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Feasibility of soil amendments and ryegrass in circum-neutral and acidic mine tailings remediation

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**FEASIBILITY OF SOIL AMENDMENTS AND RYEGRASS IN
CIRCUM-NEUTRAL AND ACIDIC MINE TAILINGS
REMEDICATION**



PRIFYSGOL
BANGOR
UNIVERSITY

A thesis submitted to Bangor University for a degree of Philosophiae Doctor (PhD) in
Environmental Science

2018

by

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SUMMARY

Abandoned metal mines and the large quantity of tailings deposited around these mines, which are rich in heavy metals, represent a significant threat to terrestrial, aquatic and atmospheric environments. The heavy metal content of mine tailings is a serious contamination issue worldwide. These elements can be transferred through the food chain and accumulate in the human body, causing considerable harm due to their carcinogenicity, mutagenicity and teratogenicity. Thus, it is extremely important to remove heavy metals from polluted media (tailings, soils and water) and/ or to inactivate them within these media in order to conserve human health and permit continued use and development of urban and rural land. Therefore, the remediation of multi heavy metal-contaminated sites was investigated using different approaches and under various conditions to reduce the phytoavailability of cadmium (Cd), zinc (Zn), lead (Pb) and copper (Cu) in mine tailings collected from Parys and Britannia copper mines, and Pandora lead mine located in North Wales, UK.

A sequential extraction protocol was applied to examine the distribution of studied heavy metals among different geochemical fractions and to evaluate the effectiveness of the remediation strategies in reducing target metal availability. The metal fractionation results obtained showed that the chemical dynamics of target metals were similar in Parys and Britannia copper mines with the majority of studied metals associated with the residual fraction. However, the exchangeable fraction retained high proportions of investigated metals in Pandora tailings. Organic and inorganic amendments individually and in combination with the phytoremediation technique were examined in terms of their ability to reduce the mobility and phytoavailability of target metals to plant and soil biota.

Application of 2% of several organic and inorganic amendments (zero valent iron (Fe^0), zeolite, green waste biochar and green waste compost) to Pandora and Parys tailings was investigated during a 1-year pot experiment under uncontrolled conditions in Henfaes research station. The application of 2% of amendments modified several physiochemical properties of treated tailings particularly highly acidic tailings with various effects on the bioavailability of target metals. The added amendments reduced the metal availability at the beginning of study, but with time the mobility of investigated metals fluctuated and was higher than in the control treatments on several occasions. We concluded that 2% of amendments was not sufficient to reduce the mobility of several metals.

A greenhouse trial using different mixtures of tailings and uncontaminated soil (US) was conducted using ryegrass as a bio-indicator of phytoremediation technology. The results

showed that applying US enhanced plant biomass production and ryegrass roots accumulated higher levels of heavy metals than shoots with lower levels of heavy metals toxicity indicated e.g., shortness of plant shoots and roots and chlorosis. Ryegrass roots accumulated higher levels of target metals with low translocation of heavy metals to the vegetative parts, with the exception of Zn. Thus, ryegrass may be an effective plant to use for the phytostabilisation of heavy metals in both sites, and a hyperaccumulator plant appropriate for the treatment of Pb in Pandora mine tailings. The metal content in grass tissue depends on the phytoavailable fraction of the metal. Plants grown in Pandora tailings accumulated higher concentrations of heavy metals in their roots than grass planted in Parys tailings, reflecting the higher occurrence of studied metals in the exchangeable fraction in Pandora tailings compared with Parys tailings.

The co-amending mine tailings experiment was conducted to examine the effect of applying phytoremediation (ryegrass) in combination with two types of amendment (Fe^0 and green waste compost, added separately to target tailings). Results indicated that the applied amendments increased the pH of severally acidic tailings (Parys tailings) more effectively and compost was more effective than Fe^0 particularly at a higher dosage to promote ryegrass biomass productivity. Fe^0 did not show significant enhancement of grass biomass production even at a higher dosage. The amendments had various effects on of target metal phytoavailability, metal uptake and translocation within plant tissues. Compost reduced the translocation of Cu, Zn, Pb and Cd within grass tissues in treated Parys mine tailings. For Pandora tailings both amendments increased Cu translocation within grass tissues, but Zn immigration from roots to shoots decreased. Also, the uptake of Zn and Cd by grass roots increased, but Pb uptake was reduced by the addition of amendments to Pandora tailings.

Pb isotopic studies suggested that geogenic routes seemed to be the main source of Pb fingerprinting in Parys tailings, but in Pandora mine tailing the ore processing of Pb appeared to be the major route of Pb pollution. In addition, the applications of compost and Fe^0 did not result in a significant alteration in the Pb isotopic content of ryegrass tissues.

Overall, application of green waste compost supported the establishment of ryegrass cover on mine sites, thus reducing the erosion of mine tailings by wind and rain. This approach also promoted the growth of deep grass roots, which increased the ability of ryegrass to take up heavy metals for storage in the roots or translocate them to aboveground parts prior to harvesting and safe disposal. The application of ryegrass and compost can be used to amend soils exposed to heavy metal contamination by any source including mine tailings. Further studies are necessary in controlled and uncontrolled conditions to examine the feasibility of

using several native plant species supported by organic matter application for phytoremediation of mine tailings.

ACKNOWLEDGMENTS

In the name of Allah, the Beneficent, the Merciful

I will always be extremely thankful for this period of my life spent in Bangor conducting my research to produce this thesis. It has been a fantastic period of my life.

I thank **ALLAH** for facilitating my study.

This study has been made possible by the funding I received from Libyan Ministry of Higher Education and Scientific Research for which I am most grateful.

I am indebted to **Dr. Paula Roberts** and **Dr. Graham Bird** for accepting me for post-graduate research at Bangor University and their guidance, advice and assistances throughout my study. As my supervisors, they have helped me to develop my ideas, supported me during my laboratory and fieldwork and acted as the necessary critics when analysing my results, developing my arguments and formulating my conclusions.

My research also benefited from the support and practical advice of the laboratory technicians working at the Thoday and Environment Centre Wales Buildings in Bangor (**Helen Simpson, Jonathan Roberts and Sarah Chesworth**) together with staff based at the Henfaes Research Centre and **Mr. Ian Harris**.

I wish to dedicate this work to my family that occupy a special place in my life. They include my **father (Irhema), mother (Salima), brothers, sisters, nephews and nieces**.

I also wish to thank all my close friends for their companionship, friendship and support throughout my time at Bangor University.

Last, but not least, I thank friends in Bangor who run Bangor Islamic Student Society for their help and support and those within The Libyan Society.

ABBREVIATIONS

Ammonium acetate	$\text{CH}_3\text{COONH}_4$
Ammonium hydroxide	NH_4OH
Ammonium- nitrogen	$\text{NH}_4^+\text{-N}$
Cadmium	Cd
Carbon- nitrogen ratio	C/N
Copper	Cu
De-ionised water	dH ₂ O
Electro-conductivity	EC
Flame Atomic Absorption Spectroscopy	FAAS
Green waste biochar	B
Green waste compost	C
Hydrochloric acid	HCl
Hydrogen peroxide	H ₂ O ₂
Hydroxyl amine hydrochloride	$\text{NH}_2\text{OH.HCl}$
Inductively Coupled Plasma Mass Spectroscopy	ICP-MS
Lead	Pb
Microgram gram ⁻¹	$\mu\text{g g}^{-1}$
Nitric acid	HNO ₃
Organic matter	OM
Part per million	ppm
Sodium acetate	CH_3COONa
Weight to volume	w/v
Weight to weight	w/w
World Health Organization	WHO
Zeolite	Z
Zero valent iron	Fe ⁰
Zinc	Zn

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1 GENERAL INTRODUCTION

1.1 Background

The pollution of the environment is one of the greatest risks to humankind. Since the industrial revolution began, the level of pollution has increased rapidly due to the rising consumption of coal, oil, natural gas and various human activities. The mismanagement of municipal, industrial and agronomic wastes, radioactive material and remains of chemical compounds as well as the waste of mines contribute to increasing levels of environmental pollution.

Generally, pollution can be defined as the addition of new substances to the natural environment leading to change several properties of ecosystem, consequently changing the functions of ecosystem and natural biodiversity (Jr *et al.*, 2009). During recent decades, there has been increased global concern of the effects of environmental pollution (Pepper *et al.*, 1996). Because pollution affects the atmosphere, all aspects of terrestrial and marine ecosystems and human health (Mani and Kumar, 2014), the World Health Organization (WHO) recorded that, about 25% of diseases which affect human beings are a result of chronic exposure to a polluted environment (Mahmoud and Abdel-Mohsein, 2015). One particular concern from a human and environmental perspective is pollution due to heavy metals.

Heavy metals pollution is a serious global environmental issue and is a result of extensive use of metal rich resources and metal concentrating processes by humankind, which led to increasing contamination levels of heavy metals in surrounding ecosystems. According to Alvarez *et al.* (2014) the main problem with this issue is that the accumulation of these elements still continues due to the rapid growth of industrial processes and their resistance to degradation. Figure 1.1 shows the global production and consumption of zinc (Zn), lead (Pb) and copper (Cu). It is estimated that there are over 10 million polluted sites globally, more than 50% of which are thought to be contaminated with heavy metal(loid)s. Most of these sites occur in developed countries. For example, the United States Environmental Protection Agency (USEPA) report that in the US around 600,000 ha of land is classified as polluted with heavy metal(loid)s and over than 8.30% of these contaminated sites required urgent remediation. In Europe approximately 250,000 sites have been classed as contaminated with heavy metals and during the last 30 years over than 32% of these sites have been treated. Heavy metal pollution has negatively affected food production with approximately 10,000 ha of agriculture land in Germany having been banned for use in crop farming due to its heavy metal content exceeding

permissible levels (Khalid *et al.*, 2017). In the UK, the metal mining industry is a major cause of heavy metal(loid)s pollution. Here, mining activities have taken place over a long period of time and contributed 75% and 50% of global copper (Cu) and lead (Pb) production, respectively in the mid of 19th century (Kossoff *et al.*, 2016). Consequently, the metal extraction process has left a large amount of mine waste on the land surrounding the mining operations resulting in high levels of heavy metal pollution, affecting an estimated 4000 ha of land and several water bodies in the UK. Extreme weather events such as the severe flooding, which affected the UK in the autumn of 2000 have also exacerbated the problem of heavy metal pollution. For instance, flooding of the River Swale in Yorkshire in 2000 led to increased levels of Cd, Zn and Pb in the overbank and channel-edge sediments, posing a high risk to the ecosystem and human health (Dennis *et al.*, 2003). Dust and aerosol particles are also expected to be highly contaminated with heavy metals as a result of mining and smelting activities and can be transported significant distances through atmospheric deposition, with implications for human health, especially in children whose hand-mouth behaviour increases the likelihood of ingestion (Dean *et al.*, 2017). Contaminated mine waters represent another hazard, especially where mismanagement has led to the contamination of drinking water sources. It has been estimated that metal mining in England and Wales has contaminated 7.3% and 3.9% of groundwater and surface water bodies, respectively (Gandy *et al.*, 2007). Globally, concern about the harmful effects of heavy metal contamination on environmental and human health have increased after widespread poisoning by heavy metals was reported following two contamination disasters. In the first case, arsenic trioxides used in wallpaper glue were converted to trimethylarsine [(CH₃)₃As], a toxic, volatile compound resulting in many cases of poisoning. Another heavy metal poisoning case was recorded in Niigata and Bay cities in Japan in the late 1950s, when the Minamata disease effected hundreds of people. The poisoning was attributed to the accumulation of mercury in the body due to the consummation of fish containing high levels of methyl mercuric (CH₃ Hg⁺) (Bolan and Duraisamy, 2003). The main fanatical implication associated directly and indirectly with the contamination of the environment with heavy metals may include; (1) reduced crop production, (2) jobs losses and unemployment, (3) foods contain levels of heavy metals are nutrient deficient which may lead to abnormal develop and grow of people exposure to heavy metal pollution and (4) increasing the poverty due to lees crop production and citizen health effects specially in developing countries (Khan *et al.*, 2015a).

1.2 Heavy metals sources

In terrestrial systems, soil is the main store of many chemical pollutants. Heavy metals can enter the environment as a result of two significant sources; first is natural sources and the other is human activities e.g., mining operations (Bolan *et al.*, 2014). Table 1.1 shows the data were summarized globally showing the levels of several metals emitted to the environment from natural sources of pollution with heavy metals (Nagajyoti *et al.*, 2010) and figure 1.2 shows the potential sources of heavy metals in soil (Mahar *et al.*, 2016).

Table 1. 1 Global input of heavy metals from natural sources into the ecosystem (from Nagajyoti *et al.*, 2010).

Sources	International production (kg ×10 ⁶)	Annual input (kg ×10 ⁶)						
		Cd	Zn	Cu	Pb	Ni	Co	Cr
Windblown dust	6 - 1100	0.25	25	12	10	20	4	5
Volcanic emission	6.5 - 150	0.5	10	4	6.4	3.8	1.4	3.9
Forest fires	2 – 200	0.01	0.5	0.3	0.5	0.6	-	-
Sea salt	300 - 2000	0.002	0.02	0.1	0.1	0.04	-	-

1.2.1 Natural sources

Heavy metals can enter ecosystems through various natural source that include

- 1- Sedimentary rocks and shale naturally contain high levels of metals, these elements can be released into the ecosystem and cause considerably damage (Alloway, 1990).
- 2- Volcanic activities can discharge large amounts of metals into different environments (Alloway, 1990) .
- 3- Natural dust can carry several heavy metals, which were emitted to the atmosphere from different sources. Heavy metals can be mixed with dust particles in the atmosphere, which may cause to redistribute them to various ecosystems by the wind and/or rain (Nagajyoti *et al.*, 2010). For example, the total precipitation of copper (Cu) due to the dust ranged between 100 g ha^{-1} to 480 g ha^{-1} (Alloway, 1990) and lead (Pb) in urban dust recorded $172 - 9600 \mu\text{g g}^{-1}$ (Dean *et al.*, 2017) in the UK.

1.2.2 Anthropogenic sources

Manmade sources are considered the main sources of heavy metal pollution in the environment and these include several types that are:

- 1- Agronomic sources including the use of pesticides fungicides and inorganic fertilizers.
- 2- Municipal and industrial wastes.
- 3- Fossil fuel combustion.
- 4- Secondary metal processing.

5- Mining industry.

Mining processes are particularly recognized as an important sources of global pollution due to the presence of high levels of heavy metals in the tailings of the mining industry (Yang *et al.*, 2016a). Metal mining such as Zn, Pb and Cu throughout human civilization lead to the accumulation of heavy metals in the lands surrounding the operated mines and smelters. For example, there are several examples of Bronze Age copper extraction on the Great Orme, Llandudno, and at Parys Mountain on Isle of Anglesey, North Wales. These were extensively worked, and ore was generally smelted on site until the 18th and 19th Century. Following, expansion during the early industrial revolution to its peak between 1750 and 1900, ore was shipped to other areas of the UK for smelting and we see a shift to pollution from extraction activities only from this time onwards (Palumbo-Roe and Colman, 2010). The mining industry depends on many process including drilling and crushing of ores, disposal the mine tailings, ore transfer, smelting, metal finishing and metal recycling. As a result, all of these procedures have a significant polluting effect on ecosystems (Nagajyoti *et al.*, 2010). For instance, 95% of Pb entering ecosystems is conceded to be a result of the use of Pb by human beings. The accumulation of Pb in environments as a result of mining operations was recorded 260×10^6 tonnes and nearly 85% of this value accumulated during 19th century (Bird, 2011). Su (2014) summarized the input of heavy metals in the soil per year globally due to human activities as table 1.2 illustrates.

Table 1. 2 The input of several sources of heavy metals within terrestrial environment globally $\times 10^3 \text{ ton a}^{-1}$ (adopted from Su, 2014).

Sources	Cd	Cu	Pb	Zn
Metallurgical activities	0 - 0.08	0.95 - 7.6	4.1 - 11	2.7 - 19
Atmospheric deposition	2.2 - 4.8	14 - 36	202 - 263	49 - 153
Agriculture and food wastes	0.0 - 0.3	3 - 38	1.5 - 27	12 - 150
Animal manure	0.2 - 1.2	14 - 80	3.2 - 20	150 - 320
Ash of coal	1.5 - 13	93 - 335	45 - 242	112 - 484
Municipal wastes	0.90 - 7.84	17.9 - 61	20.8 - 71.7	40 - 154

1.3 Justifications for research

In Wales, the mining industry was a significant commercial activity for many hundreds of years. There were more than 313 mines distributed in Wales and nearby areas of England (Davies, 1987). The operation of these mines for long periods of time lead to large inputs of mine tailings into the land around the mines. For instance, according to Walton and Johnson

(1992) and Johnson (2003) the mine tailings covered nearly 200 ha of the land around Parys copper mine. In addition, it has been estimated that, the mining operations in Parys and Mona mines in Anglesey extracted approximately 130,000 tonnes of copper by using 3000 million kg of rock (Jenkins *et al.*, 2000). These wastes are rich in copper, zinc, lead and cadmium and their ores e.g., sphalerite (ZnS), galena (PbS) and chalcopyrite (CuFeS₂), which can be harmful to the ecosystem as figure 1.3 shows. Due to the closure of the mines without a management plan for the mine waste, the areas around Parys, Britannia and Pandora mines have suffered from significant soil degradation and are poorly vegetated with the exception of some metal tolerant plants. Several environmental issues associated with the presence of mine tailings, for example, the generation of acid mine drainage containing high levels of heavy metals due to metal ore oxidation has led to the pollution of Afon Goch on Parys Mountain, and potentially downstream water bodies (Vijaya, 2015). Also, the small particles of tailings dust, which can be carried by wind pose a threat to the surrounding population either directly through inhalation and ingestion or indirectly through contact with skin and outer clothing (Okorie *et al.*, 2012). In this regard, the dominant winds on Parys Mountain come from southwest, therefore the areas located to the northeast of the mine site including areas of grassland and Amlwch town have been affected by heavy metal contaminated dust (Wilson and Pyatt, 2007). Extreme weather events such as flooding may lead to the pollution of the surrounding environment. For example, in 1964 the Snowdonia National Park was affected by a severe storm which resulted in the collapse of the tailings dam and the transfer of 13000 tonnes of mine spoils up to 1000 m along the river, polluting 11 ha of agriculture soil with Pb and Zn and causing cattle death (Davies *et al.*, 2015). Recently, in the summer of 2012, the flooding of several ancient mining sites in central and west Wales lead to the pollution of the sediments of several river catchments with Pb levels recording up to 80 times higher than the permissible Pb threshold and resulting in the poisoning and death of livestock in this area (Haresign *et al.*, 2014).

Therefore, studying the remediation of the contaminated soil in these sites should be should be a priority if the threat posed to the environment and human health by metal(loid) pollution is to be minimised. Several technologies have been applied worldwide to treat contaminated terrestrial and aquatic environments based on the use of chemical and natural amendments with the aim of reducing heavy metal mobility, consequently minimising their potential risk to the ecosystem (Fu and Wang, 2011).

In this context, a few studies have attempted to treat these sites using chemical and organic amendments to reduce the bioavailability of Cu, Pb, Fe and Zn in copper mine tailings in the area around Mynydd, Parys copper mine. For example Khan and Jones (2008) and Khan and Jones (2009) studied the ability of green waste compost, lime and diammonium phosphate to reduce the available forms of Cu, Fe, Pb and Zn in the mine tailings collected from Parys copper mine. Tandy *et al.* (2009) incorporated green waste compost, paper sludge and tertiary amended biosolids to polluted soil collected from Zn and Cu mine at Parys Mountain. In addition, Farrell and Jones (2010) used five types of compost to treat polluted soil collected from Parys Mountain. None of these studies compared green waste sourced amendments (e.g. compost and biochar) with conventional remediation methods (e.g. zeolite and zero valent iron). Here we aim to address this by exploring the ability of using organic and inorganic amendments and phytoremediation to treat the target mine sites. Accordingly, green waste biochar, green waste compost, zeolite and zero valent iron were utilised individually to stabilise Cu, Pb, Zn and Cd in mine tailings collected from Pandora lead mine and Parys copper mine. We used ryegrass (*Lolium perenne*) to study its capability in cleaning up target metals from the tailings of these mines. In addition, separate applications of compost and zero valent iron separately in combination with ryegrass to clean up these sites was examined. The identification of different Pb isotopes in plant tissue enabled us to identify the sources of Pb taken up by plants and therefore identify the sources that should be of most concern on these sites.

1.4 Research aim

The general aim of this study was to investigate the ability of biochar, compost, zeolite, and zero valent iron (Fe^0) to reduce the bio-available forms of lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) in mine wastes collected from Parys copper mine and Pandora lead mine Wales, UK. Also, the ability of using ryegrass to clean up mine sites was examined. The research is intended to evaluate the concentration of Cu, Zn, Cd and Pb in order to estimate the pollution levels, how these elements are distributed among the geochemical fractions of tailings, and suggest the most suitable remediation technology.

1.5 Research objectives

To achieve the main aim of this study the following objectives were set

1. Investigate the distribution of Pb, Cu, Zn and Cd among different geochemical phases of mine tailing soil using specific sequential extraction protocol.
2. Examine the efficiency of organic and inorganic amendments to reduce the bioavailability of Pb, Cu, Zn and Cd in mine wastes and evaluating the changes in the levels of target metals in different tailings phases after three-remediation periods by sequential extraction.
3. Determine the capability of using ryegrass in mine tailings remediation and evaluating the Pb isotope ratios to identify the geochemical routes of Pb contamination in plants tissues and mine tailings.
4. Evaluate the effect of green waste compost and zero valent Iron (Fe^0) amendments on the uptake of target metals and Pb isotopes by ryegrass planted in amended mine tailings.

1.6 Research hypotheses

1. Zn, Cu, Pb and Cd distribution and their chemical speciation are similar in all studied tailings.
2. The applications of a single 2% dosage of organic and inorganic amendments into acidic tailings will:
 - a. reduce the bioavailability of target heavy metals and
 - b. the level of reduction will depend on the type of amendment.
3. Increasing the added proportion of uncontaminated soil to mine tailings will
 - a. reduce the phytoavailable fraction of target heavy metals,
 - b. reduce uptake of studied metals by ryegrass, and
 - c. enhance the ryegrass biomass productivity.

4. The phytoavailable fraction, uptake and translocation of studied metals within ryegrass tissues will decrease with increasing the application rate of amendment and will depend on the type of amendment.
5. Pb distribution in mine tailings and plant tissues reflects that of parent material.

1.7 Thesis outline

Chapter 1: General introduction

This chapter introduces the thesis, its lay out and direction. It describes general aspects of heavy metal pollution and the main sources of this phenomenon and the study aim and objectives.

Chapter 2: Literature Review

This chapter gives the academic background to the study. It discusses the general properties of studied heavy metals and the main factors affecting heavy metal behaviour in soil. The importance of this chapter is to review the literature that has used some technologies utilised in this work and their effectiveness at reducing the availability of heavy metals to flora and soil biota. The chapter summarises current understanding and identifies areas where research should be focussed for further work.

Chapter 3: Site Descriptions

This chapter provides a brief background to the study sites (Parys copper mine, Pandora lead mine Britannia copper mine), their locations and industrial history.

Chapter 4: Assessment the distribution of Pb, Cu, Zn and Cd in different geochemical forms in mine tailings.

In this chapter, we studied the geochemical forms of investigated metals in three mine tailings by using modified European Community Bureau of Reference sequential extraction protocol (BCR) to follow the distribution of target metals in different tailings phases. This chapter also, evaluates the total levels of explored metals in these locations.

Chapter 5: Assessment the ability of green waste biochar, green waste compost, zeolite and zero valent iron for stabilization of Pb, Cu, Zn and Cd in mine tailings.

Here we study the effect of adding amendments on the availability of metals in contaminated mine tailings over time. We added a single dose of amendment to mine wastes in a pot trial and subjected the tailings to ambient UK climate conditions for one year. We evaluate the effect of addition of single dose of used amendments separately to the tailing

samples by monitoring several parameters that have influence on heavy metal mobility after 0, 4, 8 and 12 months from the beginning of experiment.

Chapter 6: Using lead isotopes to identify geochemical routes of lead contamination in mine tailings remediated with ryegrass and uncontaminated soil.

Here we use ryegrass combined with mine tailings and uncontaminated soil to examine the performance of this grass in cleaning up mine sites, and evaluate the Pb isotopes ratios to trace the route of Pb contamination in mine tailings and plant tissues.

Chapter 7: Feasibility of using zero valent iron and green waste compost combined with ryegrass in the phytoremediation of mine tailings.

In this chapter, two amendments with two different dosages was added to tailings collected from two locations with the aim of studying the effect of these materials on the availability and uptake of target metals by used plant, and the influence of these amendments on the translocation of studied metals and Pb isotopes from treated mine spoils to ryegrass tissues.

Chapter 8. Overall discussion, conclusion and further work.

This chapter represents an overall discussion of the findings of the experimental chapters, refers to the main conclusion and highlights the areas of up-coming work.

Appendices part contains additional results presented in tables and figures of all trials that are not included in the thesis chapters.

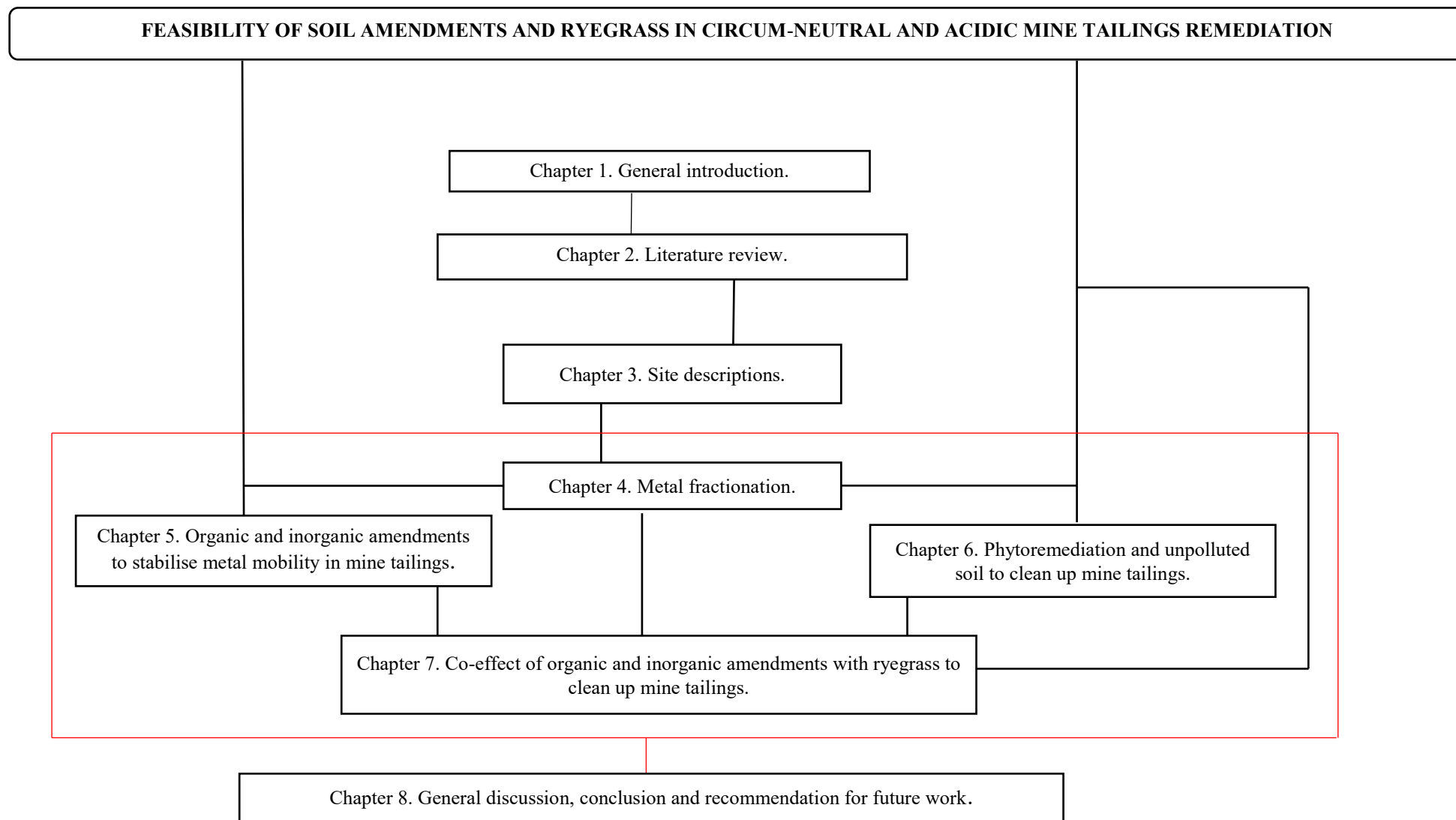


Figure 1. 1 Thesis outline.

2 LITERATURE REVIEW

2.1 Introduction

Soil and groundwater are recognised as the primary sources of heavy metals, which can be distributed in different mediums and affect both human and animal health (Nagajyoti *et al.*, 2010). Concern about the contamination of heavy metals on the environment has increased during the last decades. This has resulted from many factors, such as a greater understanding of how heavy metals accumulate in living tissues, their toxicity at elevated concentrations and the inability of soil biota to degrade or tolerate these elevated concentrations (Liu *et al.*, 2014). The enrichment of the environment with heavy metals has unfavourable effects on living creatures associated with their ability to transfer these metals through the food chain from aquatic and terrestrial mediums to animals then humans (Agnieszka *et al.*, 2014). Figure 2.1 shows the possible processes may lead to accumulate heavy metals on ecosystem and cause health issues to human bodies (Khan *et al.*, 2015a). Heavy metals, which are added to the soil from any source, can (Smith, 2009):

1. Reduce the growth of flora.
2. Enter the food chain through plant uptake.
3. Be utilised by children via compost, when used in the house garden.
4. Influence soil biota activities.
5. Bio-accumulate in plant tissues thus reducing metabolism and enzyme function.
6. Produce reactive oxygen species that may inhibit several biological functions and damage proteins, lipid, DNA and RNA (Mar Gil-Díaz *et al.*, 2014).

