415	510	9,72	4,15	5,10	6,57	Severe
	Co	40	84	99	115	2,1	2,48	2,88	3,79	Severe
	Mn	159	128	174	201	0,81	1,09	1,26	1,67	Light
	Zn	70	69	82,3	100	0,99	1,18	1,43	1,85	Light
	Pb	60	30	45,3	62	1,03	0,76	1,03	1,28	Light

6.3.2. Metal Concentration in Selected Vegetable Samples

The spatial variation of selected metal elements in the edible portion of vegetables investigated at selected sites near Chibuluma TSF, TSF15A, and TSF14 are shown in Figure 6-4 to 6-7 and in the supplemental section (Tables S6-4, S6-6, and S6-8 respectively). The findings indicate that concentration of metals in the edible part of the vegetables irrigated by water from the selected streams was considerably higher than permissible limits (WHO/FAO, 2007) (Table S6-2). Observably, accumulation of metals in the vegetables exhibited a similar trend compared to host soils, with accumulation of Cu > Mn > Zn > Co > Pb across all sites. No significant variation was observed between the selected sites in respect of Mn, Zn, and Pb concentrations. The Co concentration in sites near TSF14 (≈9.7 ppm) was lowest, compared to sites near Chibuluma TSF (≈16.6 ppm) and was highest at TSF15A ≈ 44.5 ppm). The box plot results indicated that there was significant variability in metal concentrations in vegetables from the sites (Figure 6-4 to 6-7). It can be inferred that the main probable sources for metal contamination were mine wastelands, based on high metal concentrations observed in downstream soil samples compared to upstream.

The concentration of metals under consideration was observed to be considerably higher in Amaranthus compared to other vegetables. In particular, Cu concentrations were significantly higher in Amaranthus at sites near Chibuluma TSF and TSF15A (≈540.6 ppm and ≈510.6 ppm, respectively) compared to the inactive TSF14 site (Table 6-3). The Cu concentration trend in the vegetables was as follows; Amaranthus > sweet potato leaves > pumpkin leaves > cabbage with significant high metal uptake above the permissible limit (3 ppm) (WHO/FAO, 2007) in all cases.

A comparative analysis of Mn concentration in the edible parts of vegetables, showed that Amaranthus contained the highest level from sites near TSF15A and Chibuluma TSF (≈227.8 ppm and 206 ppm), whilst cabbage recorded the lowest level of 52.2 ppm at sites near TSF15A. Manganese concentrations were observed to be above background values measured in food crops upstream. Zinc concentration in the vegetables ranged from of 11 to 117.5 ppm at sites near Chibuluma TSF, 19 to 81.6 ppm at sites near TSF14 and 15 to 102 ppm at sites near TSF15A, respectively. Notably, concentration of Zn was reported to be higher in pumpkin leaves (≈95.1 ppm) at sites near Chibuluma TSF. The edible parts of Amaranthus at sites near Chibuluma TSF accumulated the lowest Zn concentration. Accumulation of Co was equally observed to be significantly high in the vegetables. Amaranthus was reported to accumulate higher amount of Co (≈55.4 ppm) at sites near TSF15A among all the vegetables. High concentrations were equally observed in sweet potato leaves (≈50.8 ppm), pumpkin leaves (≈41.6 ppm) and cabbage (≈30.2 ppm) at sites near TSF15A compared to other sites. Overall, Co concentration was found to be in elevated levels in all the vegetables and above the permissible limit in the edible parts (WHO, 5 ppm). The concentration of Pb in vegetables ranged from 7 to 38.5 ppm at sites near Chibuluma TSF, 3.7 to 28 ppm near TSF14 and 4 to 26 ppm near TSF15A, respectively. Among the vegetables, pumpkin leaves had the highest amount of Pb (≈25.9 ppm, at sites near Chibuluma TSF). Concentration of Pb in the edible parts of all the vegetables equally exceeded the permissible safe limit of 0.3 ppm recommended by WHO/FAO.

Table 6-3: Heavy metal concentration (ppm) food crops grown near Chibuluma TSF, TSF14, & TSF15A

Sampling Sites	Number of samples	Values	Cu	Co	Mn	Zn	Pb
Near Chibuluma							
Sweet Potato Leaves	20	Range	55 - 300	3 - 36,5	51 - 144	11 - 100	8 - 38,5
		Mean ± SD	229 ± 49,5	19,1 ± 4,48	117 ± 18,3	58,3 ± 17,3	21 ± 11,2
Pumpkin Leaves	20	Range	99 - 279	10 - 47,5	41 - 163	42 - 118	9 - 37,5
		Mean ± SD	218 ± 72,5	29 ± 14,2	114 ± 56,9	95,1 ± 20,7	25,9 ± 7,22
Amaranthus	20	Range	318 - 1114	9 - 70	124 - 250	14 - 65	7 - 20,0
		Mean ± SD	540 ± 160	23,4 ± 18,9	206 ± 16	25 ± 8,52	10,9 ± 2,87
Cabbage	15	Range	109 - 211	17 - 34	48 - 89	33 - 76	8 - 23,0
		Mean ± SD	183 ± 25,1	19,7 ± 1,79	74,2 ± 6,91	54,6 ± 15,4	17,6 ± 4,34
Near TSF14							
Sweet Potato leaves	14	Range	67 - 201	9 - 21,0	24 - 114	19 - 81,6	7 - 12,7
		Mean ± SD	67,9 ± 26,3	6,38 ± 1,5	125 ± 79,5	39,6 ± 22,5	10,9 ± 2,51
Pumpkin Leaves	12	Range	78 - 132	7 - 21,0	47 - 118	37 - 61	3,7 - 11
		Mean ± SD	105 ± 20,4	11,4 ± 2,56	94,8 ± 25,6	51,3 ± 7,1	8,35 ± 1,57
Amaranthus	19	Range	84 - 350	6 - 24,0	68 - 171	19 - 66	8 - 28,0
		Mean ± SD	200 ± 86,2	11,4 ± 3,31	108 ± 28,2	51,6 ± 8,52	18,6 ± 4,8
Near TSF15A							
Sweet Potato Leaves	20	Range	101 - 358	21 - 68	99 - 282,5	29 - 102	7 - 16,0
		Mean ± SD	274 ± 58,4	50,8 ± 13,1	248 ± 70,9	76,1 ± 15,7	10,8 ± 3,88
Pumpkin Leaves	20	Range	91 - 223	18 - 59	68 - 131	23 - 99	5 - 18,0
		Mean ± SD	182 ± 35,2	41,6 ± 10,4	116 ± 10,5	70,2 ± 26,4	10,6 ± 4,13
Amaranthus	20	Range	217 - 617	13 - 70	128 - 302	23 - 95	7 - 26,0
		Mean ± SD	510 ± 116	55,4 ± 15,9	228 ± 25,9	56 ± 13,9	13,3 ± 3,77
Cabbage	14	Range	99 - 192	19 - 45	41 - 93	15 - 61	4 - 16,0
		Mean ± SD	121 ± 18,5	30,2 ± 8,09	52,2 ± 8,42	56,3 ± 23,5	10,6 ± 3,01
Mn Values Upstrem	5 Pumpkin Leaves Samples	Range	5,1 - 17	2 - 7,1	4,3 - 14	06-Feb	0,1 - 0,8
		Mean ± SD	14,4 ± 12.5	4,24 ± 1.38	10 ± 4,85	4,40 ± 1,32	0,26 ± 0,07
WHO/FAO (2007)		Limit	40	5		50 - 100	0,3

Note: Background values from pumpkin leaves upstream were used as reference points for Mn

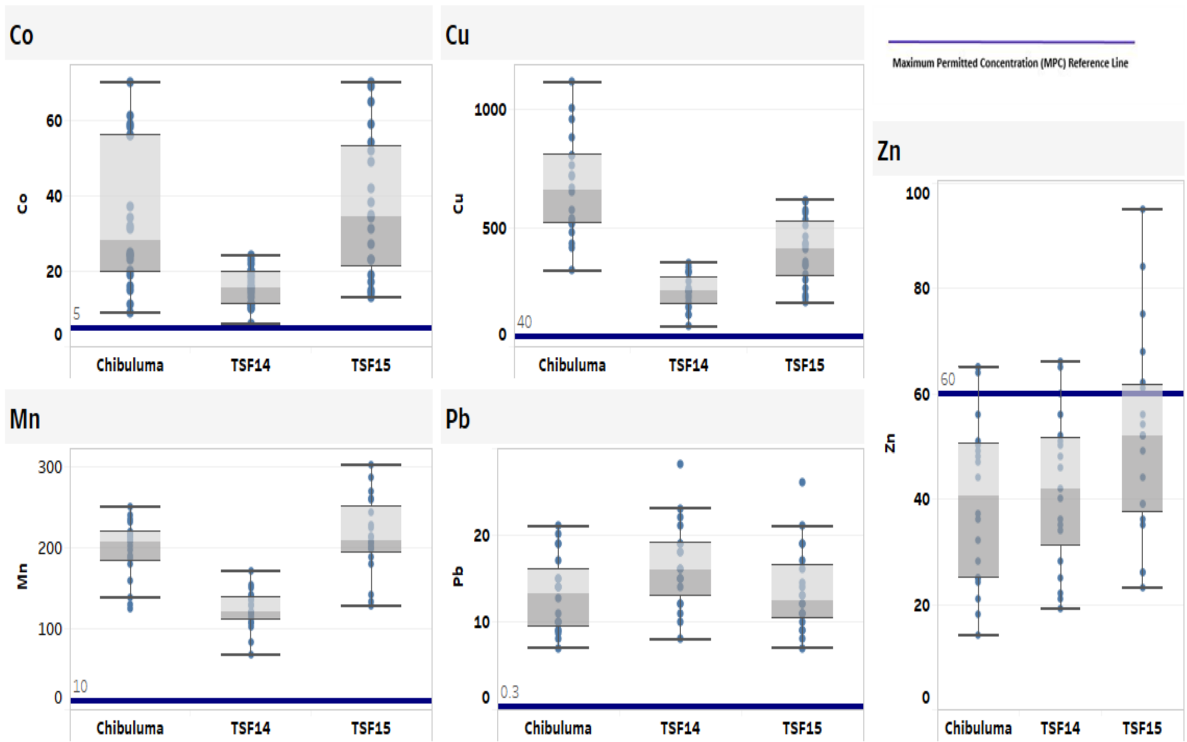


Figure 6-4: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese, (Zn) = Zinc and (Pb) = Lead, in Amaranthus in samples collected downstream of Chibuluma TSF, TSF14 and TSF15A. n = 20

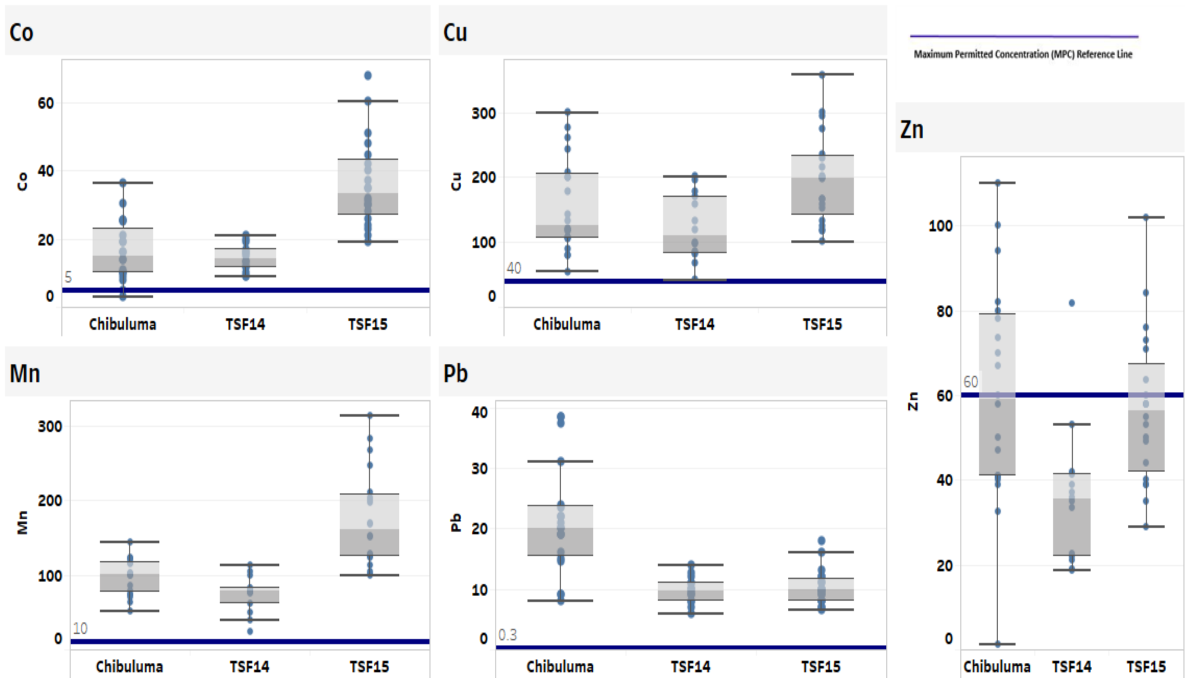


Figure 6-5: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese, (Zn) = Zinc and (Pb) = Lead, in sweet potatoes Leaves in samples collected downstream of Chibuluma TSF, TSF14 and TSF15A. n = 14 for TSF14 and 20 for Chibuluma TSF and TSF15A

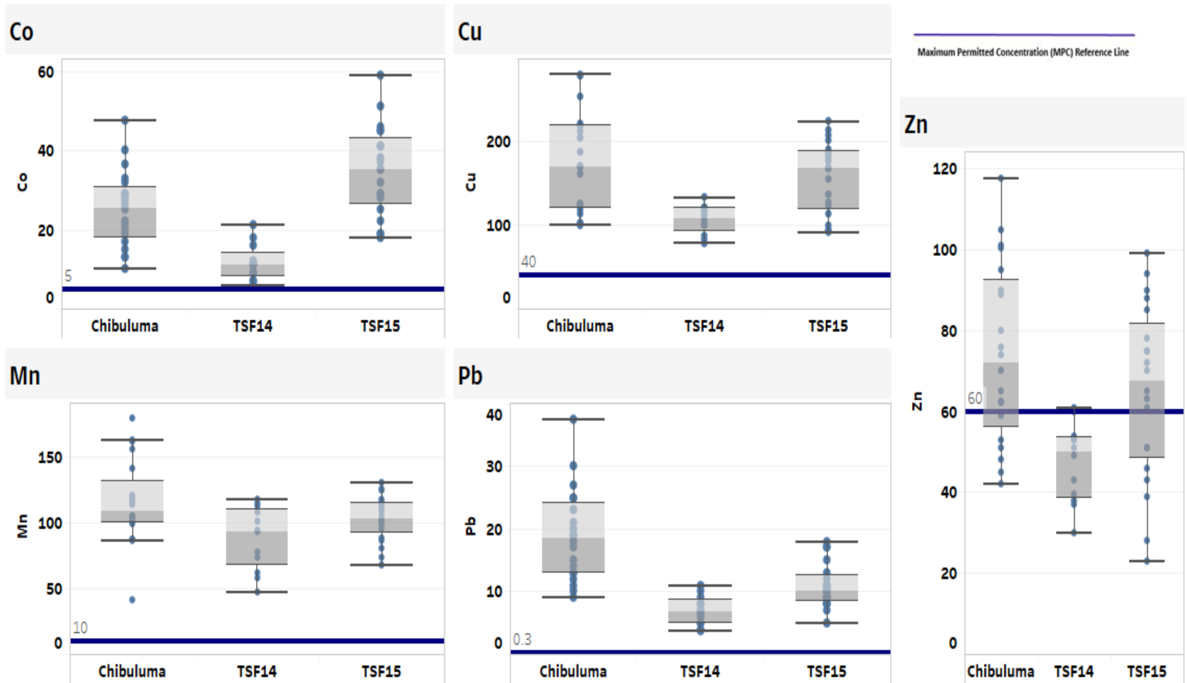


Figure 6-6: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese, (Zn) = Zinc and (Pb) = Lead, in pumpkin leaves in samples collected downstream of Chibuluma TSF, TSF14 and TSF15A. n = 12 for TSF14 and 20 for Chibuluma TSF and TSF15A

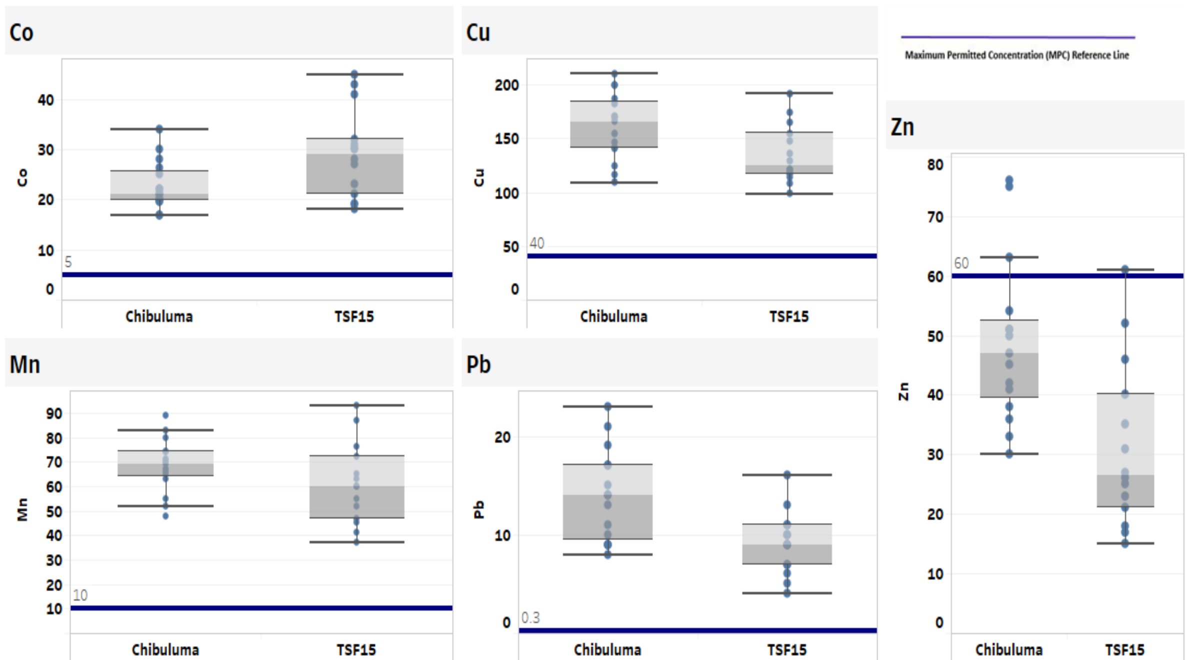


Figure 6-7: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese, (Zn) = Zinc and (Pb) = Lead, in cabbage in samples collected downstream of Chibuluma TSF (n = 15) and TSF15A (n = 14) for TSF14

Generally, the BAF values were <1 in the selected vegetables, the overall trend of BAF values for the metals in the selected vegetables was Zn > Pb > Mn > Co > Cu for potato leaves and pumpkin leaves, Pb > Cu > Zn > Mn > Co for Amaranthus leaves and Pb > Zn > Co > Cu > Mn for cabbage, respectively (Table 6-4). Within the sites near Chibuluma TSF, the highest BAF (≈0.36) of Cu was observed in Amaranthus, while Cabbage had the lowest BAF (≈0.1). The highest BAF value (≈0.42) for Co was observed in potato leaves at sites near Chibuluma TSF, showing significant difference from the other vegetables. The BAF for Mn showed no significant variation between the sampling sites, low BAF (≈0.05) were observed in cabbage at sites near TSF15A compared to the other sites. Higher Pb and Zn BAF (≈0.80 and ≈0.56, respectively) in pumpkin leaves were determined at sites near Chibuluma TSF in comparison to the other sites.

Table 6-4: Bioaccumulation factor (BAF) of potato leaves, pumpkin leaves, Amaranthus, and cabbage at sampling sites near Chibuluma TSF, TSF15A, and TSF14

Site	Chibuluma TSF			TSF14			TSF15A		
Elements	**Soil**	**Potato Leaves**		**Soil**	**Potatoe Leaves**		**Soil**	**Potato Leaves**	
	Mean	**Mean**	**BAF**	**Mean**	**Mean**	**BAF**	**Mean**	**Mean**	**BAF**
Cu	1464	229	0,16	1627	67,9	0,04	1519	274	0,18
Co	179	19,1	0,11	198	6,4	0,03	122	50,8	0,42
Mn	610	117	0,19	1056	125	0,12	1009	248	0,25
Zn	138	58,3	0,42	203	39,6	0,19	150	76,1	0,51
Pb	54,8	20,9	0,38	59	10,9	0,18	47,6	10,8	0,23
Elements	**Soil**	**Pumpkin Leaves**		**Soil**	**Pumpkin Leaves**		**Soil**	**Pumpkin Leaves**	
Cu	1654	218	0,13	1182	105	0,09	1221	182	0,15
Co	213	29	0,14	292	11,4	0,04	197	41,6	0,21
Mn	614	114	0,19	830	94,8	0,11	958	116	0,12
Zn	170	95,1	0,56	121	51,3	0,42	202	70,2	0,35
Pb	32,2	25,9	0,8	40,5	8,4	0,21	46,3	10,6	0,23
Elements	**Soil**	**Amaranthus Leaves**		**Soil**	**Amaranthus Leaves**		**Soil**	**Amaranthus Leaves**	
Cu	1510	541	0,36	1240	200	0,16	1305	511	0,39
Co	236	23,4	0,1	200	11,4	0,06	197	55,4	0,28
Mn	666	206	0,31	674	108	0,16	1147	228	0,2
Zn	233	25	0,11	190	51,6	0,27	200	56	0,28
Pb	41,6	10,9	0,26	47,5	18,6	0,39	31,7	13,3	0,42
Elements	**Soil**	**Cabbage**		**Soil**	**Cabbage**				
Cu	1806	183	0,1	1442	121	0,08			
Co	267	19,7	0,07	171,5	30,2	0,18			
Mn	710	74,2	0,1	1060	52,2	0,05			
Zn	311	54,6	0,18	204	56,3	0,28			
Pb	32,6	17,6	0,54	32,5	10,6	0,33			

Table 6-5 shows the contamination load index (CLI) of the vegetable samples from each of the sites near TSF. The results show that CLI of Pb at all the sampling sites (Chibuluma TSF, TSF14, and TSF15A) were > 6, higher than for other metal. The general trend of metal CLI observed was Pb > Mn > Co > Cu > Zn for potato leaves, pumpkin leaves, and cabbage; whilst for Amaranthus, the PI trend was Pb > Mn > Cu > Co > Zn. When comparing the vegetables from all of the sites, the trend for CLI for vegetable samples from sites near TSF15A was potato leaves > Amaranthus leaves > pumpkin leaves > cabbage, whilst the trend for TSF14 and Chibuluma TSF positioned the CLI higher in Amaranthus leaves (i.e., Amaranthus leaves > potato leaves > pumpkin leaves > cabbage).