2.2 Chemical Principles

The term heavy metals, refers to several metalloids, such as Arsenic (As), transition metals e.g., Cadmium (Cd), Zinc (Zn) and Copper (Cu), lanthanides and actinides and lead (Pb) as a post-transition metal. Heavy metals can be defined as metals with a density five times higher than water and a high atomic mass ranging from 63.50 to 200.6 such as Cu, Zn, Pb and Cd. Few of these elements have a biological function, some are non-essential, while others are essential (Fu and Wang, 2011; Nagajyoti *et al.*, 2010). For example, Pb and Cd are a hazard to flora and fauna at any levels. However, a certain amount of Zn and Cu is essential for certain biological processes Table 2.1 shows the guideline of safe limits of heavy metals in agriculture soils. Furthermore, non-essential heavy metals and metalloids and elevated levels of essential heavy metals are toxic and can cause serious health problems. The health effects of these elements depend on the metal concentration, its oxidation state and metal bioaccumulative ability (Ali *et al.*, 2013). Table 2.2 illustrates the harmful effects of studied heavy metals on human health.

Table 2. 1 International standards of heavy metals ($\mu\text{g g}^{-1}$) in agriculture soils.

Standards	Cd	Cu	Pb	Zn	Ni	References
Indian standard	3-6	135-270	250-500	300-600	75-150	Nagajyoti <i>et al.</i> (2010)
World Health Orgnization	0.35	30	35	90	50	Tembo <i>et al.</i> (2006)
United States of America	0.78	48	55	88.5	29	Su (2014)
China	0.6	100	300	300-500	50	Khan <i>et al.</i> (2015)
European unioun standards	3	140	300	300	75	Nagajyoti <i>et al.</i> (2010).

Table 2. 2 Harmful effects of studied heavy metals on human health (Ali *et al.*, 2013).

Metal	Harmful effects
Zn	Elevated levels can cause fatigue and dizziness.
Cd	Teratogenic, carcinogenic, mutagenic. Causes chronic anaemia and interferes with calcium regulation in biological systems.
Cu	Overdose can cause liver cirrhosis, chronic anaemia, kidney and brain damage, stomach and intestinal irritation.
Pb	Lead poisoning can cause health problems for children e.g., short term memory loss, reduced intelligence, learning disabilities and the development of cardiovascular disease.

The elemental bio-degradation is impossible, they can all be transformed into other more stable forms by oxidation or a reduction process and complexation by organic ligands. According to Cristaldi *et al.* (2017) heavy metals may exist in the soil with various fractions as follow:

1. Free ions.
2. Soluble complexes.
3. Bound to organic material.
4. Forms of carbonates, oxides and hydroxides.
5. Associated with silicate minerals.

Heavy metal uptake by plants and animals is most likely in soil and aquatic environments and bio-concentration through the food chain is a distinct concern (Nagajyoti *et al.*, 2010). The following sections illustrate the occurrences and some principles of the behaviour of investigated heavy metals in soil. The key processes that determine metal availability are described in figure 2.2.

2.2.1 Copper

Cu is an element that has been found in the earth's crust with natural levels ranging between 24 to 55 $\mu\text{g g}^{-1}$ of soil. Nevertheless, the total levels of Cu in the surface soil range from 0.01 to 0.6 μM . If the amount exceeds 1.5 – 4.5 μM , it could have a negative impact upon the growth of plant roots (Baker, 1990). Cu is an essential element for flora and fauna in the synthesis of protein, the activity of the cell membrane, photosynthesis processes and it works as a catalyst to stimulate many redox enzymes as a micronutrient (Baker, 1990; Maric *et al.*, 2013; Mackie *et al.*, 2012). The high level of Cu in soil may cause a detrimental effect on decreasing plant growth, development of leaf chlorosis and affecting metabolic process (Nagajyoti *et al.*, 2010). The sources of Cu in the soil are various such as, mining operations, fungicides and fertilizers, while some animal waste, which is used as natural organic fertilizers e.g., chicken manure contains Cu. Cu is found in manures due to the presence of growth

stimulating Cu compounds in the feed of poultry. Cu has been associated in the soil with organic matter, Fe and Mn oxides as well as other minerals (Kabata-Pendias and Mukherjee, 2007). Cu^{2+} and their organic complexes are considered the most mobile fractions and can have harmful impacts on soil biota and plants (Karlsson *et al.*, 2006). However, insoluble organic complexes formed by Cu reduce its bioavailability in the soil by minimising Cu proportions in soluble and exchangeable fractions (Kabirinejad *et al.*, 2014). Chemisorption is the main mechanism which can reduce Cu mobility in soil particularly with the occurrence of CO_3^{2-} , PO_4^{3-} and clay (Kumpiene *et al.*, 2008). pH has a significant influence on the mobility of Cu in soils. In an acidic soil, Cu is readily available in the soluble form of $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$. However, at $\text{pH} \geq 7$ the dominant form of Cu is copper hydroxide, an insoluble form in the soil making it unavailable for plant uptake (Baker, 1990). The different chemical forms of copper have a high influence on the toxicity of Cu which declines as follows; $\text{Cu}(\text{OH})_2 / \text{Cu}(\text{OH})^+ > \text{CuCO}_3 > \text{CuHCO}_3^+ > \text{Cu}(\text{CO}_3)_2^{2-} > \text{chloro-complexes}$ (Mackie *et al.*, 2012). It seems to be that Cu is distributed in the soil in several fractions, these are; (1) stable organic compounds in humus, (2) adsorbed onto clay minerals and Fe, Mn and Al oxides, (3) exchangeable Cu on the soil's surface, (4) soluble ion and inorganic complexes in the soil's solution (Rodríguez *et al.*, 2009; Wiszniewska *et al.*, 2016).

2.2.2 Zinc

Zn is one of the essential elements for plants and animals and has several biological functions such as, in carbohydrate and protein metabolism. It can be found in the structures of most enzymes. The Zn content in the soil ranges between $10\text{--}300 \mu\text{g g}^{-1}$, levels of $400 \mu\text{g g}^{-1}$ in plants is considered to be a hazardous dosage of Zn. The soil pollution by Zn is recognized when the soil's Zn levels ranges from thousands to tens of thousands $\mu\text{g g}^{-1}$ of Zn. The sources of Zn vary, such as decomposition of acid rocks (granite) and several anthropogenic activities (KieKens, 1990; Broadley *et al.*, 2007). Plants can uptake Zn as one or all of the following forms Zn^{2+} , ZnHCO_3^+ and ZnOH^+ (Kabata-Pendias and Mukherjee, 2007). The presence of a high content level of Zn in soil may cause interference with the metabolic pathways leading to a decrease in the growth of roots and shoots of plants (Nagajyoti *et al.*, 2010).

The behaviour of Zn in the soil depends upon its form, which affects Zn distribution in different geochemical fractions of soil. Zn can be associated with several different fractions of the soil matrix, typical associations and forms may be water soluble, exchangeable, adsorbed or as complexes with metal oxides and organic compounds, and finally, fraction bound with clay materials. This metal is found in many forms in the different fractions mentioned above

and it may occur as a free ion or complexed with organic matter in the soil solution, adsorbed or exchanged on the surface of soil colloidal, clay particles, Fe and Mn oxides and folate or humates. Further, Zn can occur as insoluble compounds such as $\text{Zn}(\text{OH})_2$ and ZnCO_3 in calcareous soils. The chemical equilibrium in the soil can influence and affect all of these forms by precipitation, dissolution, adsorption, desorption, complexation and de-complexation. All of these processes are controlled by; the concentration of Zn and other ions in the soil solution, types and numbers of charged sites on the solid phase of the soil, the concentration of the organic ligands and pH. Any changes in these factors can affect the chemical equilibrium of Zn in the soil. For instance, in acidic soil, with pH lower than 4, the levels of free ions of Zn is higher than others with a pH above 4. Generally, in an acid medium, the adsorption of Zn in the soil depends upon ion exchange and complexation by O.M, however in an alkaline medium it may be due to chemical precipitation as hydroxides, sulphides, phosphates and carbonates. Furthermore, the occurrences of several functional groups (e.g. OH, SH, COOH and C=O) on organic matter, can release the H^+ to neutralize the increase the concentration OH^- in the soil solution when the pH is increased. Therefore, hydrogen ions leave these groups with a negative charge on their surface, which can attract Zn^{2+} electronically (KieKens, 1990). On other hand, inorganic ligands such as SO_4^{2-} , Cl^- and NO_3^- can form complexes with Zn, however, these compounds have a minor effect on the behaviour of Zn in soils (Bradl, 2004).

2.2.3 Lead

Pb is one member of the 4A group of the periodic table of elements and has a high density of 11.4 g cm^{-3} . This element is found naturally in the environment in many ores such as galena (PbS) and cerussite (PbCO_3). All of these combinations can contribute to increased levels of Pb in the soil (Mulligan *et al.*, 2001). Anthropogenic activities such as mining and smelting can elevate Pb concentrations in the environment. For example, sources of soil pollution of Pb in Wales has long been recognized due to Pb mining, the evidence of this has been found in the Ystwyth river valley, where the levels of Pb were 90 – 2900 $\mu\text{g Pb/g soil}$ (Davies, 1987).

Pb tends to accumulate in atmospheric, terrestrial and aquatic environments due to the low solubility of its compounds. But, biologically available levels of Pb are negligible when it is compared with the total concentration of Pb in the soil (Davies, 1990). Pb accumulation has negative impacts on plants by inhibiting the active sites of enzymes that leads to an effect on the photosynthetic process, vegetation growth and seed germination (Nagajyoti *et al.*, 2010; Maric *et al.*, 2013). The behaviour of Pb in the soil may depend upon its form in different

fractions in the soil including soil solution, being chelated from the surface of clay minerals, making complexes with humus, precipitation forms, co-precipitation with Fe and Mn oxides and associated with carbonate. Soil organic matter plays a major role in Pb behaviour by complexation and specific adsorption processes; however, there is the presence of fulvic and humic acids, which can increase the mobility of Pb, due to the formation of dissolved organic complexes. pH and ion exchange have a considerable influence on the behaviour and distribution of Pb in the soil. With an increase in pH, the Pb could be precipitated as $\text{Pb}_3(\text{PO}_4)_2$, $\text{Pb}_5(\text{PO}_4)_3$ and $\text{Pb}(\text{OH})_2$ in soils with very low CO_3^{2-} . At pH ranging from 6 to 9, the dominant form being $\text{Pb}(\text{OH})^+$ but at a pH above 9 $\text{Pb}(\text{OH})_2$ is the main insoluble compound of Pb (Bradl, 2004). However, in acidic soil, Pb is found as a free ion with low levels of complex compounds associated with organic matter (Davies, 1990).

2.2.4 Cadmium

Cd is one of the most hazardous heavy metals. This metal is a member of group 1B in the periodic table of elements. CdS is often present in galena and sphalerite (Jarup, 2003) and Cd presence in the environment is often a by-product of the Pb and Zn extraction and smelting processes (Alloway, 1990a). Soils are considered to be uncontaminated with levels of Cd below the 2 ppm level (Clemens, 2006). The increase in the concentration of Cd in the soil can adversely affect the process of photosynthesis, inhibit plant enzymes and inhibit the translation of water and nutrients and minimise their uptake from the soil by the plant, consequently decreasing the plant's growth rate (Das *et al.*, 1997; Nagajyoti *et al.*, 2010). The behaviour of Cd and its species in the soil may depend on various factors such as the levels of Cd and solubility of Cd complexes with ligands, pH, the nature of the species and the strength of ions bound with functional groups on the surface of organic ligands.

The carboxylic and phenolic functional groups have a high ability to reduce the mobility of Cd in the soil by making bonds with Cd ions. The adsorption of Cd as a free ion on the surface of solid phases of the soil may be the dominant adsorption mechanism compared to other species and the occurrence of chloro species such as CdCl_2 and CdCl_3^- can affect the adsorption process of Cd. Cd inactivation in soils may be achieved through using several mechanisms, for instance at high concentrations of Cd the precipitation process is favourable to immobilise the Cd here. Whilst, the ion exchange process seems to be the main technique which can control Cd^{2+} behaviour in soils with low levels of Cd (Bradl, 2004). In the acid soil solution; Cd^{2+} , CdSO_4 and CdCl^+ are the main forms of cadmium, which are found; but in the basic soil solution the dominant form is CdHCO_3^+ . The distribution of Cd between different

soil phases seems to be controlled by the adsorption process rather than by precipitation. The presence of some metal ions such as Ca, Cu, Pb, Cr and Zn can affect the adsorption rate of Cd in the soil due to the variation of hydrated radius, hydration energy and electronegativity of adsorbent ions (Alloway, 1990a).

As mentioned above, there are several mechanisms that play a significant role in the availability of heavy metals and that can change the metal forms in soils. Table 2.3 shows the metal fractions and their accessibility to soil biota and plants (Intawongse, 2007; Sheoran *et al.*, 2016).

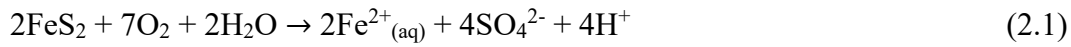
Table 2. 3 Metal fractions and their availability to flora and fauna (Intawongse, 2007; Sheoran *et al.*, 2016).

Metal fraction	Bioavailability
Exchangeable fraction	The release of metals, which occupied the exchangeable sites, may be high, due to the change of the main cationic structures.
Metal fraction associated with Fe-Mn oxides	The release of metals bound with Fe-Mn oxides might occur due to the change in redox conditions, but this change may form insoluble element sulphides if the metals' ore contains sulphides.
Metal form associated with organic matter	The decay of organic material in the soil may cause an increase in the mobility of metal associated with organic substance fraction.
Metals bound with sulphides.	In the presence of both water and oxygen, the oxidation of sulphides can affect an increase in the mobility of metals.
Metals linked to silicate and secondary soil material.	Due to weathering or the breakdown in conditions, the metals bound to this fraction may be translated to the soil's solution at a low rate.

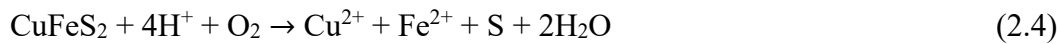
2.3 Geochemistry of metal ores in mine areas

Several metals have affinity to Fe and its oxides (siderophile elements) and others named chalcophile metals have a high attraction to S and occur as sulphide ores (Alloway 1990b). In the sites where sulphides are the dominant ore of heavy metals, such as in the case of Parys Mountain, the oxidation of the sulphide ore in a moist environment plays a significant role in the chemical behaviour of metals. Sulphide oxidisation can release heavy metal ions into the mine' spoil solution due to increasing the levels of H^+ thus generating a highly acidic

solution. For instance, pyrite (FeS₂) is the main ore in Parys tailings; this ore can be oxidised according to the following reactions (Jenkins *et al.*, 2000).



The hydrolysis of Fe³⁺ may have happened (equation 2.3), and the result of this reaction is that Fe can precipitate as ferrous hydroxide (Walton and Johnson, 1992). In the FeSO₄ solutions, the chalcopryrite oxidation rate increases due to the consumption of O₂ and H⁺, causing the discharge of Fe²⁺ and Cu²⁺ into the tailings solution. Further oxidation of Fe²⁺ and S generates H⁺, leading to a recycling of the ore oxidation process (Lindsay *et al.*, 2015):



2.4 Factors affecting the mobility of heavy elements in the soil

2.4.1 pH

pH is used as a useful indicator for the quality of mine soils (Sheoran and Sheoran, 2006). pH is the main factor influencing the mobility of metals in soils; pH affects ion exchange, complexation, reduction and oxidation. The effect of pH on the mobility of heavy metals can be direct or indirect. Direct impact is through dissolution, (co)precipitation, and the adsorption of heavy metals by adsorbents; while indirect effects such as the change of pH may cause a change in metal species e.g., hydrate some species of metals (MeOH⁺). Thus, the adsorption of new species of target metals is easier than free metal ions (Herreweghe *et al.*, 2002). For instance, in the alkaline medium pH ≥ 9 complex Cu compounds are formed. These can be dissolved in the soil solution. As a result, the amount of Cu available to the plant increases (Singh and Kalamdhad, 2013). The pH of soil and the bio-availability of most heavy metals are negatively correlated. The mobility of several metals and metalloids may decrease at lower pHs. An example of this is in acidic soils when the mobility of molybdate (MoO₄²⁻) is low and the adsorption of arsenate (AsO₄³⁻) on the surface of Fe and Mn oxides is strong (Hartley *et al.*, 2004). Zeng *et al.* (2011) observed that, the concentration of EDTA-extractable of Zn, Pb, Mn, Fe, Cu and Cr was affected by the pH value of soil. When pH increased, the EDTA-extractable concentration of these metals declined. They noted that the increase in the

pH of natural soil will results in an increase in the bioavailability and mobility of Cr because of the oxidation of insoluble form of Cr (Cr^{3+}) to a soluble form (Cr^{6+}) at higher pH. Figure 2. 3 shows the effect of soil pH on the mobile trend of heavy metal ions.

2.4.2 Organic matter (O.M.)

Namgay *et al.* (2010) reported that O.M has a significant influence on the behaviour of heavy metals in soil due to the occurrence of several functional groups, such as carbonyl, alcoholic and carboxyl groups that can chelate with metal cations and store them. These mechanisms can minimize the toxicity of heavy metals by reducing the availability of hazardous metals to plants. As stated by Zhang (2011), the movement and availability of heavy metals in the soil can be altered by forming stable organic compounds between humic acid and target metals reducing their translocation to plant and soil biota.

Several organic compounds are able to form highly stabilized complexes with Cu and Pb; this process is affected by factors such as the pH and Eh of the organic substances. Another mechanism, which can be used by O.M to reduce the phytotoxicity of metals in soil is co-precipitation (Dave and Chopda, 2014). Grybos *et al.* (2007) concluded that, soluble organic matter is a major sink for heavy metals and can form stable complexes, which can act to

minimize heavy metal adsorption by soil particles. According to Sheoran *et al.* (2016) the affinity of cations of heavy metals to O.M follow this order $\text{Cu}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Mn}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+}$. The functional groups in O.M. such as phenol, carbonyl, and carboxyl groups can be protonated in the soils with extremely low pH. This process leads to a positive charge occurring on the surface of the O.M attracting negatively charged metal species. However, with increasing the pH the organic functional groups can be deprotonated and behave as negatively charged moieties, consequently the free cations of heavy metal can be attracted to these charged species on the surface of the O.M, leading to a reduction in metal mobility in the soil solution. The protonation of organic functional groups may cause an increase in the pH of soil as a result of the concentration of OH^- ion rising, which allows the precipitation process to form metal hydroxides, hence the bioavailability of heavy metals is minimised (Farooq *et al.*, 2010).

2.4.3 Ion exchange

The ion exchange process occurs when a heavy metal ion is substituted to the surface of soil particles or any adsorbents that are added to treat polluted environments. These adsorbents are added because of their many essential characteristics e.g., a high capacity of treatment, a high heavy metal selectivity and a good ability to reduce chemical precipitation issues (Fu and Wang, 2011). Ion exchange is one of several influential mechanisms in the reduction of the concentration of plant available heavy metals. However, in some cases, this factor does not influence the adsorption process, e.g., in the case of removing Cu^{2+} from aqueous solution by using biochar made from *Spartina alterniflora*; the effect of ion exchange was very small (Li *et al.*, 2013). There are several factors that may affect the efficiency of the ion exchange process, such as chemical and physical properties of metal ion, pH, temperature, the charge and size of metal ion (Fu and Wang, 2011). In acidic soils H^+ ions replace the metals that have occupied the exchange sites in the soil matrix, which release these metals into the soil solution (Alloway, 1990c).

2.4.4 Redox potential

Seshadri *et al.*, (2016) stated that, Redox potential (Eh) has a significant effect on the behaviour of heavy metals in the soil as it has a direct effect on the distribution of these metals among soil geochemical fractions. A decline in the Eh with an increase in the pH causes a decrease in the mobility of heavy metals, but with a stable pH the reduction of Eh leads to a rise in the mobility and solubility of heavy metals, moreover Pb shows a higher sensitivity to a reduction in Eh than Zn and Cd (Charlatchka and Cambier, 2000). Figure 2. 4 illustrates the expected Eh-pH stability field for Pb and its chemical species in soils (Bradl, 2004). Ma and

Dong (2004) reported that, a change of the redox state of Fe and Mn oxides and their hydroxides can contribute to the release of heavy metals that have been bounded to this fraction. Sheoran *et al.* (2016) reported that, the oxidation states of metal can control metal mobility in soils. Several heavy metals have more than one oxidation state, the highest one is the oxidised form, which is considered as a highly soluble form, however a lower oxidised form has a lower solubility in a soil solution. For example, Cr is found in nature in a two-oxidation state, Cr^{6+} is an oxidised form and Cr^{3+} is a reduced form, and Cr^{6+} has more availability and toxicity for plants than Cr^{3+} (Shi *et al.*, 2011).

2.5 Soil remediation

A stable soil structure is important for soil productivity and minimising soil erosion. However, the physical and chemical properties of mine tailings e.g., high acidity, salinity, low organic matter content, high levels of heavy metals and low fertility make them unsuitable for natural plant growth (Wang *et al.*, 2017). Thus, the remediation of polluted soils has had considerable research and technological attention around the world during the last few decades (Yao *et al.*, 2012). For instance, about €23M was spent to amend polluted area around former metal smelter in France by conducting one of the biggest remediation project in Europe using

1% Fe⁰ (Komárek *et al.*, 2013). The treatment of contaminated lands can isolate and reduce the mobility and permeability of pollutants to less than $1 \times 10^{-7} \text{ m s}^{-1}$ (Mulligan *et al.*, 2001). Different remediation techniques have been established to clean up soil polluted with heavy metals and other pollutants (Eweis, 1998). Several methods are used to remove heavy metals from wastewater and contaminated soil (e.g., chemical precipitation, ion exchange and filtration). The remediation technologies of polluted soil are classified into two categories: ex-situ methods remove or extract pollutants from the contaminated soil by using a washing process or electro kinetic method off site; these methods are recognized as expensive and hard management procedures. The second approach is based on reducing the mobility and bioavailability of the pollutants in the polluted soil by mixing several natural or artificial materials with polluted soil on the site, which are called in-situ methods (Shi *et al.*, 2009). These methods can be inexpensive and have the least environmental impacts (Nwachukwu and Pulford, 2008). Thus, some natural amendments including compost, animal waste, sewage sludge, biochar, zeolites and residues of inking paper sludge have been applied with the aim of reducing the availability of metals in soil (Mar Gil-Díaz *et al.*, 2014). Artificial amendments, such as iron and its oxides at nanoscale have also been utilised in-situ for limiting metal availability in the soil (Manzano *et al.*, 2014).

2.5.1 Bioremediation

Bioremediation is any biological method used to mitigate the concentration and the mobility of pollutants in the environment to recapture the soil's natural condition (Mani and Kumar, 2014). The remediation techniques, which are used to treat soil, water and gases, vary and depend upon the matrix type and national reduction targets for specific contaminants. Additionally, there are many factors can affect the bioremediation process (Eweis, 1998):

1. Environmental factors, such as pH and temperature.
2. The molecular structure of the pollutants.
3. The availability of pollutants as a primary physical factor has a significant effect on the bioremediation process.

The lower cost of treatment operations is a major advantage of bioremediation in contrast to other methods, such as capping, deriding and incineration. Moreover, treating polluted soil in situ may mitigate the exposure of pollutants to the environment, when it is transported to treat it ex situ (Mani and Kumar, 2014). Bio-adsorbents, such as agricultural waste have been demonstrated to amend soil contaminated with heavy metals. This is because of several

reasons, for example, the lower cost, the higher ability to reduce pollutants and environmentally benign substances (Zhang, 2011).

2.5.1.1 Composting

Composting is a method of stabilising organic agricultural and municipal wastes that, with good management, does not harm the environment (Zhang *et al.*, 2014). According to Roberts *et al.* (2007), composting can be defined simply as an efficient process of reducing the amount of waste and it enables the use of the processed product. Using compost to treat polluted soils is a major and common method (Medina *et al.*, 2015). Many studies have indicated that compost can be used as an amendment in the in-situ treatment of soil polluted with heavy metals. These studies suggest that compost is able to immobilize the bioavailable heavy metals by several methods such as precipitation, complexation, adsorption of target metals, and increasing the pH of soil (Paradelo *et al.*, 2011).

Additionally, organic amendments can stimulate the natural process to alter the movement of heavy metals in the soil. These amendments can either raise or reduce the mobility and bioavailability of heavy metals (Karami *et al.*, 2011). However, not all composts reduce the mobility of metals in soils. For instance, compost made from some types of sewage sludge can release Zn and Cd into highly contaminated soil. Meanwhile, the compost made from garden green waste decreased the mobility of these metals (Van Herwijnen *et al.*, 2007). Gorgievski *et al.* (2013) used wheat straw to treat soil polluted with Zn^{2+} , Cu^{2+} and Ni^{2+} . They used the Langmuir isotherm equation to study the adsorption mechanisms of target heavy metals by wheat straw. They concluded that the wheat straw, as an organic amendment, has a potential to be used as an adsorbent to mitigate the movement of Zn^{2+} , Cu^{2+} and Ni^{2+} in a weak acidic solution.

Farrell *et al.* (2010) also reported on adding composted of organic waste to polluted acid soil. In this study, the pH of soil was increased; the growth rate of plants was accelerated, and the concentration of plant available contaminations was reduced. However, an important issue may be overlooked, where the further decomposition of the organic amendment results in re-release of the pollutants. This effect has been reported by Paradelo *et al.* (2011) where mobilized heavy metals, which were associated with compost, especially Cu, were re-released into the soil medium during the mineralization process of organic matter used to treat polluted soils. To avoid this possibility, highly stable compost should be used. The use of organic matter (e.g. animal waste and composted bio-solid) to treat polluted soil can decrease the uptake of heavy metals by plants and soil biota. Furthermore, they can modify the pH value of soil due

to active groups, such as OH and COOH on the surface of organic matter. These groups can immobilize heavy metals by the adsorption and complexation processes (Khan and Jones, 2008). A study conducted by Hanč *et al.* (2008) showed that the addition of organic matter to polluted soil leads to the reduction of the bioavailability and phytotoxicity of Cd in soil over three growing seasons. However, chicken waste results in the increase of free Cd^{2+} . This waste increases the adsorption rate of Cd^{2+} from the soil solution, but an uptake of Cu^{2+} declined due to a decrease in the bioavailability of Cu^{2+} by using chicken manure.

2.5.1.2 Biochar

Biochar is a natural amendment that has been used to amend polluted soil. It can improve the fertility and productivity of soil. Biochar can be defined as a solid product resulting from the pyrolysis of biomass waste in the absence of oxygen. The result is a matrix with high-surface-area, a high-cation exchange capacity, a high pH and porosity makes biochar a useful product for immobilizing heavy metals and organic pollutants in the polluted soil (Lehmann and Joseph, 2009). Beesley *et al.* (2010) concluded that hardwood biochar is more effective than green waste compost to reduce the bioavailable form of Cd and Zn in multi-metal polluted soil, due to the biochar ability to increase the concentration of dissolved organic carbon (DOC) and pH of treated polluted soil. The size of biochar particles exerts a major influence on the adsorption of Ni, Zn, Cd, Cu and Pb (Rees *et al.*, 2014). According to Lu *et al.* (2014) the addition of 5% of fine biochar made from rice straw raises soil pH, which leads to the precipitation of Cu^{2+} as $\text{Cu}(\text{OH})_2$, Pb as $\text{Pb}_5(\text{PO}_4)_3\text{OH}$ and Cd as CdCO_3 , which can cause a reduction in the mobility of Cu^{2+} , Pb^{2+} and Cd^{2+} . Forming metal oxide or hydroxide seems to be an alternative technology to remove Cu^{2+} from aqueous solution by biochar (Li *et al.*, 2013). Typically, biochar has high porosity, which can contribute to decreasing the mobility of heavy metals and organic pollutants in soil by the adsorption process and storage them inside the biochar is pore (Zhang *et al.*, 2013). The type of biochar (raw material and synthetic conditions) and the application rate that can affect the efficiency of biochars alters the mobile form of heavy metals (Lu *et al.*, 2017).

Brennan *et al.* (2014) determined the effects of two types of biochar; olive wood biochar (OB), and pine woodchip biochar (PB), on the phytoavailability of pollutants to maize roots. The research implied that OB and PB could reduce the mobility and availability of Cu and Zn to plants. Additionally, biochar derived from OB has a higher alkalinity than PB, which affects the mobility of arsenic (As) in a soil solution. Biochar is able to immobilize heavy metals and organic compounds, because the biochar contains many deprotonated organic functional

groups, which are located on the surface of the biochar such as the carboxyl group (COOH) and the hydroxyl group (OH). These groups are able to attract the free ions of heavy metals by using various chemical mechanisms. As figure 2.5 shows, the possible mechanisms can be used by sludge biochar as an adsorbent to inactivate lead ions in polluted environments; Zhang *et al.* (2013) identified these mechanisms to include:

1. Substitution that might occur between Pb^{2+} and Na^+ , K^+ , Mg^{2+} and Ca^{2+} , which are located on the surface of biochar. Pb^{2+} can make an inner complex with humic material and the oxides of elements in the biochar.
2. Surface precipitation by making a complex compound between Pb^{2+} and functional organic groups on the surface of the biochar.
3. The adsorption of Pb^{2+} physically by biochar and the precipitation of Pb^{2+} on the surface of biochar are likely to immobilize the availability of Pb^{2+} at contaminated sites.

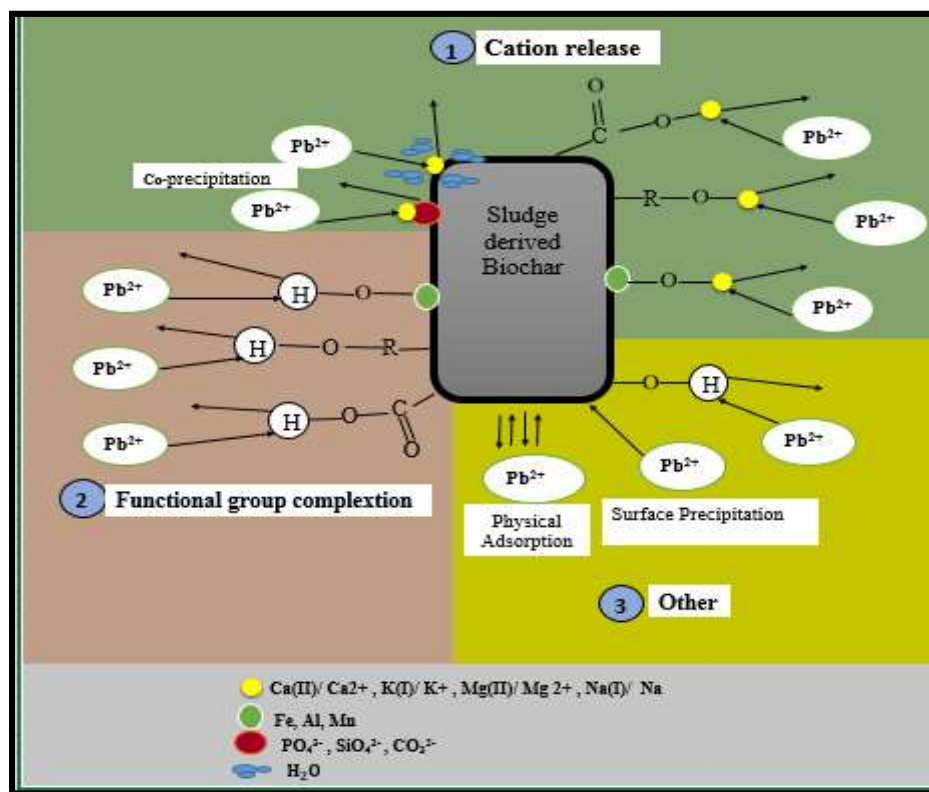


Figure 2. 1 Potential mechanisms of adsorption of Pb^{2+} on the surface of biochar (Redrawn from Zhang *et al.*, 2013)

Jones *et al.* (2016) studied the effect of green waste compost and biochar on the mobility of Cu and sunflower (*Helianthus annuus L.*) growth in contaminated soil. They concluded that, both amendments are effective in reducing the concentration of Cu in leachates and the mobility of Cu decreased with an increase in the rate of amendment. However, biochar applications were more effective than compost to enhance plant growth in the contaminated area.

Indeed, biochar should be analysed before being used as an amendment, because the initial biochar ores that may contain pollutants. For instance, according to Lucchini *et al.* (2014) the wood of *Picea abies L.*, which was used as a raw material of biochar contains As and Cr, of which the levels range from 5.2 – 16.3 $\mu\text{g g}^{-1}$ and 5.3 – 19.0 $\mu\text{g g}^{-1}$, respectively. Contaminated acidic soil collected from a Zn mine site in Brazil was treated with sugar cane straw biochar to examine its ability to reduce the available fraction of Cd, Pb and Zn. The result suggested that, biochar effectively minimized the presence of studied metals in mobile form and the adsorption process was the major mechanism used to immobilise heavy metals (Puga *et al.*, 2016). In addition, bamboo biochar (BB) is highly efficient in removing silver ions (Ag^+) from aqueous solution when it is mixed with zero-valent iron nanomaterial. As a result, the adsorption capacity of this mixture had increased twice as much as the adsorption capacity when they used only BB to Ag^+ removal (Zhou *et al.*, 2014).

Conversely, not all types of biochar are capable of reducing the mobility of all heavy metals; and they can be selective in adsorbing metals under different conditions. For instance, Li *et al.* (2013) states biochar produced from *Spartina alterniflora* (SABC) has a higher adsorption rate for Cu^{2+} than several adsorbents (e.g., zeolites, bentonite and powder of leaves). The results showed that the adsorption capacity was raised by increasing the pH of the solution, and the adsorption capacity of Cu^{2+} onto SABC was greater than biochar made from corn straw. This makes the SABC a suitable adsorbent to remove Cu^{2+} from an aqueous solution.

2.5.1.3 Phytoremediation

“Phytoremediation basically refers to the use of plants and associated soil microbials to reduce the concentration or toxic effects of contamination in the environments” (Ali *et al.*, 2013). Phytoremediation is one of the most effective bioremediation strategies to clean up mine areas based on the use of several native plants (Leguizamo *et al.*, 2017) and alternatively several plant species that have high growth rates are cultivated to clean up polluted soils, minimize the erosion of mine soils and reduce mine dust distribution (Mugica-Alvarez *et al.*,

2015). The plants are used to mitigate the availability of toxic elements have some features that are (Mendez and Maier, 2008; Adesodun *et al.*, 2010; Shabani and Sayadi, 2012):

- 1- A high resistance to the harmful effects of heavy metals.
- 2- The management of cultivation and harvesting is easy.
- 3- They can respond to changes in weather and environmental conditions.
- 4- A high productivity of both vegetative part and roots.
- 5- A high density and wide distribution of the root system.
- 6- The effective ability to translocate the stored heavy metals from roots to above ground parts.
- 7- They are not favourable for grazing animals therefor minimising the pollution of the food chain.

Phytoremediation technology is based on using several mechanisms that are phytoextraction, phytoimmobilization, phytovolatilisation, and phytodegradation (Fernández *et al.*, 2017). Figure 2.6 gives an overview of some phytoremediation strategies. The main advantages of phytoremediation that it is a cost effective and eco-friendly approach (Cristaldi *et al.*, 2017). For instance, the cost of treating 1 ton of polluted soil using plants ranges from 25 to 100 \$ in US in contrast nearly 6-8 billion US \$ have been spent annually in the remediation of contaminated lands using Chemical and physical strategies in the USA (Luo *et al.*, 2016). Phytoremediation technology is based on using two main methods phytoextraction and phytoimmobilization (Fernández *et al.*, 2017).

1. Phytostabilisation

Phytostabilisation is the technology used by plants to reduce the transfer of heavy metals to the food web and mitigate their migration to ground water. Several mechanisms are used by the plant to minimise the translocation of toxic metals within plant tissues, such as complexation of elements with organic compounds of root tissues, adsorption on the root surface, a reduction in their oxidation state to a lower valance state and precipitation in the root zone. Root accumulation and rhizosphere inactivation of heavy metals are the main mechanisms of phytostabilisation contributing to protect the surrounding environments from heavy metal leaching to surrounding aquatic bodies (Sun *et al.*, 2016b). Also, the plants that use phytostabilisation strategies should have a high resistance to the translocation of toxic metals from the roots to the above ground parts of the plant to avoid food chain contamination (Mendez and Maier, 2008). This strategy is suitable for multi-heavy metal polluted areas under different effects of various environmental factors (e.g., pH, EC and soil texture) in the tailing

medium (Yang *et al.*, 2016; Fernández *et al.*, 2017). Several plant species such as agricultural grasses have this ability (Mahar *et al.*, 2016).

2. Phytoextraction

Phytoextraction is another approach used by plants to translocate toxic elements from polluted soils through roots and store them in the parts above ground (e.g., stems and leaves) (Mugica-Alvarez *et al.*, 2015). The plants that are able to do this with more than $10,000 \mu\text{g g}^{-1}$ Zn, $1000 \mu\text{g g}^{-1}$ for Pb, Cu and $100 \mu\text{g g}^{-1}$ Cd are classified as hyper-accumulator plants (Luo *et al.*, 2016). Up to date, more than 400 plant species are classified as hyper-accumulator plants and this number is still rising. For instance, according to Kashem *et al.* (2010) 14 plant species were considered as Zn hyperaccumulators due to their abilities to store $10,000 \mu\text{g g}^{-1}$ Zn in their shoots based on the dry weight of analysed material, as well as *Arabidopsis halleri ssp. Gemmifera*, which stores $5,600 \mu\text{g g}^{-1}$ Cd in its leaves. Phytoextraction plants are suitable for use to amend polluted soils with low to moderate levels of heavy metals due to the performance of these plants to extract elevated concentrations of target metals from massive area of land and accumulate them in aerial parts with low volume relative to target soils specially after drying the harvested plants (Sun *et al.*, 2016). There are two factors that can be used to examine the effectiveness of these plants as hyperaccumulators, the element bio-concentration factor (BF), and the density of biomass (McGrath and Zhao, 2003). Table 2.4 shows several hyperaccumulators plant species utilised for cleaning up contaminated soils. The BF measures the ratio of the heavy metal level in the plant tissue to the concentration of the same metal in the soil dry weight (Leguizamo *et al.*, 2017). BF can give an indicator of the ability of flora to adsorb toxic metal and translocate it to the parts which are easiest to harvest (shoots), the BF should be more than 1 to classify these plants as hyper-accumulators (McGrath and Zhao, 2003). The use of grass that has the ability to grow in highly polluted sites and to tolerate and survive in different climate conditions with high biomass production has become common in terms of the gradual removal of heavy metals from mine sites (Mugica-Alvarez *et al.*, 2015; Sun *et al.*, 2016). In the UK, ryegrass (*Lolium perenne L. var. Cadxi*) was used to examine the ability of biochar and green waste compost to reduce the transfer of Cu and Cd to plants from mine soils (Karami *et al.*, 2011). Phytoextraction takes long time and experienced operators to clean up polluted soils, but it is a publicly acceptable, inexpensive, eco-friendly method suitable to use in amending multi-metal polluted lands (Khalid *et al.*, 2017). Figure 2.3 gives an overview of these two phytoremediation strategies.

Table 2. 4 Several hyperaccumulators plant species used for phytoremediation of heavy metal polluted sites (adopted from Mahar, 2016).

Plant species	Metal	Accumulated metals ($\mu\text{g g}^{-1}$)	References
<i>Aeolanthus biformifolius</i>	Cu	13700	Chaney <i>et al.</i> (2010)
<i>Azolla pinnata</i>	Cd	740	Rai (2008)
<i>Betula occidentalis</i>	Pb	1000	Koptsik (2014)
<i>Brassica juncea</i>	Pb	10300	Koptsik (2014)
<i>Brassica nigra</i>	Pb	9400	Koptsik (2014)
<i>Deschampsia cespitosa</i>	Cd	236	Kucharski <i>et al.</i> (2005)
	Zn	3614	Kucharski <i>et al.</i> (2005)
<i>Eleocharis acicularis</i>	Cu	20200	Sakakibara <i>et al.</i> (2011)
	Zn	11200	Sakakibara <i>et al.</i> (2011)
	Cd	239	Sakakibara <i>et al.</i> (2011)
<i>Medicago sativa</i>	Pb	43300	Koptsik (2014)
<i>Thlaspi calaminare</i>	Zn	10,000	Sheoran <i>et al.</i> (2009)

2.5.1.3.1 Plant uptake and transport of heavy metals

Several mechanisms are involved in the adsorption of heavy metals from contaminated soils by plants and their translocation within flower tissues. The uptake of heavy metals by plant roots depends on the concentration of metal, metal geochemical form and the presence of other metals or plant nutrients that can compete with each other to occupy the adsorption (uptake) surface of plant roots (Larsson *et al.*, 1991).

1. Root uptake

Plants have a natural tendency to uptake heavy metals. The transport of these metals may be obstructed by the cytosol which can act as barrier between the outside of the plant cell wall and vacuole, reducing the diffusion of metals across the cell wall. Phytoavailable forms of heavy metals can enter the root through extracellular (apoplectic) or intracellular (symplastic) routes. The intracellular route refers to the movement of a metals through the root cortex *via* plasmodesmata by travelling from one cell to next one. The extracellular route refers to the movement of metals *via* cell walls by travelling across the root cortex without crossing any membrane. Metal ions can reach the endodermis, which is the beginning of the internal space by travelling along this waterway. The metal ions can enter the xylem *via* the Casparian strip and the endodermis. The Casparian strip may limit the entry of metal ions into the xylem

(Figure 2.7). After the metal ions have been taken up by roots, they may be stored in the roots or translocated within the plant tissue *via* xylem sap (Promnim, 2012).

2. Metal translocation within plant tissue

The translocation of heavy metal ions within plant tissues can occur by diffusion *via* xylem and phloem. The rate of this process can be affected by the composition of the saps of the xylem and phloem, metal properties and the movement of other nutrients within the plant tissues. Xylem movement of metal ions is fundamentally driven by mass upward flow of water created by the transportation stream. But, the loading of sucrose into the phloem from mature, actively photosynthesising leaves and the discharging of sucrose into the sink tissues produces positive

hydrostatic pressure which can stimulate metal transport *via* the phloem (figure 2.8) (Welch and Shuman, 1995; Promnim, 2012).

2.5.1.4 Microbial remediation

Microorganisms are not able to degrade heavy metals but they can reduce metal mobility and translocation in the soil solution by changing the physiochemical properties of polluted soils. Several mechanisms are involved in the immobilization of heavy metals by microorganisms including precipitation, extracellular complexation, intracellular accumulation and oxidation-reduction reactions. These processes are effective in reducing soil solution heavy metal concentrations in soils with low levels of contamination (Yao *et al.*, 2012). Yang *et al.* (2016a) concluded that native bacteria (*Bacillus firmus*) isolated from mine tailings soil were able to increase the pH of the target tailings, promoting the precipitation and inactivation of Cu, Pb and Cd thus decreasing the levels of target metals in the exchangeable fraction and increasing the concentration of these metals in the carbonate fraction. However, several

physiochemical factors e.g., pH, moisture content, oxygen level, temperature, heavy metal concentration, and microbe species composition may affect the efficiency of microbes in amending polluted soils (Yao *et al.*, 2012).

2.5.1.5 Animal remediation

Earthworms have been used in the treatment of heavy metal polluted soils due to their ability to adsorb heavy metals (Yao *et al.*, 2012). Boughattas *et al.* (2018) reported that earthworms (*Eisenia andrei*) were effective in the stabilisation of Zn, Cu, Pb and Cd by decreasing their levels in exchangeable fraction and increasing the concentrations of target metals in the most stable fractions in contaminated soil. The presence of earthworms in polluted soil may allow other animals that consume earthworms to return to treated soils, which may help to ameliorate several soil properties (Cele and Maboeta, 2016). However, according to Li *et al.* (2018a) several studies have reported that earthworms increased metal mobility and availability in polluted soils. Also, the accumulated heavy metals in earthworms can be transferred through the food chain to the secondary consumer and eventually reach the human body, which may limit the use earthworms in the remediation of heavy metal contaminated sites (Centofanti *et al.*, 2016).

2.5.2 Zeolites

Zeolites are types of aluminium silicates that have a cage structure; the isomorphous substitution of Al^{3+} for Si^{4+} in the framework of zeolites results in a net negative charge that may be occupied by cations (Figure 2.9). This allows zeolites to be used in many applications involving exchange and the adsorption process. The zeolites group contains more than 40 types formed naturally; the international consumption of natural zeolites was 0.3Mt in the 1980s and increased to 3.5 Mt in 2006 then reached 5.5 Mt in 2010 (Wang and Peng, 2010). These materials have the ability to attract and store heavy metals and other pollutants in soils (Zorpas *et al.*, 2008). Several features of zeolites include negative charges, their size, porosity and exchange capacity, which allows them to adsorb any ion with a positive charge (Castaldi *et al.*, 2005). In nature, zeolites are weakly acidic, also the zeolite that contains sodium has selectivity for hydrogen in aqueous solutions, which causes an increase in the pH of the medium, due to the replacement of Na^+ in the zeolite by H^+ ($\text{Zeolite} \equiv \text{Na}^+ + \text{H}_2\text{O} \leftrightarrow \text{Zeolite} \equiv \text{H}^+ + \text{OH}^- + \text{Na}^+$). The increase of the pH can reduce the mobility of metals in the solution by the precipitation process, as metal hydroxide (Motsi *et al.*, 2009). However, zeolites contain high levels of sodium in their original structure may increase the salinity of the amended soil (Singh and Kalamdhad, 2013).

Many studies have been conducted exploring the use of natural and synthetic zeolites as soil amendments (Motsi *et al.*, 2009). Though, zeolites have ability to adsorb some plant nutrients specially NH_4^{1+} during the remediation period and then re-release the previously adsorbed macronutrients e.g., PO_4^{3-} , K^+ and NH_4^+ slowly to degraded soil's solution (Yilmaz *et al.*, 2014; Wijesinghe *et al.*, 2016). Li *et al.* (2009) reported that zeolites are used worldwide due to their availability and lower costs. The results of this study showed that zeolite can play an important role in modifying some soil characteristics, especially pH and CEC, which lead to the rise of the soil quality. This can decrease the available fraction of Pb when they use $\geq 10 \text{ g kg}^{-1}$ dose of natural zeolite, but a high dose of zeolite 20 g kg^{-1} can reduce the levels of Pb in the shoots of rape up to 30% of initial concentration of Pb. In highly acidic soils, the presence of hydronium ion (H_3O^+) may inhibit the efficiency of zeolite to adsorb metal cations due to the ability of H_3O^+ to compete with element cations during the adsorption process, consequently the efficiency of zeolite to remove target metal from the soil solution is reduced (Delkash *et al.*, 2015). Several recent studies have proposed two various views about the mechanisms are used to minimise metal mobility by zeolites. The first one is the increase of the pH may lead to the enhancement of metal adsorption by creating chemical complexes on the surface of the zeolite; another point is that the cation exchange can immobilize the metal rather than the pH. Moreover, they concluded that the addition of clinoptilolite (a natural type of zeolite) has a significant effect on the reduction of the available concentration of Pb by

transforming the exchange and water-soluble fraction to unavailable forms by bounding Pb with organic and residual phases of soil. As a result, zeolites can protect the edible parts of the plant from Pb pollution. Wang and Peng (2010) reported that natural zeolites have different capacity and selectivity to adsorb heavy metal cations from solutions, alternatively the modification of natural zeolites surface may improve the adsorption capacity of these zeolites. Several factors controlling the adsorption capacity of heavy metals by natural zeolites including cation suitability, hydration radius and hydration enthalpies (Motsi *et al.*, 2009). Zeolites are also high selectivity for divalent cations compared to monovalent cations and the highest charged cation has strong interaction within the zeolite lattice by ion exchange process (Edwards *et al.*, 1999).

Zorpas *et al.* (2008) used a mixture of clinoptilolite and sewage sludge to treat agronomic land polluted with heavy metals. The results showed that this mixture has a high efficiency to take up the exchangeable and carbonate fractions of heavy metals from the contaminated soil except Zn. Motsi *et al.* (2009) concluded that the adsorption efficiency of clinoptilolite to adsorb Fe^{3+} , Mn^{2+} , Zn^{2+} and Cu^{2+} from an aqueous solution was higher in the first 40 minutes of their experiment. The main adsorption techniques used by clinoptilolite are the precipitation of target metals as metal hydroxides and ion exchange processes.

Abollino *et al.* (2003) concluded that Montmorillonite which is type of clay linings with structure similar to zeolite (clay minerals 2:1 structure). This mineral uses two mechanisms to adsorb metals cation; the first is cation exchange by interaction among ions and a negative demented charge at the surface of Montmorillonite, and the second is formation complexes with deprotonated silanol and aluminol groups, these inner-sphere complexes are formed on the edges of clay grains. Additionally, pH has a high influence on these two mechanisms, for instance at $\text{pH} < 4$ the $\text{Al}-\text{OH}$ and $\text{Si}-\text{OH}$ are protonated, which leads to a decrease in the ability of Montmorillonite to attract heavy metal cations. However, the adsorption of metal anions such as AsO_4^{3-} is increased. The type of syntactic zeolite formed from coal fly ash shows a great ability to immobilize several contaminants in soils due to the application of 1000 - 2500 kg ha^{-1} coal fly ash zeolite to polluted soil by Co, Cd, Zn, Ni and Cu. As a result, the leaching levels of target metals were reduced by up to 99% of their initial concentrations. This can be attributed to the increase of the pH as a main factor due to zeolite addition led to immobilise studied metals mobility by adsorption onto the surface of zeolites and precipitation as metal hydroxide. Nevertheless, the cation exchange process in this case is not an effective mechanism to reduce metal mobility. Querol *et al.* (2006) used fly ash derived-zeolite made at a low

temperature to treat mine soils, the mobility of investigated heavy metals was reduced, due to the increase in the pH as a result of the liming effect of used zeolite.

2.5.3 Nanoparticles

Nanoparticles (NPs) are defined as small size particles, which have a diameter of less than 100 nanometres and they can take any shape. These particulates are naturally present in the ecosystems and can be found everywhere, but may also arise from the introduction of manufactured nanoparticles from industrial processes or other artificial sources (Tang and Lo, 2013). Due to their essential properties, the demand for this technology has resulted in increased production from 2000 tonnes in 2004 to estimated amounts of 58,000 tonnes for the period from 2011 to 2020. The increase in the production of nanomaterial leads to increases in doses of NPs in the environment, which may be utilized by flora and fauna in the ecosystem (Nowack and Bucheli, 2007). Recently, according to Xu *et al.* (2012), using nanomaterial such as carbon nanotubes, zero valent iron (Fe^0), active carbon and iron oxides, has become common in order to reduce the mobility and toxicity of heavy metals in aqueous solution.

2.5.3.1 NPs as adsorbents

There are several properties, such as unique construction and electro-chemical characteristics of some NPs that may enable them to be important adsorbents. Some NPs have been recommended for ecosystem remediation and might be released into the ecosystem. For example, titanium dioxide was mixed with ethylenediamine to clean up contaminated environments from anionic elements, e.g., AsO_4^{3-} and CrO_4^{2-} (Komárek *et al.*, 2013). The size and surface area of nanoparticles gives them a considerable ability to adsorb several metals (e.g., A diameter of 7nm of hematite ($\alpha\text{-Fe}_2\text{O}_3$) can adsorb Cu^{2+} at lower level of pH value than particles with diameter size of 25nm or 88nm). In the same way, Ju-Nam and Lead (2008) indicated that the adsorption of Pb^{2+} into TiO_2 NPs was greater than the adsorption capacity of bulk material of TiO_2 . As mentioned above, Fe^0 and its oxides could be used as soil amendments. For example, the use of 1% of iron grit to immobilise the available fractions of Cu, As and Cr in contaminated soils due to industrial activity; this application led to a reduction in the leachable fractions of As and Cr up to 98% and 45% of their initial levels, respectively. Nevertheless, the bioavailable fractions of Cu were still high, which lead to the concern that, the toxicity of Cu in the target soil has not reduced completely (Kumpiene *et al.*, 2006).

2.5.3.2 Iron and iron oxides

Fe and its oxides at nano-scale have been implemented as a treatment for pollution of soil and water by metals, pesticides and polycyclic aromatic hydrocarbons (Grieger *et al.*, 2010;

Komárek *et al.*, 2013). Němeček *et al.* (2014) used nano Fe⁰ for the in-situ remediation of polluted groundwater with Cr⁶⁺. Using Fe⁰ to treat the contaminated environment in-situ has drawn significant attention in the last 15 years because of many essential features. Additionally, there is a cost saving, compared with other methods, for example Fe⁰ is renewable material as the previously adsorbed elements can be re-adsorbed by using HCl and HNO₃ to renew Fe⁰ (Tang and Lo, 2013). Fe⁰ has been widely used to immobilize organic pollutants from soils and water and several heavy metals such as arsenate (Mueller *et al.*, 2012). These features are as follows:

1. They are more active than other iron compounds.
2. Fe⁰ has a highly reactive surface area due to the small size of its particles, which are less than 100 nm that can promote the adsorption of pollutants (Grieger *et al.*, 2010).

Moreover, due to these features, nearly 60% of polluted sites, which were treated with NPs were amended by Fe⁰ (Mar Gil-Díaz *et al.*, 2014). Several types of iron oxides have been used to immobilize the concentrations of organic contaminants and heavy metals in contaminated soil and water. According to Tang and Lo (2013) much work has been done in this field by using artificial Fe⁰ NPs to treat polluted sites in the USA and Europe.

The main types of iron oxides, which are used in soil treatment are included with their fundamental characteristics in Table 2.5. The major iron compound found in the soil in wet and cold climates is α -ferric oxyhydroxide (α -FeOOH). However, other oxides of iron may be found in the same mixture (e.g., α -ferric oxide (α -Fe₂O₃) and γ -ferric oxyhydroxide (lepidocrocite; γ -FeOOH)) which is formed in warm climate zones. Moreover, iron oxide (Fe₅H₈.4H₂O) can occur as a mixed ore with α -FeOOH, when α -ferric oxyhydroxide is formed in the presence of organic matter and soluble silicate [SiO₃⁻²]_n. The use of artificial oxides of iron specially, iron oxyhydroxide is more effective than natural iron oxyhydroxide in immobilizing Arsenic in soil (Komárek *et al.*, 2013).

Table 2. 5 Several properties of different Fe oxides (Rewritten from Komárek *et al.*, 2013).

Fe oxide	Molecular formula	Surface area m ² / g	pH	Log K _{sp}
Hematite	α -Fe ₂ O ₃	2 -115	7.5- 9.5	-43.9 ± 0.2
Lepidocrocite	γ -FeOOH	15 – 260	6.7 – 7.5	-38.7 to - 40.6
Goethite	α -FeOOH	8 - 20	7.5 -9.4	-44
Ferrihydrite	Fe ₅ H ₈ .4H ₂ O	100 - 700	7.8 – 8.8	-73 to -39

2.5.3.2.1 Iron and iron oxides as soil amendments

Iron and its oxides have been used for in-situ remediation approaches to remediate soil polluted with heavy metals. According to Komárek *et al.* (2013) the use of 1-2 wt. % of Fe^0 as a soil amendment has a significant effect on the stabilization of metals in soil. The researchers estimated the effect of a Fe compound to mitigate the mobility of As in polluted soil. The results showed that Fe^{3+} has the greatest effect on mitigation, followed by Fe_2SO_4 , then Fe^{2+} ; the smallest was Fe^0 .

As mentioned by Tang and Lo (2013), the oxidation process causes a few changes in the pH of the soil and creates new sites on the surface of iron oxides to adsorb the cations and anions from the soil solution. Coating the surface area of Fe^0 and Fe oxides with several organic compounds such as starch and humic acid, which contains active function groups like carboxylic group, can give a particular function and characteristics to these materials. However, the rate of reaction was mitigated, because of humic acid covering the surface of Fe and its oxide, but the efficiency of removing metals was improved due to adding more reactive sites (Komárek *et al.*, 2013).

Another study conducted by Mar Gil-Díaz *et al.* (2014) to estimate the effects of the addition of Fe^0 on two different types of soil (calcareous and acidic) polluted with Zn and Pb. This showed a considerable reduction of available Pb and Zn in both types of soil, although the chemical and physical properties of soils were varied. Tang and Lo (2013) suggested that there were three probable mechanisms, which iron and its oxides might follow to reduce the mobility and availability of ions of heavy metals (Me^{n+}) and organic compounds containing chlorine (RCl) as pollutants in the soil. For instance, Me^{n+} could be immobilized by the adsorption of another less mobile oxidation state form ($\text{Me}^{(n-x)+}$). Otherwise, the adsorbents can adsorb this ion on the subsurface of the adsorbent. The third possible process is co-precipitation of Me^{n+} with iron oxides to form (Me-Fe-OOH) a complex. Figure 2.10 shows these possible mechanisms to reduce the mobility of heavy metals by Iron and Iron oxides.

Esfahani *et al.* (2015) indicated that the presence of some anions such as nitrate, chlorine and phosphate in ground water might lead to decrease the efficiency of Fe^0 in the remediation process. To date, most research, which used Fe^0 to treat polluted soil and water has been conducted on a laboratory scale. The use of Fe^0 in the field has raised many concerns about the toxicity perspective of Fe^0 , when an excess amount of it was used (Trujillo-Reyes *et al.*, 2014). As reported by Tang and Lo (2013), the toxicity of Fe^0 in the soil biota is lower than that in the laboratory, and it is too early to give important information about the toxicity of Fe^0 in the natural soils.

2.5.4 Polluted soil replacement

Soil replacement refers to removal the polluted soil from the site and its replacement with uncontaminated soil. Alternatively, the contaminated soil may be mixed with deeper layers by digging, thereby diluting the pollutants. Contaminated soils may also be covered with a layer of uncontaminated soil, which may isolate the contaminated soil and therefore reduce the transfer of heavy metals to the surrounding environment. However, these approaches are expensive and the replacement of contaminated soil may lead to secondary pollution. These methods are also only suitable for small areas (Yao *et al.*, 2012).

2.5.5 Soil Washing

This technology refers to the use of: (1) physical separation based on the difference in several physical properties of soil and metal bearing particles e.g., magnetism, size and density or (2) chemical extraction involving the application of several agents e.g., chelating agents and acids to increase target metal solubility. Physical separation is more widely used than chemical extraction due to its lower cost (\$ 70 – 187 m⁻³ soil) compared with chemical extraction (\$ 358 – 1717 m⁻³ soil) (Dermont *et al.*, 2008). According to Chen *et al.* (2019) citric and acetic acid solutions have been used separately to wash soils polluted with Ni. Citric acid was found to perform better in Ni removal. Several factors affect the efficiency of the soil washing process e.g., the type of acid solution, reaction time and target soil characteristics. Another consideration is the fact that the remained aquatic solution needs to be treated which represents an additional cost (Ko *et al.*, 2006). In addition, a reduction in soil fertility may result from the use of chemical agents for soil washing (Yi and Sung, 2015). Another disadvantage of soil washing is that the cost may be as high as \$ 1717 m⁻³. Immobilisation approaches are less expensive, typically costing \$ 50 – 330 m⁻³ (Shen *et al.*, 2018).

2.5.6 Electro-kinetic (EK)

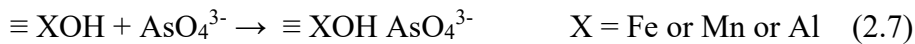
This technology is used for polluted soils with low permeability and can be operated *in situ* and *ex situ* to remove a variety of pollutants including heavy metals. This approach works by introducing two electrodes into the contaminated soil and applying an electric field which results in the migration of metals cations towards the electrode with the opposite charge (Figure 2.11). Three transport mechanisms can control the migration of metal anions: (1) transport of ionic species, (2) movement of charged particles and colloids, and (3) transport within interstitial fluid (Iannelli *et al.*, 2015). Several factors may affect the efficiency of EK including the presence of more than metal in contaminated soil matrixes, level and type of contaminant, soil pH. This approach is also limited to small sites and where specialist equipment and operators are available (Iannelli *et al.*, 2015; Chen *et al.*, 2019). Additionally, the removal efficacy of EK is significantly decreased when target soils contain carbonates and hematite as well as large amounts of gravel and rocks (Virkutyte *et al.*, 2002).