Table 6-5: Pollution Indices of heavy metals in potato leaves, pumpkin leaves, Amaranthus, and cabbage from selected sites near Chibuluma TSF, TSF15A, and TSF14

Sites	Elements	MPC	Sweet Potato Leaves			Pumpkin leaves		
			Mean/SD	CLI		Mean/SD	CLI	
Chibuluma	Cu	40	156 ± 70.5	3.91	contaminated	173 ± 57.9	4.33	contaminated
	Co	5	16.4 ± 8.57	3.28	contaminated	25.2 ± 9.45	5.03	contaminated
	Mn	10	98.2 ± 23.2	9.82	contaminated	115 ± 68.7	11.5	contaminated
	Zn	60	60.3 ± 25.7	1	critical	74.3 ± 21.6	1.24	contaminated
	Pb	0.3	21.3 ± 8.87	71.1	contaminated	18.9 ± 7.18	63.1	contaminated
TSF14	Cu	40	122 ± 48.8	3.06	contaminated	106 ± 16.4	2.64	contaminated
	Co	5	14.4 ± 3.79	2.88	contaminated	11.5 ± 4.46	2.3	contaminated
	Mn	10	74.6 ± 23.7	7.46	contaminated	88.4 ± 23.2	8.84	contaminated
	Zn	60	35.8 ± 16.1	0.6	not contaminated	47.4 ± 9.36	0.79	not contaminated
	Pb	0.3	9.8 ± 2.14	32.7	contaminated	6.95 ± 2.24	23.2	contaminated
TS15A	Cu	40	198 ± 67.6	4.94	contaminated	156 ± 42.5	3.91	contaminated
	Co	5	36.3 ± 12.7	7.25	contaminated	34.7 ± 11.0	6.94	contaminated
	Mn	10	175 ± 62.6	17.5	contaminated	103 ± 16.7	10.3	contaminated
	Zn	60	56.8 ± 17.5	0.95	not contaminated	64.8 ± 21.3	1.08	Critical
	Pb	0.3	10.3 ± 2.85	34.2	contaminated	10.9 ± 3.43	36.3	contaminated
Sites	**Elements**	**MPC**	**Amaranthus Leaves**			**Cabbage**		
			Mean/SD	**CLI**		**Mean/SD**	**CLI**	
Chibuluma	Cu	40	670 ± 207	16.8	contaminated	161 ± 30.5	4.03	Contaminated
	Co	5	34 ± 18.5	6.8	contaminated	22.8 ± 4.71	4.57	Contaminated
	Mn	10	197 ± 35	19.7	contaminated	68.4 ± 10.8	6.84	Contaminated
	Zn	60	40 ± 15.8	0.67	not contaminated	48.8 ± 13.4	0.81	not contaminated
	Pb	0.3	13.2 ± 4.21	43.9	contaminated	13.9 ± 4.55	46.4	Contaminated
TSF14	Cu	40	229 ± 72.3	5.72	contaminated			
	Co	5	15.6 ± 4.86	3.12	contaminated			
	Mn	10	123 ± 23.8	12.3	contaminated			
	Zn	60	41.9 ± 14.4	0.7	not contaminated			
	Pb	0.3	16.4 ± 4.82	54.7	contaminated			
TS15A	Cu	40	402 ± 134	10.0	contaminated	135 ± 26.4	3.39	Contaminated
	Co	5	37.5 ± 18.6	7.49	contaminated	29.1 ± 8.61	5.82	Contaminated
	Mn	10	214 ± 46.5	21.4	contaminated	60.9 ± 16.1	6,.09	Contaminated
	Zn	60	51.4 ± 18.7	0.86	not contaminated	31.2 ± 13.43	0.52	not contaminated
	Pb	0,3	13.6 ± 47	45.4	contaminated	9.07 ± 3.08	30.2	Contaminated

6.4. Discussion

6.4.1. Metal Concentration in Selected Soil Samples

The irrigation of the vegetable crops with water resources within perimeters that are susceptible to impacts from mine waste in TSFs was observed to have a significant impact on both soils and vegetables. The high pollution index (PI) and metal concentration of Cu, Co, Mn, and Zn were found to be above the permissible limits across the sampling sites, except for Pb. The observed high metal concentration could be linked to land use activities located upstream of the study sites, especially the presence of TSFs. Metal concentration trends showed no significant variations across sites downstream of TSFs but considerably lower concentrations in the upstream soils, suggesting similarities in source contaminants and migration of these contaminants from TSFs. Observably, water from the selected streams was the principal source for crop irrigation by the surrounding communities. Similar trends have been reported by Ikenaka et al. (2010), Kapungwe (2013) and Lindahl (2010), higher metal concentration was observed in arable land near mine wastelands on the Copperbelt Province of Zambia. Metals were observed to accumulate in food crops via contaminated water, particularly, Cu was observed to be high in concentration when compared to arable land outside the influence of mining related activities. In both studies, high turbidity, TDS and metal concentration was observed in water resources used for crop irrigation, supporting the streams as primary sources for contamination.

Another potential source of metal contamination could be usage of agriculture products such as chemicals (Edokpayi et al., 2017; Zwolak et al., 2019). In particular, metals can be introduced in soils used for agriculture purposes through the application of both mineral and organic fertilizer. Sampling sites near Chibuluma TSF and TSF15A were observed to have a high level of agricultural activities and informal settlers compared to the sites TSF14. Consequently, metal contamination through agriculture could be happening in the catchments. Additionally, disposal of waste material from informal communities can be another plausible source (point source) contributing to soil contamination (Jackson et al., 2007). Although the contributions of agriculture practices and waste disposal remain possible, the result from this study suggests metal mobilization from mine waste into water resources as the major source of contamination, based on the low metal concentration trends reported in upstream soil samples compared to downstream (Table S6-3). The observed high metal concentration in the soils could significantly affect plant growth and food safety, noting that this also depends on the plant type, tendency to accumulate metals and their metabolism (Srivastava et al., 2017).

6.4.2. Metal Contamination in Selected Vegetable Samples

The results from the current study showed that the concentration of selected metals in the edible parts of the vegetables was significantly high. The irrigation of vegetables using watersheds impacted by the TSFs was observed to substantially influence metal uptake in the vegetables. Although the study was limited to compare metal contamination in between downstream and upstream vegetable samples, we argue that the differences in metal concentrations in soils between upstream and downstream, suggests that mine waste contributed significantly to high metal concentration through crop irrigation. The results from this study synthesize and links the variations in metal contamination to mine waste. Given the current magnitude of the impact of mine waste on the aquatic ecosystem and agro-ecosystem, management of the mine waste must be placed thoughtfully within a wider environmental context. As observed by Sonter et al. (2018), environmental burdens associated with mining are complex, and interact with other threatening processes over multiple scales, more knowledge is needed at spatial and temporal scale to determine best suite mitigation measures against crop contamination. Continued consumption of vegetable products irrigated by contaminated water resources might result in health complications (Rai et al., 2019). However, based on the functional traits of the vegetable samples, the bioaccumulation of metals was low (BAF < 1). Despite this, the high metal loading in the soil resulted in metal concentrations in vegetables exceeding WHO limits. Thus, the BAF cannot be used as a sole indicator of metal contamination risks but must be used in conjunction with soil analysis. Further, the BAF may be used to select crops less likely to accumulate metals into the edible components. The contamination load index (CLI) was more reliable in evaluating metal contamination of edible vegetables.

Observably, high CLI was reported for metal species Pb and Mn in all the selected vegetable samples. Consumption of Pb is highly poisonous affecting renal, neurological, musculoskeletal, reproductive, ocular, and other developmental parts of the body (Green and Pain, 2019). Similarly, chronic exposure to high levels of Mn may cause permanent neurological damage as observed in former Mn miners and smelters by O'Neal and Zheng, (2015). The study equally showed that CLI for elements Cu and Co was a cause for concern, indicating Cu and Co contamination, though the values were considerably lower than Pb and Mn. Studies by Taylor et al. (2020) have shown that mobilized Co and Cu in body tissues over a long time can cause fibrosis in the lungs, asthma, respiratory difficulty, nausea, dizziness, liver and lung failure. The CLI showed that the translocation of Zn was relatively low. Incidences of Zn toxicity are rare as it is an essential element that is required in the human diet in order to sustain normal brain activity and proper functions of the immune system, thus Zn deficiency may be detrimental to human health (Prasad, 2013).

The CLI observed in selected vegetable samples suggests the need for appropriate mitigation measures aimed at minimizing the impact of mining related activities on water resources and arable land. Improved water quality in streams through reduction in metal contamination could be extenuative for communities whose livelihoods depend on the impacted streams, resulting in healthier agriculture soils and safe vegetables. There is a need to safeguard strategically placed streams that supply water to numerous communities against severe environmental decline induced by anthropogenic activities and preserve the aquatic ecosystem and its services.

6.5. Conclusion

Safety of food, security, health and wellbeing of humans and environmental pollutants are indistinguishably interwoven. In most underdeveloped nations, crop irrigation with poorly treated water resources is one of the main sources of food crop contamination. In the current study, metal concentration in soil and vegetables irrigated by watersheds were assessed to determine the risk of metal mobilization from the mine waste. High concentration of metals was reported in vegetables grown in the selected soil samples, particularly Cu (55 – 1114 ppm), Co (3 – 70 ppm), and Pb (3.7 – 38.5 ppm) were higher than the WHO (2007) permissible limits. Manganese concentration was also observed to be higher than background value (10 ppm), thereby suggesting higher uptake of the elements from the soil. Although metal uptake was observed to be high in the selected vegetables (Amaranthus leaves, sweet potato leaves, pumpkin leaves, and cabbage), the BAF in all the edible components was < 1; however, due to the high soil contamination, the high metal content in plants could cause significant health problems. Comparatively, metal contamination from the sampling sites was significant, suggesting unsuitability of the selected water resources for crop irrigation. It can be inferred that the main probable sources for metal contamination were mine wastelands, based on variability of metal concentrations upstream and downstream, presented in Chapter 4 (section 4.3.2 and 4.3.3). Although no health risk reports associated with the consumption of metal contaminated vegetables have been reported in the study areas; attention must be paid to mitigating metal mobilization into water resources used for irrigation farming. Phytoremediation strategies such as phytomining can be used as both part of the rehabilitation strategy as well as for metal recovery from the tailings. Aside from remediation strategies, accurate and rapid mapping of contaminated soils is required to minimise food contamination in the catchment. Studies aimed at identifying food crops with metal exclusion strategies need to be enhanced to minimise metal uptake by humans and increase food security.

6.6. References

Ali, H., Khan, E., Ilahi, I., 2019. Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation [WWW Document]. Journal of Chemistry 2019. https://doi.org/10.1155/2019/6730305

Anyanwu, B.O., Ezejiofor, A.N., Igweze, Z.N., Orisakwe, O.E., 2018. Heavy Metal Mixture Exposure and Effects in Developing Nations: An Update. Toxics 6, 65. https://doi.org/10.3390/toxics6040065

Attiogbe, F., Nkansah, A., 2017. The Impact of Mining on the Water Resources in Ghana: Newmont Case Study at Birim North District (New Abirem). Energy and Environment Research 7, p27. https://doi.org/10.5539/eer.v7n2p27

Edokpayi, J.N., Odiyo, J.O., Durowoju, O.S., 2017. Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. Water Quality pp 401-416. https://doi.org/10.5772/66561

Emmanuel, A.Y., Jerry, C.S., Dzigbodi, D.A., 2018. Review of Environmental and Health Impacts of Mining in Ghana. Journal of Health and Pollution 8, 43–52. https://doi.org/10.5696/2156-9614-8.17.43

Ezeofor, C.C., Ihedioha, J.N., T.Ujam, O., Ekere, N.R., Nwuche, C.O., 2019. Human health risk assessment of potential toxic elements in paddy soil and rice (Oryza sativa) from Ugbawka fields, Enugu, Nigeria. Open Chemistry 17, 1050–1060. https://doi.org/10.1515/chem-2019-0121

Gola, D., Malik, A., Shaikh, Z.A., Sreekrishnan, T.R., 2016. Impact of Heavy Metal Containing Wastewater on Agricultural Soil and Produce: Relevance of Biological Treatment. Environmental Processes 3, 1063–1080. https://doi.org/10.1007/s40710-016-0176-9

Green, R.E., Pain, D.J., 2019. Risks to human health from ammunition-derived lead in Europe. Ambio 48, 954–968. https://doi.org/10.1007/s13280-019-01194-x

Hamilton, A.J., Stagnitti, F., Xiong, X., Kreidl, S.L., Benke, K.K., Maher, P., 2007. Wastewater Irrigation: The State of Play. Vadose Zone Journal 6, 823–840. https://doi.org/10.2136/vzj2007.0026

Hooda, P., 2010. Trace Elements in Soils. ISBN: 978-1-405-16037-7 EAN: 781405160377. https://doi.org/10.1002/9781444319477

Ikenaka, Y., Nakayama, S., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy Metal Contamination of Soil and Sediment in Zambia. African Journal of Environmental Science and Technology 4, 729–739. https://doi.org/10.4314/ajest.v4i11.71339

Jackson, V.A., Paulse, A.N., Van Stormbroek, T., Odendaal, J.P., Khan, W., 2007. Investigation into metal contamination of the Berg River, Western Cape, South Africa. Water SA 33. https://doi.org/10.4314/wsa.v33i2.49057

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. Introduction, in: James, G., Witten, D., Hastie, T., Tibshirani, R. (Eds.), An Introduction to Statistical Learning: With Applications in R, Springer

Texts in Statistics. Springer, New York, NY, pp. 1–14. https://doi.org/10.1007/978-1-4614-7138-7_1
Jhariya, D., Khan, R., Thakur, G.S., 2016. Impact of Mining Activity on Water Resource: An Overview study. Conference: national Seminar on Recent Practices and Innovations in Mining Industry (RPIMI 2016). At: Raipur
Jolly, Y.N., Islam, A., Akbar, S., 2013. Transfer of metals from soil to vegetables and possible health risk assessment. Springerplus 2, 385. https://doi.org/10.1186/2193-1801-2-385
Kapungwe, E.M., 2013. Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. Journal of Geography and Geology 5, p55. https://doi.org/10.5539/jgg.v5n2p55
Kapwata, T., Mathee, A., Sweijd, N., Minakawa, N., Mogotsi, M., Kunene, Z., Wright, C.Y., 2020. Spatial assessment of heavy metals contamination in household garden soils in rural Limpopo Province, South Africa. Environmental Geochemistry and Health 42, 4181–4191. https://doi.org/10.1007/s10653-020-00535-0
Kookana, R.S., Drechsel, P., Jamwal, P., Vanderzalm, J., 2020. Urbanisation and emerging economies: Issues and potential solutions for water and food security. Science of The Total Environment 732, 139057. https://doi.org/10.1016/j.scitotenv.2020.139057
Laniyan, T.A., Adewumi, A.J., 2020. Evaluation of Contamination and Ecological Risk of Heavy Metals Associated with Cement Production in Ewekoro, Southwest Nigeria. Journal of Health and Pollution 10. https://doi.org/10.5696/2156-9614-10.25.200306
Lee, C.S., Li, X., Shi, W., Cheung, S.C., Thornton, I., 2006. Metal contamination in urban, suburban, and country park soils of Hong Kong: A study based on GIS and multivariate statistics. Science of The Total Environment 356, 45–61. https://doi.org/10.1016/j.scitotenv.2005.03.024
Lindahl, J., 2014. Environmental Impact of Mining in Zambia: Towards Better Environmental Management and Sustainable Exploitation of Mineral Resources, s.l.: s.n.
Lu, Y., Yao, H., Shan, D., Jiang, Y., Zhang, S., Yang, J., 2015. Heavy Metal Residues in Soil and Accumulation in Maize at Long-Term Wastewater Irrigation Area in Tongliao, China [WWW Document]. Journal of Chemistry. https://doi.org/10.1155/2015/628280
Environmental Impact of Mining in Zambia: Towards Better Environmental Management and sustainable exploitation of mineral resources, s.l.:s.n.Lu, Y., Yao, H., Shan, D., Jiang, Y., Zhang, S., Yang, J., 2015. Heavy Metal Residues in Soil and Accumulation in Maize at Long-Term Wastewater Irrigation Area in Tongliao, China [WWW Document]. Journal of Chemistry 2015. https://doi.org/10.1155/2015/628280
Masindi, V., Muedi, K.L., 2018. Environmental Contamination by Heavy Metals. Intechopen 2018, 115-133. https://doi.org/10.5772/intechopen.76082
Mcintyre, N., Angarita, M., Fernández, N., Camacho-Botero, L., Pearse, J., Huguet, C., Restrepo, O., Ossa Moreno, J., 2018. A Framework for Assessing the Impacts of Mining Development on Regional Water Resources in Colombia. Water 10, 268. https://doi.org/10.3390/w10030268
Mishra, P., Pandey, C.M., Singh, U., Gupta, A., Sahu, C., Keshri, A., 2019. Descriptive Statistics and Normality Tests for Statistical Data. Ann Card Anaesth 22, 67–72. https://doi.org/10.4103/aca.ACA_157_18
Mudenda, L., 2018. Assessment of Water Pollution Arising from Copper Mining In Zambia: A Case Study of Munkulungwe Stream in Ndola, Copperbelt Province. Master's thesis, University of Cape Town, South Africa.
Nakayama, S.M.M., Ikenaka, Y., Muzandu, K., Choongo, K., Oroszlany, B., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy metal accumulation in lake sediments, fish (Oreochromis niloticus and Serranochromis thumbergi), and crayfish (Cherax quadricarinatus) in Lake Itezhi-tezhi and Lake Kariba, Zambia. Archives of Environmental Contamination and Toxicology 59, 291–300. https://doi.org/10.1007/s00244-010-9483-8
Nemerow, N.L., 1991. Stream, lake, estuary, and ocean pollution, 2nd edition. New York: Van Nostrand Reinhold

Nolde, E., 2005. Greywater recycling systems in Germany--results, experiences and guidelines. Water Science and Technology 51, 203–210.

Nwankwo, J.N., Elinder, C.-G., 1979. Cadmium, lead and zinc concentrations in soils and in food grown near a zinc and lead smelter in Zambia. Bulletin of Environmental Contamination and Toxicology 22, 625–631. https://doi.org/10.1007/BF02026998

Obasi, P.N., Akudinobi, B.B., 2020. Potential health risk and levels of heavy metals in water resources of lead–zinc mining communities of Abakaliki, southeast Nigeria. Applied Water Science 10, 184. https://doi.org/10.1007/s13201-020-01233-z

Ochieng, G., Seanego, E., Nkwonta, O., 2010. Impacts of mining on water resources in South Africa: A review. Scientific Research and Essays 5, 3351–3357.

O'Neal, S.L., Zheng, W., 2015. Manganese Toxicity Upon Overexposure: A Decade in Review. Current Environmental Health Reports 2, 315–328. https://doi.org/10.1007/s40572-015-0056-x

Oti, W.O., 2015. Bioaccumulation Factors and Pollution Indices of Heavy Metals in Selected Fruits and Vegetables from a Derelict Mine and Their Associated Health Implications. International Journal of Environment and Sustainability 4. https://doi.org/10.24102/ijes.v4i1.548

Papapreponis, P., Robertson, R., Roworth, M., Ogston, S. A., & William, F. L. R. (2006). Levels of inorganics and organics in soils: When is high, high? Environment and Health International, 8(1), 4-12.

Prasad, A.S., 2013. Discovery of Human Zinc Deficiency: Its Impact on Human Health and Disease. Advances in Nutrition 4, 176–190. https://doi.org/10.3945/an.112.003210

Rai, P.K., Kumar, V., Lee, S., Raza, N., Kim, K.-H., Ok, Y.S., Tsang, D.C.W., 2018. Nanoparticle-plant interaction: Implications in energy, environment, and agriculture. Environment International 119, 1–19. https://doi.org/10.1016/j.envint.2018.06.012

Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F., Kim, K.-H., 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment International 125, 365–385. https://doi.org/10.1016/j.envint.2019.01.067

Saldías, C., Speelman, S., Drechsel, P., Van Huylenbroeck, G., 2017. A livelihood in a risky environment: Farmers' preferences for irrigation with wastewater in Hyderabad, India. Ambio 46, 347–360. https://doi.org/10.1007/s13280-016-0824-3

Shakir, E., Zahraw, Z., Al-Obaidy, A.H.M.J., 2017. Environmental and health risks associated with reuse of wastewater for irrigation. Egyptian Journal of Petroleum 26, 95–102. https://doi.org/10.1016/j.ejpe.2016.01.003

Sonter, L.J., Ali, S.H., Watson, J.E.M., 2018. Mining and biodiversity: key issues and research needs in conservation science. Proceedings of the Royal Society: Biological Sciences 285. https://doi.org/10.1098/rspb.2018.1926

Srivastava, V., Sarkar, A., Singh, S., Singh, P., de Araujo, A.S.F., Singh, R.P., 2017. Agroecological Responses of Heavy Metal Pollution with Special Emphasis on Soil Health and Plant Performances. Frontiers in Environmental Science. 5. https://doi.org/10.3389/fenvs.2017.00064

Suruchi, Khanna, P., 2011. Assessment of Heavy Metal Contamination in Different Vegetables Grown in and Around Urban Areas. Research Journal of Environmental Toxicology 5, 162–179. https://doi.org/10.3923/rjet.2011.162.179

Taylor, A.A., Tsuji, J.S., Garry, M.R., McArdle, M.E., Goodfellow, W.L., Adams, W.J., Menzie, C.A., 2020. Critical Review of Exposure and Effects: Implications for Setting Regulatory Health Criteria for Ingested Copper. Environmental Management 65, 131–159. https://doi.org/10.1007/s00267-019-01234-y

Tóth, G., Hermann, T., Da Silva, M.R., Montanarella, L., 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environment International 88, 299–309. https://doi.org/10.1016/j.envint.2015.12.017

United Kingdom Regulation. (1989). Statutory Instrument 1989 No. 1263 Sludge (Use in Agriculture) Regulation. London: Ministry of Agriculture Food and Fisheries.

WHO / FAO. Expert committee on food additives. Cambridge: Cambridge University Press; 2007. p. 329–36.
Wuana, R.A., Okieimen, F.E., 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation [WWW Document]. ISRN Ecology 2011, 1-20. https://doi.org/10.5402/2011/402647
Zwolak, A., Sarzyńska, M., Szpyrka, E., Stawarczyk, K., 2019. Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: a Review. Water, Air and Soil Pollution 230, 164. https://doi.org/10.1007/s11270-019-4221-y

6.7. Supplementary Material

Table S6-1: Grouping Criterion for Soil Analysis (Hooda, 2010)

Grade division	NIPI	Contamination level	Contamination degree
1	NIPI ≤ 0.7	Safe	Clean
2	0.7 ≤ NIPI ≤ 1	Alert	Still clean
3	1 < NIPI ≤ 2	Light contamination	soil slightly contaminated, crop beginning to be contaminated
4	2 < NIPI ≤ 3	Moderate contamination	Soil and crops moderately contaminated
5	NIPI > 3	Severe contamination	Soil and crops seriously contaminated

Table S6-2: WHO (2007) Allowable Limits for Vegetables and Soils

Metals	Units	Criteria		Source
		Vegetables	Soil	
Copper	ppm	40	100	WHO, FAO
Cobalt	ppm	5	40	WHO, FAO
Manganese	ppm	Background Value (10)	Background Value (10)	
Zinc	ppm	60	70	WHO, FAO
Lead	ppm	0,3	60	WHO, FAO

Table S6-3: Concentration of metals in ppm, observed in soil samples from control points near Chibuluma TSF, TSF15A, and TSF14

Control Sites	Cu	Co	Mn	Zn	Pb
Near Chibuluma TSF					
Site 1	109	49,6	196	25	30
Site 2	287	71	210	40	11
Site 3	113	86	204	70	62
Mean ± SD	170 ± 83	78,5 ± 7,5	203 ± 5	45 ± 18,7	34,3 ± 21,1
Near TSF14					
Site 1	210	16	185	14	21
Site 2	91	45	164	34	26
Site 3	170	68	191	27	18
Mean ± SD	157 ± 49,4	43 ± 21,3	180 ± 10	25 ± 8,3	21,7 ± 3,3
Near TSF15A					
Site 1	91	44	153	69	30
Site 2	243	105	135	100	44
Site 3	210	98	189	78	62
Mean ± SD	181 ± 65,3	82,3 ± 27,3	159 ± 22,5	82,3 ± 13	45,3 ± 13,1

Table S6-4: Metal concentrations in selected vegetable samples across the sampling sites near Chibuluma TSF

Sample No.	Site 1 - Sweet Potato Leaves					Site 3 - Amaranthus Leaves				
	Cu	**Co**	**Mn**	**Zn**	**Pb**	**Cu**	**Co**	**Mn**	**Zn**	**Pb**
1	208	16	102	50	14,5	719	58	213	14	12,7
2	179	21	99	41	9	517	20	230	25	8
3	260	14	115	80	23,9	670	9	189	37	15
4	300	19	144	47	19	479	11	201	28	10
5	200	25,5	124	73,5	38,5	318	19	197	21	9
6	244	16	79	32,5	19	540	24	206	25	10
7	277	36,5	118	100	37,5	572	32	159	18	8,7
8	122	10	101	41	19	412	16	218	64	19
9	200	25,5	73,5	94	38,5	431	15	186	44	21
10	132	10	74	82	20	534	24	124	65	14
11	105	8	117	11	22	523	31	208	24	11
12	55	3	86	110	20	804	25	129	36	9
13	80	5	51	40,8	22	1001	23	137	47	14
14	108	19	121	60	9	1114	59	239	49	15
15	79	11	64	39	15	716	56	216	56	19
16	117	25	101	78	21	764	34	234	65	17
17	120	9	122	67	8	804	37	179	32	14
18	90	14	71	70,1	23,4	955	61	212	51	20
19	142	10	103	40	16	651	70	250	48	7
20	107	30,3	99	58	31	877	56	218	50	10
	Site 2 - Pumpkin Leaves					**Site 4 - Cabbage**				
1	279	47,5	162,5	62,5	27	211	19,5	71	76	13
2	277	36,5	180	118	37,5	187	17	80	51	17
3	122	10	41	101	19	147	20	67	47	21
4	160	22	87	105	25	170	22	83	63	14
5	253	29,1	101	90	21	200	20	70	36	23
6	218	27	114	95,1	25	182	19,7	74	54	17
7	211	32	99	62	30	166	21	69	51	19
8	118	26	103	45	23	141	22	63	30	9
9	186	19	88	51	18	124	17	52	45	11
10	125	20	105	74	13	155	34	55	42	14
11	102	13	142	80	11	116	26,2	89	38	9
12	113	29	163	65	14	171	25	66	50	10
13	204	17	119	76	12	142	30	65	41	15
14	217	15	121	101	19	200	21	48	75	9
15	169	21	88	48	17	109	28	74	33	8
16	123	40	101	53	20					
17	99	25	115	42	15					
18	102	28	117	89	9					
19	168	33	156	70	10					
20	220	13	105	59	13					

Table S6-5: Metal concentrations in selected soil samples hosting vegetables near Chibuluma TSF

Sample No.	Site 1 - Sweet Potato Leaves						Site 3 - Amaranthus Leaves					
	Cu	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**
1	1 895	295	490	145	36	5,5	990	380	512	90	50	6,2
2	1 230	105	420	135	34	6	1124	269	653	103	26	6,8
3	1 390	185	450	110	24	6,8	1001	173	490	185	40	7,3
4	1 001	215	500	99	31	6,1	1400	200	830	127	41	6,9
5	1 589	219	397	113	42	7	1278	215	387	115	37	6,3
6	1761	190	1002	200	44	6,9	1158	267	414	264	38	6,7
7	1059	235	475	175	38	6,8	1781	263	393	217	42	5,9
8	2008	251	900	125	50	7,3	1110	111	522	201	45	5,5
9	1042	108	634	132	50	7,4	2041	227	678	132	34	7,2
10	2201	250	1002	98	54	7,5	1674	326	659	251	57	6,6
11	1370	301	811	112	74	7,4	1832	231	890	397	41	5,7
12	1275	102	460	140	56	6,5	2001	140	779	319	55	6
13	1810	154	712	132	112	6,8	1731	247	705	220	46	6,3
14	998	100	589	150	86	6,7	2072	209	815	365	61	6,2
15	1135	92	585	122	112	6,8	1019	183	901	300	38	7,5
16	1560	116	601	104	63	7,1	1577	262	1013	198	29	7
17	1374	121	444	95	54	7,1	2016	131	709	212	33	5,8
18	2001	215	510	118	54	5,6	1475	292	633	229	35	6,4
19	1570	201	758	210	39	6	1417	401	742	370	47	7
20	1009	118	450	236	43	5,3	1500	193	599	361	36	6,8
	Site 2 - Pumpkin Leaves						**Site 4 - Cabbage**					
1	1198	184	322	112	15	7,5	2100	370	600	220	26	6,6
2	2150	400	759	183	30	6,3	2003	415	586	303	31	6,3
3	998	328	570	105	22	5,9	1570	289	417	212	19	5,9
4	2300	270	620	152	34	7	998	260	509	312	40	7
5	1260	205	480	100	20	7,9	1320	300	411	410	41	7,2
6	1581	277	550	130	24	7,5	1598	326	504	391	34	6,6
7	1792	208	896	99,3	38	6,3	1868	163	695	489	23	5,4
8	1600	307	700	204	41	6,9	1981	235	865	213	39	6,1
9	1543	202	511	109	32	7,1	1967	301	1265	345	36	7
10	1670	309	603	181	37	7	2018	189	709	187	42	5,9
11	2001	180	519	119	50	7	2567	201	716	292	38	6,6
12	1402	218	587	260	46	6,5	1997	168	863	411	27	6,7
13	1781	175	900	156	57	7,1	2106	218	983	289	34	7,1
14	2341	162	706	213	30	7	1432	313	923	371	30	7
15	1086	215	621	269	29	7,7	1560	256	610	225	29	5,1
16	1510	99	628	190	25	7						
17	1877	182	892	215	19	6						
18	1659	114	420	191	32	6,1						
19	1186	123	527	165	36	6,2						
20	2152	109	461	249	27	5,9						

Table S6-6: Metal concentrations in selected vegetable samples across the sampling sites near TSF14

Sample No.	Site 1 - Sweet Potato Leaves					Site 3 - Amaranthus Leaves				
	Cu	Co	Mn	Zn	Pb	Cu	Co	Mn	Zn	Pb
1	132,2	13,5	99	81,6	12	214	6	118	46	28
2	41,8	16,8	83,1	41,4	12,7	134	11,5	68	48	18
3	83,6	15,9	77,6	18,7	7,8	334	14	142	56	15
4	81,9	9,05	49,1	22,6	8,1	235	10	82	42	16
5	100	11,5	114	33,5	14	84	15,5	129	66	16
6	67	10	24	39	9	200	11	107	51	18
7	86	13	79	22	11	186	10	118	52	8
8	120	16	75	21	9	312	15	135	60	11
9	158	9	61	37	7	309	22	121	35	15
10	201	12	104	53	10	231	23	149	22	23
11	195	19	82	35	11	165	24	112	25	21
12	178	21	78	42	9,6	162	19	102	36	19
13	170	15	80	36	10,1	235	20	128	50	12
14	97	20	39	19	6	313	16	136	65	14
	Site 2 - Pumpkin Leaves					271	13	107	21	19
1	111	11	101	53,2	6,54	350	11	115	28	22
2	78	16	93	49	8,1	235	17	153	19	15
3	86	9	47	61	9	247	20	171	34	12
4	120	12	115	39,4	7,1	131	18	139	40	10
5	132	9,1	118	54	11					
6	105	11	94	51	8					
7	99	6	58	38	4					
8	119	7	62	60	3,7					
9	82	6,8	108	37	5					
10	100	11	74	43	10					
11	116	18	78	53	5					
12	121	21	113	30	6					

Table S6-7: Metal concentrations in selected soil samples near TSF14

Sample No.	Site 1 - Sweet Potato Leaves						Site 3 - Amaranthus Leaves					
	Cu	**Co**	**Mn**	**Zn**	**Pb**	**pH**	**Cu**	**Co**	**Mn**	**Zn**	**Pb**	**pH**
1	2382	255	1292	185	112	7	1110	412	522	107	50	5,5
2	1542	200	1000	133	63	7,2	2114	271	876	231	64	7,7
3	1400	210	980	244	54	6,6	1460	300	659	152	47	7,6
4	2210	250	1115	308	54	7,9	1238	189	520	97	51	7,5
5	1200	230	1600	225	70	7,7	2000	305	635	119	42	6,1
6	1756	229	1197	139	76	7	1584	295	642	141	50	6
7	1592	215	899	182	55	6,4	2162	99	905	204	49	6,1
8	1617	181	974	181	32	6	998	113	531	119	51	5,8
9	1009	119	794	129	74	6,1	846	85	664	86	32	7
10	1676	165	1036	165	40	7	901	160	708	121	37	5,7
11	1479	211	1095	132	61	5,9	899	129	813	192	58	6,3
12	2115	153	1124	237	48	6,7	1000	217	724	312	41	7,1
13	1579	165	1004	268	51	6,4	928	140	688	431	43	6,8
14	1227	184	678	317	36	7,1	1123	168	1000	189	39	6,6
Site 2 - Pumpkin Leaves							875	208	450	309	50	6,5
1	1279	128	689	100	68	6,7	1002	162	526	201	62	6,9
2	1800	370	810	84	43	7,1	916	137	417	158	61	7
3	1345	408	470	119	28	7,3	1326	203	870	172	44	6,8
4	1076	203	1006	128	39	5,6	1072	212	654	265	31	7,3
5	1010	165	951	99	62	5,6						
6	1302	254	785	106	48	6,4						
7	878	112	943	63	45	7,2						
8	911	418	632	217	34	7						
9	1512	324	1001	158	20	5,6						
10	839	532	705	130	19	5,8						
11	623	270	820	123	28	6,7						
12	1612	315	1148	127	52	7						

Table S6-8: Metal concentrations in selected vegetable samples across the sampling sites near TSF15A

Sample No.	Site 1 - Sweet Potato Leaves						Site 3 - Amaranthus Leaves				
	Cu	Co	Mn	Zn	Pb		Cu	Co	Mn	Zn	Pb
1	200	44,5	125,5	102	9,5		606	65	269	68	12
2	358	30	314	73	6,5		617	42	243	75	19
3	301	60,5	204,5	58	9		464	31	198	52	11
4	295	68	282,5	63,5	18		305	70	224	49	9
5	216	51	211	84	11		561	69	205	36	14
6	274	48	247	76	8		510	54	227	56	13
7	158	32	170	49	7		279	59	258	39	8
8	201	26	105	50	10		421	38	211	62	7
9	119	35	99	39	8		341	15	286	84	19
10	230	30	151	58	13		433	27	214	95	16
11	153	42	129	40	16		532	49	128	26	17
12	167	19	124	53	10		184	35	201	61	21
13	132	28	169	71	12		202	14	187	54	26
14	118	31	201	44	7		245	23	142	52	13
15	236	23	267	60	8,1		358	34	207	44	14,5
16	198	37	114	29	12		217	52	132	35	9
17	199	40	127	35	11,3		576	13	198	39	12
18	167	35	153	39	10		347	17	302	26	11
19	126	21	197	55	9		431	19	179	23	10
20	101	24	101	58	9,8		406	23	260	52	10,9
	Pumpkin Leaves						Cabbage				
1	207	35	113	99	10		121	32	60	18	16
2	189	41	102	78	17		114	18	41	25	7
3	223	59	131	63	8		155	43	52	61	10
4	167	45	125	23	5		99	31	63	40	9
5	122	28	110	88	13		117	27	45	35	11
6	181	46	116	70,2	10		121	30,2	55	46	10
7	154	35	104	75	9		108	45	76	52	9
8	201	37	101	61	11		165	28	47	26	11
9	187	51	89	39	8		148	19	60	21	13
10	168	19	74	46	9		174	23	37	17	6
11	99	22	95	43	11		117	30	93	23	9
12	113	18	108	72	7		129	21	87	31	7
13	91	32	98	51	8		192	19	72	27	5
14	92	25	87	90	12		136	41	65	15	4
15	176	45	126	65	17						
16	127	41	118	75	10						
17	212	38	114	28	18						
18	136	29	68	51	15						
19	185	19	81	94	11						
20	94	29	100	85	9						

Table S6-9: Metal concentrations in selected soil samples near TSF15A

Sample No.	Site 1 - Sweet Potato Leaves							Site 3 - Amaranthus Leaves					
	Cu	Co	Mn	Zn	Pb	pH		Cu	Co	Mn	Zn	Pb	pH
1	1760	80	741	86	47	5,5		1901	83	1520	100	25	5,5
2	950	99	882	95	22	6,1		775	126	1553	298	26	7,8
3	1766	85	603	211	24	6,7		1600	131	907	112	29	5,2
4	1639	101	947	100	36	5,8		754	92	1383	129	23	6,6
5	1551	90	1724	101	35	7		1000	101	1427	302	31	7,3
6	1533	91	979	110	32	6,2		1206	106	1358	108	26	6,8
7	1302	116	1013	152	41	6		1766	288	904	175	28	6,6
8	1 598	129	1094	154	36	5,9		1301	314	1025	204	21	6
9	2 130	151	942	113	43	6		1057	227	1260	213	19	7,1
10	1 903	115	1005	171	55	6,4		964	318	756	225	40	7,1
11	999	86	900	104	60	7		1483	198	925	279	35	7
12	1 190	101	793	138	39	6,1		2014	152	814	191	14	6,9
13	1823	98	1229	201	77	7,2		1446	249	905	142	29	5,8
14	1452	120	1011	173	82	7,4		1641	312	1299	181	37	5,9
15	1204	119	896	184	45	7,3		1002	160	1512	109	30	6,3
16	2012	150	1415	128	43	7		998	296	863	206	32	6,1
17	1874	200	1233	142	70	7		1100	137	1092	170	25	7
18	1209	217	966	215	67	6,9		1270	211	864	312	60	6,9
19	1600	154	865	247	51	6,9		1500	165	1178	325	55	6,2
20	891	138	937	174	46	7		1315	264	1402	214	48	6,7
	Site 2 - Pumpkin Leaves							Site 4 - Cabbage					
1	1000	111	670	213	40	7,5		1890	125	806	99	44	7,1
2	860	235	1621	269	29	6,9		1769	107	1685	101	29	8
3	1310	97	826	90	31	6,8		1800	98	907	278	43	6,2
4	1778	182	1298	115	50	7		2365	104	1617	192	30	7,1
5	965	94	720	119	33	6,4		1979	86	1398	111	32	7,9
6	1182,6	123	1027	115,6	66	6,9		1960	104	1283	96,2	36	7,2
7	1152	360	961	149	65	5,8		1117	127	1091	199	31	6,5
8	972	182	986	102	70	6		1003	273	798	331	40	6,3
9	1008	307	1081	294	34	6,2		984	212	865	206	26	6,2
10	894	204	704	321	82	6		1257	308	1041	234	28	6,9
11	1670	153	992	416	41	6,6		1098	263	905	161	34	7
12	1452	265	1015	209	26	5,9		897	285	641	303	21	7,1
13	1203	218	857	212	42	6,4		1204	162	846	224	24	5,9
14	998	174	943	315	45	7		868	147	956	327	37	5,6
15	1019	148	722	102	35	7,2							
16	1215	200	959	231	39	7,1							
17	1375	223	705	252	52	6,5							
18	1111	172	862	153	43	6,7							
19	1581	254	1048	234	60	6,2							
20	1677	242	1152	133	42	6,7							

CHAPTER 7: POTENTIAL REHABILITATION OF ECOLOGICAL INFRASTRUCTURE THROUGH PHYTOMINING IN RESPONSE TO METAL MOBILIZATION FROM MINE TAILINGS

Copper mining changes the natural landscape and generates large volumes of wastes which, when disposed of without sufficient containment or treatment, become a source of environmental burden, as shown in previous chapters relating to the Zambian Copperbelt. Consequently, the recent two decades have witnessed a surge in research at a global scale on landscape restoration post-mining, yielding a suite of mostly phytoremediation and some phytomining techniques. The application of phytomining technologies in recovery of residual metals in mineralized soils such as tailings has been suggested as a viable alternative for minimising metal migration and landscape restoration as it both enables valorisation of the waste and reduces long-term liabilities.

This study investigated potential to extract value from copper tailing storage facilities (TSFs) by studying the plant species thriving in metal rich areas and classification of these plants based on their functional traits (e.g., bioconcentration factors, translocation factors etc). The minor quantities of metals and toxic waste in tailings can be removed using adapted hyperaccumulator plant species, thus reducing the environmental burden. Using this approach, we propose suitable plant species for phytomining technologies based on their ability to either exclude or accumulate metals on contaminated sites and augment the economic prospects of this method. The desired outcome of the study was to provide innovations in the handling of regions surrounding the TSF to speed up rehabilitation and minimize impact of metal mobilization.

7.1. Introduction

The impact of activities like copper mining is associated with a decline in ecological services provided by naturally functioning ecosystems (ecological infrastructure) (Andrade et al., 2006; Castilla and Nealler, 1978; Haddaway et al., 2019). In essence, indirect and direct ecosystem services are tangible and usually linked to a broader catchment basin like regulation of several ecosystem processes that contribute to the rectitude or wholeness of a positive environment (Böck et al., 2018; Small et al., 2017). The Kafue River catchment is a good example of an ecosystem impacted by, inter alia, heightened copper mining related activities (Norrgren et al., 2000; Sracek et al., 2012). Consequently, the Kafue River catchment has been identified as one with adulterated watersheds in the southern region, especially due to the effects of metal mobilization and siltation (Ikenaka et al., 2010; Kambole, 2003). These impacts are ongoing owing both to legacy sites and to copper being an important strategic resource in Zambia with its exploitation likely to continue to support the country's development (Sikamo et al., 2016). The various land use activities in the Kafue River catchment depend mainly on ecosystem services provided for by the river and its tributaries, also known as streams (Cowx et al., 2018; Deines et al., 2013). The catchment provides diverse ecosystem services such as water resources for crop irrigation to numerous communities in the Copperbelt region (Nachiyunde et al., 2013; Volk and Yerokun, 2016). However, by virtue of its position in the landscape and relationship to stream networks, the catchment is frequently affected by copper mining activities such as mine waste, as demonstrated in Chapters 4 to 6.

The benefits of investing in the restoration and rehabilitation of ecological infrastructure has become a subject of research focus globally (Koelmel et al., 2015; Mahar et al., 2016). Several different approaches (e.g. rhizofiltration, phytotransformation, phytostabilization) have been suggested for the rehabilitation of sources of environmental burden such as TSFs (Festin et al., 2019; Martínez-Pagán et al., 2009; Wilson-Corral et al., 2012). Precious, and other low-grade metals (e.g., Ni, Ag, Pt, Co and Cu depending on ore deposit) are contained in these TSFs in substantial quantities. The application of hyperaccumulators in the recovery of these residual metals left in mineralized soils has been suggested as a viable alternative for both rehabilitating such sites and recovering value from them while enhancing resource efficiency, through phytomining (Parker et al., 2014; van der Ent et al., 2015, 2013)

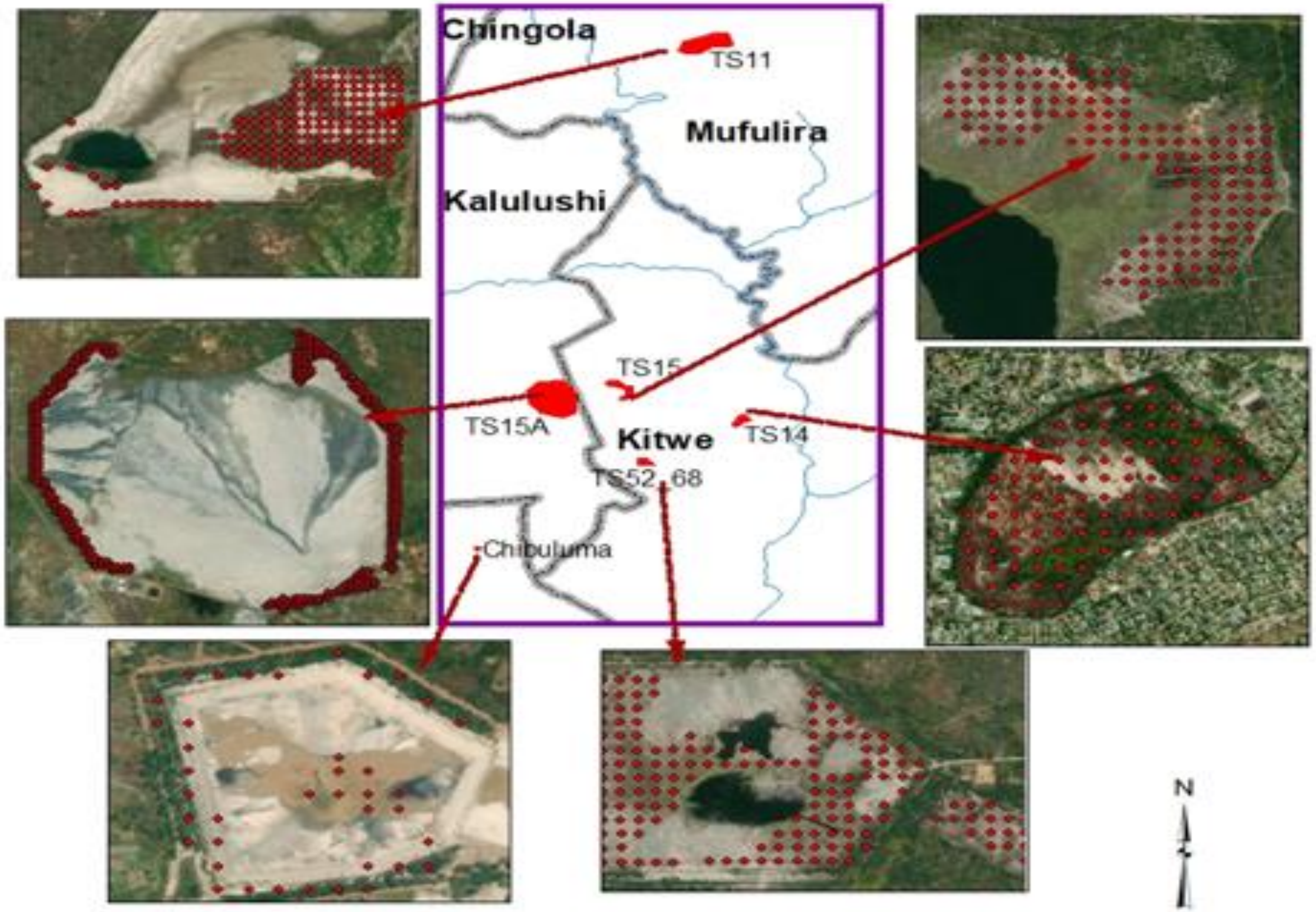
Phytomining creates opportunities for rehabilitation of ambient environments whilst realizing new income streams when suitable accumulator plants are used to recover valuable metals (Sheoran et al., 2013; Sinkala, 2018). It is an important driver for cost effective remedial solutions aimed at mitigating environmental risks that are apparent for TSFs and other wastelands (Chaney et al., 2007). Hyperaccumulators are grown on metal enriched contaminated soils, then harvested, and the biomass is processed to recover the metal. The application of phytomining requires the use of plants with attributes such as large biomass, high metal tolerance, deep roots, high metal bioaccumulation from the soil or TSF, high metal translocation rate from below-ground biomass to above-ground biomass, fast growth, and metal specificity (Hunt et al., 2014). Several response patterns have been observed by plants thriving on metal contaminated sites (Chaney et al., 2007). While many plants are not receptive to elevated metal concentration, some have developed tolerance and resistance strategies, and are able to accumulate inordinate amounts of metals, sometimes with specificity (Sheoran et al., 2013; Suman et al., 2018). These plant species are called hyperaccumulators (Hunt et al., 2014; van der Ent et al., 2013). Selection and identification of such species are among the important factors in their application for phytomining.