2.6 The chemical mechanisms of metal immobilisation

2.6.1 Adsorption

Adsorption can be defined as a phenomenon of transformation of several molecules and /or ions from the liquid phase to the surface of a solid adsorbent that being added to the medium or being found naturally (Yadla *et al.*, 2012). Metals are observed to adsorb onto amendments or soil by 2 key mechanisms cation exchange and chemisorption. Moreover, electronic attraction may be used by clay and organic matter to bind the cations of metals. The heavy metal forms might be changed over the time to other forms, as the soil condition changes and causes a conversion in the species of element to a more or less available fraction (Wießner *et al.*, 2005; Sheoran and Sheoran, 2006). The adsorption process can act on the surface of metal oxides due to the existence of many actively charged sites there; this may lead to a chemical reaction, and the adsorption of organic and inorganic compounds taking place (Malamis and

Katsou, 2013). Generally, there are two types of adsorption that could be used to mitigate the mobility of pollutants in the soil solution. The first one is chemical adsorption. This type is selective and irreversible. Moreover, chemical adsorption can create a stable inner complex by bonding strongly with metal oxides. These mechanisms may depend on the reactions of functional groups, which are located on the surface of metal oxides and the ion of target metal (Me^{2+}). The following chemical equations show how this reaction acts (Bardl, 2004).



Physical adsorption is another mechanism used by metal oxides to adsorb the ions of hazardous metals. It can be defined as the attraction between the metal ions and the surface of adsorbent as a result of Van der Waals forces to form outer complex compounds that make a weak bond between adsorbants and the adsorbent (Yadla *et al.*, 2012). This type has a lower selectivity and stability than chemical adsorption (Grillo *et al.*, 2015). Many factors have an effect on physical and chemical mechanisms, for instance, organic substances in soil (e.g., oxalate and malate) and inorganic material, such as sulphate and phosphate. pH has a considerable influence upon the adsorption process of heavy metals on soils or by amendments. An example of this is, the adsorption of Pb rising noticeably with the increase of pH up to 5 and slightly between 6-8, when it reaches the maximum adsorption at pH=8; this may depend on the nature of adsorbents. Other factors that can control the adsorption process are redox potential, cation/ anion exchange capacity and soil composition including organic matter, Fe, Mn and Al oxides, silicate and clay. The competition between different metal ions to adsorb on the surface of adsorbents can affect adsorption efficiency (Dave and Chopda, 2014).

Alongside the factors mentioned above, the high porosity of several adsorbents e.g., biochar and zeolite can allow the adsorption process to occur in the walls of pores in the side of the amendment. This process seems to be affected by the rate of liquid diffusion into the adsorbent, as well as, the size, shape and polarity of adsorbants (Yadla *et al.*, 2012). The addition of amendments can stimulate the adsorption of pollutants, such as heavy metals indirectly by altering several chemical and physical characteristics of amended soil, such as pH, O.M. content and Eh (Huang *et al.*, 2016). The main disadvantage of adsorption is that sometimes the adsorption process is a reversible reaction, thus the adsorbents might be regenerated by the appropriate desorption procedure (Fu and Wang, 2011).

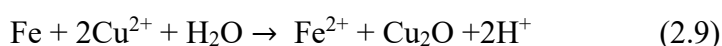
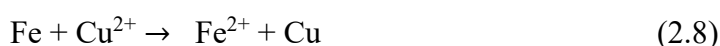
2.6.2 Precipitation and co-precipitation

Precipitation and co-precipitation are significant techniques to form indissoluble compounds of heavy metals, which can reduce the mobile fraction of these elements within the environmental medium. These processes can be controlled by the solubility products of compounds, the concentration of elements and other anions in soils such as phosphate, pH and Eh (Park *et al.*, 2011). For example, according to Seshadri *et al.* (2016), the availability of Cd to rice grain decreases due to the formation of CdS as a result of a sharp decrease in the redox condition, leading to a drop in the redox potential (Eh) of soil in the rice field. Another example of this is using hydroxide precipitation to remove heavy metals from waste water, which is an effective technology, because it is cost effective, simple and it is easy to control the pH (Fu and Wang, 2011). Alternatively, several organic substances that contain chelating agents to bind with heavy metal ions are used effectively, such as di-propyl di-thiophosphate, which has shown a high efficiency in removing Pb, Cd, Cu and Hg from water up to 99% of their initial value as reported by Ying and Fang (2006). The anionic form of metals such as AsO_4^{3-} can co-precipitate on the surface of Fe and Mn oxides, such as ferric oxyhydroxide, due to the occurrence of the positive charges on the surface of these oxides in the acid condition. However, where pH is above 7, a negative charge is the dominant charge on the surface of Fe and Mn oxides, which allows to them to adsorb heavy metals cations. In the case of Pb pollution, the majority of studies have used amendments containing phosphorus in their components, which allows to them to mitigate the mobile fraction of Pb by ionic exchange and precipitation reaction as $[\text{Pb}_5(\text{PO}_4)_3\text{X}]$; where X = OH, Br, Cl or F], yet the common precipitated complexes are hydroxymorphite or chloropyromorphite (Park *et al.*, 2011). In the soils with high levels of sulphide in reduced conditions, precipitation of heavy metals as sulphides could play a major role in the metal dynamic (Sheoran *et al.*, 2016).

2.6.3 Reduction reaction

Several heavy metals are engaged in reduction reaction, which can affect their biogeochemical conductance. The reduction reaction seems to occur as a result of adding materials to the soil solution that can act as a donor electrons (Park *et al.*, 2011). Due to this donation process, the oxidation state of the target metal is reduced to another form which is less mobile and, therefore less hazard for plants and soil contents (Wang *et al.*, 2010). An example of this is the reduction of Cr^{6+} to a less dangerous state Cr^{3+} in the soil solution because of Cr^{3+} has lower solubility therefore less mobility in soil solution (Lasat, 1997; Lukina *et al.*, 2016). This reaction depends upon the standard reduction potential (E°) of both adsorbants and

adsorbent materials. The elements that have E° are more positive than the amendment can be reduced electrochemically by reduction reaction. For example, the adding of Fe^0 , which has $E^\circ = -0.44 \text{ V}$ to polluted soil. In this particular case, heavy metals with E° are more positive than that of Fe^0 such as $\text{Pb}^{2+}/\text{Pb}^0$ with $E^\circ = -0.126 \text{ V}$ and $\text{Cu}^{2+}/\text{Cu}^0$ with $E^\circ = 0.34 \text{ V}$ can be reduced to less bioavailable forms by acting as electron acceptors of electrons that provided by the oxidation of Fe^0 to Fe^{2+} and later to Fe^{+3} (Li *et al.*, 2016). Especially, Cu^{2+} may be quickly reduced when Fe^0 is added, as the following chemical reactions show (Zhang *et al.*, 2011; Li *et al.*, 2016).



Dissolved organic matter contain several organic functional groups e.g., Quinone moieties, that work as an electron acceptor where electrons are donated by heavy metal anions with mutable valance. Thus, the oxidation state of the electron acceptor ion is reduced to a less toxic form, due to a redox reaction occurring among these constituents (Huang *et al.*, 2016).

2.7 Advanced methods in metal pollution studies

2.7.1 Sequential extraction procedure for exploring heavy metal behaviour in soils

The chemistry of heavy metals in the soil is influenced by many factors that have a considerable effect upon their toxicity and bioavailability, such as total concentration and environmental conditions e.g., pH, OM, the metal chemical form and element properties. Several chemical fractions can control the chemical behaviour of toxic elements in the soil such as (Gleyzes *et al.*, 2002):

- 1- Water-soluble fraction, this fraction can dissolve in a tailings solution and easily translate to soil biota and flora. For that reason, including this fraction in geochemical studies is favourable, to provide a pontifical guide to study the environmental risk of soluble heavy metals.
- 2- Exchangeable fraction, this refers to the level of metal substituted on the surface of tailings by a weak electronic interaction and weak adsorption on the surface. These elements may be extracted by using several reagents that deliver cations e.g., Mg^{2+} and NH_4^+ that can replace the heavy metals from the occupied sites with an ion-exchange process in an acid medium to avoid re-adsorption of extracted metals.
- 3- Fe-Mn oxides, this fraction is favourable for several heavy metals and by using selected reagents in specific conditions of pH and Eh, the oxides of Fe and Mn can be dissolved

and their contents of heavy metals are released into the solution. The reagents used here should not have the ability to attack OM and the sulphide fraction.

- 4- OM and sulphides fraction, OM and sulphides are a good sink for some heavy metals. The degradation of these materials by oxidising reagents such as H_2O_2 or NaOCl leads to a release of the metals fixed with OM and sulphide.
- 5- Residual fraction, the element associated with soil primer and the second material in their crystalline lattice can be extracted with concentrated acids that have the ability to dissolve these compounds and release the metals that are retained in their structure (Singh *et al.*, 2011).

Evaluating the geochemical fractions of heavy metals may provide beneficial information about the chemical interaction and availability of hazardous elements in soils. The assessing of a bioavailable form of pollutants can indicate the environmental risk that affects plant growth and causes ground water pollution. To evaluate the geochemistry of heavy metals in soils and sediments several sequential extraction protocols have been developed and employed over the last 40 years (Rodríguez *et al.*, 2009). These approaches are based on the use of several reagents have different abilities to dissolve the fractions of metal associated with different geochemical fractions and are developed by simulating the variation of environmental conditions (Gleyzes *et al.*, 2002). According to Zimmerman and Weindorf (2010) the modified sequential extraction is similar to that described by Tessier *et al.* (1979), and Community Bureau Reference (BCR) is used to study the highly polluted soil by acid mine drainage in Spain. Rodríguez *et al.* (2009) carried out the sequential extraction protocol in 3 steps (acid soluble, reducible, oxidisable and residual fractions) to study the geochemistry of mine tailings collected from a historic Pb-Zn mine in Spain.

2.7.2 Lead isotopes to identify the geochemical routes of lead pollution

The interaction of Pb within the environments is attributed to two sources; first, anthropogenic activities such as mining and smelting Pb ores, ceramics, nuclear and battery industries, and leaded gasoline. Other sources of Pb pollution are geogenic sources, such as the weathering process of rocks containing Pb (Dean *et al.*, 2017). The establishing of the emission sources of Pb suggested that atmospheric deposition and human activities are a significant cause of lead pollution. For instance, the lead input was $80.000 \text{ t year}^{-1}$ during the period from the Roman era up to the 17th century, in contrast at the beginning of the 20th century; this amount had increased up to 10^6 t year^{-1} . However, leaded petrol has become the main source of Pb pollution since the 1960s (Komárek *et al.*, 2008). According to Bird (2011), the

identification of the geochemical routes and distinguishing between different sources of Pb pollution has been done by using Pb isotopes as one of the most effective tracers. Within the natural ecosystems, Pb has four isotopes that are; ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb with an abundance 1%, 24%, 23% and 52%, respectively. ^{206}Pb , ^{207}Pb and ^{208}Pb are generated as a result of the decay of ^{238}U , ^{235}U and ^{232}Th , yet ^{204}Pb is recognised as being stable and having a lengthy half-live time isotope (Brewer *et al.*, 2016). As reported by many researchers, the radiogenic ratios of anthropogenic Pb isotopes are lower than those of geogenic Pb isotopes. The use of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ ratios are more common in differentiating between lead pollution sources and identifying the fingerprint of Pb within the environment (Bird, 2011). For example, the $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in natural soil, Pb ores and coal in the UK ranged from 1.17 to 1.19 and in the leaded gasoline they were 1.06-1.09 (Bellis *et al.*, 2004).

2.8 Dealing with contaminated lands in the UK

The issue of contamination of lands remained on the schedule of restoration and environmental agendas in most European countries. There are two distinct areas of policy relating to polluted lands (Ferguson, 1999):

- 1- The protection view refers to the effects and impacts of polluted lands on environmental quality and human health.
- 2- The planning view refers to managing and treating contaminated sites (e.g., restoration of mine sites).

The report published by Environment Agency (EA) in 2016 stated that the EA, the Scottish Environment Protection Agency (SEPA) and the Coal Authority (CA) are working together to deal with heavy metal contamination caused by metal mining activities and reduce the discharge of mine waters containing hazardous levels of heavy metals into surface waters and aquifers. To this end, the EA, SEPA and CA have established the biggest mine water treatment project in Britain, preventing approximately 670 tonnes of Fe and 150 tonnes of Zn from discharging into Restrounguet Creek in Cornwall. In Wales, research has been carried out to identify the main pollution sites efforts have been made to decide on appropriate treatment technologies to amend these sites (Sc *et al.*, 2008). The EA (2016) concluded that further work needed to be carried out to: (1) set up a national strategy aimed at cleaning up abandoned metal mines, (2) identify sustainable treatment approaches to amend metal mines, (3) monitor the quality of mine water, (4) find new techniques to recover energy and resources from remediation residues and mine water and (5) study the effect of earlier mine input on sediment and environmental health (EA, 2016) .

2.8.1 Land legislation and policy

Part 2A (Part 2A is the primary legalisation on contaminated land that come into force in England and Wales at the beginning of April 2000 and aims to (EA, 2016):

- a. Identify and removing unacceptable risks to ecosystems and human health.
- b. Ensure that the polluted terrestrial environments are completely appropriate for their current use.
- c. Ensure that the burdens faced by individual, firms and societies as a whole are proportionate, manageable and compatible with the principles of sustainable development.

In the EP Act 1990 polluted land was defined as “Any land which appears to the local authority in whose area is situated to be in such a condition, by reason of substances in, on or under land that: (a) significant harm is being caused or there is a significant possibility of such harm being caused; or (b) pollution of controlled waters is being, or is likely to be caused”.

The local authority was required by the act to investigate any land that fitted this definition, and to order the persons responsible to treat it. The party responsible for remediating the site may be: (A) the person who polluted or allowed the disposal of pollutants on the land in question, or (B) the current landowner. However, if there is no person or company have been identified causing the pollution, than the local authority (e.g., Welsh Assembly or Scottish Governments) have to facilitate the financial sources for remediation projects of polluted lands (Johnston *et al.*, 2008). Once the contaminated land identified and the founding of remediation optioned, the remediation can be done by: (1) cleaning up or decreasing the pollution levels, (2) blocking the route between the pollution sources and receptor, (3) alleviating the exposure to the contamination, and (4) removing all the receptor. Part 2A legalisation states a requirement to identify the most suitable option from the above. The remediation strategy must be set out in a remediation declaration, a remediation notice and a remediation to make the remediation decisions (EA, 2016).

2.9 Conclusion

The ecosystem and mainly soils suffer from pollution from heavy metals and metalloids. These metals have accumulated in the soil from many sources, some of them are natural, others artificial sources. Mining activities are one of the significant sources of soil contamination by heavy metals, which can affect human health and the stability of the environment. The mismanagement of mine tailings, which are rich with heavy metals leads to an increase in the hazardous level of these metals in the surrounding areas, where the mines are located. The

literature above summarized the use of organic and inorganic amendments in the case of soil contaminated with heavy metals as effective approaches to immobilize the availability of heavy metals. Adding several materials to the polluted soils in the same place (in-situ) where the pollution has occurred is cost effective and has lower environmental impacts than treating these soils elsewhere (ex-situ). Several organic and inorganic amendments such as biochar, compost, zeolite, and Fe^0 have the ability to reduce the mobility and bioavailability of heavy metals in the soil medium. The effectiveness of these applications to immobilize the metal in the soil depends upon the use of some mechanisms such as chemical reduction, surface precipitation, physical adsorption, co-precipitation and ion exchange. However, some amendments were not sufficient to reduce the bioavailability of heavy metals. This may be due to the lower proportion of amendments that were added to the polluted soils and/or treatment conditions. Alternatively, the use of several plants that can tolerate and survive in extreme conditions due to pollution with heavy metals may be better choices to stabilize these metals in the root zone or accumulate the target metals in harvestable parts of plants. The reinforcement of all introduced remediation technologies by government environmental policies is necessary to ensure effective mine tailings remediation.

3 SITE DESCRIPTIONS

3.1 Historical view

Metal mining in the UK started 4500 years ago, and later during the 18th and 19th centuries, the UK was the main global producer of several metals. As a result, more than 140,000 UK sites are recorded as mine areas (Figure 3.1), and nearly 400 km² are estimated to be polluted due to the metallurgical industry in this country (Davies *et al.*, 2015). Several of these mining sites are in Wales (Figure 3.2) and mining waste heaps are observed in many provinces. These are rich in elevated levels of heavy metals and pose considerable threats to soils, sediments and plants. Smelting metal ores took place near the mines during early stages of exploitation. With decreasing wood supply but increased use of coal for smelting, smelting centres were established nearer to coal mines in the 19th century and ores were exported to these areas. For example, there were 20 main smelters that operated around Swansea in Tawe Valley, Wales. The metallurgical industry has polluted several agricultural areas, e.g. Aberystwyth in the middle of the 19th century. Farmers reported that their agronomic lands received amounts of mine tailings due to the flooding of local rivers. Later the analysis process estimated that 6.8 tons of metal ores had leaked into the ecosystem for every 100 tonnes of ore that have been discovered (Davies, 1987). Similarly, Parc Mine in Gwydyr Forest generated 10,000 tonnes of Pb and 4000 tonnes of Zn during its investment time (1855 – 1963). This led to the creation of 250,000 tonnes of tailings, which covered the area that surrounded the mine (Davies *et al.*, 2015).

3.2 Geological view

The main host rocks in Wales and the UK are limestone and carboniferous limestone underlies several old lead mines. Other sites are based on non-calcareous sedimentary rocks that generate acidic soil. However, natural or weak acidic soil is generated when the limestone is not associated with glaciers drifting or heavy rain (Davies and Roberts, 1975). Particularly, the Parys Mountain rocks were geologically categorised to have an age from the Silurian and Ordovician eras. The mineralisation occurred initially due to the association of the sediments and volcanic activities on the bed of the sea. After that, with several earth movements, some minerals were distributed between different types of rocks. The main ores found in high abundance are sphalerite (ZnS), galena (PbS) and chalcopyrite (CuFeS₂) as sources of Zn, Pb, Cu and Fe (Vernon, 1996). Table 3.1 shows the minerals composition of studied mines.

Table 3. 1 Primary and secondary minerals formed in Parys, Britannia and Pandora mines.

Primary minerals		Secondary minerals	
Mineral	Formula	Mineral	Formula
1- Primary and secondary minerals formed in Parys Mountain (adopted from Jenkins <i>et al.</i> , 2000).			
Copper	Cu	Allophane	Al ₂ O ₃ /SiO ₂ /H ₂ O
Gold	Au	Anglesite	PbSO ₄
Silver	Ag	Antlerite	Cu ₃ (SO ₄).17H ₂ O
Bornite	Cu ₅ FeS ₄	Brochantite	Cu ₄ (SO ₄)(OH) ₆
Bourmonite	CuPbSbS ₃	Basaluminitite	Al ₄ (SO ₄)(OH) ₁₀ .5H ₂ O
Cuprite	Cu ₂ O	Calcite	CaCO ₃
Chalcocite	Cu ₂ S	Chalcanthite	CuSO ₄ .5H ₂ O
Chalcopyrite	CuFeS ₂	Goethite	α -FeO(OH)
Covellite	CuS	Gunningite	ZnSO ₄ .H ₂ O
Galena	PbS	Gypsum (selenite)	CaSO ₄ .2H ₂ O
Kobellite	Pb ₂₂ Cu ₄ (Bi,Sb) ₃₀ S ₂₉	Jarosite	K ₂ Fe ₆ (SO ₄) ₄ (OH) ₁₂
Malachite	Cu ₂ (CO ₃)(OH) ₂	Pisanite	(Cu,Fe)(SO ₄).7H ₂ O
Minium	Pb ₃ O ₄	Pyromorphite	Pb ₅ (PO ₄) ₃ Cl
Pyrite	FeS ₂	Rozenite	FeSO ₄ .4H ₂ O
Sphalerite	ZnS		
Tenorite	CuO		
Tetrahedrite	(Cu,Ag,Fe,Zn) ₁₂ As ₄ S ₁₃		
2- Primary and secondary minerals formed in Britannia mine (Hudson, 2018a).			
Chalcopyrite	CuFeS ₂	Brochantite	Cu ₄ (SO ₄)(OH) ₆
Marcasite	FeS ₂	Calcite	CaCO ₃
Pyrite	FeS ₂	Chalcoalumite	CuAl ₄ (SO ₄)(OH) ₁₁ .3H ₂ O
		Connellite	Cu ₁₉ (SO ₄)(OH) ₃₂ Cl ₄ .3H ₂ O
		Georgeite	Cu ₂ (CO ₃)(OH).6H ₂ O
		Goethite	α -FeO(OH)
		Gypsum (selenite)	CaSO ₄ .2H ₂ O
		Linarite	PbCu(SO ₄)(OH) ₂
		Malachite	Cu ₂ (CO ₃)(OH) ₂
		Posnjakite	Cu ₄ (SO ₄)(OH) ₆ .H ₂ O
3- Primary and secondary minerals formed in Pandora mine (Hudson, 2018b).			
Chalcopyrite	CuFeS ₂	Allophane	(Al ₂ O ₃)(SiO ₂) _{1.3-2} .5H ₂ O
Galena	PbS	Cerussite	PbCO ₃
Sphalerite	ZnS	Calcite	CaCO ₃
		Dundasite	PbAl ₂ (CO ₃) ₂ (OH) ₄ .H ₂ O
		Quartz	SiO ₂

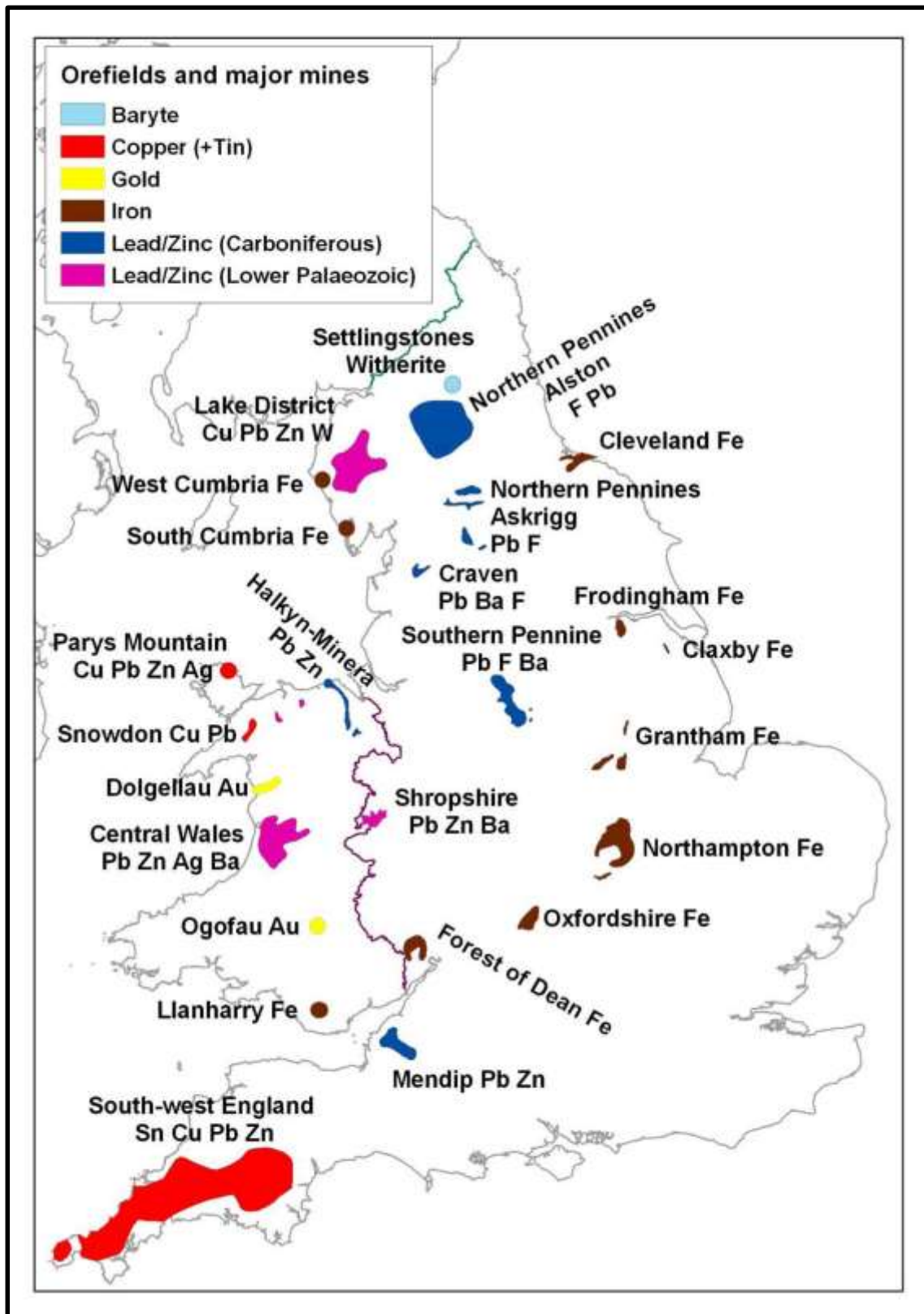


Figure 3. 1 Abandoned metal mines in the UK (Represented by permission from British Geological Survey; Palumbo-Roe and Colman, 2010).

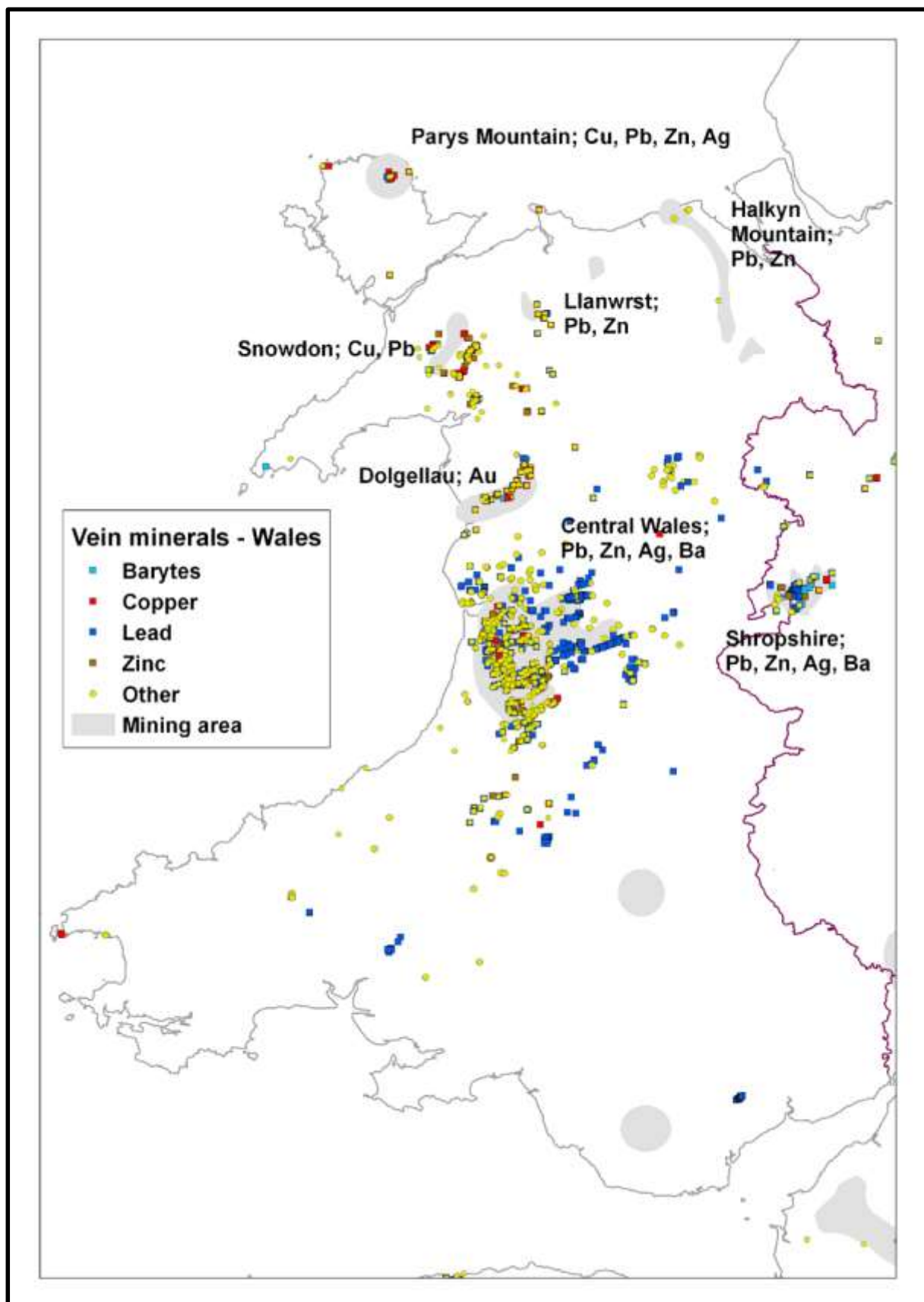


Figure 3. 2 The distribution of mining sites in Wales (Represented by permission from British Geological Survey Palumbo-Roe and Colman, 2010).

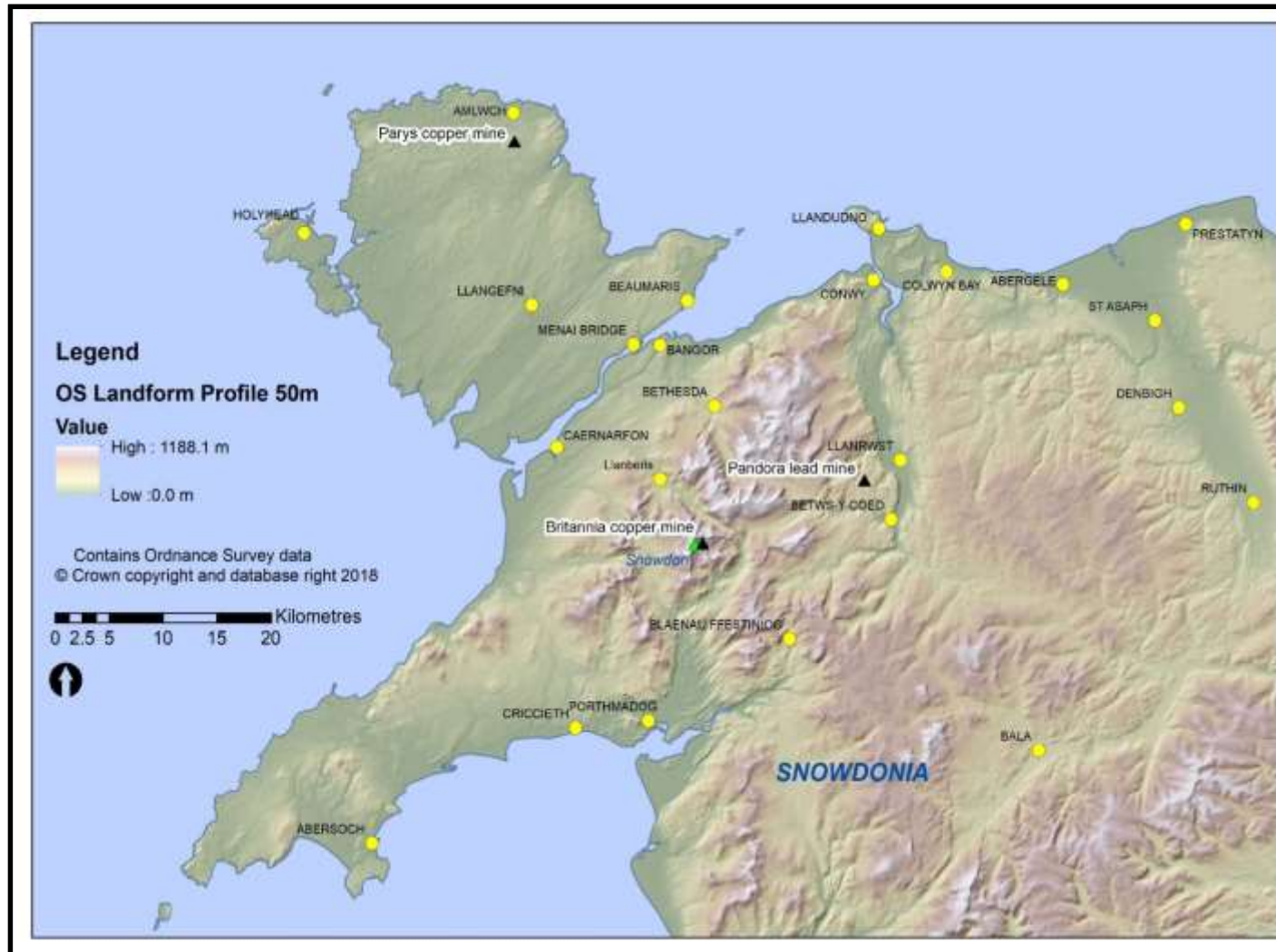


Figure 3. 3 Map showing the locations of the Parys copper mine, Pandora lead mine and Britannia copper mine.

3.3 Site description

Three mine sites were selected based on their current known pollution issues to study in this research; we adopted a random sampling approach to select tailings from each of these sites. In each of the mine sites studied, there is a tendency for tailings particles to be large and the sediment appear rocky in texture. Within each mine site, areas where sufficient amounts of sediment of smaller particle sizes, < 2mm, were identified and random samples taken from each of these sites.

3.3.1 Parys copper mine

Parys copper mine is situated near Amlwch town in the north of Anglesey Island in Wales, UK (53° 14' 45" N, 04° 11' 45" W) as figure 3.3 shows. This mine has been operating since the Roman era, and it was most in use and the main copper mine worldwide during the 18th century. This mine closed in 1904 and operated again shortly in 1984 (Walton and Johnson, 1992; Davies *et al.*, 2015). Copper was extracted initially from the surface and later the underground mine opened. Sulphide minerals, e.g. pyrite, galena, sphalerite and chalcopyrite were the dominant ores at this mine. The smelting process took place primarily near the mine then at the nearby town of Amlwch, the copper was shipped to other sites in the UK via Amlwch harbour. Due to this process, a massive amount of mine tailings were placed in the surrounding environment as the mine waste covered nearly 200 ha around the Parys copper mine (Wilson and Pyatt, 2007). Figures 3.4 and 3.5 show part of the Parys area covered with mine tailings.



Figure 3. 4 Part of the Parys mountain area around the copper mine. Source: Photo taken by the researcher 2015.



Figure 3. 5 View of mine spoils at Parys mine. Source: Photo taken by the researcher 2017.

3.3.2 Pandora site

The lead and zinc mining industry at Gwydyr Forest started in the 17th century and it reached its peak between 1850 – 1900s, when 25,000 tonnes of Pb and Zn were extracted from

their ores. Several mines are distributed in this region such as the Parc, Hafna, Llanrwst, Pandora, Betws-y-Coed and Cyffy mines (Dutton *et al.*, 1994; Ukcaving.com, n. d.).

3.3.2.1 Pandora lead mine

This mine is situated in the south east of Llyn Geirionydd in the Conwy Valley ($53^{\circ} 12' 45''$ N, $03^{\circ} 84' 27''$ W) as figure 3.3 shows. The mining of lead in the Pandora mine was established at the end of the 19th century, and it was most in use during the period from 1840 – the 1920s. Historically, the Pandora mine has been recorded with different names Eagle, Welsh-Foxdale, Foel Ddu, Willoughby and Pandora, however Pandora was the most used name (Dutton *et al.*, 1994; Robinsonmaps.blogspot.blogspot.co.uk, 2013). Due to the long-term operation of this mine, spoil covered the area around the mine and affected the vegetation and the forest as figures 3.6, 3.7 and 3.8 illustrate.



Figure 3. 6 Mine spoils around the Pandora lead mine. Photo taken by the researcher 2017.



Figure 3. 7 Revegetation part of Pandora lead mine tailings. Photo taken by the researcher 2017.



Figure 3. 8 Sampling from Pandora lead mine. Photo taken by the researcher's supervisor 2017.

3.3.3 Llanberis or Britannia copper mine

Also known as Cwmaelhir, Gwaith Nant Peris or Dol Ithel mine. This mine is situated on the A4086 road, between Llanberis and Nant Peris close to Llyn Peris lake on Snowdon Mountain (53° 4 16" N, 4° 3 57" W) as figure 3.3 shows. The mining of copper started here at the beginning of the 1800s and during its investment time, it was operated by several companies. This mine closed in 1916, however effects on the ecosystem continued, due to the massive amount of tailings that remained and were distributed in the area around the mine.

3.4 Study background

In the UK, many studies have been conducted with the aim of evaluating the environmental impacts of mining activities on terrestrial and aquatic ecosystems. Davies (1987) and Davies and White (1981) stated that, as early as 1919, Griffiths explored the effect of mining on the soil and animals health in Cardiganshire. Moreover, they reported that, later in 1969, Alloway conducted a comprehensive investigation of the impact of lead mining on soil and plants in the Ystwyth Valley. Since then, many studies have been carried out addressing this issue such as Alloway and Davies (1971), and Davies and Roberts (1975 and 1978). Furthermore, Davies (2015) reported that much research has assessed the environmental impacts of the mining industry, such as Harrison (1984), Filipek *et al.* (1987), Johnson (1991), Walton and Johnson (1992), Palmer (2006), Atkinson (2010) and Poonia *et al.* (2010). More recently, Bird (2016) studied the effect of mining activities and the remediation process on the level of contamination of rivers and sediments with heavy metals.

3.5 Conclusion

Parys copper mine, Pandora lead mine and Britannia copper mine are some of many abandoned mines that have been operating in Wales, and then closed many decades ago. These mines are located in different areas and surrounded with various environments. The investment in these mines led to distributing the mine waste that was rich with hazardous metals to terrestrial and aquatic ecosystems posing damage to the ecosystem's content, including soil, plant, animal and human health. Many researchers have assessed the impact of mine tailings on neighbouring systems, such as soil, plants, catchments and rivers. Therefore, studying the appropriate approaches to treat these sites may help the local authorities to make a proper decision to protect and recover the ecosystems around the Parys, Pandora and Britannia mines and other mining effected locations.

4 ASSESSING THE DISTRIBUTION OF LEAD (Pb), COPPER (Cu), ZINC (Zn) AND CADMIUM (Cd) IN DIFFERENT GEOCHEMICAL FORMS IN MINE TAILINGS

4.1 Introduction

Heavy metal pollution is considered a global issue, representing a hazard for flora and fauna, accumulating in the food chain and ultimately posing a risk to human health (Singh *et al.*, 2011). Many human activities, in particular, prospecting for and extracting metals from their raw materials, have contributed to an increase in the levels of heavy metals in the surrounding areas. The behaviour of heavy metals in these soils can be affected by several environmental conditions including pH, organic matter content and redox conditions. For instance, at low pH levels, heavy metals may be found as cations, e.g. Pb^{2+} , Cu^{2+} , Zn^{2+} , which allows to them to be transferred through soil medium, in the water-soluble fraction, to plants and microorganisms, leading to degradation in the quality of the chemical, physical and biological properties of the soil (Arenas-Lago *et al.*, 2014). Heavy metals can also accumulate in other geochemical forms in the soil, e.g., exchangeable, oxidisable, reducible and residual forms (Rodríguez *et al.*, 2009; Zeng *et al.*, 2011). The water-soluble and exchangeable fractions are the available forms, whilst the oxidisable and reducible forms might become available to plant and soil biota under specific conditions such as changing the redox condition and pH (Fernández-Ondoño *et al.*, 2017). Details of the chemical fractions of heavy metals should therefore be included in heavy metal pollution studies since this may provide beneficial information about the behaviour and distribution of heavy metals in soils (Ahmadipourv *et al.*, 2014; Lago-Vila *et al.*, 2014). Furthermore, such information could help to control pollution levels and protect the environment by facilitating the development of effective remediation techniques to treat polluted areas (Gabarrón *et al.*, 2017).

The concentrations of heavy metals associated with different fractions can be measured by a sequential extraction procedure, used for more than thirty years to detect the distribution of heavy metals in different soil and sediment phases. This technique was developed by examining the use of a variety of reagents and different laboratory conditions to extract the metals associated with different geochemical fractions in soils, mine tailings and sediments (Gleyzes *et al.*, 2002; Zeng *et al.*, 2011). In the literature, different versions of the protocol employ different reagents with different degrees of dissolving power to extract the metals accumulated in a specific geochemical fraction (Alvarez *et al.*, 2014).

To our knowledge, there are a lack of studies in recent years which focus on exploring the distribution of heavy metals in different mine tailings phases. A number of studies have been published examining the contamination and evaluating the bioavailable form of several toxic metals at Parys Mountain, however, these studies do not investigate the distribution of heavy metals in different geochemical forms (Farrell *et al.*, 2010; Farrell and Jones, 2010; Khan and Jones, 2009). Similarly, in the case of Pandora lead mine and Britannia copper mine, no published work has examined the distribution of heavy metals in different tailings phases. Therefore, studying the distribution of heavy metals in different geochemical fractions of mine tailings is essential to evaluate the environmental risk of toxic elements in multi- metal polluted sites.

4. 2 Objectives

This work investigated the distribution of copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd) in mine tailings by:

- 1- Analysing the chemical and physical properties of tailings, which have a significant influence on heavy metal behaviour in mine areas.
- 2- Determining the total concentration of studied metals.
- 3- Detecting the distribution of target metals in different geochemical forms in mine tailings collected from three mines in Wales.

By achieving these aims, the study seeks to answer the following questions:

- 1- Are there any difference in the total concentrations of Cu, Cd, Zn and Pb between different sampling sites?
- 2- Does the distribution of heavy metals in different mine tailings fractions vary between sites?

4. 3 Material and Methods

4. 3. 1 Study sites

Mine tailing samples were collected in March 2015 randomly from the three locations described on sections 3.3.1, 3.3.2 and 3. 3.3, five samples were selected from each site (the weight of each sample approximately 1.0 kg), making a total of 15 samples from all sites.

4. 3. 2 Sample preparation

The samples were transferred to the laboratory, air-dried at room temperature for a week, sieved to < 2 mm to remove the stones and gravel then kept in polyethylene bags in a cool room at 4°C until further analysis.

4.3.3 Analytical methods

4.3.3.1 Control and assurance the result quality

Precision and accuracy of results was achieved using standard laboratory procedures including the use of analytical grade chemicals, and ultra-pure water to prepare a 0.1 N HNO₃ solution to clean equipment. Also, all treatments were run in triplicate. Heavy metal analysis was carried out using a Varian flame atomic absorption spectrometer (FAAS) model 220-FS fitted with an impact bead, appropriate hollow cathode lamps and an air- acetylene grate. The analytical conditions and linear range for the instrument fitted with the impact bead are illustrated in Table 4.1. The multi-metal standard solutions (8 solutions) used for calibration were prepared in the same matrixes used to extract target metals in different steps set out in the sequential extraction scheme. They made up from a 50 mg L⁻¹ working solution for each target metal separately. The working solutions were prepared from stock solutions (bought from Sigma-Aldrich; the certified reference material (CRM) was produced and certified in accordance with ISO/IEC 17025 and ISO Guide 34) with a concentration of 1000 mg L⁻¹ within the linear ranges set out in Table 4.1. During the analysis processes the blank solution were injected after 10-15 unknown samples were analysed (zeroing the instrument). The dilution factors for extracts were determined by trial and error.

Table 4. 1 Analytical conditions of Varian flame atomic absorption spectrometer (FAAS) instrument.

Metal	Wavelength (nm)	Range of working solution (mg L ⁻¹)	Calibration Equation $y = bx + a$	R ²
Cd	228.8	0.01 – 2.0	$y = 0.03685x + 0.005$	0.9982
Cu	327.4	0.1 - 24	$y = 0.0455x + 0.008$	0.9986
Pb	217.0	0.1 - 30	$y = 0.016x + 0.091$	0.9883
Zn	213.9	0.01 - 2	$y = 0.0281x + 0.018$	0.9918

R² refers to correlation coefficient of calibration curve.

Eight multi-metal calibration solutions were used to calibrate the FAAS. The limits of detection were calculated after injecting five blank solutions (15 ml of dH₂O + 5 ml concentrated nitric acid) to the instrument after the linear curve was obtained by FAAS. The FAAS limits of detection (LOD) were calculated as follows (Miller and Miller, 2005):

$$\text{Limit of detection (LOD)} = Y_B + 3S_B \quad (4.1)$$

Where Y_B refers to the mean concentration of target metal in the blank and S_B is the standard deviations of the blank. The FAAS LOD for Cu was 0.38 $\mu\text{g g}^{-1}$, Zn 0.15 $\mu\text{g g}^{-1}$, Cd

0.13 $\mu\text{g g}^{-1}$ and Pb 1.27 $\mu\text{g g}^{-1}$. For statistical analysis, where values were recoded below the LOD, a value of half the calculated LOD was used (USEPA, 2000). To assess the efficiency of the sequential extraction protocol used, the % recoveries of the studied elements were calculated and most of the proportions were reasonable as section 4.4.4 illustrates (Naj *et al.*, 2010; Rinklebe and Shaheen, 2014).

4.3.3.2 Sample analyses

4.3.3.2.1 Mine tailings pH and electrical conductivity (EC)

The tailing suspensions were prepared for measuring both EC and pH by adding 5g of air-dried and sieved (2mm) tailings to 10 ml of deionised water (dH_2O) in a 50 ml centrifuge tube (1:2 tailings: water ($^w/v$) ratio). Three replications for each sample were used for pH and EC analyses. The samples were shaken for 30 min at 250 rpm (SW2 Shaker Table, Edmund Buhler GmbH). The pH of the tailing suspensions were measured using a pH-metre (Hanna Instruments pH 209). This was conducted after the device had been calibrated by using buffer solutions (pH = 7 and 4). The EC of the tailings solutions were measured immediately after the determination of the pHs, by using a conductivity meter (Jenway 4520) at 21 ± 0.5 °C after the calibration of the machine using a 0.01 M KCl, which had an EC of 1413 $\mu\text{S cm}^{-1}$ (Ryan *et al.*, 2007).

4.3.3.2.2 Evaluation the mine tailings moisture and organic matter

A clean dry porcelain crucible was weighted (W_1) and then 10g of wet sieved mine tailings (at filed capacity) was placed in the crucible and weighed (W_2). The sample was put in the oven at 105 °C for 24 hours. After that, the temperature of the oven was allowed to decline and then the crucible was allowed to cool at room temperature. Following this, the crucible was weighed accurately (W_3). In order to measure the total organic matter, the tailings sample (W_3) was put in an oven at 450 °C for 24 hours and then the oven temperature was allowed to settle, after which the sample was removed and cooled to room temperature and then weighed (W_4) (Faithfull, 2002 ; Abdalgader, 2014). The analyses were run in triplicates, and the percentage of moisture and organic matter were calculated using the following equations:

$$\text{Tailings moisture (g g}^{-1}\text{)} = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \quad \text{Equation 4.2}$$

$$\text{Total organic matter (g g}^{-1}\text{)} = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \quad \text{Equation 4.3}$$

4.3.3.2.3 Cation exchange capacity (CEC)

4 g of air-dried and sieved (2 mm) mine tailing samples were placed in 50ml polyethylene centrifuge tubes, ($n = 3$ for each point), then 33 ml of 1 M sodium acetate (CH_3COONa) were added to each sample. The samples were shaken (SW2 Shaker Table, Edmund Buhler GmbH) for 5 min and after that, the samples were centrifuged at 2000 rpm for 15min (Eppendorf AG centrifuge 5810 R.), then the matrix was discarded. This step was run four times. Afterwards, 33 ml of 95% ethanol (v/v) was added to the samples to wash the tailings, the samples were then shaken for 5 min at 300 rpm and then centrifuged at 2000 rpm and the suspensions were spilt. This process was repeated three times until the EC of the last washing counted at less than $200 \mu\text{S cm}^{-1}$. The remains of the previous step were used to replace the sodium ions adsorbed on the tailings' surface by adding 33 ml of 1 M ammonium acetate (NH_4OAc) solution and following the same procedures that were used to saturate the samples with CH_3COONa as stated above. However, the suspensions were placed in 100 ml volumetric flasks and filled up with 1 M NH_4OAc . The standard solutions of Na^+ ranged from 0 to 200 mg L^{-1} (0, 10, 20, 40, 80, 120, 160 and 200 mg L^{-1}) and were prepared by using a stock solution 1000 mg L^{-1} NaCl to make a calibration curve (calibration equation; $y = 7.12x + 103$), and $R^2 = 0.9874$). A flame photometer model Sherwood 410 was used to evaluate the Na^+ concentration (Ryan *et al.*, 2007; Abdalgader, 2014).

The following equation is used to calculate CEC in $\text{meq} / 100\text{g}$ of tailings.

$$\text{CEC}_{\text{meq}/100\text{tailings}} = \frac{\text{mg L}^{-1} \text{Na (from calibration curve)} \times \text{Df} \times \text{A} \times 100}{\text{Equivalent weight Of Na (22.99 mg meq}^{-1}) \times \text{Wt} \times 1000} \quad \text{Equation 4.4}$$

Where: Df = dilution factor, A= the volume of volumetric flask (100 ml), Wt. = weight of the air-dry tailings (4 g).

4.3.3.2.4 Evaluating the tailings texture by the sedimentation method

In 600 ml beaker, 40g of air-dried and sieved (2mm) tailings were weighed and three replications were used for every point. This involved 60ml of mixture being dispersed with 3% sodium hex-metaphosphate ($(\text{NaPO}_3)_6$) and 0.1 M of sodium carbonate (Na_2CO_3), then the samples were left for 24 h, after which they were covered with a watch glass. After that, the tailing solutions were mixed thoroughly, by using a magnetic stirrer for 3 min, which was then transferred to the 1L calibrated cylinders, and filled up to 1L with dH_2O . After this, the solutions were mixed by a special paddle and this was promptly following by the hydrometer and thermometer being inserted in the cylinder. A reading was taken of the silt (R_{sc}) and the

temperature was taken at 40s after the hydrometer and the thermometer were put in. The blank was performed by using the same protocol, but without tailings; the reading and temperature were taken directly after mixing sodium hex-metaphosphate and sodium carbonate (R_b) in a 1L cylinder. The sample solution was left for 4 hours and then the clay reading (R_c) and temperature were taken. The suspension was passed through a sieve ($63\mu\text{m}$), and then washed with tap water, until the water-passing sieve was pure. The remains of the tailings were transferred from the sieve to 50 ml of the known weight beaker. The solid material was allowed to precipitate and the excess water was discarded. After this the beaker was placed in an oven at 105°C for 24 hours and finally the beaker was weighed and the sand weight was recorded. The room temperature was 21°C and that of the tailings solutions was $21 \pm 0.5^\circ\text{C}$. The proportion of clay, silt and sand were calculated as the following equations show (Ryan *et al.*, 2007; Abdalgader, 2014).

$$\% [\text{Silt} + \text{Clay}] (w/w) = \frac{(R_{sc} - R_b) \times 100}{Wt.} \quad \text{Equation 4.5}$$

$$\% \text{Clay} (w/w) = \frac{(R_c - R_b) \times 100}{Wt.} \quad \text{Equation 4.6}$$

$$\% \text{Silt} (w/w) = [\text{Silt} + \text{Clay} (w/w)] - [\text{Clay} (w/w)] \quad \text{Equation 4.7}$$

$$\% \text{Sand} (w/w) = \frac{(\text{Sand weight (g)}) \times 100}{Wt.} \quad \text{Equation 4.8}$$

The mine tailings were classified using a USDA textural triangle.

4.3.3.2.5 Nitrate ($\text{NO}_3^- - \text{N}$) and Ammonium ($\text{NH}_4^+ - \text{N}$) determination in tailings

extraction

10 g of air-dried tailings were placed in a 50 ml polyethylene centrifuge tube and 25 ml of dH_2O was added to the sample (1: 2.5 w/v ratio). The sample was shaken for 30 min at 300 rpm (SW2 Shaker Table, Edmund Buhler GmbH) and then the suspension was filtered through Whatman® 42 filter paper, following which the substrate was centrifuged for 15 min at 4000 rpm. Finally the supernatant was filtered and stored at -20°C to analyse $\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$ (Mulvaney, 1996).

1. Nitrate ($\text{NO}_3^- - \text{N}$) analyses

The three required reagents were prepared separately as follows: 0.16g of vanadium chloride (VCl_3) was dissolved in 20 ml of 1 M HCl, and 0.02 g of N-(1-Naphthyl) ethylenediaminedi-hydrochloride (NEDD) was dissolved in 20 ml dH_2O . The Sulfanilamide solution was prepared by dissolving 0.4 g in 20 ml 1 M HCl. All the reagents were kept in the dark at 4°C . A range of standard solutions of $\text{NO}_3^- - \text{N}$ were prepared ranging from 0 to 10 mg

L⁻¹ by using the NO₃⁻ - N 1000 mg L⁻¹ stock solution. The determination of NO₃⁻ - N in the stored supernatant was indicated by using a microplate reader and 96 well microplates as follows: 100 µL of the sample and standard were placed in each microwell plate. After that, 100 µL of VCl₃ solution was added to all the micro wells, followed by 50 µL of NEDD solution. Next, 50 µL of Sulfanilamide solution was added to the micro wells and the mixture was mixed by micropipette. This was allowed for a pink colour to develop for 10 – 15 min. The absorbance was read at 540 nm on the plate-reader (Mulvaney, 1996).

The concentration of NO₃⁻ - N was calculated using equation 4.9.

$$\text{NO}_3^- - \text{N } \mu\text{g g}^{-1} = \frac{\text{NO}_3^- - \text{N } \mu\text{g mL}^{-1} \times A}{\text{wt.}} \quad \text{Equation 4.9}$$

Where: A = the volume of extract (25ml), and wt. = the weight of the tailings (10g).

2. Ammonium (NH₄⁺ – N) analyses

The three reagents were prepared separately as follows: 1.5626g of sodium salicylate and 0.025 g of sodium nitroprusside were dissolved in 20 ml dH₂O. 0.296 g of sodium hydroxide and 0.996 g of dibasic potassium phosphate (KH₂PO₄) were dissolved in 9 ml dH₂O and 1 ml of sodium hypochlorite was added (hypochlorite reagent). Next, 0.6g of Ethelendiaminetetraacetic acid (Na₂EDTA) was dissolved in 10 ml of dH₂O. All the reagents were kept at 4°C until they were used. The range of standard solutions ranging from 0 to 10 mg L⁻¹ of NH₄⁺ – N were prepared using stock solution 1000 mg L⁻¹ of NH₄⁺ – N using dH₂O. The evaluation of NH₄⁺ – N in the sample was conducted by using a microplate reader and 96 well micro plates and the next steps were followed: 150 µL of samples and standards were added to the well of the micro plate, then 15 ml of Na₂EDTA was added to each well and mixed accurately with a micro pipette. 60 ml of sodium salicylate and sodium nitroprusside solution were added and the mixtures were mixed, followed by 30 ml of hypochlorite reagent being mixed with the matrixes in all the wells. The 96 well micro plates were left at room temperature for 15 min to allow for the green colour to develop. After 15 minutes, the reading of the absorbance was taken at 667 nm on the plate-reader (Mulvaney, 1996).

The concentrations of NH₄⁺ – N were calculated using equation 4.10.

$$\text{NH}_4^+ - \text{N } \mu\text{g g}^{-1} = \frac{\text{NH}_4^+ - \text{N } \mu\text{g mL}^{-1} \times A}{\text{wt.}} \quad \text{Equation 4.10}$$

Where: A = the volume of extractant (25ml), and wt. = the weight of tailings (10g).

4.3.3.2.6 Determining total carbon and nitrogen

To evaluate the total carbon and nitrogen 0.1g of air-dried tailings were weighed in tin sheet and analysed for carbon / nitrogen ratio using CHN-2000 analyser, Leco Corp , St Joseph MI) (Abdalgader, 2014).

4.3.3.2.7 H₂O-extractable PO₄³⁻ – P determination in tailings extraction

10 g of air-dry sieved tailings were weighed in a 50 ml polyethylene centrifuge tube and 25 ml of dH₂O was added. The sample was shaken for 30 min at 250 rpm (SW2 Shaker Table, Edmund Buhler GmbH) and after that, the sample was centrifuged at 2000 rpm for 15min (Eppendorf AG centrifuge 5810 R.), then the suspension was filtered through Whatman® 42 filter paper, following which, the substrate was centrifuged for 15 min at 4000 rpm. Finally, the supernatant was filtered and stored at - 20°C in order to analyse PO₄³⁻- P calorimetrically on a Biotek [™]® Powerwave XS microplate spectrophotometer, using the molybdate method (Murphy and Riley, 1962). The concentrations of PO₄³⁻- P were calculated using equation 4.11.

$$PO_4^{3-} - P \mu g g^{-1} = \frac{PO_4^{3-} - P \mu g mL^{-1} \times A}{Wt.} \quad \text{Equation 4.11}$$

Where: A = the volume of extractant (25ml), and wt. = the weight of tailings (10g).

4.3.3.2.8 Pseudo-total levels of heavy metals analysis

The total concentration of heavy metals in mine tailings were estimated using the digestion by Aqua Regia matrix by following procedures:

One gram of sieved air-dried tailings placed in 50 ml polyethylene centrifuge tube then 10 ml of concentrated HNO₃ was added. The tube was put in water bath at 85°C and left for 1 hour. Then 5 ml of concentrated HNO₃ was added, the digestion was allowed to continue until there is no brown fumes emitted, then 10 ml of concentrated HCl was added and the sample was left to digest for half a hour. The mixture was left to cool and filtered through Whatman® 42 filter paper in 50 ml volumetric flask and the volume made up to 50 ml with ultra-pure water. The sample labelled and kept at 4°C to evaluate the concentrations of target metals by Atomic Absorption Spectrometer type Varian-220 FS.

The concentration of metals was calculated as follows (Lindsay and Norvell, 1978):

$$C_m \mu g g^{-1} = \frac{C_m \mu g mL^{-1} \text{ (from calibration curve)} \times V \times Df}{Wt.} \quad \text{Equation 4.12}$$

Where: C_m = The concentration of target metal, v = Volume of the volumetric flask, Df = dilution factor and $wt.$ = Weigh of mine tailings sample.

4.3.3.2.9 Metal fractions determination

The metal fractions were determined by conducting a modified sequential extraction protocol following the procedure shown in Figure 4.1. The protocol was used in this study developed from (Dold, 2003; Zimmerman and Weindorf, 2010; Favas *et al.*, 2011; Torres and Auleda, 2013). The water-soluble fraction is referred to as F1. The exchangeable metals that can dissolve in a slightly acidic medium is referred to as the exchangeable fraction (F2), also reducible fraction (F3) indicating the proportion of metals bound with Fe and Mn oxides, Oxidisable fraction (F4) refers to the percentage of metals associated with OM and sulphide in mine tailings. Last fraction is residual fraction (F5) that refers to the part of metals bound to the crystalline structure of tailings contents (Favas *et al.*, 2011).

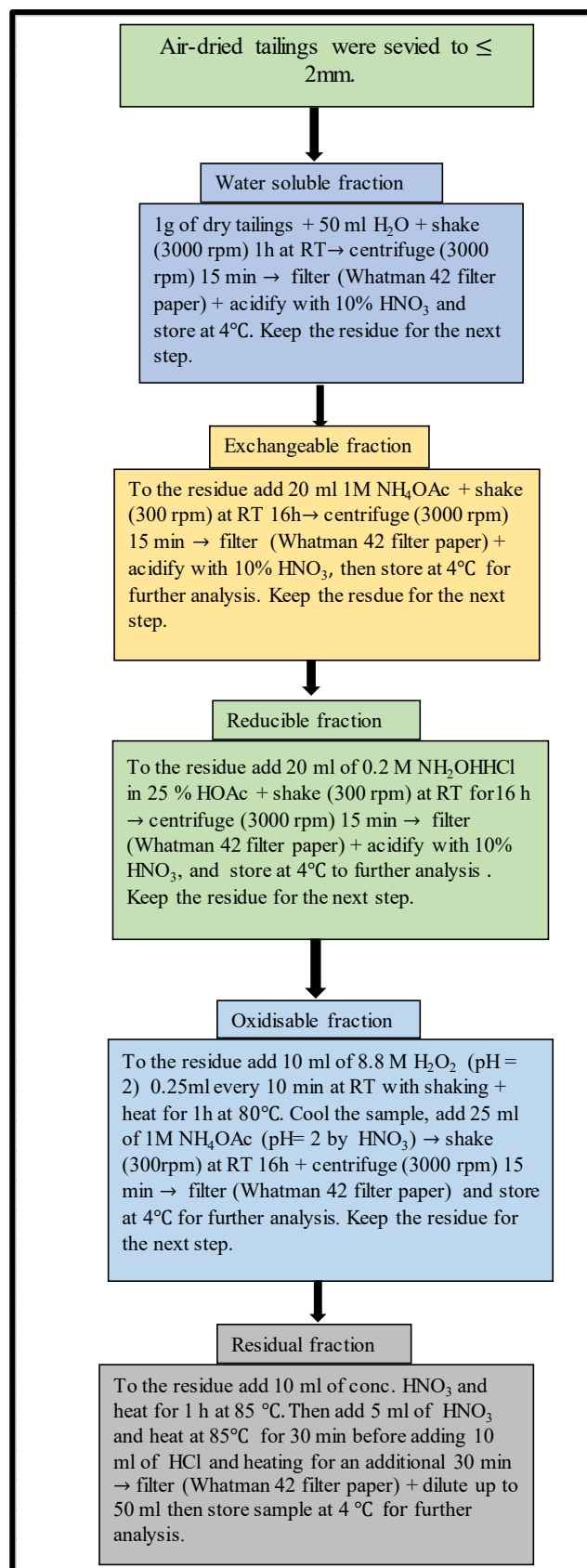


Figure 4. 1 Schematic representation of the sequential extraction protocol employed in this study.