In spite of the long rich history of mining in Africa, the availability of large copper and gold deposits in the Democratic Republic of Congo (DRC), and the presence of historical mine sites in South Africa, Zimbabwe, and Zambia; the pace and applications of phytomining in Africa is sluggish compared to selected parts of the global south (Festin et al., 2019; Sandell, 2020). This study therefore aimed at identification and evaluation of native herbaceous plants thriving on mine wastelands with potential for phytoextraction technologies. We hypothesize that there is a correlation between the herbaceous plant communities colonising TSFs and the chemical characteistics of soils, and that the presence of contaminants influences the type of plants colonising the TSFs.

7.2. Materials and Methods

7.2.1. Site Selection and Description

The study was conducted in selected mining towns of the Copperbelt Province in Zambia (Figure 7-1). The study sites were strategically selected to enhance comparison of plants colonising the TSFs. The objective of site selection was to obtain an understanding in the behaviour patterns of native plants with respect to metal accumulation.



B

Figure 7-1: Location of the study area (A) and selected sampling sites (B), on the Copperbelt Province of Zambia

7.2.2. Sample Collection

Field surveys were carried out from November 2018 to March 2019 across five decommissioned sites TSFs (TSF11, TSF14, TSF15, TSF52, and TSF68 (Kitwe), and two active TSFs (TSF15A and Chibuluma TSF) (Figure 7-1). These sites are mainly characterised by low grade Cu, Mn, and Co deposists in substantial quantities (Golder Associates, 2011). The behaviour tendencies of herbaceous plants with respect to accumulation of selected metal species was compared across all sampling sites.

The study adopted the systematic random method of sampling owing to the need to assess broader vegetation variability (Larson et al., 2019). Random and systematic sampling reveals a more reliable correlation strategy of community of species to soil factors (Swacha et al., 2017). Specifically, 100 m by 100 m sampling plots were established on the TSFs; these were not dependent on the vegetation cover (Figure 7-2). Bias was reduced by randomly selecting the starting point (plot) and thereafter sampling at 200 m intervals, as recommended by Mártin et al. (2012). At each site, 15 sampling plots were selected. A circular plotwith a 25 m radius was established at each sampling plot from which samples of roots, stem, and leaves were collected. Additionally, soil samples from the rhizosphere were collected at 0-30 cm depth at each sampling point. This depth was chosen because biological factors and nutrient active zones which influence plant growth and the active root zone mostly occur within this depth (Crépin and Johnson, 1993; Lei and Duan, 2008). On each circular plot, GPS coordinates were taken from the centre of the plot for location purposes and description.

Biomass below-ground was separated from biomass above-ground. Tap water was used to thoroughly wash the plant samples to remove attached soil particles on plant parts surfaces; thereafter, distilled water was used to rinse the collected samples as recommended by Moraghan and Mascagni (1991) and Richards (1993).

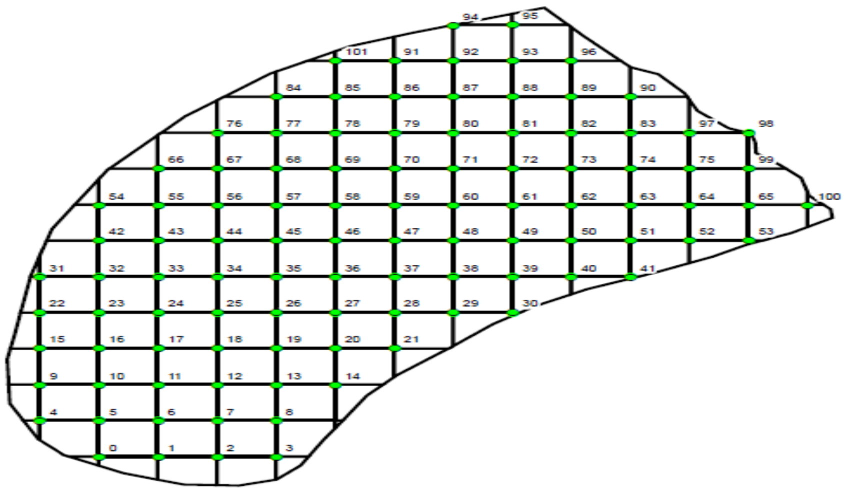


*Figure 7-2: Division of tailings storage facility into 100 m *100 m plots for sampling - Kitwe tailings storage facility (TSF14)*

7.2.3. Rhizosphere and Biomass Analysis

The collected rhizospheric soils were dehumidified and sieved to obtain samples with particle size of < 75 µm. The metal concentration in the rhizosphere were determined by digesting 1 g of a rhizospheric sample in concentrated 30 ml HNO_3 and 3 ml HF. This was then heated with an electronic plate set at 120^{o}C for 20 minutes. The solution was allowed to cool after boiling, and thereafter filtered through a 50 ml volumetric flask; and to add up to the mark, deionized water was used. The filtered solution was analysed for the concentrations of Cu, Co, Zn, Mn, Ni, Pb, and Zn using PinAAcle 900T Atomic Absorption Spectrometer (AAS).

The collected native herbaceous plant tissue samples were thoroughly washed with tap water in order to detach soil particles. Thereafter, the plant samples were rinsed using distilled water and separated into below-ground and above-ground biomass before being oven dried for 24 hours at 70^0 C. The oven dried samples were ground using a mortar to fine powder and sieved through a 0.18 µm sieve. Two grams of dry sample was added into 250 cm^3 conical flasks with 30 ml of nitric acid (HNO_3) was added. The solution was heated for 30 minutes. Thereafter, 10 ml of perchloric acid ($HClO_4$) was added. The solution was transferred into 100 ml flask and the volume made up using deionized water. The concentration of selected metals Cu, Co, Zn, Mn, Ni, and Pb in the filtered digestates were determined by AAS.

7.2.4. Ecological and Phytomining Analysis

The herbaceous plant species that occurred in the sampling plots were identified. Thereafter, the importance value index (IVI) was determined for each of those species (Naidu and Kumar, 2016). IVI was used to give an indication of relative density, frequency and dominance for the species observed on TSFs (Kacholi, 2014; Zerbo et al., 2016).

IVI=Relative frequency + Relative density + Relative dominance (1)

Where:

$$\text{Relative frequency, RF} = \frac{Frequency\ of\ a\ species}{Total\ frequencies\ of\ all\ species} \quad (2)$$

$$\text{Relative density, RDe} = \frac{Number\ of\ individuals\ of\ one\ species}{Total\ number\ of\ all\ individuals\ counted} \quad (3)$$

$$\text{Relative dominance, RDo} = \frac{Basal\ area\ per\ species}{Total\ basal\ area} \quad (4)$$

The IVI was converted to relative Importance Value Index (Relative IVI) as follows:

$$\text{Relative IVI}=\frac{\text{IVI of a species}}{\text{IVI of all species}} \times 100 \quad (5)$$

The diversity of native herbaceous plant community colonizing the TSFs was assessed using the Shannon diversity index (*H'*). The higher the values of H', the higher the diversity of reported species in the selected community. The lower the value of H', the lower the diversity. A value of H' = 0 indicates a community that has only one species. The diversity index was necessary to understand plant adaptability to the metal contaminated sites.

$$H' = -\sum_{i=1}^{s} P_{\mathrm{i}} \ln P_{\mathrm{i}} \quad (6)$$

In which *Σ* is the summation of computations, *s* represents the sum of species, P_i represents the ratio (*n/N*) of number of individuals of a specific organism (n) against the sum total of identified individual organisms (*N*) and ln represents the natural log.

To determine the phytoextraction or phytomining potential of plant species occurring on the TSFs, the bioconcentration and translocation factors were determined. The bioconcentration factor (BCF) was calculated as a ratio of elements in below-ground tissues to rhizospheric soils while for the translocation factor (TF) was calculated as a ratio of elements in the above-ground tissues to below-ground tissues (Ali et al., 2013; Usman et al., 2012, 2019) (Equation 7 and 8).

$$\mathrm{BCF} = \frac{\text{Concentration of metals in belowground biomass}}{\text{Concentration of metals in rhizosphere)}} \quad (7)$$

$$\mathrm{TF} = \frac{\text{Concentration of metals in aboveground biomass}}{\text{Concentration of metals in roots}} \quad (8)$$

7.2.5. Statistical Analyses

The normality and homogeneity of variance of results generated was tested using Shapiro-Wilk and Levene's and Brown tests to ensure that distribution of outcomes for independent groups are comparable. Two-way ANOVA was used to determine the interactions between metal concentration in above-ground and below-ground plant tissues. The multidimensional scaling (MDS) was used as a means of visualizing the level of similarity in metal concentration in the root and shoot system of collected plants (Borg and Groenen, 2005; James et al., 2013). MDS focusses on creating mappings of items based on distance or similarity, enabling end-user to compare items based on the mappings or clusters. This approach allows representation of high dimensional data in a low dimensional space with preservations of similarities. This method was adopted in this study to compare the high dimensional data of metal concentration in herbaceous plant species collected from 7 different TSFs.

7.3. Results

7.3.1. Species Composition

A total of 622 indigenous herbaceous species from 21 families and 46 genera were collected from the TSFs (Table 7-1). These families have varying importance value indeces (IVI) that ranged from 0.2% for *Euphorbiaceae* to 28.8% for *Poaceae*. Among the families observed, *Poaceae* had both the highest number of genera and species (Table 7-1). At a species level, different species exhibited a variation in importance value index. Species dominance on the TSFs was exhibited by *A. eucomus* (IVI = ≈9.19%), *H. filipendula* (IVI = ≈8.31%), *C. floribunda* (IVI = ≈5.9%), and *V. Glabra* (IVI = ≈5.49%), respectively (Table S7-1). A high diversity and species richness was observed on the TSFs (H' = ≈3.0) except for Chibuluma TSF and TSF15A sites which reported lower values (H' = ≈2.32) (Figure 7-3). It was observed that 72.9% of plants found on the sites were perennial while 27.1% were annual (Table S7-1).

Table 7-1: Dominant families on Copperbelt TSFs based on IVI, number of species, genera, and relative density

No	Family	Number of Genera	Number of Species	Relative Frequency	Relative Density	Relative IVI
1	Acanthaceae	1	2	0,32	0,23	0,27
2	Amaranthacea	4	8	0,64	1,84	1,24
3	Asperagaceae	1	3	0,24	0,99	0,62
4	Asteraceae	15	193	15,68	31,28	23,48
5	Cyperaceae	3	41	3,95	5,51	4,73
6	Convovulaceae	1	2	0,20	0,67	0,44
7	Dioscoreaceae	2	6	0,60	0,09	0,34
8	Euphorbiaceae	1	1	0,10	0,30	0,20
9	Filicies	2	5	0,51	1,73	1,12
10	Iridaceae	1	13	1,32	0,40	0,86
11	Liliaceae	1	20	2,06	3,58	2,82
12	Orchidaceae	1	13	1,37	1,66	1,51
13	Papilionaceae	1	7	0,75	2,62	1,68
14	Pedaliaceae	1	2	0,22	0,27	0,24
15	Poaceae	15	242	27,70	29,85	28,76
16	Polygonaceae	2	18	2,62	2,09	2,36
17	Rubiaceae	1	10	1,50	6,15	3,82
18	Scrophulariaceae	1	20	3,04	2,68	2,86
19	Solanaceae	1	1	0,16	0,69	0,42
20	Tiliaceae	1	2	0,31	0,99	0,65
21	Vitaceae	2	13	2,05	6,39	4,22
	Total	58	622	100,00	100,00	

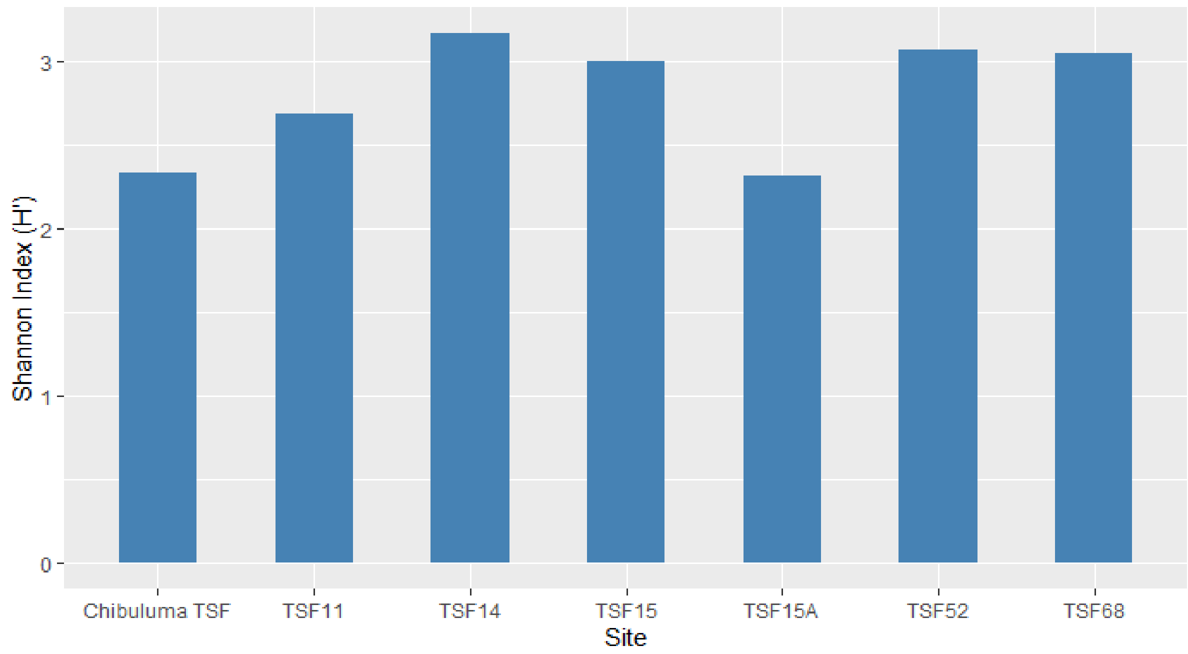


Figure 7-3: The Shannon's diversity (H') index results for the herbaceous plant species colonizing the TSFs

7.3.2. Concentration of Metals in the Rhizosphere

The spatial distribution of metal concentration and pH of the rhizospheres of different plant species at the selected sites are displayed in Table 7-2. The soil pH for collected samples from the rhizosphere of herbaceous plant species from the TSFs sites were observed to range from 6.0 to 8.1 (Table 7-2). The variation in pH between the sites was not significant. Based on a comparative analysis of metal concentrations, a consonant pattern can be observed with reference to the mapping of the selected metal concentrations in the rhizosphere of individual herbaceous plants, detailed in Table 7-2 and Figure 7-4. TSF52 and TSF68 reported the highest concentrations of Cu (≈11018 ppm and ≈3855 ppm respectively), and Co (≈2204 ppm and ≈849 ppm respectively). TSF 11 also reported high Cu (3712 ppm) but reduced Co (438 ppm) while the remaining sites lay in the range 362 – 3002 ppm Cu and 245 – 613 ppm Co. Concentrations of Mn and Zn did not differ substantially between the sites (Figure 7-4). The highest concentration of Zn was found at TSF52 and TSF15 (≈382 ppm and ≈118ppm respectively), while the concentration of Mn at TSF15 stood at ≈2351 ppm. Furthermore, the highest mean metal concentration analysed from the rhizosphere of herbaceous plants from a specific TSF equalled (in descending order) 11018 ± 1270 ppm for Copper at TSF52, 2351 ± 639 ppm for Manganese at TSF15, 2204 ± 2474 ppm for Cobalt at TSF52, and 382 ± 1197 ppm for Zinc at TSF52.

Table 7-2: Total metal concentrations in the rhizospheric soil of herbaceous plants at each TSF site. Element concentrations values are all given in ppm

Site	Cu	Co	Mn	Zn	pH
Chibuluma TSF					
Range	750 - 1890	140 - 620	520 - 1050	30 - 50	6 - 8.1
Mean+SD	1111 ± 301	245 ± 113	745 ± 120	35.8 ± 6.67	7.02 ± 0.56
TSF11					
Range	400 - 6930	200 - 800	770 - 2500	20 - 440	6.1 - 8
Mean+SD	3712 ± 1416	438 ± 231	1488 ± 363	84.1 ± 88.1	7.19 ± 0.49
TSF14					
Range	71 - 4800	170 - 7700	50 - 3400	30 - 800	6 -8.1
Mean+SD	362 ± 458	613 ± 1120	1368 ± 795	69.9 ± 93.8	7.1 ± 0.55
TSF15					
Range	320 - 5930	80 - 1010	340 - 6300	30 - 2570	6.2 -8.1
Mean+SD	3005 ± 1054	444 ± 164	2351 ± 639	118 ± 321	7.17 ± 0.48
TSF15A					
Range	1180 - 4440	190 - 680	1870 - 2630	30 - 100	6.2 - 8
Mean+SD	1995 ± 800	386 ± 99.9	2218 ± 159	42.5 ± 12.5	7.09 ± 0.49
TSF52					
Range	1830 - 39700	310 - 8700	330 - 5200	30 - 8100	6.3 - 8
Mean+SD	11018 ± 12720	2204 ± 2474	1822 ± 754	382 ± 1197	7.1 ± 0.51
TSF68					
Range	510 - 10900	280 - 2190	830 - 3190	40 -160	6.1 - 8
Mean+SD	3855 ± 1590	849 ± 365	1907 ± 430	77.8 ± 24.8	7.13 ± 0.5

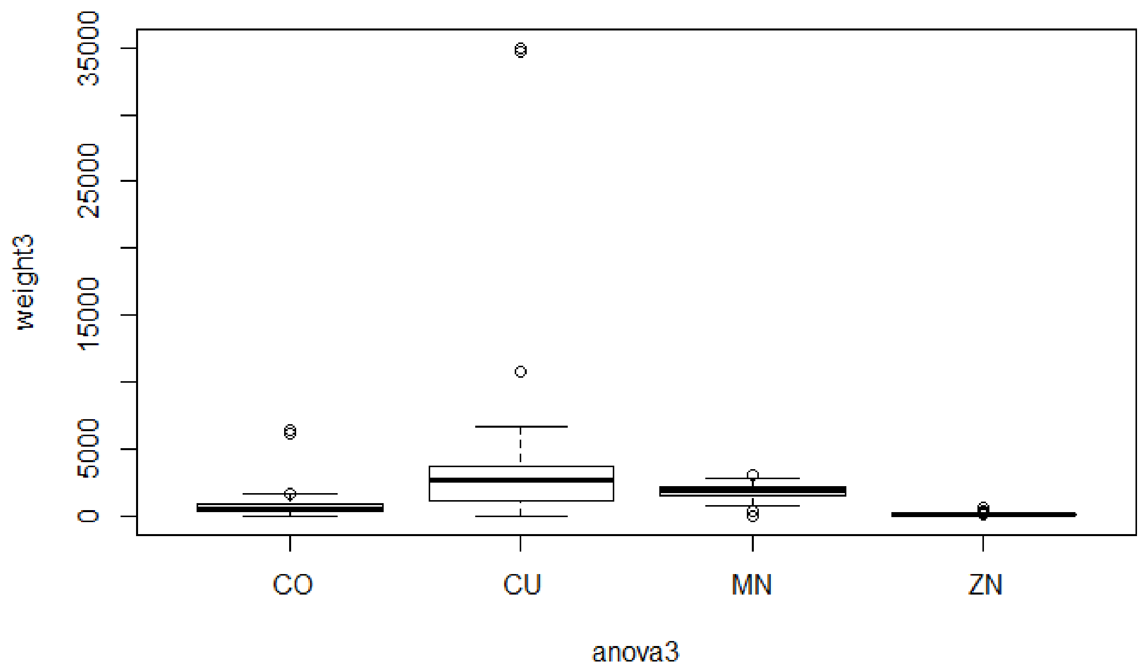


Figure 7-4: The boxplot shows the range and distribution of metal concentrations in ppm of (Cu) = Copper, (Co) = Cobalt, (Mn) = Manganese and (Zn) = Zinc, in the rhizosphere of selected

sites at Chibuluma TSF, TSF11, TSF14, TSF15A, TSF15, TSF52 and TSF68. Significant differences are indicated by the variability in the box plot sizes

7.3.3. Uptake of Metals by Plants

The spatial variation of selected metals determined in the above-ground biomass (AGM) and below-ground biomass (BGM) of native herbaceous plant species harvested are presented in Table S7-2 and Figure 7-5. Additional information on metal concentration in plants colonising the mine wastelands can be accessed on https://drive.google.com/drive/folders/1TXutLlyfWfYb4keDwAkDdBP4cMbgxZsI?usp=sharing. Copper concentrations within the biomass were significantly different than those of Co, Mn, and Zn in most of the species, with *C. Trothae* accumulating the highest Cu concentration (≈4052 ppm) in BGM, while *H Filipendula* accumulated the highest Cu concentration (≈1542 ppm) in AGM (Table S7-2). Overall, higher Cu concentration were measured in the BGM compared to AGM, although higher values in AGM were observed in *C. Anua, I. Obscura, A. Schinzi, A. Ciliate and G. Schinzi.* The plants *A. lanata, A. eucomus, C. trothae, C. ductylon, C. alternifolius, C. rotundus, D. Debilis, H. Filipendula, and S. uomblei* showed extraordinarily high concentrations of Cu in their below-ground biomass (above 2000 ppm) and AGM (above 1000 ppm), suggesting high translocation abilities and potential for phytomining.

Cyperus alternifolius accumulated the highest amount of Co (≈570 ppm) in BGM among the plant species harvested while *B. steppia* had the lowest amount of Co in the roots (≈102 ppm) and shoots (≈32 ppm) (Table S7-2). In the shoots *C. ductylon* had the highest Co concentration (≈346 ppm). Co concentrations, on average were observed to be higher in the BGM compared to AGM, except in *B. Alata, B. Brevipes, C. floribunda, G. unguculatus, P. pulchrum, S. spacelate* and *V. glabra.* Manganese concentrations were observed to vary significant among the plant species. High Mn concentration in the BGM of *D. debilis* (≈1590 ppm) were reported, ranging from 520 ppm to 6300 ppm while in the AGM, *B. brevipes* recorded the highest Mn concentration (≈1068 ppm) ranging from 310 ppm to 6600 ppm (Table S7-2). *A. eucomus* (3150 ppm), *C. ductylon* (6490 ppm), *C. alternifolius* (3120 ppm), *D. debilis* (2140 ppm), *H. filipendula* (2420 ppm), *S. spacelate* (3780 ppm), *S. uomblei* (2350 ppm), *S. cameronii* (2270 ppm), and *V. glabra* (4200 ppm) reported the highest Mn concentration in AGM. Concentration of Zn in the BGM and AGM where relatively low compared to Cu and Mn. It was observed that in the BGM, Zn concentrations on average were higher compared to the

AGM, except for A. eucomus (≈321 ppm), G. simplex (≈283 ppm), *H. filipendula* (≈205 ppm), D*. heterostachyum* (≈358 ppm), C. floribunda (≈245 ppm) and *S. panicoides* (≈1778 ppm) (Table S7-3). Overall, the results showed *A. eucomus, C. ductylon, C. floribunda* and *H. filipendula* (Figure 7-6) were more endemic to metal enriched soils compared to other plants. These plants were associated with high metal content (≥ 1000 ppm) in their BGM and AGM, and high IVI values (Table S7-1 and S7-2).

The multi dimension scaling (MDS) plots indicate that most of the metal concentrations in the herbaceous plant species study sites grouped together, whilst metal concentration in plants *A. eucomus (6*), *A. burtti (7*), *A. stenostachya (8*), *C. nudicaulis (24*) and *S. cameronii (50*) were noticed to be different from others (Figure 7-7). The plants corresponding to the numbers presented in the MDS plot are reported in Table S7-3. This dissimilarity correlated with high metal concentrations observed in these plants compared to the remaining plants (Table S7-2). The different colours show the plants as they are grouped in different clusters according to their similarity. Essentially, plants with higher Cu concentration in their biomass were coded black, whilst those coded red had higher Mn concentrations. Plants associated with high values in both Co and Zn were coded green, although the concentrations are not as high as Cu and Mn values.

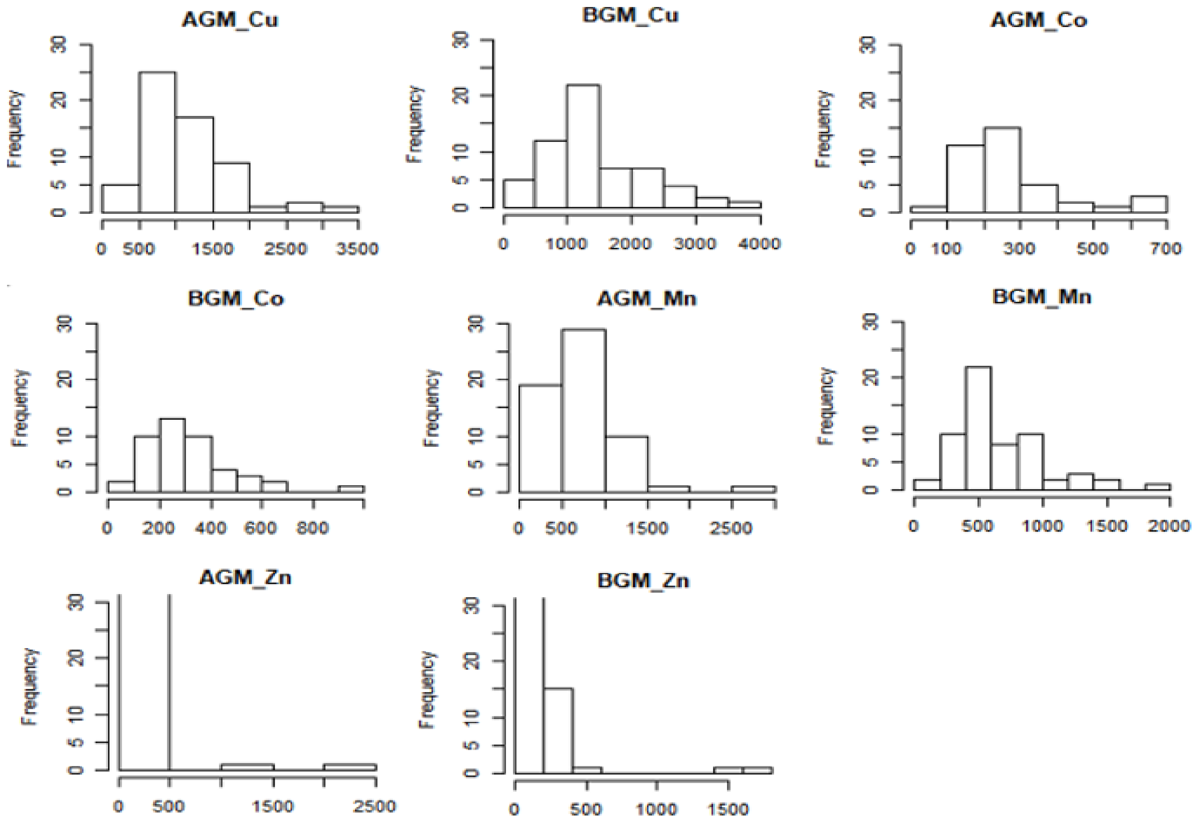


Figure 7-5: Distribution of metals variables Cu, Co, Mn, and Zn (ppm) in below-ground biomass (BGM) and above ground biomass (AGM) within the different habitats of native herbaceous plant species colonizing TSFs in Zambia (Copperbelt Province)

Figure 7-6: Photos of native herbaceous plant species endemic to the selected TSFs

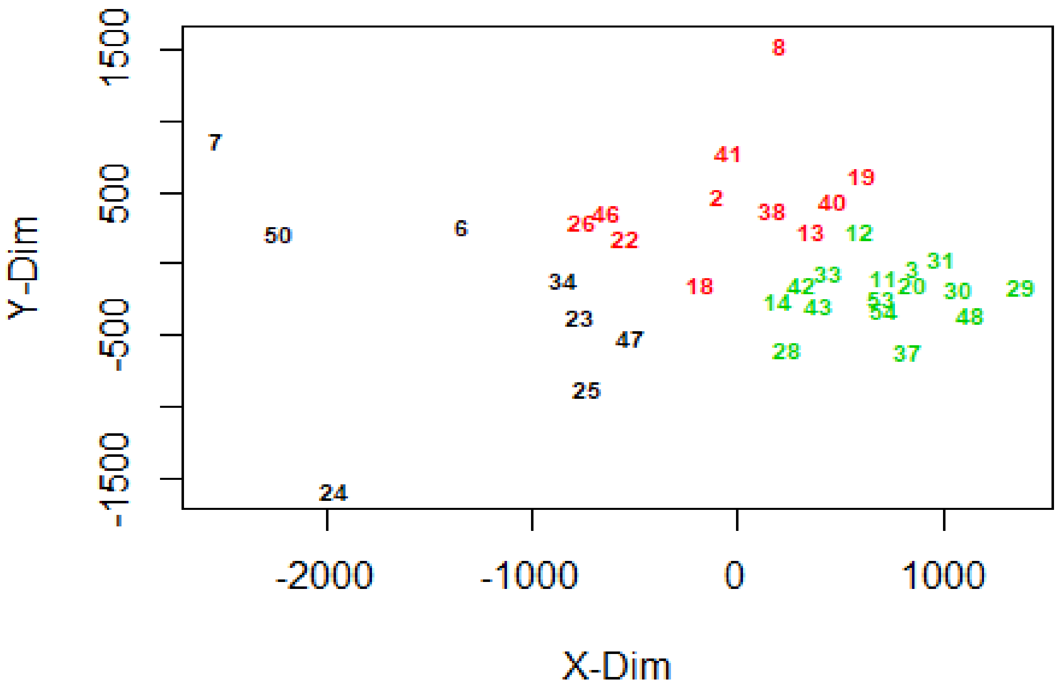


Figure 7-7: Multi Dimension Scaling (MDS) indicating the (dis)similarities of the plant species from Chibuluma, TSF, TSF11, TSF14, TSF15, TSF15A, TSF32, and TSF68 derived from the selected metals measured at the TSF sites. The plants are identified in Table S7.3. Plants with high Cu concentration are shown in black, high Mn in red and high Zn and Co in green.

7.3.4. Heavy Metal Influence on Species Abundance

The results for elemental analysis of the soil and plant species showed that the correlation coefficient between Cu concentration in soil and AGM_Cu (0.75), and BGM_Cu (0.63) was significant (Table 7-3). We can infer that Cu had significant influence on the type of plant species colonising the TSFs. On the other hand, a strong negative correlation (-0.54) was reported between Mn concentration in soil and AGM_Mn). Observably, no other significant correlations were reported. Regression analysis between AGM (dependent) variables and BGM (independent) and Soil (independent) variables was undertaken. The R^2 of the models were less than 0.5, thus signifying that the independent variables, (i.e., the respective elemental concentrations in soils and BGM values) do not explain most of the variation in dependent variable (AGM values) (Table 7-3). A comparison of the variables using a pairwise test for AGM and BGM showed that at 5% level of significance, the means of Co and Cu are different, Mn and Co are different, Zn and Co are different, Cu and Mn are different, Zn and Cu are different, and Mn and Zn are different. It can inferred that the influence of selected metals on species abundance was different, with Cu reporting significant influence on plant species abundance compared to Co, Mn and Zn.

Table 7-3: Correlation of elemental concentration in soils (Co, Cu, Mn, and Zn) and plant tissues (BGM_Ni, AGM_Ni, BGM_Zn, AGM_Zn, BGM_Mn, AGM_Mn, BGM_Co, AGM_Co, BGM_Cu and AGM_Cu

Features	AGM_Cu	BGM_Cu	AGM_Co	BGM_Co	AGM_Mn	BGM_Mn	AGM_Zn	BGM_Zn	AGM_Ni	BGM_Ni	Cu	Co	Mn	Zn
Zn	0.86	0.79	0.42	-0.49	0.65	0.91	-0.06	-0.01	-0.57	0.48	0.94	0.13	0.27	1
Mn	-0.19	-0.04	-0.01	0.06	-0.54	0.04	-0.28	-0.62	-0.74	0.52	0.49	0.78	1	0.27
Co	-0.19	-0.09	0.37	0.11	-0.5	-0.08	-0.11	-0.04	-0.22	0.39	0.32	1	0.78	0.13
Cu	0.75	0.63	0.5	-0.54	0.47	0.77	-0.24	-0.17	-0.68	0.59	1	0.32	0.49	0.94
BGM_Ni	0.38	0.62	0.14	0.23	0.08	0.54	-0.8	-0.14	-0.35	1	0.59	0.39	0.52	0.48
AGM_Ni	-0.16	-0.18	0.13	0.32	0.08	-0.35	0.15	0.81	1	-0.35	-0.68	-0.22	-0.74	-0.57
BGM_Zn	0.33	0.27	0.48	0.05	0.45	0.16	0.24	1	0.81	-0.14	-0.17	-0.04	-0.62	-0.01
AGM_Zn	-0.15	-0.23	-0.1	-0.17	0.01	-0.11	1	0.24	0.15	-0.8	-0.24	-0.11	-0.28	-0.06
BGM_Mn	0.88	0.96	0.21	-0.21	0.73	1	-0.11	0.16	-0.35	0.54	0.77	-0.08	0.04	0.91
AGM_Mn	0.93	0.68	0.45	-0.54	1	0.73	0.01	0.45	0.08	0.08	0.47	-0.5	-0.54	0.65
BGM_Co	-0.53	0.01	-0.65	1	-0.54	-0.21	-0.17	0.05	0.32	0.23	-0.54	0.11	0.06	-0.49
AGM_Co	0.55	0.15	1	-0.65	0.45	0.21	-0.1	0.48	0.13	0.14	0.5	0.37	-0.01	0.42
BGM_Cu	0.82	1	0.15	0.01	0.68	0.96	-0.23	0.27	-0.18	0.62	0.63	-0.09	-0.04	0.79
AGM_Cu	1	0.82	0.55	-0.53	0.93	0.88	-0.15	0.33	-0.16	0.38	0.75	-0.19	-0.19	0.86

Features

Correlation Meter -1.0 -0.5 0.0 0.5 1.0

7.3.5. Bioconcentration and Translocation Factors of Native Herbaceous Plants

Spatial distribution of the bioconcentration (BCF) and translocation (TF) factors of herbaceous plant species at the selected TSF sites are presented in Table 7-4. From these results, it was observed that most of the species had a TF > 1 for Zn and Cu compared to other metals. Higher Zn TF were measured in *E. scuber* (≈14.4) and *A. lanata* (≈4.22) in comparison to the other plants. With Cu, *S. calycinum* had the highest TF for Cu (≈3.86) followed by *A. leucura* (≈2.55), *B. mollis* (≈2.16), *I. obscura* (≈2.04), *S. spacelate* (≈2.0), *A. stenostachya* (≈1.87), *T. leotopetaloides* (≈1.85), *P. pulchrum* (≈1.64), *P. mauritanus* (≈1.54), *C. anua* (≈1.39), *C. purrdopappa* (≈1.38), *E. scuber* (≈1.37), *G. schinzi* (≈1.36), *H. scapose* (≈1.32), *D.debilis* (≈1.30), *G. simplex* (≈1.29), *N. auriculata* (≈1.26), *G. unguculatus* (≈1.17), *C. floribunda* (≈1.15), *B. alata* (≈1.14), *L. zambeziaca* (≈1.05), *A. schinzi* (≈1.04), *B. brizantha* (≈1.03), *A. africana* (≈1.02) and *C. ductylon* (≈1.02). Selected species were equally observed to have TF > 1 for metal species Co, Mn, and Zn. *A. stenostachya* (≈2.64) and *A. lanata* (≈1.74) reported high TF for Co, whilst *I. obscura* (≈5.30) and *Nidorella Auriculata* (≈3.46) had the highest TF for Mn.

Results from the BCF_{roots} and BCF_{shoots} analyses showed that the BCF for Cu and Zn was >1 for most plant species (Table 7-4). Observably, higher Cu BCFs were reported in *S. uomblei* (BCF_{roots} = ≈8.47 and BCF_{shoots} = ≈6.59 respectively), *A. schinzi* (BCF_{roots} = ≈6.07 and BCF_{shoots} = ≈6.32 respectively), *H. scapose* (BCF_{roots} = ≈5.64 and BCF_{shoots} = ≈3.92 respectively), while *T. rotundifolia* (BCF_{roots} = ≈9.81 and BCF_{shoots} = ≈4.11 respectively), *E. scuber* (BCF_{roots} = ≈1.81 and BCF_{shoots} = ≈14.4 respectively), *H. filipendula* (BCF_{roots} = ≈7.19 and BCF_{shoots} = ≈2.16 respectively) higher Zn BCF respectively. The BCF for Mn and Co were observed to be <1; although, a high BCF for Mn was observed in D. *debilis* (BCF_{roots} = ≈1.79 and BCF_{shoots} = ≈1.62 respectively).

Table 7-4: Translocation (TF) and Bioconcentration (BF) factors of indigenous herbaceous plant species colonizing TSFSs

Plant Species	Cu			Co			Mn			Zn		
	BCF (Root	BCF (Shoot	TF	BCF (Root	BCF (Shoot	TF	BCF (Root	BCF (Shoot	TF	BCF (Root	BCF (Shoot	TF
Cynodon ductylon	2,76	1,48	1,02	0,85	0,59	0,99	0,62	0,77	1,68	2,89	3,62	3,03
Aerva lanata	3,80	1,49	0,77	0,40	0,34	1,78	0,35	0,37	1,12	1,02	2,48	4,22
Gloriosa simplex	0,72	0,76	1,29	0,33	0,19	0,82	0,22	0,30	1,34	5,68	2,66	2,96
Andropogon eucomus	2,63	2,14	0,96	1,33	0,88	1,41	0,85	0,84	1,58	3,41	1,78	0,88
Bidens steppia	1,52	0,89	0,93	0,49	0,22	1,16	0,51	0,42	1,16	2,17	2,38	1,47
Conyza floribunda	0,95	0,62	1,15	0,31	0,54	1,44	0,41	0,60	1,62	9,52	2,17	2,20
Hyparrhenia filipendula	1,79	1,47	0,88	0,89	0,46	0,64	0,85	0,69	1,06	3,68	7,19	2,16
Vernonia glabra	1,34	1,39	0,82	0,30	0,29	0,89	0,27	0,53	1,27	7,66	4,05	1,17
Setaria spacelate	0,50	0,53	2,00	0,35	0,47	1,64	0,42	0,53	1,44	2,53	1,90	1,67
Blumae brevipes	0,46	0,30	0,88	0,25	0,36	0,74	0,29	0,68	1,78	2,03	2,08	1,12
Tithonia rotundifolia	0,72	0,47	0,73	0,34	0,71	1,41	0,34	0,34	0,88	2,46	9,81	4,11
Blumae alata	0,58	0,49	1,14	0,44	0,61	1,28	0,60	0,45	1,67	3,59	2,35	1,83
Digitaria debilis	3,82	2,85	1,30	0,95	0,95	0,74	1,79	1,62	1,27	3,98	3,63	1,47
Elephantropus scuber	0,51	0,46	1,37	0,19	0,21	1,75	0,38	0,34	1,22	1,05	1,81	14,41
Lactuca zambeziaca	0,79	0,39	1,05	0,22			0,36	0,31	1,05	2,27	2,59	2,27
Anthephara burtti	0,93	0,72	0,84	0,67	0,62	0,52	0,97	0,74	0,86	2,09	2,03	1,03
Cissus trothae	1,76	0,43	0,76	1,58			0,22	0,29	1,72	2,48	1,64	0,80
Panicum heterostachyum	0,59	0,26	0,46	0,54			0,26	0,21	0,78	2,80	1,52	0,89
Disa heterostachyum	0,50	0,29	0,71	0,52	0,24	0,90	0,33	0,24	0,90	1,78	1,61	0,77
Setaria pallidifusca	0,83	0,35	0,58				0,62	0,58	1,86			0,78
Sporobolus panicoides	0,98	0,61	0,65				0,58	0,33	0,66	15,00	6,25	0,82
Harplocapha scaposa	5,64	3,92	1,32	0,75	0,57	1,12	0,71	0,71	1,52	1,68	3,29	1,56
Grewia schinzi	2,41	3,28	1,36				0,20	0,40	2,00	1,25	3,00	2,40
Sopubia ramosa	0,85	0,63	0,81	0,30	0,28	1,32	0,31	0,25	0,79	2,99	2,57	1,36
Gladiolus unguculatus	1,80	1,81	1,17	0,25	0,20	2,00	0,39	0,32	1,18	1,93	5,86	2,57
Cyperus rotundus	2,18	1,20	0,43	0,41	0,32	0,28	0,43	0,30	0,82	2,36	1,65	1,01
Tacca leotopetaloides	0,71	1,32	1,85				0,25	0,32	2,12	1,52	0,86	0,72
Dioscorea buchanani	0,97	1,21	0,94				0,14	0,21	1,20	0,83	0,67	1,12
Nephrolepis undulata	2,27	1,18	0,60				0,10	0,13	0,91	1,11	1,00	1,35
Crotalaria anua	1,10	1,65	1,39		0,41		0,28	0,31	1,26	0,94	2,03	2,18
Setaria uomblei	8,47	6,59	0,79	0,58	0,46	0..953	0,74	0,58	1,02	11,17	5,54	1,24
Cyperus alternifolius	1,37	0,77	0,46	0,81	0,22	0,26	0,52	0,32	0,66	2,02	1,70	2,44
Bidens pilosa	3,74	1,41	0,54				0,90	0,42	0,85	1,95	1,64	1,18
Ipomoea obscura	1,84	3,75	2,04		0,80		0,11	0,56	5,30	0,67	0,83	1,25
Adropogon schinzi	6,07	6,32	1,04	0,61	0,79	1,30	0,64	0,72	1,13	1,75	2,25	1,29
Barleria spinulosa	0,70	0,57	0,84	0,23			0,91	1,53	0,96	5,08	3,58	0,85
Brachlaria brizantha	2,38	2,45	1,03				0,30	0,29	0,96	4,25	1,38	0,32
Acalypha ciliata	3,28	2,24	0,68				0,63	0,46	0,73	1,50	1,33	0,89
Stereochlaena cameronii	7,05	4,16	0,63	0,65	0,65	0,63	0,94	0,60	0,61	0,86	1,00	0,83
Phragmites mauritanus	0,35	0,42	1,54	0,61	0,43		0,23	0,30	2,10	1,80	5,12	1,76
Conzya welwitschii	0,20	0,16	0,79	0,14	0,20	1,50	0,20	0,25	1,27	1,20	4,20	3,50
Agava siciliana	0,30	0,16	0,56	0,19			0,22	0,10	0,45	0,57	1,86	1,75
Nidorella auriculata	0,59	0,66	1,26	0,68	0,65	0,84	0,26	0,52	3,46	2,26	1,30	1,24
Sesamum calycinum	0,22	0,85	3,86				0,15	0,48	3,13			1,06
Richardia scabra	0,51	0,30	0,81	0,82	0,33	0,34	0,32	0,27	1,01	1,76	2,42	
Aristida stenostachya	0,56	1,04	1,87	0,24	0,63	2,64	0,21	0,70	3,33			1,67
Celosa trygina	0,29	0,19	0,68				0,12	0,19	0,97	1,18	2,63	2,28
Pellaea longipilosa			0,09			0,60			0,41			1,00
Polygonum senegalense	0,84	0,57	0,81	0,98	0,81	0,99	0,28	0,37	1,66	1,60	2,18	1,58
Amaranthrus graecizans	0,08	0,04	0,54	0,08	0,03	0,42	1,03	0,45	0,43	0,17	0,28	1,64
Aerva leucura	0,03	0,07	2,55		0,06		0,18	0,29	1,63	0,10	0,39	3,83
Ampelocissus africana	0,38	0,39	1,02				0,19	0,50	2,67	3,50	1,00	0,29
Conyza purrdopappa	0,36	0,50	1,38		0,91		0,15	0,48	3,21	1,40	5,40	3,86
Polygonum pulchrum	0,11	0,18	1,64	0,09	0,08	0,86	0,08	0,18	2,25	0,28	0,61	2,20
Nephrolepis undulata	0,32	0,31	0,96				0,25	0,26	1,05			2,67
Cyperus nudicaulis	1,00	0,31	0,25	1,01	0,30	0,15	0,88	0,26	0,28	1,18	0,82	0,79
Polygonum pulchrum	0,51	0,45	0,88	0,36	0,31	0,86	0,40	0,23	0,57	1,11	1,11	1,00
Blumae mollis	0,55	0,22	2,16	0,39	0,25	0,42	0,61	0,41	1,71	0,65	0,85	1,48

7.4. Discussion

Application of ecological infrastructure restoration focuses on returning a particular ecosystem to similar pristine conditions prior to impacts influenced by anthropogenic activities, and avert further ecological degradation (de Klerk et al., 2016) by taking advantage of the various ecological attributes of either fauna or flora species. It is aimed at restoring the ecosystem function, structure, biotic composition and other services associated with the ecosystem (Carrick and Forsythe, 2020; Sheoran et al., 2009). In this study, the potential of using native herbaceous plant species to recover metals and rehabilitative copper mine wastelands was investigated.