4.3.4 Statistical analysis

The software SPSS v 22 for Windows was performed to analyse the obtained data, and SigmaPlot v 12.5 was used to present the data graphically. A Shapiro-Wilk test was used to check the normality of data. To analyse the mean difference between the parameters that have influence on the metal mobility (pH, CEC and OM) and moisture %, H₂O-extractable NO₃⁻ – N and C/N in three sites, Tukey's Honestly Significant difference (HSD) post hoc test was used, when the homogeneity of variances were not obtained, consequently Welch's ANOVA test were conducted. For non-parametric data (data did not normally distributed), Kruskal-Wallis and Mann-Whitney U tests were performed to differentiate between H₂O-extractable PO₄³⁻-P and EC. The results measured below the detection limit of AAS are reported in tables as BD (below detection limit), but were assumed to be half of AAS detection limit and treated as non-parametric data (USEPA, 2000), when statistical analysis was performed, presenting the data graphically and for discussion where necessary.

4.4 Results and Discussion

4.4.1 Tailings characteristics

Properties of mine tailings that have a significant effect on the behaviour of heavy metals investigated are shown in table 4.2.

The pH of the mine tailing samples at Parys copper mine (PCM) and Britannia copper mine (BCM) were highly acidic, measuring 3.29 ± 0.02 and 4.26 ± 0.04 , respectively. By contrast, samples at Pandora lead mine (PLM) were weakly acidic, measuring 6.28 ± 0.02 . There were statistically significant difference ($P < 0.05$) between the means of the pHs in all studied samples. According to Mendez and Maier (2008) the pH of ancient mine waste ranged between 2.0 and 9. The high acidity of PCM and BCM samples may be due to the existence of ore metal as sulphides such as CuS and ZnS ores (Vernon, 1996). Moreover, when these ores are exposed to both oxygen and water at the same time the oxidation process can affect the sulphide. As a result this reaction can produce 4 moles of H⁺ when 1 mole of sulphide is oxidised, leading to a decrease in pH according to the following equation (Dang *et al.*, 2002):

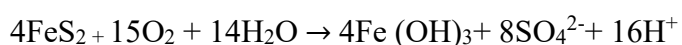


Table 4. 2 Selected properties of mine tailings (measurements presented as mean \pm SEM; n = 5).

Parameter / Location	(BCM) ^a	(PLM) ^b	(PCM) ^c
pH _{H2O}	4.26 \pm 0.04 ^a	6.28 \pm 0.02 ^b	3.29 \pm 0.02 ^c
EC μ S cm ⁻¹	67.21 \pm 5.82 ^A	346.80 \pm 42.43 ^B	425.40 \pm 44.18 ^B
Moisture %	18.89 \pm 1.96 ^a	40.89 \pm 1.47 ^b	17.34 \pm 0.88 ^a
O.M. %	6.11 \pm 0.83 ^{a, b}	7.92 \pm 0.39 ^b	4.91 \pm 0.24 ^a
CEC meq/ 100gtailings	17.89 \pm 0.49 ^a	45.26 \pm 2.25 ^b	13.58 \pm 0.57 ^a
H ₂ O-extractable PO ₄ ³⁻ -P μ g g ⁻¹	0.27 \pm 0.03 ^A	0.27 \pm 0.10 ^A	0.28 \pm 0.08 ^A
H ₂ O-extractable NO ₃ ⁻ N μ g g ⁻¹	2.62 \pm 1.11 ^a	0.46 \pm 0.08 ^a	0.22 \pm 0.01 ^a
H ₂ O-extractable NH ₄ ⁺ N μ g g ⁻¹	4.54 \pm 0.49 ^a	23.74 \pm 4.55 ^b	11.65 \pm 1.62 ^c
C/N	13.66 \pm 1.22 ^a	44.12 \pm 7.72 ^b	38.15 \pm 3.15 ^b
Clay %	9.22	5.13	11.38
Silt %	27.15	35.83	23.57
Sand %	63.63	57.82	62.34

^{a)} Britannia copper mine, ^{b)} Pandora Lead mine, ^{c)} Parys copper mine.

- Measurements with different lower case letter within the same row indicating significantly difference according to the Tukey's HSD test $P < 0.05$.

- Values labelled with different upper case letter within the same row indicating significant difference among marked parameters at $P < 0.05$ according to Kruskal-Wallis test and Mann-Whitney U tests.

The electrical conductivity (EC) of samples at BCM, PCM and PLM recorded 67.21 \pm 5.82 μ S cm⁻¹, 425.40 \pm 44.18 μ S cm⁻¹ and 346.80 \pm 42.18 μ Scm⁻¹, respectively and were significantly different ($P < 0.05$) for that of BCM and others. The moderately high conductivity of tailings at PCM and PLM could be due to the effects of low pH and a temperate maritime climate characterised by high rainfall which leads to flooding of the sites and the dissolution of the inorganic salts of several metals (Wilson and Pyatt, 2007). The percentage moisture was 40.89 \pm 1.47 % in PCM samples, more than double (higher significantly; $P < 0.05$) of the value recorded for the PCM and BCM samples which measured 18.89 \pm 1.96 % and 17.34 \pm 0.88 %, respectively. The classification of tailings was conducted using the USDA textural triangle. The results indicate that the mine tailings at the three locations were sandy loam.

Organic matter content was low for all three sites, measuring 4.91 \pm 0.24 %, 6.11 \pm 0.83 % and 7.92 \pm 0.39 % for PCM, BCM and PLM samples, respectively. These relatively low percentages of OM specifically in PCM may be caused by high levels of heavy metals in

investigated sites due to long history of mining activities minimising the input of OM (Epelde *et al.*, 2015).

The cation exchange capacity (CEC) of the mine tailings were 17.89 ± 0.49 meq /100 g_{tailings} and 13.58 ± 0.57 meq /100 g_{tailings} in the samples collected from BCM and, PCM, respectively, but for PLM were significantly ($P < 0.05$) higher by approximately 3-fold than others and recorded 45.26 ± 2.25 meq /100g_{tailings} .

The $\text{PO}_4^{3-}\text{-P}$ content of the samples were nearly similar at all three sites (no significant difference indicated; ($P > 0.05$)) with 0.27 ± 0.03 $\mu\text{g g}^{-1}$, 0.27 ± 0.10 $\mu\text{g g}^{-1}$ and 0.28 ± 0.08 $\mu\text{g g}^{-1}$ for BCM, PLM and PCM samples, respectively.

The $\text{NO}_3^{-}\text{-N}$ levels were not significantly different between all sites ($P > 0.05$) recording 2.62 ± 0.11 $\mu\text{g g}^{-1}$, 0.46 ± 0.08 $\mu\text{g g}^{-1}$ and 0.22 ± 0.01 $\mu\text{g g}^{-1}$ for BCM, PLM and PCM samples, respectively. Ammonium levels were 4.54 ± 0.49 $\mu\text{g g}^{-1}$ in the BCM samples, 23.74 ± 4.55 $\mu\text{g g}^{-1}$ in the PLM samples and 11.65 ± 1.62 $\mu\text{g g}^{-1}$ in the PCM samples. The concentration of $\text{NH}_4^{+}\text{-N}$ was found to be higher than the $\text{NO}_3^{-}\text{-N}$ concentration in all three locations. This is likely to be due to the fact that $\text{NH}_4^{+}\text{-N}$ tends to accumulate in these tailings due to change the nitrification rate leads to declining the levels of nitrate in the samples at all sites (Ge *et al.*, 2010). In addition, Pardo *et al.*, (2014) reported that, the reduction of total nitrogen in the pore water of treated mine tailings with organic material may due to the leaching of $\text{NO}_3^{-}\text{-N}$ to deep layers of mine wastes, but $\text{NH}_4^{+}\text{-N}$ can be adsorbed through exchangeable process on the surface of tailings and OM . Low levels of plant available forms of N and P can be a direct result of low plant and microbial biomass. In systems that receive no additional inputs in the form of fertiliser, the concentrations of N and P are directly linked to their cycling from, plant and microbial residues. High levels of Cu, Cd, Zn, Pb and extremely acid conditions in BCM and PCM mines will restrict plant growth and microbial activity thus resulting in soils with low levels plant available forms of N and P (Wong, 2003; Bian *et al.*, 2015).

4.4.2 Heavy metal analysis

4.4.2.1 Pseudo-total levels of heavy metal in mine tailings

Table 4. 3 Total concentration of target metals (concentrations in $\mu\text{g g}^{-1}$ and data presented as mean \pm SEM; n = 5).

Location / Element	Cu	Zn	Cd	Pb
Britannia mine (BCM)	4255 \pm 506	115.44 \pm 6.76	0.11 \pm 0.03	243.2 \pm 36.51
Pandora mine (PLM)	198 \pm 10.70	49444 \pm 1176	95.53 \pm 5.06	109190 \pm 5609
Parys mine (PCM)	585 \pm 63.10	2407 \pm 58.67	5.91 \pm 0.34	2913 \pm 253

The total concentration of studied elements is listed in table 4.3. The concentration of Cu was higher in BCM (4255 \pm 506 $\mu\text{g g}^{-1}$) than the other locations. The levels of Cu in the samples collected from PCM were much lower at 585 \pm 63.10 $\mu\text{g g}^{-1}$ even though this mine was used as a source of Cu. This may be because of its highly acidic tailings leading to Cu being present in its soluble form $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ (Baker, 1990). This complex may then be washed from the tailings during rainfall events. This is particularly likely given that the samples were collected in March 2015 after a period of heavy rain. The lowest levels of Cu (198 \pm 10.70 $\mu\text{g g}^{-1}$) was found in the sample collected from the PLM site. The concentration of Cu in PCM tailings was lower than the results obtained by Khan and Jones (2008) and Farrell *et al.* (2010) for Cu in the same site. The difference may be related to the area of PCM that covered with tailings being large (>200 ha) and the sampling for current study was from a different site to previous studies were sampled or / and the sampling times differed seasonally (Hanauer *et al.*, 2011). The variability of pseudo-total levels of heavy metals in industrial sites is expected due to the heterogeneity of wastes disposal (Herreweghe *et al.*, 2003; Epelde *et al.*, 2015).The concentration of zinc varied substantially between sites. The concentration recorded at the PLM site was by far the highest (49444 \pm 1176 $\mu\text{g g}^{-1}$).

Cadmium concentration was highest at the PLM site. This may be due to the association of Cd with Zn ore in nature, but with very low percentage of Cd as reported by Alloway, (1990a) and Davies, (1987), and wherever the concentration of Zn is high, the Cd must be measured, their presence together may due to the similarity of chemical behaviour of both metals (Kossoff *et al.*, 2014). The results obtained in this study agree with this observation, since the levels of Cd in this study tended to correlate with Zn levels.

The levels of Pb was very high in samples at the PLM site (109190 \pm 5609 $\mu\text{g g}^{-1}$) compared with the PCM and BCM samples. This was expected as Pb (and Zn) was actively

extracted from this site until 1931. Mendez and Maier (2008) reported that in old mine areas, levels of Zn, As, Pb and Cu may be higher than 50,000 $\mu\text{g g}^{-1}$.

Heavy metals are classified as hazardous elements when they are found at levels higher than those set out by the World Health Organisation (WHO) (Tembo *et al.*, 2006). The acceptable limit for the analysed metals in soils are 35 $\mu\text{g g}^{-1}$ for Pb, 90 $\mu\text{g g}^{-1}$ for Zn, 0.35 $\mu\text{g g}^{-1}$ for Cd and 30 $\mu\text{g g}^{-1}$ for Cu. Accordingly, a comparison between these values and the results obtained in the current study suggests that the levels of all metals (except Cd in BCM) exceeded the acceptable levels stipulated by the WHO. As a result, these tailings can be classified as a terrestrial environment heavily polluted by heavy metals. More attention must be paid to the issue of finding suitable economic and environmentally friendly approaches to prevent the transfer of heavy metals from mine tailings to surrounding environments such as groundwater and croplands.

4.4.2.2 Levels of pollution

To evaluate the levels of pollution in urban soil the geo-accumulation index (I_{geo}) has been widely used. This value can be calculated using the following equation (Zhan *et al.*, 2014; García-Giménez and Jiménez-Ballesta, 2017):

$$I_{\text{geo}} = \log_2 (C_n / 1.5 B_n)$$

Where C_n is the concentration of target metal in the studied tailings, and B_n is the background level of the target elements in soils that are 35 $\mu\text{g g}^{-1}$ for Pb, 90 $\mu\text{g g}^{-1}$ for Zn, 0.35 $\mu\text{g g}^{-1}$ for Cd and 30 $\mu\text{g g}^{-1}$ for Cu (Tembo *et al.*, 2006).

The I_{geo} value has been divided in to 7 categories signifying the degree of soil pollution. $I_{\text{geo}} < 0$: no pollution, 1-0: polluted but not moderate, 1-2: moderate, 2-3: moderate to heavy, 3-4: heavy, 4-5: heavy to extreme, >5 : extreme pollution (JI *et al.*, 2008; Wei *et al.*, 2009).

Table 4. 4 I_{geo} values of investigated metals in studied sites.

Location	Cu	Zn	Cd	Pb
Britannia mine	6.56	-0.23	-2.25	2.21
Pandora mine	2.14	8.52	7.51	11.02
Parys mine	3.70	4.16	3.49	5.79

Table 4.4 shows the I_{geo} values for single elements in different sampling locations. The geo-accumulation index (I_{geo}) indicated that the tailings of BCM were extremely polluted by Cu and moderately by Pb. However, there was no pollution from Cd and Zn. In the PLM samples, extreme pollution was recorded by Pb, Cd and Zn as I_{geo} at 11.02, 7.51 and 8.52 respectively and the moderate polluted by Cu ($I_{geo} = 2.14$). The mine tailings at PCM can be classified as heavily contaminated tailings by Cu and Cd, as having heavy to extreme pollution by Zn and as extremely polluted by Pb with I_{geo} values of 3.70, 3.49, 4.16 and 5.79, respectively.

Overall, the tailings collected from the upper 20 cm at the mine sites can be described as being polluted with Cu, Zn, Cd and Pb except for the mine tailings at BCM, which did not record any pollution for Zn and Cd.

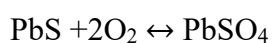
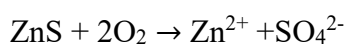
4.4.3 Metal fractionation in mine tailings

The fraction levels of investigated elements were calculated in concentrations and illustrated in tables 4.5 - 4.7, and the results in these tables was used to calculate metal percentages (Figures 4.2 - 4.5) from the summation of the sequential extraction steps for individual metal. The distribution of target metals in different geochemical fractions in BCM and PCM tailings are generally similar, with only slight differences in the proportions of the investigated fractions in the samples at these two sites where the majority of the metals associated with soil minerals occur in the residual fraction (F5). Similarly, Kerolli–Mustafa *et al.* (2015) found that the dominant geochemical form of heavy metals in mine tailings is the residual fraction, which gives heavy metals high stability and a resistance to leaching by rain water into ground water. However, the water soluble, exchangeable, reducible and oxidisable fractions are recognised as being more mobile fractions when a change in environmental conditions occurs such as a decrease in pH (Rodríguez *et al.*, 2009). The most mobile fractions had low proportions of metals associated with them in samples at both PCM and BCM. This may be due to several factors as Conesa *et al.* (2006) noted including low pH, high sand content and relatively low CEC which may cause the transfer of free metal ions through the soil profile to ground water and with runoff to surface water bodies. The high acidity, significant proportion of sand and relatively low CEC recorded in the samples at BCM and PCM may therefore have facilitated the leaching of the potentially mobile geochemical forms of the heavy metals resulting in their transfer to nearby environments via runoff. This is likely to explain the low levels of heavy metals in potentially mobile fractions and specifically the absence of Cu and Cd in the water-soluble fraction in samples at PCM and BCM. Nevertheless, there is

noticeable variation in the distribution of target metals between the investigated fractions in the case of PLM samples. The exchangeable fraction is the most abundant fraction for studied elements in PLM tailings. This is the result of substitution on exchangeable sites due to the high cation exchange capacity of tailings at this site. The difference in the distribution of studied elements among sites may be explained by differences in pH. pH at the Pandora site was substantially higher (6.28) than at the Parys (3.29) and Britannia (4.26) sites. The difference in pH may be explained the difference in the geochemistry of investigated metals in current study as supported by Arenas-Lago *et al.* (2014) who observed that low pH values in mine tailings can control the distribution and retention of heavy metals between different fractions.

It is worth stating that, as illustrates in figure 2, the proportion of Cu is the same in the water-soluble fraction (F1) in all studied tailings (below the limit of AAs detection). The very low presence of Cu in F1 may be linked to the acidic conditions leading to a dissolution and leaching of soluble organic complexes from the soil (Yu *et al.*, 2004). Also, the percentage of Pb associated with the F3 fraction was almost the same at the three studied sites. The stable proportions of Pb bound with Fe and Mn oxides in all samples in the current study agree with those reported by Favas *et al.* (2011). They found that the Fe and Mn fraction are an important sink for Pb and can control its mobility in mine tailings.

The low proportions (except for Cu and Pb in the PLM samples) of studied metals associated with the OM and sulphide fraction (F4) may be attributed to the oxidation of metal sulphide ore leading to the release of heavy metals bound to this fraction, as shown by the following equation (Min *et al.*, 2013):



In general, the results of the metal fractionation in samples at PCM and BCM obtained in this study are in agreement with the results obtained by Arenas-Lago *et al.* (2014). They concluded that a major proportion of Zn, Cu and Pd was associated with the residual fraction in copper mine tailings. Also, Armienta *et al.* (2016) concluded that the lowest proportions (3.6% of Cu, 3.6% of Pb and 4.1% of Zn) were detected in the soluble fraction in soils collected from sites surrounding various ancient mines in Zimapan, Mexico, but the residual fraction had the highest percentage of these metals. In addition, the high levels of investigated metals in the exchangeable fraction in samples at PLM (Table 4.6) indicates that this geochemical fraction

plays an important role in the behaviour of heavy metals in these tailings. However, the fraction associated with crystalline material (F5) seems to be the main fraction influencing the heavy metal distribution in BCM and PCM. The high levels of heavy metals with oxidation conditions, combined with highly acidic conditions and heavy rain in BCM and PCM may have led to a change in the geochemical form of heavy metals being potentially available and leaching from tailings into the surrounding environments (Min *et al.*, 2013). Cation exchange capacity plays an important role in the distribution of heavy metals in different sites. CEC in the BCM and PCM tailings was approximately 3-fold lower than that in the PLM wastes. Accordingly, the exchangeable fraction in the PLM site retained significant levels of heavy metals, a result supported by Arenas-Lago *et al.* (2014) who reported that soils with low CEC contain lower levels of heavy metals associated with the exchangeable fraction.

According to Gabarrón *et al.* (2017), in industrial sites when most of the Zn and Pb is present in the residual fraction, this indicates pollution resulting from geological source, whereas pollution caused by human activity is associated with metals with a high proportions of potentially mobile fractions. Therefore, the pollution in PLM seems to be related to mining operations resulting from the remains of extracted metals process, but at PCM and BCM sites may be linked to mineralization of metal ores as the dominated material seems to be as a raw material before metal extraction procedure were operated. Similarly, Bird *et al.* (2005) reported that high proportions of heavy metal supplied to the sediments and Pb in soil (Dean *et al.*, 2017) by non-indigenous sources tended to associate with most mobile geochemical fractions (exchangeable and water soluble fractions). However, the high percentage of metals in residual fraction of sediment and soils may reflects the mineralisation of metal ores and parent material.

Table 4. 5 Distribution of metal species in Britannia mine tailings ($\mu\text{g g}^{-1}$, measurements presented as mean \pm SEM; n = 5).

	Cu		Zn		Cd		Pb	
Fraction	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range
F1	BD		1.31 \pm 0.46	0.53 – 3.17	BD		15.10 \pm 1.98	11.67 – 22.83
F2	58.18 \pm 5.55	41.98 – 68.98	2.18 \pm 0.44	1.12 – 3.40	BD		BD	
F3	71.64 \pm 8.70	52.29 – 93.20	BD		BD		49.50 \pm 26.11	4.67 – 150
F4	449 \pm 42.68	340 - 561	BD		BD		39.27 \pm 11.35	11.00 – 65.33
F5	3261 \pm 344	2468 - 4433	125.53 \pm 9.54	98.87 – 154.21	0.30 \pm 0.09	BD – 0.47	252 \pm 27.93	172 - 316
SF	3840 \pm 376		130 \pm 9.41		0.30		356 \pm 51.8	

F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF= (F1+F2+F3+F5+F5), and BD = below the limit of the detection.

Table 4. 6 Distribution of metal species in Pandora mine tailings ($\mu\text{g g}^{-1}$, measurements presented as mean \pm SEM; n = 5).

Fraction	Cu		Zn		Cd		Pb	
	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range
F1	BD		130 \pm 28.80	61.10 - 203	1.92 \pm 0.21	1.27 – 2.45	21.97 \pm 1.22	18 – 25.33
F2	107.65 \pm 10.23	74.27 – 135.78	17125 \pm 1167	13190 - 19806	37.14 \pm 2.03	31.24 – 40.80	85979 \pm 6126	73253 - 103867
F3	7.78 \pm 2.94	BD – 18.12	22559 \pm 5090	2461 - 29909	13.14 \pm 1.96	8.22 – 18.80	18893 \pm 2747	14067- 29133
F4	40.71 \pm 6.79	20.58 – 57.08	2183 \pm 442	468 - 2787	0.18 \pm 0.12	BD - 0.64	72293 \pm 14159	35200 - 112333
F5	26.28 \pm 4.87	0.83 – 50.43	9555 \pm 907	6864 - 11502	31.0 \pm 2.68	25.00 – 39.55	7645 \pm 1714	3697 - 13590
SF	183 \pm 15.24		51098 \pm 6147		83.38 \pm 4.60		184832 \pm 18890	

F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF= (F1+F2+F3+F5+F5), and BD = below the limit of the detection.

Table 4. 7 Distribution of metal species in Parys mine tailings ($\mu\text{g g}^{-1}$, measurements presented as mean \pm SEM; n = 5).

	Cu		Zn		Cd		Pb	
Fraction	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range	Mean \pm S.E.M	Range
F1	BD		2.57 ± 0.58	1.46 – 4.44	BD		14.70 ± 0.94	12.67 – 18.00
F2	BD		2.65 ± 1.42	BD – 7.39	BD		26.96 ± 10.99	3.47 – 68.07
F3	2.77 ± 0.72	1.11 – 4.73	2.11 ± 0.98	BD - 5.26	BD		499 ± 33.02	387 - 588
F4	62.68 ± 7.07	44.97 – 88.27	95.67 ± 11.03	66.18 - 118	BD		735.5 ± 128	309 - 1037
F5	341 ± 26.54	257 - 425	1941 ± 126	1672 -2308	4.27 ± 0.58	3.10 – 6.05	2468 ± 123	2115 - 2875
SF	407 ± 28.21		2078 ± 122		4.27		3744 ± 228	

F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF= (F1+F2+F3+F5+F5), and BD = below the limit of the detection.

4.4.3.1 Copper speciation

The Cu proportions in the studied tailings at all sites was plotted on figure 4.2. The distribution of copper shows a very similar pattern for the BCM and PCM samples. Overall, the occurrence of Cu in different fractions followed the order $F5 > F4 > F3 > F2 > F1$ in both these sites. $85 \pm 0.93\%$ of Cu occurred predominantly in the residual fraction ($3261 \pm 344 \mu\text{g g}^{-1}$) and $84 \pm 1.69\%$ ($341 \pm 26.54 \mu\text{g g}^{-1}$) in BCM and PCM, respectively, this is associated with crystalline silicate structures in the tailings (Rodríguez *et al.*, 2009; Favas *et al.*, 2011; Liao *et al.*, 2016). Cu appears to have an affinity to OM and the sulphide fraction rather than the reducible, exchangeable (except in PLM) and water fractions in all the tailings investigated (tables 7, 8 and 9). The oxidisable fraction recorded $16 \pm 1.75\%$ ($62.68 \pm 7.07 \mu\text{g g}^{-1}$) in samples at PCM and $12 \pm 0.77\%$ ($449 \pm 42.68 \mu\text{g g}^{-1}$) in tailings at BCM. This may be due to the presence of OM and sulphide ores. Kabirinejad *et al.* (2014) reported that Cu has a high tendency to bond with OM, as well as with sulphides which exist as galena (PbS) and sphalerite (ZnS) that are considered a significant ores of Pb and Zn along with lower existence of chalcopyrite CuFeS_2 (Davies, 1987). There are two possible explanations for Cu being below detection limits in the exchangeable fraction in the PCM and BCM samples. Firstly, this may be due to the occurrence of other exchangeable ions, which can occupy the exchangeable sites faster than Cu or replace it (Alloway, 1990c). Secondly, the low pH values recorded in the tailings at the BCM and PCM may cause metals to dissolve and then be transferred to the surrounding area (Rodríguez *et al.*, 2009). The Cu distribution results in the PCM and BCM samples are in agreement with those obtained by Favas *et al.* (2011) who reported that Cu appears to preferentially bind to primary and secondary soil minerals in the residual fraction. Similarly, Zhou *et al.* (2007) found that the majority of heavy metals are associated with the residual fraction in mine soils in China. There did however appear to be a difference in the behaviour of Cu at the PLM samples where Cu was distributed in the order $F2 > F4 > F5 > F3 > F1$. Additionally, almost all of the Cu was found in the exchangeable, oxidisable and residual fractions ($60 \pm 6.90\%$; $107.65 \pm 10.23 \mu\text{g g}^{-1}$), $21.84 \pm 2.38\%$ ($40.71 \pm 6.79 \mu\text{g g}^{-1}$) and $13.79 \pm 4.18\%$ ($26.28 \pm 4.87 \mu\text{g g}^{-1}$), respectively). Thus, in this site Cu seems to be more available than in the other sites. This may be because the CEC in the Pandora tailings was the highest ($45.26 \pm 1.24 \text{ meq}/100\text{g}_{\text{tailings}}$) leads to metal retention in exchangeable fraction.

A significant proportion of Cu was associated with the oxidisable fraction in the PLM samples and was higher than Cu in this fraction at the other sites. This may be due to the ability of Cu to bond with OM at high pH levels at weak acidic mediums (Park *et al.*, 2011). However, the proportions of Cu associated with the residual fraction were nearly 6 times lower in the PLM samples compared to the proportions bound with the same fraction in BCM and PCM samples. Very low levels of Cu bound with Fe and Mn oxides can be attributed to the acidity of the tailings at all locations. According to Yu *et al.* (2004); Contin *et al.* (2007) and Seshadri *et al.* (2016), high concentrations of Cu under acidic and/or reduction conditions can lead Fe-Mn oxides to dissolve resulting in the release of heavy metals into tailings solution. Likewise, the existence of OM in acidic soil can deliver organic ligands that can form soluble compounds leading to a rise in the availability of heavy metals in the soil (Arenas-Lago *et al.*, 2014). This may explain the lower levels of Cu and other metals in the oxidisable fraction observed in the current study, excluding Pb in the PLM samples.

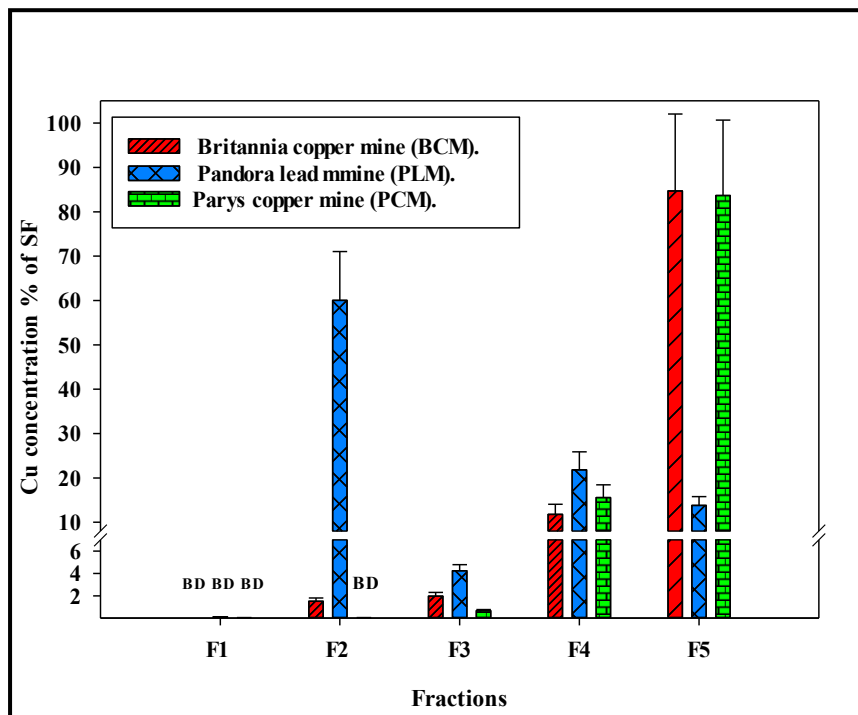


Figure 4. 2 The distribution of Cu in different tailing fractions in studied sites. F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF (Summation of Cu concentration in all fractions) = F1+F2+F3+F4+F5, BD indicates values below detection limits; bars represent mean \pm SEM (n = 5).