7.4.1. Species Composition

The identification and selection of species colonizing the mine wastelands is essential in determining the outcomes of ecological restoration. The potential restoration technique evaluated during the present study allowed for identification of plants adaptive to the environment as well as having abilities to translocate relatively high amount of metal content in the AGM. The study observed a significant difference in plant species adaptation to the mine wastelands, with species from Asteraceae and *Poaceae* families showing high dominance. Studies by Gratão et al. (2005), Hasnaoui et al. (2020), Pandey et al. (2019) and Salas-Luevano et al. (2008) have shown that *Asteraceae* and *Poaceae* plants are adaptive to contaminated areas. This is supported by the relatively high IVI values reported in this study, compared to other families. Species dominance and abundance are an indication of good growth performance and adaptability (Roeling et al., 2018). Gajić et al. (2018) reported that native plants thriving on contaminated sites over time devoid of human interference have attributes best suited to local environments, thus making them valuable alternatives for phytoremediation technologies. Selected plant species such as *A. eucomus, B. alata, C. floribunda, C. ductylon, C. alternifolius, H. filipendula, E. scuber* and *V. glabra* had relatively high IVI values, indicating resilience to local climate, tolerance to adverse physio-chemical conditions and encouraging growth patterns.

The high IVI values coupled with substantial quantity of Cu in their AGM and BGM, makes a compelling case in their application for phytomining technologies. Various studies have shown that plants with such attributes are good candidates for phytomining (Perlatti et al., 2015; van der Ent et al., 2015, 2013). Although other plant species such as *C. anua, I. obscura, A. schinzi etc.,* exhibited similar traits with regards to metal translocation and concentration, they may not be suitable for phytomining owing to their relatively low IVI values across the studied TSFs (Baker, 1981; Baker and Brooks, 1989). Their relative low index values or frequency show that they may not adapt in other places.

7.4.2. Heavy Metal Concentration in the Rhizosphere

Tailings storage facilities (TSF) are normally characterized with high metal contents, with low nutrient and water holding capacity, high acidity or salinity and compromised structural stability (Festin et al., 2019). The results indicated that the occurrence of metals Cu, Co, Mn, and Zn in the TSFs was high compared to the allowable limit in soils. According to Ballabio et al. (2018) and WHO (2007); the permissible limit for Cu concentration in soils globally is about 5 to 30 ppm, while for Co 40 ppm and Zn 70 to 120 ppm. In the case of Mn, soil background values from potentially uncontaminated site are often adopted as reference values (Ikenaka et al., 2010). Concentration of metals in the collected rhizospheric soil samples in the current study (Table 7-2) was observed to be high, suggesting potential for exploiting phytomining technologies. The residual base metals reported in this study can be harnessed using this approach, thus providing access to resources that cannot be easily accessed using conventional mining techniques.

Copper concentration was reported to be substantially higher than any other metal in the TSFs. In comparison with other studies, residual Cu concentration in the TSFs was slightly above the values reported from an abandoned As TSF in North-East district of Botswana, whose concentration ranged from 865 ppm to 2125 ppm (Vogel and Kasper, 2002), Nyala Magnesite mine, Limpopo, South Africa (26.3 ppm) (Jeleni et al., 2012), and in historical TSF site in Hokkaido, Japan whose concentration ranged from 1400 to 6500 ppm (Tabelin et al., 2019). The high concentration of Cu observed in the rhizospheric soils could be accredited to the processing efficiency and geology of the study area.

The profiles of Cu, Co, Mn, and Zn in soils were observed to vary between sampling sites. The spatial variations pertaining to concentration of selected metals in the rhizospheric soils were observed to be site specific. For instance, the study showed that comparatively, Cu concentrations in soils where higher at TSF52 (≈11018 ppm), TSF68 (≈3855 ppm) and TSF11 (≈3712 ppm) respectively, compared to the rest (≈1618 ppm).

7.4.3. Plant Uptake of Metals

The accumulation of metals (Cu, Co, Mn, and Zn) by plants differed significantly among species (Table S7-2), thus suggesting differences in response to their exposure to metals. Most of the herbaceous plants accumulated higher metal content in their BGM than in their AGM, indicating the existence of metal exclusion strategy among them. Similar patterns of metal accumulation in BGM and AGM were also observed by Nouri et al. (2009) in plant species on the Hame Kasi Mine area in Iran. Such species tend to develop a mechanism of limiting metal uptake to aerial parts as a strategy to mitigate against phytotoxicity (van der Ent et al., 2015). The high concentration of metals in BGM plant parts and low uptake to AGM plant parts suggests that such plant species have wide ecological amplitudes that make them survive in contaminated edaphic environments (Gabbard and Fowler, 2007; Wang et al., 2019). Observably, above-ground accumulation of metals also varied among plant species. Some plants accumulated higher metal content in their AGM compared to their BGM. The high translocation factor, ratio of metal concentration between AGM and BGM, indicated internal detoxification ability or metal resilience mechanisms that makes such plants suitable for phytomining applications (DalCorso et al., 2019). Phytomining potential among plant species has been observed by a number of researchers across a number of metal contaminated wastelands namely Bor, Serbia (Antonijević et al., 2012), Kerman, Southeast Iran (Ghaderian and Ravandi, 2012), and southern Spain (Conesa and Faz, 2011). The concentration of Cu in the studied plant species were reported to be above 1000 ppm, the limit above which plant species qualifies to be considered hyperaccumulators (Baker, 1981; van der Ent et al., 2015). Particularly, most of the dominant herbaceous plants reported in the current study may be considered Cu hyperaccumulators as the Cu concentration in their below-ground biomass and above-ground biomass were above the recommended thresholds (Table 7-5). The accumulation of Mn and Zn was above 300 – 500 ppm level, making phytomining potential for Mn and Zn less attractive. Studies by Forte and Mutiti, (2017), and Mehes-Smith et al., (2013) have shown that hyperaccumulation of Mn and Zn requires threshold amounts > 10000 ppm, while the thresholds of Co and Ni are > 1000 ppm. Based on this, the plant species reported in this study may not be considered for phytomining of Co, Mn, Ni, and Zn as they are below the required thresholds. However, potential exists to further explore the processing flowsheets to ascertain the techno-economic cut-off.

Table 7-5: Plant species that were observed to have relatively high IVI, Cu accumulation and potential use of phytomining technologies. The colours show plants with similar attributes

No.	Plant Species	Relative IVI	Life form	AGM_Cu	BGM_Cu	BCF (Root)	BCF (Shoot)	TF
1	*Andropogon eucomus*	9,2	Perenial	2036	2655	2,63	2,14	0,96
2	*Cynodon ductylon*	3,9	Perenial	1608	2118	2,76	1,48	1,02
3	*Cyperus alternifolius*	3,2	Perenial	1281	2433	1,37	0,77	0,46
4	*Hyparrhenia Filipendula*	8,3	Perenial	1491	2355	1,79	1,47	0,88
5	*Richardia scabra*	3,8	Annual	1053	1473	0,51	0,30	0,81
6	*Blumae Alata*	2,8	Annual	1171	1158	0,58	0,49	1,14
7	*Blumae brevipes*	2,8	Perenial	1106	1324	0,46	0,30	0,88
8	*Conyza floribunda*	5,9	Perenial	1288	1968	0,95	0,62	1,15
9	*Gloriosa Simplex*	3,4	Perenial	913	1053	0,72	0,76	1,29
10	*Tithonia Rotundifolia*	2,5	Perenial	840	1304	0,72	0,47	0,73
11	*Vernonia Glabra*	5,5	Perenial	735	1256	1,34	1,39	0,82
12	*Sopubia ramosa*	2,9	Perenial	620	908	0,85	0,63	0,81
13	*Elephantropus scuber*	3,1	Perenial	649	754	0,51	0,46	1,37

7.4.4. Heavy Metal Influence on Species Abundance

The correlation analysis (Table 7-3) showed the inter-element relation on the pathways and sources of metals. A strong positive correlation is indicative of likely influence of particular metals on the structure of plant communities while a negative correlation entails the influence of metal on indices of diversity may not be significant (Liu et al., 2014). Overall, Cu concentration in the soil were positively and significantly correlated with AGM (0.76) and BGM (0.63) (Table 7-3), while Mn negatively and strongly correlated with AGM (-0.53). This strong positive correlation suggests that the influence of Cu on the structural composition of plant species on the TSFs is significant. This could be attributed to the relative abundance of Cu on the TSFs compared to other metals. Studies by Koptsik et al. (2003) and Beattie et al. (2018) have shown that, in metal contaminated soils, the structure of the major diversity indices is largely related to specific dominant metals in the soil, thus, suggesting that metal content is one of the best soil-related predictors of diversity of species in contaminated areas. Nevertheless, other factors such as bioavailability, toxicity and mobility of metals in soils tend to influence vegetation growth on sites contaminated by metals (Wuana and Okieimen, 2011). The interplay between plants and metals depends on metal sequestration systems of different plants, reactivity and solubility with organic and inorganic molecules of individual plant species (Chibuike and Obiora, 2014). In addition, the mobility of metals may be influenced by the pH of soil and organic matter (Adamczyk-Szabela et al., 2015).

Marginally negative correlation between the metals Co and Zn with AGM and BGM values was observed in most of the plant species. This could be attributed to the relatively low concentration of Co and Zn reported on the TSFs. Several studies have linked high Zn and other metals to reduced diversity and density of plant communities on contaminated sites (Cabot et al., 2019; Nguyen et al., 2019; Rout and Das, 2003). Mapaure et al. (2011) in their investigations of Cu, Zn, Pb, As, and Cr in soils near Kombat mine wastelands in Namibia observed changes in structure and composition of vegetation in close proximity to mine dumps, particularly species sensitive to pollution disappeared. It is noteworthy that metals in low concentration are essential for plant growth and establishment, however, with high concentration of metals, selected metal species such as Zn can be toxic and inhibit plant growth (Morkunas et al., 2018).

7.4.5. Bioconcentration and Translocation Factors of Plant Species

The ecological tolerance of divergent categories of plant species differ depending on their behaviour characteristics and habitat (Suman et al., 2018). The plants exhibited different behaviour tendencies concerning the capability to accumulate metals in BGM and AGM.The observed metal concentration in the BGM and AGM were used to measure the BCF and TF to assess if the plant species is an accumulator or excluder (Baker, 1981). Excluder plant species are those that accumulate metals in their BGM and inhibit their migration to AGM while plant accumulators are those that translocate high metals from BGM to AGM. In addition, excluders are preferable for phytostabilization while accumulators are preferred for phytomining (Usman et al., 2012). In this study, better translocation for Cu was observed within *A. eucomus, B. alata, C. floribunda, C. ductylon, C. alternifolius, H. filipendula, E. scuber* and *V. glabra.* Their BCF_{root}, BCF_{shoot}, and TF were found to be > 1, with Cu content above 1000 ppm, traits that are seemingly promising for phytomining technologies. In contrast to the other plant species, these could be considered as hyperaccumulators of Cu because of the elevated concentrations (Lange et al., 2017). The BCF and TF for Zn was equally observed to be >1 for most of the plant species, however the metal content was below the acceptable limit (above 10000 ppm) for the plants to be considered for phytomining (Suman et al., 2018). Similarly, selected plant species were observed to have excellent BCF and TF for Mn and Co, however, the low metal accumulation augments their unsuitability for phytomining (Balafrej et al., 2020; Suman et al., 2018).

Additional criteria for phytomining as proposed by Vangronsveld et al. (2009) include: effective translocation, fast growth and high biomass. Through the different assessments done during this study, it was observed that some plants exhibited effective metal translocation mechanisms, with 72.9% of the plant life form indicating that they can be grown throughout the year. Although potential for high biomass production was not investigated, it was evident that phytomining as a rehabilitation measure for TSFs in Zambia, has potential as a viable technology.

7.5. Conclusion

The present study of active and historical TSFs provides insight into the ability of native herbaceous plant species to interact with metal concentrations present in the rhizosphere. The results suggest that *A. eucomus, B. alata, C. floribunda, C. ductylon, C. alternifolius, H. filipendula, E. scuber* and *V. glabra* are Cu hyperaccumulators as evident from the BCF, TF, and metal content in the below-ground and above-ground biomass. We suggest the use of these native plant species has a better edge over the other identified species in this study. In addition, they provide an opportunity for metal recovery, thus creating a new resource base and employment opportunities. The accumulation of metal contaminants by the plants may lead to a reduction in metal mobilization and impacts on the aquatic ecosystem. These findings are of global importance in addressing the challenges of sustainable management of water resources that need to recover from environmental shocks and stresses induced by anthropogenic activities. This is important in Zambia where the impacts of metal mobilization from mine wastes have been confirmed. Therefore, mitigating water quality problems in the Kafue River catchment using phytomining technologies holds a promise for the environmental sustainability of this area.

7.6. References

Adamczyk-Szabela, D., Markiewicz, J., Wolf, W.M., 2015. Heavy Metal Uptake by Herbs. IV. Influence of Soil pH on the Content of Heavy Metals in Valeriana officinalis L. Water, Air and Soil Pollution 226. https://doi.org/10.1007/s11270-015-2360-3

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals—Concepts and applications. Chemosphere 91, 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Andrade, S., Moffett, J., Correa, J.A., 2006. Distribution of dissolved species and suspended particulate copper in an intertidal ecosystem affected by copper mine tailings in Northern Chile. Marine Chemistry 101, 203–212. https://doi.org/10.1016/j.marchem.2006.03.002

Antonijević, M.M., Dimitrijević, M.D., Milić, S.M., Nujkić, M.M., 2012. Metal concentrations in the soils and native plants surrounding the old flotation tailings pond of the Copper Mining and Smelting Complex Bor (Serbia). Journal of Environmental Monitoring 14, 866–877. https://doi.org/10.1039/C2EM10803H

Armstrong, C.W., Vondolia, G.K., Foley, N.S., Henry, L.-A., Needham, K., Ressurreição, A., 2019. Expert Assessment of Risks Posed by Climate Change and Anthropogenic Activities to Ecosystem Services in the Deep North Atlantic. Frontiers in Marine Science 6. https://doi.org/10.3389/fmars.2019.00158

Baker, A., 1981. Accumulators and Excluders Strategies in Response of Plants to Heavy Metals. Journal of Plant Nutrition 3, 643–654. https://doi.org/10.1080/01904168109362867

Baker, A., Brooks, R., 1989. Terrestrial Higher Plants Which Hyperaccumulate Metallic Elements, A Review of Their Distribution, E. Ecol. Phytochem. Biorecovery 1, 81-86. http://dx.doi.org/10.1080/01904168109362867

Balafrej, H., Bogusz, D., Triqui, Z., Abdelkarim, G., Bendaou, N., Abdelaziz, S., Mouna, F., 2020. Zinc Hyperaccumulation in Plants: A Review. Plants 9, 562. https://doi.org/10.3390/plants9050562

Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P., Montanarella, L., 2018. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Science of The Total Environment 636, 282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268

Beattie, R.E., Henke, W., Campa, M.F., Hazen, T.C., McAliley, L.R., Campbell, J.H., 2018. Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased. Soil Biology and Biochemistry 126, 57–63. https://doi.org/10.1016/j.soilbio.2018.08.011

Böck, K., Polt, R., Schülting, L., 2018. Ecosystem Services in River Landscapes, in: Schmutz, S., Sendzimir, J. (Eds.), Riverine Ecosystem Management: Science for Governing Towards a Sustainable Future, Aquatic Ecology Series. Springer International Publishing pp. 413–433. https://doi.org/10.1007/978-3-319-73250-3_21

Borg, I., Groenen, P.J.F., 2005. Modern multidimensional scaling: Theory and applications, 2nd ed, Modern multidimensional scaling: Theory and applications, 2nd ed. Springer Science + Business Media, New York, NY, US.

Cabot, C., Martos, S., Llugany, M., Gallego, B., Tolrà, R., Poschenrieder, C., 2019. A Role for Zinc in Plant Defense Against Pathogens and Herbivores. Frontiers in Plant Science 10, 1171. https://doi.org/10.3389/fpls.2019.01171

Carrick, P.J., Forsythe, K.J., 2020. The species composition—ecosystem function relationship: A global meta-analysis using data from intact and recovering ecosystems. PLOS ONE 15, e0236550. https://doi.org/10.1371/journal.pone.0236550

Castilla, J.C., Nealler, E., 1978. Marine environmental impact due to mining activities of El Salvador copper mine, Chile. Marine Pollution Bulletin 9, 67–70. https://doi.org/10.1016/0025-326X(78)90451-4

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. Journal of Environmental Quality 36, 1429–1443. https://doi.org/10.2134/jeq2006.0514

Chibuike, G.U., Obiora, S.C., 2014. Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods [WWW Document]. Applied and Environmental Soil Science 2014. https://doi.org/10.1155/2014/752708

Conesa, H.M., Faz, Á., 2011. Metal Uptake by Spontaneous Vegetation in Acidic Mine Tailings from a Semiarid Area in South Spain: Implications for Revegetation and Land Management. Water, Air and Soil Pollution 215, 221–227. https://doi.org/10.1007/s11270-010-0471-4

Cowx, I.G., Lungu, A., Kalonga, M., 2018. Optimising hydropower development and ecosystem services in the Kafue River, Zambia. Mar. Freshwater Res. 69, 1974–1982. https://doi.org/10.1071/MF18132

Crépin, J., Johnson, R.L., 1993. Soil Sampling for Environmental. In Carter R, M. (Ed) Soil sampling and methods of analyses.

DalCorso, G., Fasani, E., Manara, A., Visioli, G., Furini, A., 2019. Heavy Metal Pollutions: State of the Art and Innovation in Phytoremediation. International Journey of Molecular Science 20, 3412. https://doi.org/10.3390/ijms20143412

de Klerk, A.R., Oberholster, P.J., van Wyk, J.H., Truter, J.C., Schaefer, L.M., Botha, A.-M., 2016. The effect of rehabilitation measures on ecological infrastructure in response to acid mine drainage from coal mining. Ecological Engineering 95, 463–474. https://doi.org/10.1016/j.ecoleng.2016.06.070

Deines, A.M., Bee, C.A., Katongo, C., Jensen, R., Lodge, D.M., 2013. The potential trade-off between artisanal fisheries production and hydroelectricity generation on the Kafue River, Zambia. Freshwater Biology 58, 640–654. https://doi.org/10.1111/fwb.12055

Festin, E.S., Tigabu, M., Chileshe, M.N., Syampungani, S., Odén, P.C., 2019. Progresses in restoration of post-mining landscape in Africa. Journal of Forestry Research 30, 381–396. https://doi.org/10.1007/s11676-018-0621-x

Forte, J., Mutiti, S., 2017. Phytoremediation Potential of Helianthus annuus and Hydrangea paniculata in Copper and Lead-Contaminated Soil. Water, Air and Soil Pollution 228, 77. https://doi.org/10.1007/s11270-017-3249-0

Gabbard, B.L., Fowler, N.L., 2007. Wide Ecological Amplitude of a Diversity-Reducing Invasive Grass. Biological Invasions 9, 149–160. https://doi.org/10.1007/s10530-006-9012-x

Gajić, G., Djurdjević, L., Kostić, O., Jarić, S., Mitrović, M., Pavlović, P., 2018. Ecological Potential of Plants for Phytoremediation and Ecorestoration of Fly Ash Deposits and Mine Wastes. Frontiers in Environmental Science 6, 124. https://doi.org/10.3389/fenvs.2018.00124

Ghaderian, S.M., Ghotbi Ravandi, A.A., 2012. Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. Journal of Geochemical Exploration, Phytoremediation of polluted soils 123, 25–32. https://doi.org/10.1016/j.gexplo.2012.06.022

Golder Associates, 2011. Mineral experts report: Mopani Copper Mines Plc

Gratão, P.L., Prasad, M.N.V., Cardoso, P.F., Lea, P.J., Azevedo, R.A., 2005. Phytoremediation: green technology for the clean-up of toxic metals in the environment. Brazilian Journal of Plant Physiology 17, 53–64. https://doi.org/10.1590/S1677-04202005000100005

Haddaway, N.R., Cooke, S.J., Lesser, P., Macura, B., Nilsson, A.E., Taylor, J.J., Raito, K., 2019. Evidence of the impacts of metal mining and the effectiveness of mining mitigation measures on social–ecological systems in Arctic and boreal regions: a systematic map protocol. Environmental Evidence 8, 9. https://doi.org/10.1186/s13750-019-0152-8

Hasnaoui, S.E., Fahr, M., Keller, C., Levard, C., Angeletti, B., Chaurand, P., Triqui, Z.E.A., Guedira, A., Rhazi, L., Colin, F., Smouni, A., 2020. Screening of Native Plants Growing on a Pb/Zn Mining Area in Eastern Morocco: Perspectives for Phytoremediation. Plants 9, 1458. https://doi.org/10.3390/plants9111458

Hunt, A.J., Anderson, C., Bruce, N.C., Garcia, A.M., Graedel, T., Hodson, M.E., Meech, J., Nassar, N., Parker, H.L., Rylott, L., Sotiriou, K., Zhang, Q., Clark, J.H., 2014. Phytoextraction as a tool for green chemistry. Green Processing and Synthesis 3, 3–22. DOI:10.1515/gps-2013-0103

Ikenaka, Y., Nakayama, S., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Heavy Metal Contamination of Soil and Sediment in Zambia. African Journal of Environmental Science and Technology 4, 729–739. https://doi.org/10.4314/ajest.v4i11.71339

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. Linear Regression, in: James, G., Witten, D., Hastie, T., Tibshirani, R. (Eds.), An Introduction to Statistical Learning: With Applications in R, Springer Texts in Statistics. Springer, New York, NY, pp. 59–126. https://doi.org/10.1007/978-1-4614-7138-7_3

Jeleni, M.N., Gumbo, J.R., Muzerengi, C., Dacosta, F.A., 2012. An assessment of toxic metals in soda mine tailings and a native grass: a case study of an abandoned Nyala Magnesite mine, Limpopo, South Africa. Presented at the Water Pollution 2012, New Forest, UK, pp. 415–426. https://doi.org/10.2495/WP120361

Kacholi, D.S., 2014. Analysis of Structure and Diversity of the Kilengwe Forest in the Morogoro Region, Tanzania [WWW Document]. International Journal of Biodiversity 2014. https://doi.org/10.1155/2014/516840

Kambole, M.S., 2003. Managing the water quality of the Kafue River. Physics and Chemistry of the Earth, Parts A/B/C 28, 1105–1109. https://doi.org/10.1016/j.pce.2003.08.031

Koelmel, J., Prasad, M.N.V., Pershell, K., 2015. Bibliometric Analysis of Phytotechnologies for Remediation: Global Scenario of Research and Applications. International Journal of Phytoremediation 17, 145–153. https://doi.org/10.1080/15226514.2013.862207

Koptsik, S., Koptsik, G., Livantsova, S., Eruslankina, L., Zhmelkova, T., Vologdina, Z., 2003. Heavy metals in soils near the nickel smelter: chemistry, spatial variation, and impacts on plant diversity. Journal of Environmental Monitoring 5, 441–450. https://doi.org/10.1039/B210397B

Lange, B., Ent, A. van der, Baker, A.J.M., Echevarria, G., Mahy, G., Malaisse, F., Meerts, P., Pourret, O., Verbruggen, N., Faucon, M.-P., 2017. Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytologist 213, 537–551. https://doi.org/10.1111/nph.14175

Larson, L., Larson, P., Johnson, D.E., 2019. Differences in Stubble Height Estimates Resulting from Systematic and Random Sample Designs. Rangeland Ecology & Management 72, 586–589. https://doi.org/10.1016/j.rama.2019.03.007

Lei, D., Duan, C., 2008. Restoration potential of pioneer plants growing on lead-zinc mine tailings in Lanping, southwest China. Journal of environmental sciences (China) 20, 1202–9. https://doi.org/10.1016/S1001-0742(08)62210-X

Liao, K., Bai, Y., Huo, Y., Jian, Z., Hu, W., Zhao, C., Qu, J., 2018. Integrating microbial biomass, composition and function to discern the level of anthropogenic activity in a river ecosystem. Environment International 116, 147–155. https://doi.org/10.1016/j.envint.2018.04.003

Liu, G., Xue, W., Tao, L., Liu, X., Hou, J., Wilton, M., Gao, D., Wang, A., Li, R., 2014. Vertical Distribution and Mobility of Heavy Metals in Agricultural Soils along Jishui River Affected by Mining in Jiangxi Province, China. Clean – Soil, Air, Water 42, 1450–1456. https://doi.org/10.1002/clen.201300668

Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R., Zhang, Z., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotoxicology and Environmental Safety 126, 111–121. https://doi.org/10.1016/j.ecoenv.2015.12.023

Mapaure, I., Chimwamurombe, P.M., Mapani, B.S., Kamona, F.A., 2011. Impacts of mine dump pollution on plant species diversity, composition and structure of a semiarid savanna in Namibia. African Journal of Range and Forage Science 28, 149–154. https://doi.org/10.2989/10220119.2011.647753

Mártin, Kambing'a, Stephen, Syampungani, 2012. Performance of Tree Species Growing on Tailings Dam Soils in Zambia: A Basis for Selection of Species for Re-vegetating Tailings Dams [WWW Document]. undefined. URL /paper/Performance-of-Tree-Species-Growing-on-Tailings-Dam-M%C3%A1rtin-Kambing%27a/f0a409b15be7816fdf5add21cc86098b46ce8dc9 (accessed 12.15.20).