4.4.3.2 Lead speciation

Pb concentration percentage in studied fractions in samples from three studied sites is presented in figure 4.3. The behaviour of lead in the mine tailings appears to be similar in the samples at BCM and PCM. Our data show that the residual fraction is the dominant fraction at both sites, measuring $72.16 \pm 4.24\%$ ($252 \pm 27.93 \mu\text{g g}^{-1}$) and $66.34 \pm 2.59\%$ ($2468 \pm 123 \mu\text{g g}^{-1}$), respectively. The main ore in these mines is sulphides; even though the concentration of Pb in the oxidisable fraction was relatively low, this can be attributed to the weathering process and environmental conditions such as a low pH leading to the degradation of OM and sulphides dissolution (Favas *et al.*, 2011). The distribution of Pb in the tailings at the BCM and PCM mines agrees with data reported by Favas *et al.* (2011); Sungur *et al.* (2014) and Lu *et al.* (2015) who found that a high proportion of Pb was found in the residual fraction. In mine tailings sample collected from PCM Tandy *et al.* (2009) found that residual fraction retained 49% of total Pb and was the highest among other fractions. However, when the tailings are weakly acidic, as in the samples at PLM, moderate levels ($47.41 \pm 2.73\%$; $85979 \pm 6126 \mu\text{g g}^{-1}$) of total Pb were found in the exchangeable fraction, and $37.68 \pm 4.20\%$ ($72293 \pm 14159 \mu\text{g g}^{-1}$) of total Pb was associated with the OM and sulphide fraction (F4). The moderate levels of Pb found in the F4 fraction can be explained by the ability of OM to retain heavy metals due to the negative charges which occur when the H^+ ion is released from the surface organic functional groups in a weakly acidic medium such as was present in the Pandora samples ($\text{pH} = 6.28$), in contrast with the other sites. The weak acidity of the Pandora tailings leads to increased attraction of metal cations to organic acid anions (Park *et al.*, 2011; Rinklebe and Shaheen, 2014). The weak pH medium may have caused the oxidation of sulphides with the consequence of forming secondary Pb compounds with low solubility in soil solution (Anju and Banerjee, 2010), which may explain the lower levels of Pb in water-soluble fraction.

The results indicated that Pb in tailings at the PLM is likely to be more available than Pb in the tailings at PCM and BCM due to the moderate occurrence ($47.41 \pm 2.73\%$) of Pb in the exchangeable fraction. Although, the concentration of Pb in the Fe and Mn oxides fraction (F3) differed between the sampled sites. However, the percentage of Pb in the F3 fraction was very similar between the sites, measuring $12.54 \pm 4.71\%$, $13.36 \pm 0.53\%$ and $10.68 \pm 1.73\%$ for BCM, PCM and PLM, respectively. This result may be attributed to the affinity of Pb to Fe and Mn oxides (Yu *et al.*, 2004; Komárek *et al.*, 2008).

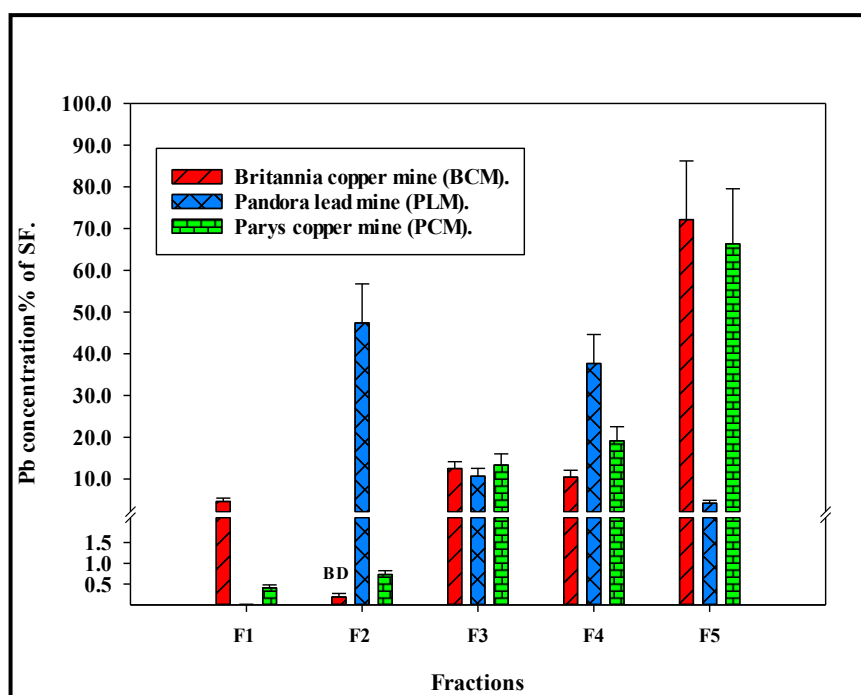


Figure 4. 3 The distribution of Pb in different tailing fractions in studied sites. F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF (Summation of Cu concentration in all fractions) = F1+F2+F3+F5+F5, BD indicates values below detection limits; bars represent mean \pm SEM (n = 5) in different tailings fractions in studied sites.

4.4.3.3 Cadmium speciation

Figure 4.4 shows Cd percentage in different geochemical fractions in samples from all investigated sites. The behaviour of Cd in mine tailings was the same for the BCM and PCM samples where most of Cd was associated with silicate and soil minerals in the residual fraction. This may be attributed to the weak adsorption of Cd to OM, clay and mineral oxides at pH's lower than 6. The lower pH led to dissolve Cd and increase its mobility (Favas *et al.*, 2011). Thus, soluble Cd is readily transferred by rainwater to deep soils and aquatic environments leading to it being present in mineral lattices in BCM and PCM tailings. The oxidation conditions in the surface of tailings can protect Cd from co-precipitation and/or adsorption by Fe (hydr)oxides due to the formation of dissolved organic complexes as a result of the abundance of OM in soils (Rinklebe and Shaheen, 2014). Similarly, it was noted by Alloway (1990a) and Cappuyns *et al.* (2007) that at lower pH Cd appears to be more weakly bound with humic acid than Pb and Cu. This may explain the absence of Cd in the organic fraction of mine tailings collected from BCM and PCM (Arenas-Lago *et al.*, 2014; Rinklebe and Shaheen, 2014). By contrast, the concentration of Cd in the PLM was distributed in the following order;

F2>F5>F3>F1>F4. As a result, most of the Cd was distributed between three main fractions: the exchangeable fraction ($44.82 \pm 2.57\%$), the residual fraction ($37.17 \pm 2.36\%$) and the reducible fraction ($15.58 \pm 1.66\%$). Additionally, Cd was preferentially bound to exchangeable sites ($44.82 \pm 2.57\%$, $37.14 \pm 2.03 \mu\text{g g}^{-1}$). This may due to the similarity between the ionic radius of Cd^{2+} and Ca^{2+} , which makes the replacement of Ca^{2+} by Cd^{2+} easier in ion exchange sites (Lu *et al.*, 2015) since the CEC of Pandora tailings is high with high proportion of Cd associated with it, consequently Cd is available and easily mobilised, especially in acidic soil. At lower pH's Cd seems to be weakly adsorbed to the surface of OM and Fe-Mn oxides (Favas *et al.*, 2011; Arenas-Lago *et al.*, 2014). This may explain the lower proportion of Cd associated with the Fe and Mn oxides and oxidisable fraction. Likewise, the lower concentration or absence of Cd in all samples in the F3 and F4 fractions may attributed to the same reason. The non-specifically adsorbed (exchangeable) fraction of metal is easy to extract from the site where the ions of the element are substituted due to the weak electron interaction of the metal on the solid surface of tailings (Cappuyns *et al.*, 2007). Therefore, Cd in Pandora tailings seems to be more available than Cd in the other sites, because of its moderate presence ($44.82 \pm 2.57\%$) in the exchangeable fraction in PLM samples.

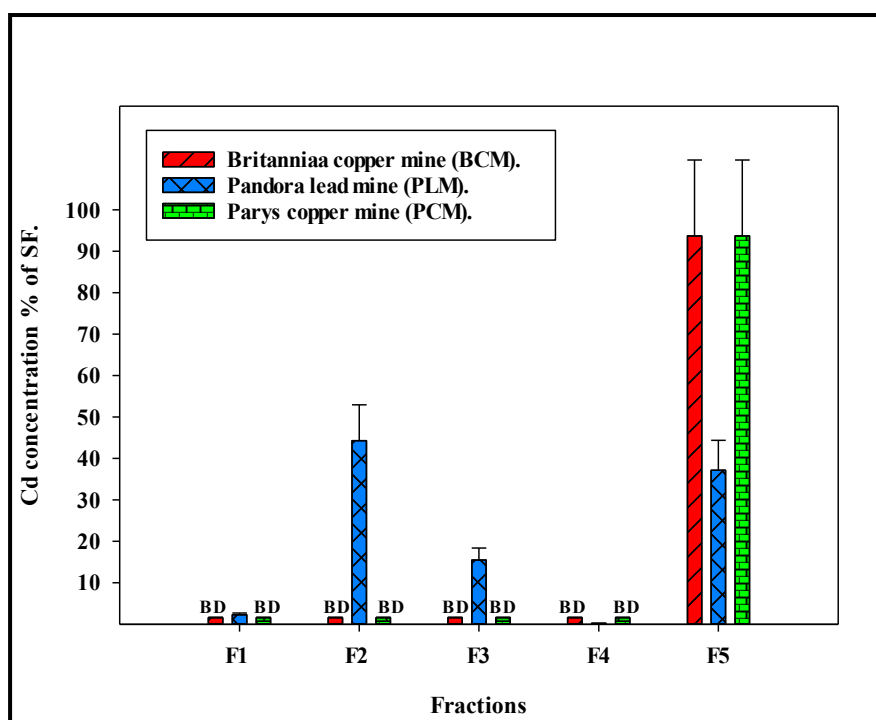
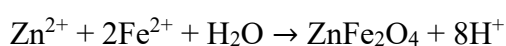


Figure 4. 4 The distribution of Cd in different tailing fractions in studied sites. F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF (Summation of Cu concentration in all fractions) = F1+F2+F3+F5+F5, BD indicates values below detection limits; bars represent mean \pm SEM (n = 5) in different tailing fractions in studied sites.

4.4.3.4 Zinc speciation

The concentration percentage of Zn in the tailings fraction from the studied sites is plotted on figure 4.5. The distribution of Zn in samples at BCM and PCM was almost the same. Zn was found in very low quantities in the exchangeable and water soluble fractions in both locations. The presence of Zn in different geochemical forms followed the order $F5 > F2 > F1 > F3 = F4$ in samples at the BCM site, but at the PCM samples followed the sequence $F5 > F4 > F2 > F1 > F3$. In addition, in both sites the dominant fraction was the residual form, due to the high presence of Zn in primary and secondary soil material. The results obtained are similar to those reported by Arenas-Lago *et al.* (2014) who found that most Zn was associated with the residual fraction in copper mine tailings. Similarly, Favas *et al.* (2011) found that a high proportion of Zn was associated with the residual fraction. The proportion of Zn in the oxidisable fraction was lower than expected, possibly due to high rates of sulphide oxidation at these sites resulting in a lowering of the pH and a consequent release of the metal associated with the oxidisable fraction (Rodríguez *et al.*, 2009). Similarly, Tandy *et al.* (2009) also found that 78% of Zn associated with residual fraction in mine spoil collected from Parys Mountain. The behaviour of Zn in the PLM samples was distinct with the exception of the proportion of Zn associated with the oxidisable fraction which was very similar to that measured at the PCM. Zn in PLM tailings showed highest affinity to the Fe-Mn oxides ($40.67 \pm 8.05\%$, $22559 \pm 5090 \mu\text{g g}^{-1}$). Arenas-Lago *et al.* (2014); Rodríguez *et al.* (2009) and Favas *et al.* (2011) stated that adsorption and co-precipitation are important mechanisms by which Fe-Mn oxides control the mobility and availability of Zn in soils and tailings, as illustrated by the following reaction:



Dang *et al.* (2002) in their study attributed the high proportion of heavy metals associated with the Fe-Mn oxides fraction to the complexation of released metals resulting from the decomposition of sulphide ore by Fe-Mn oxides.

Moderately high levels of Zn were associated with the exchangeable fraction ($35.81 \pm 5.52\%$, $17125 \pm 1167 \mu\text{g g}^{-1}$) in PLM, suggesting that Zn is available and less stable in the Pandora tailings compared with Zn in Britannia and Parys tailings. On the other hand, the proportion of Zn in the residual fraction in the Pandora samples was nearly 5-fold lower than its levels in the same fraction in the samples at other two locations.

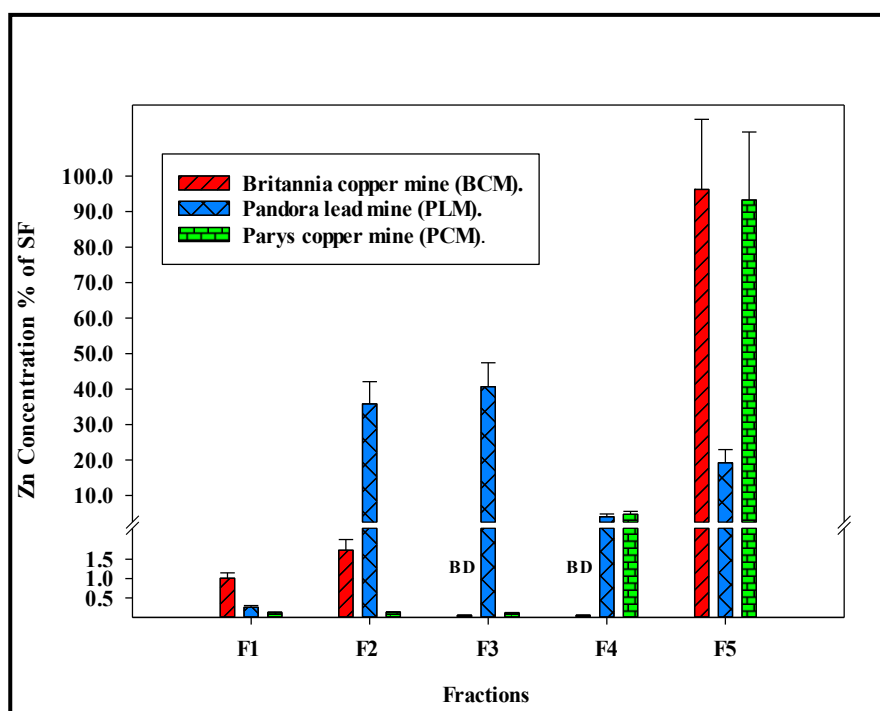


Figure 4. 5 The distribution of Zn in different tailing fractions in studied sites. F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF (Summation of Cu concentration in all fractions) = F1+F2+F3+F4+F5, BD indicates values below detection limits; bars represent mean \pm SEM (n = 5).in different tailing fractions in studied sites.

4.4.4 Recovery

To evaluate the efficiency of the protocol in extracting the target metals, the percentage recovery was calculated using the following equation (Cappuyns *et al.*, 2007; Rinklebe and Shaheen, 2014):

$$\text{Recovery \%} = \frac{\text{Sumation of the concentrations of metal in the 5 sequential extraction steps (SF)}}{\text{Total concentration of element}} \times 100$$

Table 4. 8 Recovery % of Cu, Zn, Cd and Pb determined in mine tailings from Britannia, Pandora and Parys mines.

Location	Cu %	Zn %	Cd %	Pb %
Britannia mine (BCM)	98 \pm 13.90	115 \pm 8.60	78 \pm 4.41	151 \pm 14
Pandora mine (PLM)	93 \pm 8.35	104 \pm 11.57	88 \pm 2.38	169 \pm 11.30
Parys mine (PCM)	72 \pm 4.41	87 \pm 4.07	78 \pm 8.04	138 \pm 14.69

The results listed in table 4.8 show the recovery of investigated metals. Cu percentage recovery ranged from 72 \pm 4.41% to 98 \pm 13.90%. The percentage recovery of Zn ranged from 87 \pm 4.07% to 115 \pm 8.60% and that of Cd ranged from 78 \pm 8.04% to 88 \pm 2.38%. The lower

proportion recovery of Cu, Zn and Cd in the PCM samples may be due to the loss of some of the sample during the experiment, or possibly the presence of some particles of gravels that could not be removed by the sieving process as their diameter was less than 2 mm. The percentage recoveries for Pb were high in all investigated samples, but the highest ($169 \pm 11.30 \%$) was in Pandora samples. This may be explained by the high concentration of lead in the samples ($109190 \pm 5609 \mu\text{g g}^{-1}$) and the five extraction stages which may have led to higher levels of Pb extracted compared with the single extraction by acid digestion used to measure the total concentration of heavy metals (Herreweghe *et al.*, 2003). The high percentage recoveries for Zn in the Britannia and Pandora samples, and Pb in all sites establishing that the protocol was used to extract metals has good efficiency (Wen *et al.*, 2016). The variability of extraction percentage recoveries in the samples collected from these highly polluted sites may also be attributed to the heterogeneity of the subsamples (Herreweghe *et al.*, 2003).

4.4.5 Heavy metal mobility

The mobility of heavy metals in soils can be estimated by calculating the individual concentration factor (C_f). The C_f was calculated by using the following equation:

$$C_f = \frac{F1+F2+F3+F4}{F5}$$

This factor indicates the degree of heavy metal pollution in relation to retention time with low C_f indicating high retention time and low environmental risk (Ahmadipour *et al.*, 2014).

Table 4. 9 The concentration factor for studied elements.

	C_f			
	Cu	Zn	Cd	Pb
Britannia mine (BCM)	0.18 ± 0.01	0.03 ± 0.01	0.95 ± 0.43	0.41 ± 0.09
Pandora mine (PLM)	9.92 ± 4.29	4.32 ± 0.45	1.73 ± 0.17	28.20 ± 7.29
Parys mine (PCM)	0.20 ± 0.03	0.05 ± 0.01	0.06 ± 0.01	0.52 ± 0.06

As table 4.9 illustrates C_f is very low for all investigated metals at the BCM and PCM sites, but higher in the PLM samples. This indicates that the heavy metals at the Britannia and Parys sites have long retention time in mine tailings. However, heavy metals in the Pandora site pose a high risk to the environment. Indeed, as proposed by Rodríguez *et al.* (2009) low concentrations of heavy metals in the mobile fractions and high concentrations of total

elements, as was observed in PCM and BCM, may be pose significant risk to the surrounding ecosystem under changing environmental conditions.

4.4.6 Evaluating the potential environmental risk

Mine samples can be categorised in terms of threat by following the Risk Assessment Code (RAC), which has been used by several researchers to categorise the degree of pollution from heavy metals (Favas *et al.*, 2011). According to Rodríguez *et al.* (2009) the ability of an element to be transported and reach the food chain is dependent on the strength of the bond between the element and the different soil solid phases. These factors are incorporated into the RAC when calculating the threat of heavy metal pollution. In the present study the RAC was calculated by taking into account the water-soluble and exchangeable fraction as a percentage of the total concentration of heavy metals in all fractions. The classification of investigated areas to estimate the pollution risk is carried out using the following rules; if the water-soluble and exchangeable fractions were less than 1 % of the total concentration, the RAC indicates no risk. However, when these fractions ranged from 1 to 11 % then a low risk is assumed. A medium risk is considered to occur when these values range between 11 to 30 %, from 30 to 50%, a high risk is proposed, and over 50% a very high risk is assumed. Table 4 shows the categorisation of mine tailings according to RAC for the target metals. This value was calculated according the equation proposed by Rodríguez *et al.* (2009) and Favas *et al.* (2011):

$$RAC = \frac{\text{Water Soluble fraction of elements} + \text{Exchangeable fraction of elements}}{\text{The summation of metal concentrations of Sequential extraction}} \times 100$$

Table 4. 10 The RAC for the studied elements.

Location	Cu	Zn	Cd	Pb
Britannia mine (BCM)	1.55 ± 0.15 (low risk)	2.7 ± 0.68 (low risk)	0.81 ± 0.01 (no risk)	4.84 ± 0.88 (low risk)
Pandora mine (PLM)	60 ± 6.90 (very high risk)	36.08 ± 5.56 (high risk)	47.11 ± 2.51 (high risk)	47.42 ± 1.72 (high risk)
Parys mine (PCM)	0.11 ± 0.01 (no risk)	0.26 ± 0.05 (no risk)	0.82 ± 0.32 (no risk)	1.14 ± 0.28 (low risk)

From table 4.10 it can be seen that the RAC of Cu ranged from no risk in the case of the PCM mine up to very high risk in the PLM mine. By contrast, the associated values of Zn showed high environmental risk at the PLM, but no risk in the mine tailing samples from the

PCM. There was a low risk associated with the presence of Zn in the BCM. Cd showed no risk at the Britannia and Parys sites but a high risk was recorded at the Pandora mine. Low risk associated with presence the Pb in BCM and PCM, and high risk of Pb may be posed to ecosystem from PLM mine.

Overall, mine tailings at the Pandora site showed higher RAC values than the other sites. This is a serious concern given the very high levels of bioavailable metals in this site, which can damage the surrounding area. Even the RAC factors in general showed low pollution risk at BCM and PCM, but as mentioned earlier, high levels of several target metals in residual fraction and the impact of weather conditions may enhance the oxidation process of metal ores posing high environmental risk to areas surrounded BCM and PCM.

4.5 Conclusion

This study has explored mine tailings with high levels of heavy metal contamination. PCM and BCM tailings were extremely acidic and all the tailings contained low levels of several nutrients. The application of a sequential extraction procedure provided useful information about the distribution and behaviour of Cd, Pb, Zn and Cu in the investigated mine tailings. The results showed that the high proportion of the target metals associated with residual fraction in both the BCM and PCM may be due to low tailings pH. As a result, the metals tended to occur in an ionic form which was readily soluble and the metals were readily mobilised in the tailings solution. In addition, heavy rain prior to sampling and low tailings pH may have contributed to the transportation of heavy metal ions to deep soil horizons or to surface water via runoff. In the case of the PLM, the exchangeable fraction was the main fraction controlling the behaviour of most of the investigated metals. This may be due to the higher CEC and pH of the tailings in this site than others. At the PLM, where the pH recorded 6.27, the adsorption of heavy metals to different fractions was high compared to the other sites. This result is likely to be due to the fact that at higher pH levels, the adsorption of heavy metal to tailings fractions tends to increase. A combination of elevated levels of total metals and changes in environmental conditions may lead to increased bio-availability and the transfer of target elements from residual form to other fractions which are available to flora and soil biota, therefore more work is needed by aim to amend PLM, PCM and BCM sites using several amendments and different remediation approaches.

5 EVALUATING THE ABILITY OF GREEN WASTE BIOCHAR, GREEN WASTE COMPOST, ZEOLITE AND NANOSCALE ZERO VALENT IRON TO REDUCE THE AVAILABILITY OF COPPER, LEAD, ZINC AND CADMIUM IN MINE TAILINGS

5.1 Introduction

Mining activities have polluted 240,000 km² of land surface worldwide (Epelde *et al.*, 2015). Specifically, Europe's Environment Agency reported that 20,000 sites contaminated with heavy metals need to be treated and another 350,000 sites have been investigated (Mueller *et al.*, 2012). In the UK, 400 km² have been categorised as contaminated land, much of which relates to the 140,000 deserted mines and quarries distributed across the UK, including in Wales (Davies *et al.*, 2015).

At natural levels, soils can minimise the mobility of heavy metals via a number of mechanisms including adsorption, precipitation, co-precipitation and redox reaction. However, at higher levels soils are unable to limit the translocation of heavy metals (Alloway, 1990c; Park *et al.*, 2011). Several technologies have been used for decades as in-situ remediation methods based on application of chemical amendments such as lime and phosphate to reduce the mobile form of heavy metals in polluted soils. However, several of chemical approaches have some drawbacks such as surface eutrophication in the case of phosphate. Recently, a number of organic and inorganic applications have sought to reduce these side effects. For example, zeolite, compost and biochar have been developed as alternative in-situ techniques along with nanoparticles e.g. nano zero valent iron (Fe⁰). These amendments can avoid some negative effects associated with the use of lime and phosphate. The ability of Fe⁰ zeolite, compost and biochar to reduce the mobility of heavy metals is based on several mechanisms such, precipitation, co-precipitation and adsorption process (Kumpiene *et al.*, 2008; Shi *et al.*, 2009). Using zeolite, biochar and compost avoids the addition of new pollutants to the amended soils, and can improve some soil properties e.g. by adjusting soil pH and increasing soil organic matter content. According to Beesley *et al.* (2010), incorporation of organic matter into polluted soils improves soil fertility, enhances plant growth, decreases soil compaction, reduces soil erosion and stabilises heavy metals. However, the application of biochar has also been associated with several adverse effects on soil biota, a reduction in nutrient mobility and the loss of native soil organic matter. For soils with low fertility on the other hand, including mine tailings, application of biochar was found to improve soil fertility due to its ability to reduce

soil acidity and heavy metal mobility, and ameliorate soil structure (Jones *et al.*, 2012; Kuppusamy *et al.*, 2016). In addition, nanoparticles have showed ability to immobilise heavy metals thus reducing their risk to the environment by converting the mobile form of target elements to other fractions which are more stable and unavailable to plants and soil biota (Jumnam and Lead, 2008; Beesley *et al.*, 2010). For instance, Fe⁰ has shown high efficiency in reducing the availability of heavy metals in multi-metal polluted soils (Simon, 2005; Li *et al.*, 2017), due to its fast reactivity and high surface area. In addition, Jones *et al.* (2016) found that the addition of biochar and compost individually and in combination to polluted soil with Cu minimized the available levels of Cu. These materials have the additional benefits of being less expensive and more environmentally friendly than other several methods such as lime applications (Zhou *et al.*, 2014). Several studies recommended that long-term field study is required to study the effectiveness of Fe⁰ in the reduction of heavy metal mobility in polluted soils (Cundy *et al.*, 2008; Komárek *et al.*, 2013) and water (Calderon and Fullana, 2015). Inefficiency in mitigating the bioavailability of heavy metals with some amendments has been observed by several studies. For example, Giannatou *et al.*, (2016) reported that natural zeolite was ineffective in the reduction of Pb mobility. Clinoptilolite zeolite was also found to be ineffective in immobilising Cd, Cu, Zn and Mn. The application of hardwood derived biochar and green waste compost separately to the polluted soil increased the available fraction of Cu resulting from the formation of soluble organic complexes with humic acid supplied by the amendments (Beesley *et al.*, 2011). Re-release of heavy metals in amended soils with time may become one of the drawbacks of several of these amendments. For example, Farrell *et al.* (2010) reported that the degradation of OM that has been applied as an amendment to acidic multi-heavy metals contaminated soils may result in the increased mobility of some metals with time due to releasing the previously stored heavy metals into the soil solution. It was expected that the application of Fe⁰ would have no effect on the mobility of several heavy metals in polluted soils (Hartley *et al.*, 2004).

5.2 Objectives

The objectives of this study are:

- To examine the ability of biochar (B), compost (C), zeolite (Z) and Fe⁰ to reduce the availability and redistribution of Cu, Cd, Zn and Pb in mine tailings.
- To investigate the effect of incubation time of treated tailings on the levels of target metals in the bioavailable fractions.

To achieve the above goals, experiments were conducted in order to answer of following questions:

- Does the efficiency of various amendments in stabilising heavy metals differ from one amendment to another?
- How does the incubation of tailings with amendments for 1 year influence the presence of target metals in the bioavailable fractions?

5.3 Materials and Methods

5.3.1 Sampling sites

The results of experiment 1 (chapter 4) showed that the distribution of investigated metals was very similar in samples from the Britannia and Parys mines. Therefore, Parys mine was selected, along with Pandora mine to conduct the treatment trial. The samples were collected randomly from Parys and Pandora mines (approximately 50.0 kg of wet material from each site) in November 2016 from the top 10 cm of the tailings since the addition of amendments to the soil usually takes place up to a depth of 10 cm (Farrell *et al.*, 2010). The collected tailings were transported to the laboratory and then air-dried for two weeks and homogenised during the drying period by hand mixing twice a day. The dried tailings were sieved through a 2 mm sieve.

5.3.2 The sources of amendments

Green waste compost (C) was provided by Penhesgyn household waste recycling centre (LL59 5RY) in Anglesey, North Wales, UK. Biochar (B) was made in the laboratory following the technique described by (Jones and Quilliam, 2014). The raw material (green waste) to make B was obtained from Bangor University garden. The feedstock was washed and dried in the oven at 105 °C for 3 h, then placed in a crucible and covered with Pyrex glass plates to minimise the exposure to oxygen during the pyrolysis process. The crucibles were put in the furnace with other opened crucibles contain the feedstock without covering to reduce the abundance of oxygen in the furnace. In order to meet the criteria for slow pyrolysis methods, furnace temperature was increased at a rate of 10 °C /min up to 450 °C and maintained at this temperature for 1 h. Furnace temperature was then allowed to decrease to room temperature and samples removed. The yield (Figure 5.1) was weighed than the B kept in a polyethylene bag at 4°C until further use and analysis.

Fe⁰ was bought from Hepure technological, Inc and Z in the form of a molecular sieve Type X (Na₂O.Al₂O₃.2.5SiO₂.6H₂O) was bought from Sigma-Aldrich.



Figure 5. 1 The yield of green waste pyrolised biochar.

5.3.3 Experimental design

1- Additive doses of amendments

The amendment dosage chosen (2%) was based on the recommendations of several previous studies which used Fe^0 as the stabilising agent with applications of 1 – 2% (w/w) (Komárek *et al.*, 2013). Lee *et al.* (2011) used a 2% Fe^0 dosage to treat polluted soil collected from a gold mine site in Korea and Kumpiene *et al.* (2011) applied 2% Fe^0 to contaminated soils containing $2600 \mu\text{g g}^{-1}$ Cu. The use of a low dosage was intended to avoid the possible toxic effects of Fe^0 on soil biota and plants (Saccà *et al.*, 2014; Lefevre *et al.*, 2015) and to avoid the aggregation of its particles, resulting in the reduction of Fe^0 reactivity and surface area and consequently reduce its ability to adsorb pollutants (Tang and Lo, 2013). Also, organic amendments contain high levels of nutrients and the use of B and C by high rate may have negative effects on the created biological medium in treated soils (Jones and Healey, 2010). Application of Z at a high dosage may be detrimental due to the consequent delivery of high levels of Na to the target soil and the associated increase in salinity (Singh and Kalamdhad, 2013). Therefore, to avoid all of these negative impacts, Fe^0 , Z, B and C was applied with low rate of 2%.

2- Experiment set-up

The tailings incubation trial was conducted during the period from the beginning of December 2015 to the end of November 2016 using 4 amendments and untreated tailings (UT) as a control. Three replicates were used for each treatment and sampling undertaken on four occasions, making a total of 120 pots for both sites (Table 5.1).