Martínez-Pagán, P., Faz Cano, Á., Aracil, E., Arocena, J.M., 2009. Electrical Resistivity Imaging Revealed the Spatial Properties of Mine Tailing Ponds in the Sierra Minera of Southeast Spain. Journal

of Environmental and Engineering Geophysics 14, 63–76. https://doi.org/10.2113/JEEG14.2.63
Mehes-Smith, M., Nkongolo, K., Cholewa, E., 2013. Coping Mechanisms of Plants to Metal Contaminated Soil. Environmental Change and Sustainability. https://doi.org/10.5772/55124
Moraghan, J.T., Mascagni, H.J., 1991. Environmental and Soil Factors Affecting Micronutrient Deficiencies and Toxicities, in: Micronutrients in Agriculture. John Wiley & Sons, Ltd, pp. 371–425. https://doi.org/10.2136/sssabookser4.2ed.c11
Morkunas, I., Woźniak, A., Mai, V.C., Rucińska-Sobkowiak, R., Jeandet, P., 2018. The Role of Heavy Metals in Plant Response to Biotic Stress. Molecules 23, 2320. https://doi.org/10.3390/molecules23092320
Nachiyunde, K., Ikeda, H., Okuda, T., Nishijima, W., 2013. Assessment of Dissolved Heavy Metal Pollution in Five Provinces of Zambia. Journal of Environmental Protection 4, 80–85. https://doi.org/10.4236/jep.2013.41B015
Naidu, M.T., Kumar, O.A., 2016. Tree diversity, stand structure, and community composition of tropical forests in Eastern Ghats of Andhra Pradesh, India. Journal of Asia-Pacific Biodiversity 9, 328–334. https://doi.org/10.1016/j.japb.2016.03.019
Nguyen, T.D., Cavagnaro, T.R., Watts-Williams, S.J., 2019. The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. Scientific Reports 9, 14880. https://doi.org/10.1038/s41598-019-51369-5
Norrgren, L., Pettersson, U., Örn, S., Bergqvist, P.-A., 2000. Environmental Monitoring of the Kafue River, Located in the Copperbelt, Zambia. Archives of Environmental Contamination and Toxicology 38, 334–341. https://doi.org/10.1007/s002449910044
Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A.H., Yousefi, N., 2009. Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. Environmental Earth Sciences 59, 315–323. https://doi.org/10.1007/s12665-009-0028-2
Pandey, J., Verma, R.K., Singh, S., 2019. Suitability of aromatic plants for phytoremediation of heavy metal contaminated areas: a review. International Journal of Phytoremediation 21, 405–418. https://doi.org/10.1080/15226514.2018.1540546
Parker, H.L., Rylott, E.L., Hunt, A.J., Dodson, J.R., Taylor, A.F., Bruce, N.C., Clark, J.H., 2014. Supported Palladium Nanoparticles Synthesized by Living Plants as a Catalyst for Suzuki-Miyaura Reactions. Plos One 9, e87192. https://doi.org/10.1371/journal.pone.0087192
Perlatti, F., Ferreira, T.O., Romero, R.E., Costa, M.C.G., Otero, X.L., 2015. Copper accumulation and changes in soil physical–chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: Implications for restoration of mine sites. Ecological Engineering 82, 103–111. https://doi.org/10.1016/j.ecoleng.2015.04.085
Richards, M.P.M., 1993. The new genetics: some issues for social scientists. Sociology of Health and Illness 15, 567–586. https://doi.org/10.1111/j.1467-9566.1993.tb00363.x
Roeling, I.S., Ozinga, W.A., van Dijk, J., Eppinga, M.B., Wassen, M.J., 2018. Plant species occurrence patterns in Eurasian grasslands reflect adaptation to nutrient ratios. Oecologia 186, 1055–1067. https://doi.org/10.1007/s00442-018-4086-6
Rout, G., Das, P., 2003. Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc. Agronomie 23, 3–11. https://doi.org/10.1051/agro:2002073
Salas-Luevano, M.A., Manzanares-Acuña, E., Vega-Carrillo, H.R., Letechipia de Leon, C., Mercado, G.A., 2008. Study of Phytoremediation Features of Plants Growing in Tailing Soils. Epidemiology 19, S223. https://doi.org/10.1097/01.ede.0000291913.67412.43
Sandell, E., 2020. Post-Mining Restoration in Zambia [WWW Document]. URL https://pub.epsilon.slu.se/16846/ (accessed 12.15.20).
Sheoran, V., S.Sheoran, A., Poonia, P., 2013. Phytomining of gold: A review. Journal of Geochemical Exploration 128, 42–50. https://doi.org/10.1016/j.gexplo.2013.01.008
Sheoran, V., Sheoran, A.S., Poonia, P., 2009. Phytomining: A review. Minerals Engineering 22, 1007–1019. https://doi.org/10.1016/j.mineng.2009.04.001

Sikamo, J., Mwanza, A., Mweemba, C., 2016. Copper mining in Zambia - history and future. Journal of the Southern African Institute of Mining and Metallurgy 116, 491–496. https://doi.org/10.17159/2411-9717/2016/v116n6a1

Sinkala, T., 2018. Integrated phytomining and ethanol production in the Zambian Copperbelt to minimize mine decontamination costs and environmental and social impacts: a review. Journal of the Southern African Institute of Mining and Metallurgy 118, 815–824. https://doi.org/10.17159/2411-9717/2018/v118n8a4

Small, N., Munday, M., Durance, I., 2017. The challenge of valuing ecosystem services that have no material benefits. Global Environmental Change 44, 57–67. https://doi.org/10.1016/j.gloenvcha.2017.03.005

Sracek, O., Kříbek, B., Mihaljevič, M., Majer, V., Veselovský, F., Vencelides, Z., Nyambe, I., 2012. Mining-related contamination of surface water and sediments of the Kafue River drainage system in the Copperbelt district, Zambia: An example of a high neutralization capacity system. Journal of Geochemical Exploration 112, 174–188. https://doi.org/10.1016/j.gexplo.2011.08.007

Suman, J., Uhlik, O., Viktorova, J., Macek, T., 2018. Phytoextraction of Heavy Metals: A Promising Tool for Clean-Up of Polluted Environment? Frontiers in Plant Science 9. https://doi.org/10.3389/fpls.2018.01476

Swacha, G., Botta-Dukát, Z., Kącki, Z., Pruchniewicz, D., Żołnierz, L., 2017. A performance comparison of sampling methods in the assessment of species composition patterns and environment–vegetation relationships in species-rich grasslands. Acta Societatis Botanicorum Poloniae 86, 3561. https://doi.org/10.5586/asbp.3561

Tabelin, C., Sasaki, A., Igarashi, T., Tomiyama, S., Villacorte-Tabelin, M., Ito, M., Hiroyoshi, N., 2019. Prediction of acid mine drainage formation and zinc migration in the tailings dam of a closed mine, and possible countermeasures. MATEC Web of Conferences 268, 06003. https://doi.org/10.1051/matecconf/201926806003

Usman, A.R.A., Lee, S.S., Awad, Y.M., Lim, K.J., Yang, J.E., Ok, Y.S., 2012. Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites, Korea. Chemosphere 87, 872–878. https://doi.org/10.1016/j.chemosphere.2012.01.028

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H., 2019. The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant Tetraena qataranse. Science Reports 9. https://doi.org/10.1038/s41598-019-42029-9

van der Ent, A., Baker, A.J.M., Reeves, R.D., Chaney, R.L., Anderson, C.W.N., Meech, J.A., Erskine, P.D., Simonnot, M.-O., Vaughan, J., Morel, J.L., Echevarria, G., Fogliani, B., Rongliang, Q., Mulligan, D.R., 2015. Agromining: farming for metals in the future? Environmental Science and Technology 49, 4773–4780. https://doi.org/10.1021/es506031u

van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research 16, 765–794. https://doi.org/10.1007/s11356-009-0213-6

Vogel, D.H., Kasper, B., 2002. Mine soils on abandoned gold mine tailings in Francistown. Report, North-East District, Republic of Botswana.

Volk, J., Yerokun, O., 2016. Effect of Application of Increasing Concentrations of Contaminated Water on the Different Fractions of Cu and Co in Sandy Loam and Clay Loam Soils. Agriculture 6, 64. https://doi.org/10.3390/agriculture6040064

Wang, P., Sun, Z., Hu, Y., Cheng, H., 2019. Leaching of heavy metals from abandoned mine tailings brought by precipitation and the associated environmental impact. Science of The Total Environment 695, 133893. https://doi.org/10.1016/j.scitotenv.2019.133893

Wang, Y., Feng, Q., Chen, L., Yu, T., 2013. Significance and Effect of Ecological Rehabilitation Project in Inland River Basins in Northwest China. Environmental Management 52, 209–220. https://doi.org/10.1007/s00267-013-0077-x

WHO / FAO. Expert committee on food additives. Cambridge: Cambridge University Press; 2007. p. 329–36.

Wilson-Corral, V., Anderson, C.W.N., Rodriguez-Lopez, M., 2012. Gold phytomining. A review of the relevance of this technology to mineral extraction in the 21st century. Journal of Environmental Management 111, 249–257. https://doi.org/10.1016/j.jenvman.2012.07.037

Wuana, R.A., Okieimen, F.E., 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation [WWW Document]. ISRN Ecology. https://doi.org/10.5402/2011/402647

Zerbo, I., Bernhardt-Römermann, M., Ouédraogo, O., Hahn, K., Thiombiano, A., 2016. Effects of Climate and Land Use on Herbaceous Species Richness and Vegetation Composition in West African Savanna Ecosystems. Journal of Botany 2016, 1-11. https://doi.org/10.1155/2016/9523685

7.7. Supplementary Material

Table S7-1: Species richness, abundance, IVI, and life form

Family	Species	Number of Species	Relative Frequency	Relative Dominance	Relative IVI	Life form	
Euphorbiaceae	Acalypha ciliata	1	0,16	0,29	0,23	Perennial	
Poaceae	Adropogon schinzi	1	0,16	0,42	0,29	Perennial	
Amaranthacea	Aerva lanata	3	0,48	0,61	0,54	Perennial	
Amaranthacea	Aerva leucura	1	0,16	0,6	0,38	Perennial	
Asperagaceae	Agava Siciliana	3	0,48	0,98	0,73	Perennial	
Amaranthacea	Amaranthrus graecizans	1	0,16	0,14	0,15	Perennial	
Vitaceae	Ampelocissus africana	2	0,32	5,32	2,82	Perennial	
Poaceae	Andropogon eucomus	53	8,52	9,86	9,19	Perennial	
Poecea	Anthephara burtti	14	2,25	1,92	2,08		Annual
Poecea	Aristida stenostachya	1	0,16	0,42	0,29	Perennial	
Acanthaceae	Barleria spinulosa	2	0,32	0,22	0,27	Perennial	
Asteraceae	Bidens pilosa	2	0,32	1,2	0,76	Perennial	
Asteraceae	Bidens steppia	20	3,22	0,12	1,67	Perennial	
Asteraceae	Blumae Alata	26	4,18	1,33	2,76		Annual
Asteraceae	Blumae brevipes	16	2,57	2,94	2,76	Perennial	
Asteraceae	Blumae Mollis	4	0,64	1,73	1,19		Annual
Poaceae	Brachlaria brizantha	1	0,16	0,29	0,23	Perennial	
Amaranthacea	Celosa trygina	3	0,48	0,46	0,47		Annual
Vitaceae	Cissus Trothae	5	0,8	0,96	0,88	Perennial	
Asteraceae	Conyza floribunda	33	5,31	6,5	5,9	Perennial	
Asteraceae	Conyza purrdopappa	3	0,48	0,6	0,54		Annual
Asteraceae	Conzya welwitschii	5	0,8	1,32	1,06	Perennial	
Papilionaceae	Crotalaria anua	6	0,96	1	0,98		Annual
Poaceae	Cynodon ductylon	44	7,07	0,62	3,85	Perennial	
Cyperaceae	Cyperus alternifolius	32	5,14	1,22	3,18	Perennial	
Cyperaceae	Cyperus Nudicaulis	1	0,16	3,34	1,75	Perennial	
Cyperaceae	Cyperus rotundus	8	1,29	0,85	1,07	Perennial	
Papilionaceae	Desmodium repandum	1	0,16	1,57	0,87	Perennial	
Poaceae	Digitaria Debilis	10	1,61	1,42	1,52		Annual
Dioscoreaceae	Dioscorea buchanani	1	0,16	0,08	0,12	Perennial	
Orchidaceae	Disa welwitschii	8	1,29	1,63	1,46	Perennial	
Asteraceae	Elephantropus scuber	22	3,54	2,59	3,07	Perennial	
Iridaceae	Gladiolus unguculatus	13	2,09	0,39	1,24	Perennial	
Liliaceae	Gloriosa Simplex	20	3,22	3,52	3,37	Perennial	
Asteraceae	Gnaphalium lutea-album	1	0,16	0,96	0,56		Annual
Tiliaceae	Grewia schinzi	2	0,32	0,97	0,65	Perennial	
Asteraceae	Harplocapha scaposa	12	1,93	0,12	1,03	Perennial	
Poecea	Hyparrhenia Filipendula	65	10,45	6,17	8,31	Perennial	
Convolvulaceae	Ipomoea obscura	2	0,32	0,66	0,49		Annual
Asteraceae	Lactuca zambeziaca	17	2,73	1,56	2,15	Perennial	
Filicies	Nephrolepis undulata	4	0,64	0,88	0,76		Annual
Asteraceae	Nidorella Auriculata	8	1,29	1,6	1,44		Annual
Poecea	Panicum Heterostachyum	4	0,64	2,37	1,51	Perennial	
Filicies	Pellaea longipilosa	1	0,16	0,85	0,51	Perennial	
Poaceae	Phragmites mauritanus	4	0,64	0,78	0,71	Perennial	
Polygonaceae	Polygonum pulchrum	2	0,32	1,28	0,8	Perennial	
Polygonaceae	Polygonum Senegalense	16	2,57	0,78	1,67	Perennial	
Rubiaceae	Richardia scabra	10	1,61	6,04	3,83		Annual
Pedaliaceae	Sesamum calycinum	2	0,32	0,27	0,29		Annual
Poecea	Setaria Pallidifusca	3	0,48	1,13	0,81	Perennial	
Poaceae	Setaria Spacelate	11	1,77	2,46	2,11	Perennial	
Poaceae	Setaria uomblei	9	1,45	0,48	0,96		Annual
Solanaceae	Solanum indicum distichum	1	0,16	0,67	0,42	Perennial	
Scrophulariaceae	Sopubia ramosa	20	3,22	2,64	2,93	Perennial	
Poaceae	Sporobolus Panicoides	5	0,8	0,13	0,47		Annual
Poaceae	Stereochlaena cameronii	4	0,64	0,85	0,75	Perennial	
Dioscoreaceae	Tacca leotopetaloides	5	0,8	1,69	1,25	Perennial	
Asteraceae	Tithonia Rotundifolia	13	2,09	2,81	2,45		Annual
Asteraceae	Vernonia Glabra	35	5,63	5,35	5,49	Perennial	
	Total	**622**	**100**	**100**	**100**		

Table S7-2: Heavy metal concentration in the below-ground biomass (BGM) and above-ground biomass (AGM) of herbaceous plant species colonizing the TFSs

Plant Species	Cu	Co	Mn	Zn	Cu	Co	Mn	Zn
	Root	Root	Root	Root	Shoot	Shoot	Shoot	Shoot
Cynodon ductylon								
Range	600 - 9950	120 - 1250	90 - 2420	30 - 3500	450 - 4430	80 - 1370	120 - 6490	30 - 1720
Mean + SD	2166 ± 1647	385 ± 302	756 ± 526	227 ± 519	1677 ± 937	346 ± 267	1083 ± 1332	241 ± 292
Aerva lanata								
Range	600 - 9000	210 - 550	120 - 1310	20 - 140	380 - 3030	30 - 510	200 - 1180	40 – 600
Mean + SD	2344 ± 2524	177 ± 206	547 ± 334	49 ± 41	1095 ± 757	139 ± 193	524 ± 282	167 ± 161
Andropogon eucomus								
Range	540 - 16800	190 - 2370	50 - 2440	20 - 2150	520 - 5040	220 - 800	360 - 3130	10 – 900
Mean + SD	3011 ± 2612	280 ± 427	1204 ± 627	338 ± 495	1952 ± 1375	129 ± 226	1067 ± 642	152 ± 145
Bidens steppia								
Range	640 - 4120	60 - 600	200 - 1140	70 - 600	640 - 2000	30 - 160	390 - 860	90 - 280
Mean + SD	1252 ± 1085	102 ± 184	621 ± 350	164 ± 168	1027 ± 518	32 ± 60.7	587 ± 182	143 ± 65.3
Hyparrhenia Filipendula								
Range	390 - 8060	160 - 1140	200 - 3600	40 - 1720	490 - 5400	80 - 550	250 - 2420	40 - 3040
Mean + SD	2458 ± 1762	460 ± 250	1213 ± 767	175 ± 237	1542 ± 1027	319 ± 140	923 ± 477	205 ± 478
Vernonia Glabra								
Range	620 - 6400	100 - 300	210 - 1060	30 - 800	390 - 1480	130 - 560	220 - 4200	60 - 230
Mean + SD	1237 ± 1178	207 ± 70.1	426 ± 260	164 ± 186	748 ± 279	234 ± 129	684 ± 798	139.57 ± 66.4
Digitaria Debilis								
Range	160 - 3900	400 - 430	520 - 6300	40 - 940	860 - 2150	210 - 400	800 - 2140	60 -370
Mean + SD	2148 ± 1255	415 ± 21.2	1590 ± 1808	220 ± 291	1698 ± 429	305 ± 134	1220 ± 409	184 ± 97.2
Stereochlaena Cameronii								
Range	1790 - 5840	90 - 420	940 - 2410	60 - 210	950 - 3420	60 - 290	470 - 2270	60 - 570
Mean + SD	3378 ± 1786	283 ± 150	1453 ± 653	135 ± 81	2568 ± 1135	180 ± 98.3	1045 ± 826	198 ± 249
Cissus Trothae								
Range	520 – 19300		230 – 1050	40 – 330	590 – 2860		460 - 1250	30 - 220
Mean + SD	4052 ± 7478	680	493 ± 311	153 ± 104	1200 ± 846	0	713 ± 303	108 ± 64.3
Harplocapha scaposa								
Range	540 – 2200	150 – 250	180 - 1280	50 - 220	540 – 5000	140 - 240	210 - 1180	80 - 250
Mean + SD	1393 ± 585	186 ± 42	618 ± 342	103 ± 51.2	1455 ± 1282	167 ± 33	684 ± 305	170 ± 67.8
Gladiolus unguculatus								
Range	280 - 4150	90 – 270	140 – 1710	50 -120	350 – 1340	70 - 600	130 - 620	50 - 900
Mean + SD	969 ± 1148	180 ± 127	389 ± 489	82 ± 24.4	824 ± 286	255 ± 235	326 ± 211	251 ± 297
Cyperus rotundus								
Range	1100 - 4130	140 - 770	400 - 1670	70 - 800	560 - 1830	140 - 300	310 - 1220	50 - 280
Mean + SD	2733 ± 1177	342 ± 265	905 ± 473	276 ± 248	957 ± 45475	188 ± 75.4	569 ± 287	130 ± 71.7
Crotalaria anua								
Range	360 - 680		150 - 560	30 - 50	360 - 1400	110 - 180	280 - 750	70 - 110
Mean + SD	498 ± 149	0	388 ± 196	42 ± 8.37	652 ± 429	140 ± 27.4	438 ± 190	90 ± 15.8
Cyperus alternifolius								
Range	420 - 1900	90 -2360	190 - 2400	40 - 500	430 - 4030	100 - 600	230 - 3120	30 - 600
Mean + SD	2884 ± 3180	570 ± 638	1064 ± 576	115 ± 92.3	1336 ± 938	286 ± 155	785 ± 605	121 ± 106
Bidens pilosa								
Range	620 - 2410		230 - 1540	80 - 290	400 - 560		300 - 620	140 - 150
Mean + SD	1515 ± 1266	125 ± 177	885 ± 926	185 ± 149	480 ± 113	0	460 ± 226	145 ± 7.07
Nephrolepis undulata								
Range	530 - 2250		180 - 1180	50 - 100	410 - 940		240 - 570	90 - 240
Mean + SD	1170 ± 941	300	590 ± 524	80 ± 26.5	683 ± 265	600	413 ± 166	140 ± 86.6
Setaria uomblei								
Range	1570 - 3390	130 - 440	400 - 1800	80 - 900	590 - 6850	140 - 300	370 - 2350	130 - 600
Mean + SD	2820 ± 763	250 ± 119	1088 ± 575	362 ± 322	2518 ± 2506	212 ± 69.8	982 ± 787	284 ± 189
Ipomoea obscura								
Mean + SD	560	0	200	40	1140	280	1060	50
Adropogon schinzi								
Mean + SD	1500	200	960	70	1560	260	1080	90
Brachlaria brizantha								
Mean + SD	910	0	470	340	940	0	450	110
Acalypha ciliata								
Mean + SD	570	0	220	90	390	0	160	80
Grewia schinzi								
Mean + SD	840	0	290	50	1140	0	580	120

Table S7-3: Plant information used to plot Multidimension Scaling (MDS)

	Plant Name	AGM_Cu	BGM_Cu	AGM_Co	BGM_Co	AGM_Mn	BGM_Mn	AGM_Zn	BGM_Zn	AGM_Ni	BGM_Ni
1	Acalypha ciliata	390	570	NA	NA	160	220	80	90	NA	NA
2	Adropogon schinzi	1560	1500	260	200	1080	960	90	70	NA	NA
3	Aerva lanata	957	1119	233	260	475	448	128	49	NA	120
4	Agava Siciliana	443	797	NA	90	315	457	100	70	NA	NA
5	Ampelocissus africana	1290	1260	NA	NA	960	360	60	210	NA	NA
6	Andropogon eucomus	2036	2655	436	445	1126	1209	157	321	187	215
7	Anthephara Burtti	3003	3304	418	283	1469	1826	157	169	100	230
8	Aristida stenostachya	1890	1010	290	110	1930	580	100	60	NA	NA
9	Barleria Spinulosa	650	917	NA	225	385	477	193	273	NA	NA
10	Bidens pilosa	480	1515	NA	250	460	885	145	185	NA	NA
11	Bidens steppia	919	1181	145	240	563	579	134	181	NA	NA
12	Blumae Alata	1171	1158	399	322	815	591	175	132	130	130
13	Blumae Brevipes	1106	1324	163	148	1068	612	113	287	NA	80
14	Blumae Mollis	990	1595	180	370	670	790	70	60	NA	NA
15	Brachlaria brizantha	940	910	NA	NA	450	470	110	340	NA	NA
16	Celosa trygina	690	920	NA	NA	400	230	93	35	NA	NA
17	Cissus Trothae	1200	1002	NA	680	713	493	108	153	200	NA
18	Conyza floribunda	1288	1968	254	244	765	624	172	245	NA	160
19	Conyza purrdopappa	1510	1070	560	240	860	490	180	125	NA	NA
20	Conzya welwitschii	930	1180	180	120	520	410	210	60	NA	NA
21	Crotalaria anua	652	498	143	NA	438	388	90	42	NA	NA
22	Cynodon ductylon	1608	2118	334	351	1119	767	245	240	115	NA
23	Cyperus Alternifolius	1281	2433	275	520	765	1122	123	94	115	260
24	Cyperus Nudicaulis	1020	4000	270	950	410	1440	85	110	NA	NA
25	Cyperus rotundus	1002	2756	188	393	591	866	139	214	NA	NA
26	Digitaria Debilis	1698	2148	305	415	1220	1001	184	220	175	110
27	Dioscorea buchanani	885	1120	NA	160	825	850	105	95	NA	NA
28	Disa Heterostachyum	876	1747	215	332	384	658	89	358	NA	NA
29	Elephantropus scuber	649	754	140	146	366	385	422	71	NA	NA
30	Gladiolus unguculatus	816	969	255	180	362	449	251	82	NA	NA
31	Gloriosa simplex	913	1053	90	130	501	377	197	283	NA	NA
32	Grewia schinzi	1140	840	NA	NA	580	290	120	50	NA	NA
33	Harplocapha scaposa	1061	1437	164	180	682	638	174	109	NA	NA
34	Hyparrhenia Filipendula	1491	2355	313	438	926	1230	233	193	130	130
35	Ipomoea obscura	1140	560	280	NA	1060	200	50	40	NA	NA
36	Lactuca zambeziaca	757	1061	NA	100	486	555	258	124	90	NA
37	Nephrolepis undulata	683	1170	660	300	413	590	140	80	NA	NA
38	Nidorella Auriculata	1301	1428	640	503	948	638	117	137	NA	NA
39	Panicum Heterostachyu	827	1973	NA	555	635	825	2327	1590	NA	NA
40	Phragmites Mauritanus	1397	1186	215	360	946	544	184	104	NA	100
41	Polygonum Pulchrum	1955	1575	265	260	598	520	80	63	NA	NA
42	Polygonum Senegalense	823	1523	270	348	901	570	119	72	NA	NA
43	Richardia Scabra	1053	1473	283	472	517	628	167	123	140	NA
44	Sesamum calycinum	1700	440	NA	NA	1220	390	180	170	NA	NA
45	Setaria Pallidifusca	943	2633	NA	NA	740	933	197	250	NA	NA
46	Setaria Spacelate	1609	2099	388	326	1225	986	149	333	170	NA
47	Setaria uomblei	1288	2356	165	283	634	890	208	528	NA	NA
48	Sopubia Ramosa	620	908	170	219	419	508	133	160	NA	70
49	Sporobolus Panicoides	1776	2988	NA	NA	570	986	1396	1778	NA	NA
50	Stereochlaena Cameron	2568	3378	255	347	1045	1453	198	135	NA	NA
51	Tacca leontopetaloides	630	460	NA	NA	550	170	80	180	NA	NA
52	Tacca leotopetaloides	503	293	NA	NA	233	245	85	98	NA	NA
53	Tithonia Rotundifolia	840	1304	185	195	520	534	282	123	NA	NA
54	Vernonia Glabra	735	1256	234	201	658	512	143	202	NA	220

CHAPTER 8: CONCLUSION AND RECOMMENDATIONS

8.1. Introduction

Mine waste characterization and investigation of possible ecological risks are essential before selecting suitable disposal strategy and remediation technique. Driven by the proposition of industrial ecology coupled with the desire to circularise economies, remediation of wastelands is increasingly preferred to be integrated with valorisation of wastes and protection of ecosystems services. More importantly, the ecological risk of waste material should be meticulously quantified prior to embarking on remediation strategies. In the case of sulphidic mine waste materials, basic ecological burdens potentially include the generation of acid rock drainage (ARD) and associated metal mobilization. Typically, standard static tests of acid base accounting are used to characterize ARD generation potential, however, they do not consider the relative rate of acidification and neutralization. These limitations have resulted in the development of the biokinetic tests that provide for the relative acidification and neutralization under microbial action and can be conducted within a relatively short period of three months.

Through this study, the potential for copper tailing material to generate ARD was explored using the standard static tests in conjunction with the UCT biokinetic test by considering the pH-controlled and non-pH-controlled batch tests. In addition to this, the potential and ecological risk of metal deportment from the mine waste was investigated through column bioleach tests. The study attempted to increase understanding of factors that control a multitude of environmental variables in a copper mining affected region (i.e., the Kafue River catchment). Using this information, our knowledge base on potential impact from copper tailing storage facilities (TSFs) was expanded through the usage of the datasets generated from ARD tests, column bioleach tests and increased intensity of water, sediment, macroinvertebrate, soil, and plant monitoring. The study was set up to provide potential for improved monitoring of water quality associated with active and historic TSFs on the Zambian Copperbelt; and to provide opportunity to better understand potential interventions to reduce impact of mining activities on water resources and associated arable land through comparison of environmental burden, particularly on aquatic systems, across a range of co-located tailings facilities. This was facilitated through the collection of rigorous data sets over a three-year period, during both the rainy and post rainy season. Particularly, the Nselaki Stream, Fikondo Stream and Mululu Stream, including nearby arable lands, all located within the Kafue River catchment on the Zambian Copperbelt, were selected as case studies as they have potential to be impacted by a range of active and historic TSFs. The information generated from this study may help in contributing to the sustainable management of water resources associated with copper mining. Further, the essence of this study was broadened by testing the potential of phytomining as one of the possible means to mitigate mobilization of metals through detailed case studies of seven copper mine wastelands on the Zambian Copperbelt.

Through this, we investigated both potential to extract value from these low-grade resources and their use as natural rehabilitation systems, addressing the potential to extend these through biomimicry by studying the plant species thriving in metal rich areas (TSFs) and classification of these plants based on their functional traits (i.e., bioconcentration factors, translocation factors). Using this knowledge, we proposed suitable plant species for phytomining technologies based on their ability to either exclude or accumulate metals on contaminated sites. The information generated from this study may influence the strategies for managing ecosystems linked to copper mining impacts, to such an extent that affected ecosystems might be capable of coping and recovering from shocks and stresses, as well as maintaining their integrity.

8.2. Assessing Ecological Risks Associated with Tailings Storage Facilities on the Zambian Copperbelt

Through the analysis of standard static and biokinetic tests for ARD, data was collected from mine tailing material from three tailings facilities in the Kafue River catchment. The results from the static test data were validated by the biokinetic test data, thus reducing uncertainty of inconclusive results on ARD generation potential of the copper mine waste. Useful information on expected ARD generation under microbial colonisation of the tailing material can be obtained using the biokinetic tests. Conditions likely to mitigate or aggravate ARD generation can be ascertained. The standard static tests were conducted using acid base accounting (ABA) and net acid generation (NAG) tests, so as to determine the behaviour of tailing samples under chemical conditions. The ABA results reported indicated that the tailing samples from Chibuluma TSF, TSF14 and TSF15A were non-acid forming with NAPP values of

-114 kg H_2SO_4, -229 kg H_2SO_4 and -409 kg H_2SO_4, respectively. Observably, the copper tailing samples were identified using mineralogical techniques to have high acid consuming minerals like mica, chlorite, kaolinite, and other slow weathering minerals. Owing to this, high negative NAPP values were reported, with NAG pH observed to be neutral (≈6.82). These results were validated by the biokinetic tests conducted to account for the effect of microbial activities under pH-controlled and non-pH-controlled conditions.

The influence of microbial activities presented by inoculum culture of 1×10^9 cells of a mesophilic iron-oxidising culture dominated by *Leptospirillum ferriphilum* (ATC 49881), and 1×10^9 cells of an *Acidithiobacillus caldus* (DSM 8485) culture on copper tailing samples (100 per cent < 150 µm) was investigated. These laboratory microorganisms are indicative of the species typical in natural acidophilic bioleaching environments. Further, studies (Harrison et al., 2019) on metagenomic analysis of microbial communities associated with mine waste have shown that endemic organisms can be found in all mine waste samples. To provide a time correlated attestation on the acid generating and acid neutralizing behaviour, the pH controlled, and non-pH controlled biokinetic tests were conducted. The reported results from the non-pH-controlled biokinetic tests showed that the pH increased to pH 7, due to the dissolution of acid neutralizing minerals. There was minimal effect of the inoculum on the tests, as the resultant increase in pH impedes the onset for microbial activity of acidophiles. While it is plausible that the quality of drainage from the mine waste under consideration is likely to be neutral, the batch test does not allow the washout of dissolved neutralising potential with time as found in an open system in practice. This potential was explored by removing this capacity through acidification. Under pH controlled biokinetic tests, the rise in pH was controlled adding 0.5M sulphuric acid to resume pH 2 daily, thus depleting dissolved neutralisation potential, limiting the precipitation of ferric iron and creating conducive conditions for the acidophilic inoculum to thrive. A steady rise in redox potential was observed under pH-controlled conditions up to ≈660 mV compared to ≈350 mV under non-pH-controlled conditions. The concentration of ferrous iron was relatively low and comparable, ranging from 0 to 250 mg/L tests. The low ferrous oxidation could be attributed to the high carbonate and low sulphur content in the tailing samples, confirming low acidification potential.

In addition to the biokinetic test, the possible ecological burden beyond ARD generation potential was considered. Particularly, the potential for metal mobilization from the mine tailing samples. This was assessed using the column bioleach experiments, permitting metal mobilization to be ascribed to certain environmental conditions, from neutral aqueous conditions to acidic conditions. The bioleach columns were used to simulate conditions similar to the tailing storage facilities (TSFs); while it is noteworthy that this may not fully represent TSF conditions, it is useful in predicting the quality of drainage expected from the TSF. Furthermore, an ecological risk assessment based on the analysis of leachates collected from the bioleach columns was conducted to form management strategies aimed at mitigating ecological risks associated with copper mine waste such as tailings. The results reported higher mobilities of elements Ca, Fe, Cu, Al, Mg and Mn under acidic conditions compared to non-acidic conditions. Solubilization of Fe and Cu was equally notable in the leachates from non-acidic column though at lower concentrations. It was observed that the mobilization of Fe was the antecedent to the release of metal species of interest (Cu, Co, Mn, Ni, Zn and Pb). The probable ecological risks associated with metal deportment from the bioleach column tests was assessed using a risk evaluation analysis developed by Broadhurst and Petrie (2010), to identify elements of particular interest within each tailing sample. Under high acidic conditions, Fe and Cu exhibited high ecological risk while the risk was moderate under non-acidic conditions. The ecological risk under acidic conditions for Ca, Al, Mg and Mn was observed to vary from low to moderate, while negligible ecological risk profiles were observed with elements of interest Pb, Co, Zn, and Ni. Low to negligible ecological risk was reported at high pH in elements of particular interest. However, over time, cumulative effects of low metal mobilization may cause significant ecological degradation.

8.3. A Watershed Approach in Investigating Essential Abiotic Causes in Support of Aquatic Resource Management: A Case Study of Nselaki, Mululu and Fikondo Streams

Based on the objectives set to compare the key abiotic drivers in the watersheds impacted by copper mining, the following conclusions were made. First and foremost, the selected streams were comparable with regards to similarity in land use activities that they are exposed to and other environmental aspects such as climatic conditions and geology. It was evident from the current study that the various changes reported in the sediment and water quality when contrasting Nselaki, Mululu and Fikondo streams; aquatic abiotic drivers like pH, TDS and turbidity were observed to be important. The impact of the TSFs on the streams was generally observed to be similar and significant. Hence, the importance of proper monitoring and management of the streams and TSFs was shown.

Certain metals (e.g., Cu and Mn) were observed to be in high concentration in the streams, particularly in the sediments. Since metals are not permanently bound to sediments, this represents a source of secondary pollution as metals can be released due to changes in environmental conditions (e.g., pH and temperature) or when biological or physical disturbances occur in the sediments. Comparatively, there was low variability in concentration of selected metal species in the sediments across the sampling sites downstream. However, significant differences were observed between upstream and downstream sampling sites. This observation augments the influence of TSFs on water resources. Additionally, the sediments were characterised by medium to fine grained material similar to the material found in the toe drains of the TSFs. The results from this study suggest that the TSFs had significant influence on water and sediment quality in Nselaki Stream, Mululu Stream and Fikondo Stream as they compromise the ability of the streams to sequester metal contamination. The study showed a significant link between potential mobilization of metal species of interest and metals found in the water and sediment samples downstream.

8.4. A Contrastive Analysis of Macroinvertebrate Community Structures from Nselaki, Mululu and Fikondo Streams (Copperbelt – Zambia) using Multifarious Lines of Attestation

The statistical approaches and multifarious lines of evidence adopted to characterize the composition of macroinvertebrate community structures in the watersheds helped to understand the influence of mine wastelands on biological integrity. Water and sediment quality, taxa richness, abundance and biotic index score were used to detect changes between the upstream condition and deteriorated downstream stream segments. Characterization of environmental features and macroinvertebrate assemblages of the impacted streams sites and reference upstream condition were important to assess the degree of anthropogenic (TSFs) impact on streams and to propose mitigation measures. Different habitat conditions can be exhibited by streams due to natural variations or anthropogenic influences. In the case of the selected streams, habitat deterioration was mainly caused by TSFs impact. The biotic index score was used to assess the ecological health status (Gonçalves and Menezes, 2011; Water-Monitoring, 2007); status is influenced by water quality and macroinvertebrate community structures. This method was considered a suitable assessment tool for ecological health status in Zambia, a country with limited professionals in river health assessment fields. Additionally, data can be collected by individuals with minimal field and theoretical knowledge.

A comparative analysis of the macroinvertebrate diversity (H') and richness (D), shown through the Shannon and Simpson indices indicated that Mululu Stream had relatively higher values, and hence is less impacted, than Nselaki Stream and Fikondo Stream, which appear to be substantially influenced by mine wastelands. Macroinvertebrate community analysis revealed that the streams were dominated by macroinvertebrate species semi-tolerant and tolerant to pollution. Particularly, amphipod and leech were the most dominant species in the streams, with the stream condition varying from fair (relatively contaminated) to poor (highly contaminated). Interestingly, there was no observed clear trend pertaining to changes in diversities of macroinvertebrate community structures between the sampling points downstream. However, notable differences were observed between upstream and downstream sites based on the grouping of the macroinvertebrate community structures. Species tolerant to pollution dominated downstream sampling sites, this could be attributed to the deteriorated stream segments observed downstream. Based on the biotic index score, the sites were characterised poor, fair, good and excellent, with the most diverse macroinvertebrate assemblages observed under excellent habitat conditions; this occurred upstream of the TSF. The information generated using macroinvertebrates showed that TSFs had significant influence on water and sediment quality.

8.5. Assessment of Metal Accumulation in Soils and Vegetables Irrigated by Watersheds Impacted by Mine Wastelands

Most mines in developing countries are located in areas where agriculture plays an important economic role in the livelihood of communities. Mining related activities have potential to significantly impact farmers for key inputs like water and land. We used agricultural or gardening lands close to the TSFs in the Kafue River catchment to compare spatial variation of mining impacts on agriculture. Soils and food crops irrigated by Nselaki, Fikondo and

Mululu streams were investigated to increase understanding on the influence of TSFs on water quality and associated livelihoods. The results from this study indicated that metal content in both the soils from this arable land and the vegetables irrigated by the impacted watersheds were above allowable limits by WHO/FAO. Metal uptake in the edible parts of the plants was remarkably higher than allowable limits, particularly in Amaranthus across all sampling sites. The metal contamination load index in vegetables ranged from 2.64 to 16.8 times higher than the acceptable limit for Cu, 2.3 to 7.49 for Co, 6.09 to 21.4 for Mn, 0.6 to 1.24 for Zn and 23.3 to 71.1 for Pb. In soils, the NIPI contamination was severe for Cu (≈27.8), Co (≈10.3), Mn (≈11.5) and Zn (≈3.43), and light for Pb (≈1.31). Although low metal concentration in water samples used for irrigation was observed, the effect was overshadowed by high metal content observed to have accumulated in the soil and its associated accumulation in vegetable samples. This suggests that, over time, low metal contamination in the irrigation water can cause significant environmental degradation. Consequently, soil resources need to be protected from slow but insidious metal contamination from mine waste. In addition, there is also need for mapping of arable land with regards to metal contamination with the view of increasing awareness of its impact on edible crops and of designing suitable remediation measures. The results from this study will be communicated to increase consciousness of metal contamination from mine waste in decision makers and result in improved monitoring of agricultural lands, improved public awareness and the will for effective remediation of soils as well as of pollution mitigation.

8.6. Potential Rehabilitation Measure through Phytomining of Ecological Infrastructure in Response to Metal Mobilization from Mine Wastelands

The potential use of native herbaceous plant species through phytomining or phytoextraction technologies to mitigate metal mobilization was investigated in copper mine wastelands in the Kafue River catchment. In the recent past, global interest in the topic has increased as phytomining technology is associated with environmental, social, and economic benefits. However, it is noteworthy that the economics of phytomining essentially depends on metal content in the rhizospheric soil, plant biomass and metal uptake, as well as price of metal. The potential of extracting value from native plant species thriving on TSFs rich in copper and cobalt was evaluated. Observably, the results suggest that *A. eucomus, B. alata, C. floribunda, C. ductylon, C. alternifolius, H. filipendula, E. scuber* and *V. glabra* are Cu hyperaccumulators based on the BCF, TF and metal content in the below-ground and above-ground biomass. Furthermore, the relatively high value of the importance value index (IVI) illustrates the wide-spread nature these plants, showing potential for rehabilitation.

The present study has shown that there is clear potential for phytomining to achieve both value recovery (Cu) and remediation (Cu, Co, Mn, Zn) interventions. It is proposed that this approach can be piloted as a treatment step in an effort to address the environmental pollution orchestrated by metal mobilization from mine wastelands. The ecological integrity can be improved significantly as implementation of this technology will reduce potential contamination issues in the future thus services offered by the current ecosystem can be improved.

8.7. Recommendations

The following recommendations are proposed based on the findings from this study.

8.7.1. Application of Sequential Chemical Extraction

In addition to the detailed mineralogical assessment of metal dissociation under standard static (ANC and NAG), UCT biokinetic and column bioleach tests, the sequential chemical extraction should be conducted as well as reductive conditions in soils and sediments. This would help to highlight the environmental burden beyond salinization and acidification. Particularly, the potential for metal mobilization from mine waste, allowing mobilization to be attributed to specific environmental conditions from strong acid conditions, through oxidative conditions, weak acidic conditions to neutral aqueous conditions. To date, only the extremes of these conditions have been explored. Using this approach, a rigorous understanding of metal mobilization together with an environmental risk assessment may demonstrate the ecological burden posed by mine waste.

8.7.2. Increased Monitoring and Management of Mine Wastelands

From the results generated through this study, it is recommended that the quality of drainage seeping from mine wastelands be monitored regularly and more intensively. The results from this study have shown that there is potential for metal mobilization, through the high metal content in sediments, arable land, and crops. The wastelands need to be managed well to ensure that there is minimal impact on the ambient environment. This calls for improved disposal approaches, mitigation measures on existing TSFs and improved monitoring.

It is also recommended that abiotic monitoring be blended with biomonitoring to increase awareness of the effects of contaminants, and implementation of fast mitigation measures (active and passive treatment of discharges from mine wastelands) aimed at reducing metal mobilization from the TSFs into compartments of the environment. Particularly, macroinvertebrate community composition should be used as focal points in biomonitoring. Contamination impacts usually result in replacement of sensitive macroinvertebrate species, rather than general reduction in diversity. Macroinvertebrate communities are readily monitored by semi-skilled personnel and correlate well with the physico-chemical analysis requiring skilled personal. Hence, they provide a rapid system of alert.

There is need to increase monitoring of crops irrigated by impacted water resources, with the view of recommending the choice of food crops with metal exclusion strategies. Currently, there are limited studies on food crops suitable for cultivation in mining regions. The development of suitable crops will reduce metal uptake by humans and increase food security. Treatment of irrigation water should also be considered to protect soil and plants (and health of people).

8.7.3. Phytomining: A Promising Tool for Sustainable Remediation Strategies

The results from this study have shown, based on plant accumulation and exclusion strategies, that some of the plants thriving on the mine wastelands are copper hyperaccumulators. This development is important in the advancement of knowledge for potential phytomining technologies on copper wastelands. It is recommended that native herbaceous plant species be used as a treatment step in reducing metal mobilization and in recovering or removing removal of metals from the wastelands or both. The impact of such vegetation-based approaches is already demonstrated by the lesser impact on the ecosystem integrity as TSF14 which is well-vegetated. Copper phytomining holds a promising future that will result in not only environmental benefits but as well as social and economic benefits. It is proposed that techno-economic analysis of this potential be undertaken with the identified plants and that a pilot study be commissioned to identify timelines and efficiency of recovery.

Equally, other remediation strategies such as phytostabilization can be exploited in tandem with phytomining. Most of the plants reported in this study are excluders.