In order to mimic natural conditions, the experiment was set up under natural field conditions at Henfaes Research Station, Abergwyngregyn, Gwynedd, UK (53° 14' N, 4° 01' W) (Figure 5.2). For each mine site, 5 treatments were set up and three replicates were used for each treatment. For amended tailings, each treatment used 12 pots (3 replicate for each sampling time). To each pot 5 g of amendment (8.64 t ha⁻¹) was mixed by hand with 245 g of mine tailings for 60 seconds to make sure the amendment was thoroughly distributed within the tailings sample. At the beginning of experiment, 30 pots were collected and the remaining pots (90) were left outside in neuter to monitor the effect of amendments on the availability of heavy metals after a period of 4, 8 and 12 months from the beginning of experiment. At each of these time points, 30 pots were collected to do the required analysis.

Table 5. 1 The treatments used in the experiment, including quantities of amendment and mine waste (g) for each location. The values in the brackets refer to the total amount of tailings and amendments utilised.

Amendment	ratio of amendment	quantity used of amendment	Weight of tailings	Total pots	sampling time
N-zero valent Iron (Fe ⁰)	2%	5g (60g)**	245g (2940g)*	12	0,4,8,12 months
Compost (C)	2%	5g (60g)**	245g (2940g)*	12	0,4,8,12 months
Biochar (B)	2%	5g (60g)**	245g (2940g)*	12	0,4,8,12 months
Zeolite (Z)	2%	5g (60g)**	245g (2940g)*	12	0,4,8,12 months
Untreated tailings (UT)	0	0	250g (3000g)*	12	0,4,8,12 months

The values between the brackets tagged with (*) and (**) refer to the total quantity of tailings and amendment respectively, which was used for one site.



Figure 5. 2 The experimental setup on the 1st of December 2015.

5.3.4 Column Leaching Experiment

At the time of analysis, part of air-dried sample (50 g) was packed in a 20 cm long, 3 cm diameter polyethylene column with small hole at the bottom of the column. A 2 mm layer of glass wool was placed at the bottom of the column, and over it a 2 mm layer of washed and calcined quartz sand to prevent the loss of tailings. A polyethylene tube was fitted to at the bottom of column to collect the leachate (Figure 5.3). Distilled water pH 5.5 (Jalali and Arfania, 2010; Sangiumsak and Punrattanasin, 2014) was pumped through the column using a mechanic peristaltic pump type ISMATEC® at a rate of 20 µl /min to simulate the average rainfall at Abergwyngregyn area (Abaas *et al.*, 2012). The leachate was collected after 24 h, and the pH and EC measured immediately as described in section 4.3.3.2. Part of the leachate was kept at -20°C to analyse NO₃⁻-N, NH₄⁺-N and the other part (15 - 20 ml) was acidified by 0.1 M NHO₃ and kept at 4°C for determining the total concentrations of target heavy metals.



Figure 5. 3 Performing the column experiment.

5.3.5 Amendments characterisation and sample analysis

pH and EC were measured in tailings suspensions of 1:2.5 tailings: water (^w/_v) ratio, CEC determined using the ammonium acetate method. Moisture and organic matter content evaluated by the loss-on-ignition method. A CHN-2000 analyser, Leco Corp, St Joseph MI was used to measure total carbon and nitrogen ratios and total content of investigated heavy metals was measured by acid digestion and analysed using atomic absorption spectroscopy following protocols described in section 4.3.3.2. The geochemical fractions of studied metals were investigated in the treatments following the five step sequential extraction protocol described in section 4.3.3.2.9.

5.3.6 Statistical analyses

The results we obtained are illustrated as a mean value of three replicates \pm standard error. Statistical analysis of the data was performed using SPSS v 22 for Windows and SigmaPlot v 12.5 was used to create the graphs. The Shapiro-Wilk test was used to assess the

normality of obtained data. The analysis of the mean difference between the parameters pH, OM and the proportion of target metals in the bioavailable fraction was carried out using one-way analyses of variances (ANOVA) with least significant difference (LSD) test ($P < 0.05$) and in some causes ($P < 0.01$, 0.001) or the Welch test where the assumption of homogeneity of variance was violated. Two-way ANOVA was used to compare the effect of time and treatment type on the target metal proportion in F1 and F2 together. When the data were not normally distributed a Scheirer-Ray-Hare test was performed (Dytham, 2011). The results measured below the detection limit of AAS are reported as BD (below detection limit), but were assumed to be half of AAS detection limit (USEPA, 2000) for discussion where necessary and presenting the data graphically.

5.4 Results

5.4.1 The geochemistry and total content of heavy metals in studied sites

Table 5.2 shows the pseudo-total content of studied metals in untreated Parys tailings (PT) and Pandora tailings (PaT). High levels of Pb, Cu and Zn were recorded in PT and extreme concentration of Pb and Zn were measured in PaT. For example, total Cu was over 7 times higher in PT than in PaT, even though the availability of Cu was 12 times lower in PT than that in PaT. These results are consistent with the previous chapter's result. The geochemistry of studied metals in the two sites was different. Most of the metals were associated with the residual fraction (F5) in PT. By contrast, high proportions of target metals were found in exchangeable fraction (F2) in PaT making these metals more available than those in PT.

Table 5. 2 Pseudo-total content and distribution of investigated metals in untreated Parys and Pandora tailings ($\mu\text{g g}^{-1}$, measurements presented as mean \pm S.E.M; n = 3).

Parys mine tailings							Pandora mine tailings					
Metal	Pseudo-total	F1	F2	F3	F4	F5	Pseudo-total	F1	F2	F3	F4	F5
Cu	1516 \pm 56.52	1.50 \pm 0.42	5.71 \pm 0.33	144.3 \pm 12.45	71.2 \pm 2.26	1301 \pm 27.3	207.5 \pm 6.31	BD	86.58 \pm 1.47	6.13 \pm 0.39	63.85 \pm 0.29	37.1 \pm 2.01
Zn	2065 \pm 96	15.41 \pm 0.4	8.52 \pm 0.63	19.28 \pm 1.71	21.89 \pm 0.51	1923 \pm 113	37476 \pm 797	120.8 \pm 2.2	18987 \pm 497	1861.3 \pm 86.4	14712 \pm 40.8	16700 \pm 4687
Cd	7.60 \pm 0.35	BD	0.75 \pm 0.09	0.43 \pm 0.16	BD	6.47 \pm 0.17	102.67 \pm 3.35	2.85 \pm 0.1	57.6 \pm 1.67	12.17 \pm 0.51	2.75 \pm 0.13	35.93 \pm 8.54
Pb	15360 \pm 324	408.67 \pm 32.27	5847 \pm 397	1663 \pm 90	282.8 \pm 35.5	8547 \pm 420	74533 \pm 1572	7.50 \pm 1.04	78560 \pm 1212	8107 \pm 559	3793 \pm 65.7	2487 \pm 291

F1= Water-soluble fraction, F2= Exchangeable fraction, F3= Reducible fraction, F4= Oxidisable fraction, F5= Residual fraction, SF= (F1+F2+F3+F5+F5), and BD = below the limit of the detection.

5.4.2 Amendments characterisation

Selected amendment properties are presented in table 5.3. The pHs of all amendments except compost (pH = 6.76) were basic. The organic matter contents of B ($76.54 \pm 0.16\%$) was the highest of the amendments used and in the case of C was substantially lower at $19.61 \pm 0.17\%$. The levels of studied heavy metals in the amendments were low in the case of Cu and Cd but were slightly higher for Zn and Pb in all amendments. The levels of Zn in the amendments were much lower than in the tailings. This, in addition to the fact that the amendment dosage was just 2% of the total weight of treatments, the levels of studied metals in the amendments did not significantly alter the total concentration of elements in the treated tailings.

Table 5. 3 Characteristics of amendments (measurements presented as mean \pm S.E.M; n = 3).

Parameter	Biochar	Compost	Fe ⁰	Zeolite
pH _{H2O}	9.86 ± 0.10	6.76 ± 0.01	9.5 ± 0.12	12.39 ± 0.05
EC mS cm ⁻¹	7.93 ± 0.24	5.59 ± 0.12	0.10 ± 0.01	1.32 ± 0.09
Bulk density g cm ⁻³	0.26 ± 0.01	0.66 ± 0.08	2.3*	ND
CEC meq/100g amendment	46.45 ± 1.91	37.78 ± 2.75	2.86 ± 0.45	344 ± 4.16
O. M %	76.54 ± 0.16	19.61 ± 0.17	ND	ND
TC g kg ⁻¹	416 ± 17.4	234 ± 136	16.4 (18*)	0.77 ± 0.16
TN g kg ⁻¹	24.33 ± 3.07	17.98 ± 0.33	0.21 ± 0.02	1.13 ± 0.36
C/N	18 : 1	13 : 1	81 : 1	0.68 : 1
Total elements $\mu\text{g g}^{-1}$				
Cu	13.20 ± 0.90	22.62 ± 0.30	30*	0.6 ± 0.60
Zn	222 ± 7.10	213 ± 1.15	31*	3.98 ± 0.35
Cd	BD	BD	2.96 ± 0.42	0.25 ± 0.15
Pb	57.3 ± 7.1	155 ± 12.86	123 ± 13.6	BD
Extractable elements $\mu\text{g g}^{-1}$				
Cu	BD	BD	BD	BD
Zn	2.06 ± 0.11	5.16 ± 0.08	7.46 ± 2.65	0.49 ± 0.08
Cd	BD	BD	0.07 ± 0.05	0.15 ± 0.01
Pb	BD	BD	BD	BD

ND = Did not measure, BD = below limit of detection, (*) Manufacturer information.

5.4.3 Effects of amendments on pH and OM of treated tailings

5.4.3.1 The effect of amendments on the pH of tailings

pH plays an important role in the geochemistry of heavy metals in mine tailings (Rieuwerts *et al.*, 2006; Rodríguez-Vila *et al.*, 2015; Tang *et al.*, 2015). Therefore, the pHs of all treatments were measured at the beginning of the experiment, and after 4, 8 and 12 months. At the beginning of treatment period, the addition of amendments led to a significant increase in the pH of all Parys tailings (PT) samples ($P < 0.05$) (Figure. 5.4 A). Zeolite was the most effective amendment in terms of pH adjustment, with a 2-fold increase from 2.96 ± 0.02 in untreated Parys tailings (UPT) to 6.17 ± 0.03 in PT+Z. This is due to the extreme alkalinity ($\text{pH} = 12.39$) of the raw zeolite material. After 4 months, the pH of the PT samples amended with zeolite (PT+Z) rose to 7.86 ± 0.02 before decreasing gradually with time to 7.12 ± 0.02 by the end of the study. The initial pH of PT samples amended with compost (PT+C) and PT samples amended with biochar (PT+B) was not significantly different ($P < 0.05$) from the values recorded later in the experiment at 2nd collection in the same treatments. Whereas, the pH of PT treated with Fe^0 was slightly lower after 4 months and no significant change in pH was observed at the third and fourth sample collection for the same treatments. At the end of trail the pH of PT+ Fe^0 (3.71 ± 0.01), PT+B (3.84 ± 0.02) and PT+Z (7.12 ± 0.02) were significantly higher than UPT ($P < 0.05$), but the pH of PT+C (3.55 ± 0.02) was not significantly higher ($P > 0.001$) than that of UPT. Two-way ANOVA using Scheirer- Ray- Hare test showed no significant ($P > 0.05$) effect of time on the pH of treatments when we tested for the whole study period.

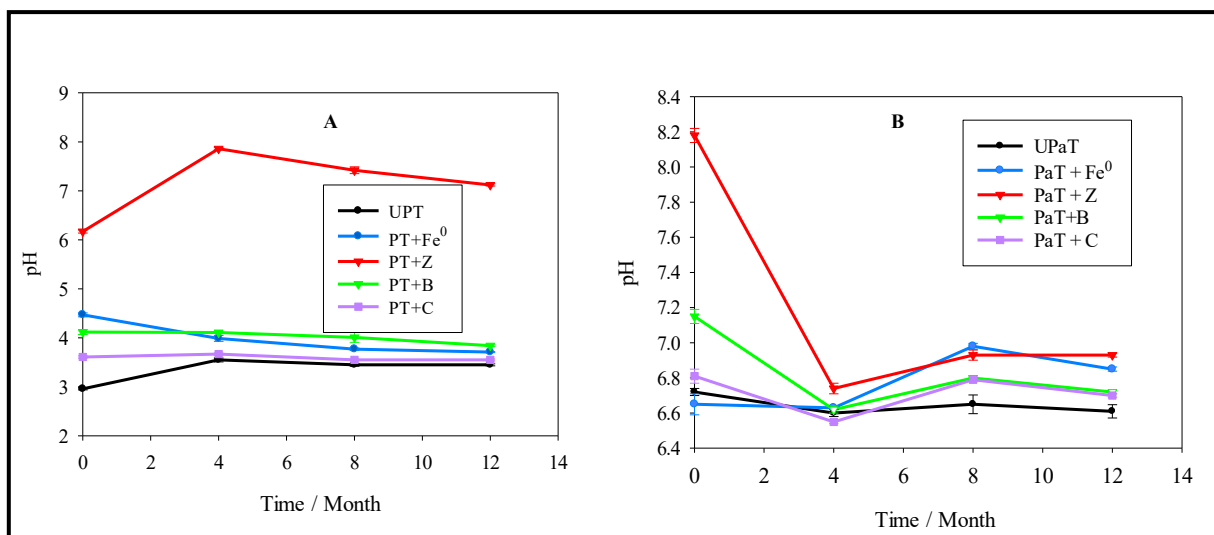


Figure 5. 4 Treatments pH at 4 collection times during the trial duration in; A (UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar and PT+C= Parys tailings amended with compost). And, B (UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora amended with zero valent iron, PaT+Z= Pandora amended with zeolite, PaT+B= Pandora amended with biochar and PaT+C= Pandora amended with compost). (Measurements presented as mean \pm S.E.M; n = 3).

For the first set of samples (time = 0), the incorporation of amendments to Pandora tailings (PaT) (Figure 5.4 B) caused a significant ($P < 0.05$) increase in the pH of PaT+Z (which increased to 8.14 ± 0.06) and PaT+B (which increased to 7.02 ± 0.01) and a negligible increase (up to 6.81 ± 0.04) in the PaT+C compared to the untreated Pandora tailings (UPaT; 6.72 ± 0.02). Conversely, the addition of Fe⁰ to PaT led to slight decrease in pH to 6.65 ± 0.06 .

After 4 months, the pH decreased in tailings treated with Z, B and C. The most substantial reduction ($P < 0.05$) was in the pH of PaT+Z. Between 4 and 8 months the pH of all treated PaT samples increased (at 8 months UPaT = 6.65 ± 0.05 , PaT+Fe⁰ = 6.98 ± 0.02 , PaT+ Z = 6.93 ± 0.03 , PaT+B = 6.80 ± 0.01 and PaT+C = 6.79 ± 0.01) and was higher than of UPaT. Between 8 months and 12 months pH levels decreased once again (UPaT = 6.61 ± 0.04 , PaT+Fe⁰ = 6.85 ± 0.01 , PaT+B = 6.72 ± 0.01 and PaT+C = 6.70 ± 0.01) except for PaT+Z (6.93 ± 0.01). The change of the pHs were not significant ($P > 0.05$) in comparison between the pH of the same treatments in both collection times (8 and 12 months). At the end of the trial, the pH of all treated tailings were significantly ($P < 0.05$) higher than that in UPaT. The Scheirer-Ray-Hare test of variance indicated that there was a significant ($P < 0.05$) change in the pH of treatments with time.

5.4.3.2 The effect of amendments on the pH of tailings leachate

Figure 5.5 (A) shows the pHs recorded in the leachates of PT treatments. At the beginning of experiment the pHs of treated PT leachates were higher than the pH of UPT. The zeolite amendment showed a significant increase ($P < 0.05$) in pH; up to 6.33 ± 0.05 compared to UPT (2.99 ± 0.02). After 4 months, changes in pHs were variable; pH decreased slightly in the leachates of tailings treated with Fe^0 and biochar, and increased in the leachates of the zeolite, compost treated tailings and UPT. In addition, the most significant increase ($P < 0.05$) was observed in the pH of PT+C relative to that of PT+C recorded at the beginning. At month 8, the pH in the leachate of UPT and PT treated with B and Fe^0 showed a significant increase relative to their pHs at the 4 months collection. At the end of trial, all of the amended PT recorded a higher pH than UPT. One exception is the pH of PT+C was lower than that of UPT, though the difference was not statistically significant ($P > 0.05$). These differences were not statistically significant ($P > 0.05$) except in the case of PT+Z whose pH was significantly higher ($P < 0.01$) than that of UPT.

The pH of all treated PaT leachates were higher than for the UPaT with the most significant increase found with the addition of zeolite (7.99 ± 0.02), as figure 5.5. B shows. After 4 months, the pH values of UPaT leachate and that treated with B and Fe^0 increased slightly. However, the pH of the PaT+Z leachate dropped sharply from 7.99 ± 0.02 to 7.23 ± 0.03 and slightly in PaT+C. After 8 months, the pHs declined slightly in all treated and untreated samples of PaT. At the end of study, the pHs of all treated PaT increased significantly relative to their pH at 8 months. In addition, the pH of all amended PaT leachates at the end of trial were significantly ($P < 0.05$) higher than in UPaT.

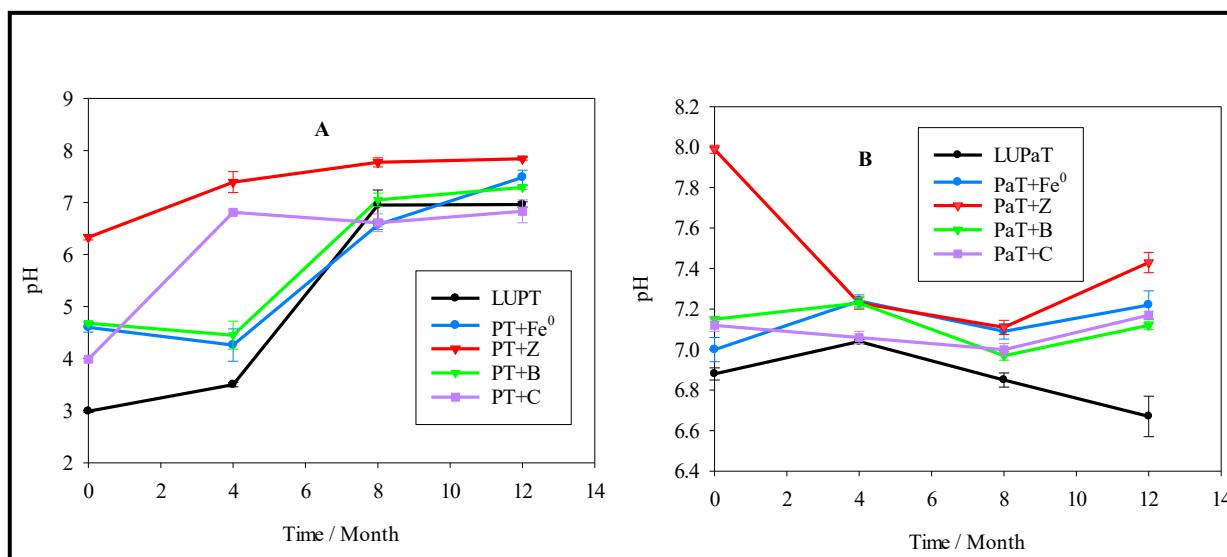


Figure 5. 5 Leachate pH at 4 collection times during the trial duration in the leachates of UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar and PT+C= Parys tailings amended with compost (A). And, UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora amended with zero valent iron, PaT+Z= Pandora amended with zeolite, PaT+B= Pandora amended with biochar and PaT+C= Pandora amended with compost (B). (Measurements presented as mean \pm S.E.M; n = 3).

5.4.3.3 The effect of amendments on organic matter content

Organic Matter is another parameter that can play significant role in the geochemistry of heavy metals in tailings as it may reduce or increase the bioavailability of heavy metals in contaminated soils. Figure 5.6 A shows the OM content in PT treatments. At the beginning of the study, the application of B and C significantly increased ($P < 0.05$) the pool of OM from $4.65 \pm 0.17\%$ in UPT to $6.81 \pm 0.04\%$ and $5.91 \pm 0.04\%$ in PT+B and PT+C, respectively. However, Fe⁰ and Z did not show significant change ($P \geq 0.05$) in the percentage of OM compared to the control (UPT). After 4 months, the OM content in all treatments was reduced. This reduction was significant ($P < 0.05$) in the PT+B and PT+C treatments compared to the OM % in the same treatments at time = 0. Percentage of OM after 4 months was still significantly ($P < 0.05$) higher in PT+B and PT+C ($5.51 \pm 0.07\%$ and $4.45 \pm 0.09\%$, respectively) than that in other treatments (UPT = $3.98 \pm 0.03\%$, PT+Fe⁰ = $3.99 \pm 0.03\%$ and PT+Z = $3.87 \pm 0.03\%$). The proportion of OM increased in PT+Z ($4.10 \pm 0.04\%$) and PT+C ($4.75 \pm 0.12\%$) and decreased in UPT ($3.81 \pm 0.04\%$), PT+Fe⁰ ($3.96 \pm 0.08\%$) and PT+B ($5.43 \pm 0.015\%$) after 8 months compared to that in the same treatments at the 2nd sample collection. Between 8 and 12 months, the % of OM increased significantly compared to previous collection (8 months) in all treatments, and OM contents being higher significantly ($P < 0.05$) in PT+B $7.28 \pm 0.14\%$ and PT+C $6.48 \pm 0.09\%$ than that in UPT

5.76 \pm 0.09 %. There was a significant effect ($P < 0.05$) of incubation time on OM content in all treatments.

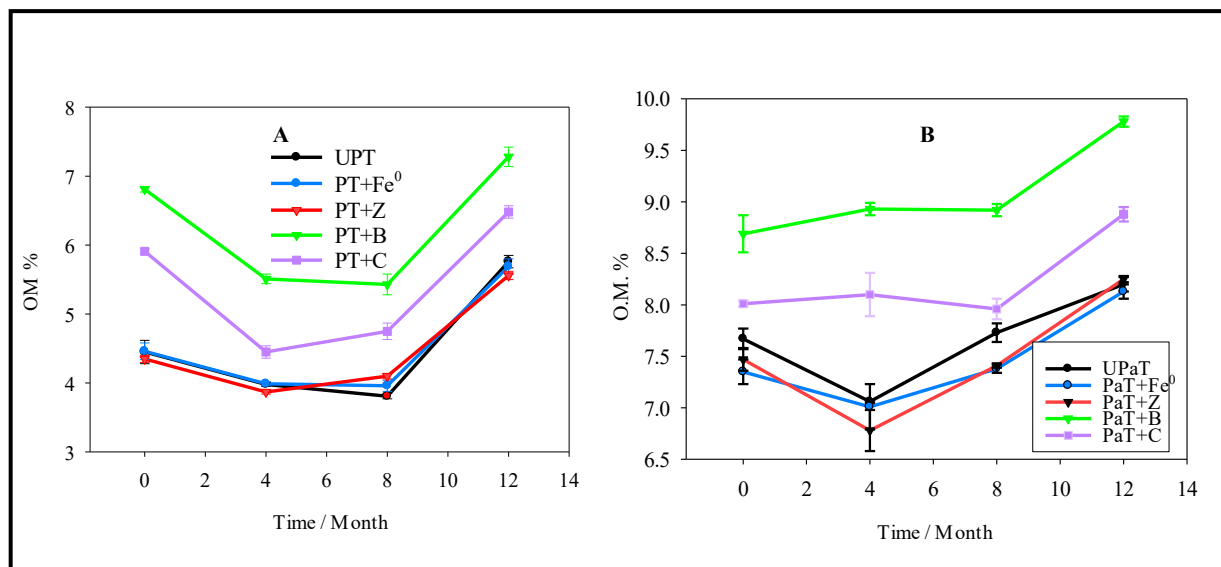


Figure 5. 6 OM content in all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar and PT+C= Parys tailings amended with compost) and (A). And, UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora amended with zero valent iron, PaT+Z= Pandora amended with zeolite, PaT+B= Pandora amended with biochar and PaT+C= Pandora amended with compost (B). (Measurements presented as mean \pm S.E.M; n = 3).

In terms of OM content, a similar pattern for PaT to that of PT was observed (Figure 5.6 B). At the beginning of the trial, where the addition of C and B increased the OM content, though this increase was significant ($P < 0.05$) only in PaT+B (8.69 \pm 0.18%) compared to UPaT (7.67 \pm 0.10 %). By contrast, the addition of Z and Fe⁰ reduced the OM pool slightly to 7.47 \pm 0.11% and 7.35 \pm 0.12%, respectively. In the samples collected after 4 months and with comparison to the previous collection time (time = 0) the OM content decreased (though not significantly) in UPaT and PaT+Fe⁰, whereas OM content decreased significantly ($P < 0.05$) in PaT+Z. Despite these changes, OM content in the different treatments at the second sample collection (4 months) still showed the same order of increasing OM as at the beginning. After 8 months, The OM content increased in UPaT, PaT+Z and PaT+Fe⁰ and still lower significantly in PaT+Z and PaT+Fe⁰ than of UPaT. Between 8 and 12 months, the OM content increased up to 8.20 \pm 0.07% in UPaT, 8.13 \pm 0.07 % in PaT+Fe⁰, 8.25 \pm 0.03% in PaT+Z, 9.78 \pm 0.05% in PaT+B and 8.88 \pm 0.07 % in PaT+C at the end of study. In addition, OM % in PaT+C and PaT+B was still higher significantly ($P < 0.05$)

than the other treatments. Two-way ANOVA showed a significant ($P < 0.05$) effect of incubation time on the content of OM in different treatments.

Overall, an increase in the content of OM in the samples for both sites was observed by the end of trial. This may be attributed to plant material introduced to the trial pots from the surrounding area by wind during autumn (September to November 2016). Similarly, the growth of some grass seeds and algae on the surface of the tailings, observed to different extents in the trial pots by the end of the experiment, may have introduced OM content.

5.4.4 The effect of amendments on the bioavailability of studied metals

Heavy metals associated with the water-soluble (F1) and exchangeable (F2) geochemical forms have been classified as the most unstable, readily soluble and plant available fractions and most toxic forms of heavy metals (Edwards *et al.*, 1999; Rodríguez *et al.*, 2009; Wen *et al.*, 2016; Liu *et al.*, 2018). Because the studied tailings are extremely contaminated with heavy metals and with variation of metal distribution and chemical speciation among sites, a study conducted in uncontrolled conditions may cause fluctuations in the most available fraction of heavy metals in treated and un-amended tailings. Therefore, the proportion of available metals was used to interpret the trial results, the percentage available of target metals was calculated by dividing the concentration of metals in the most mobile fractions (F1+F2) by the total concentration of this metal in all steps of sequential extraction (SF) ($SF = F1 + F2 + F3 + F4 + F5$) (Lee *et al.*, 2011; Chotpantarat *et al.*, 2015). The concentration percentage of investigated metals of SF is shown in figures 5.7 to 5.14.

5.4.4.1 Effects of amendments on the bioavailability of studied metals in Parys tailings

1. Bioavailability percentage of Cu in Parys tailings

Applying the amendments to PT reduced the percentage of available fractions of Cu in the samples collected at the beginning of trial compared to the control (UPT) as figure 5.7 shows. The most effective amendment at the beginning was C, the availability of Cu in PT+C measured $0.10 \pm 0.02 \%$ in comparison with $0.47 \pm 0.01 \%$ in UPT. After 4 months, the Cu availability in UPT and tailings treated with B decreased significantly ($P < 0.05$) compared to their percentage at the beginning of the trial. However, the mobility of Cu increased in the samples of UPT, PT+C and PT+ B during the remainder of the trial (third and fourth sample collections). By contrast, in the PT+ Fe^0 treatment, the proportion of available Cu increased and was higher than all other treatments for the second and third sample collection times. At the end of the experiment, tailings treated with C, B and Fe^0 showed lower availability with $0.35 \pm 0.06 \%$, $0.41 \pm 0.06 \%$ and 0.41

± 0.13 % recorded, respectively, in comparison to 0.54 ± 0.05 % for UPT. The percentage of available Cu in PT+Z was higher than that in UPT at 4, 8 and 12 months.

Overall, the most effective amendment was compost as the percentage of mobile Cu were the lowest of all treatments for the duration of the study.

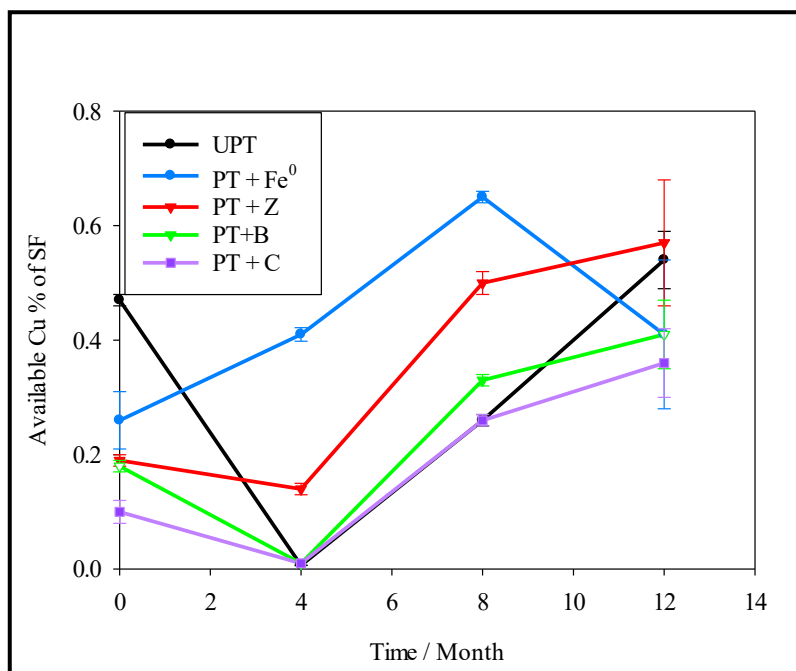


Figure 5. 7 The available % of Cu in all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar, PT+C= Parys tailings amended with compost SF the summation of Cu concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

2. Bioavailability percentage of Cd in Parys tailings

At the beginning of experiment, the incorporation of Z, B and C resulted in a significantly ($P < 0.05$) reduction in the proportion of available Cd with 0.67 ± 0.02 %, 0.56 ± 0.03 % and 0.50 ± 0.06 % recorded, respectively compared to 9.73 ± 0.88 % for UPT (Figure 5.8). By contrast, Fe⁰ (9.09 ± 2.79 %) did not affect the percentage of available fractions of Cd as it as this was not significantly different from the value recorded for UPT. During remainder of the trial, the proportion of available Cd fell to levels blow the detection limit of AAS (0.02 mg L^{-1}) in all treatments.

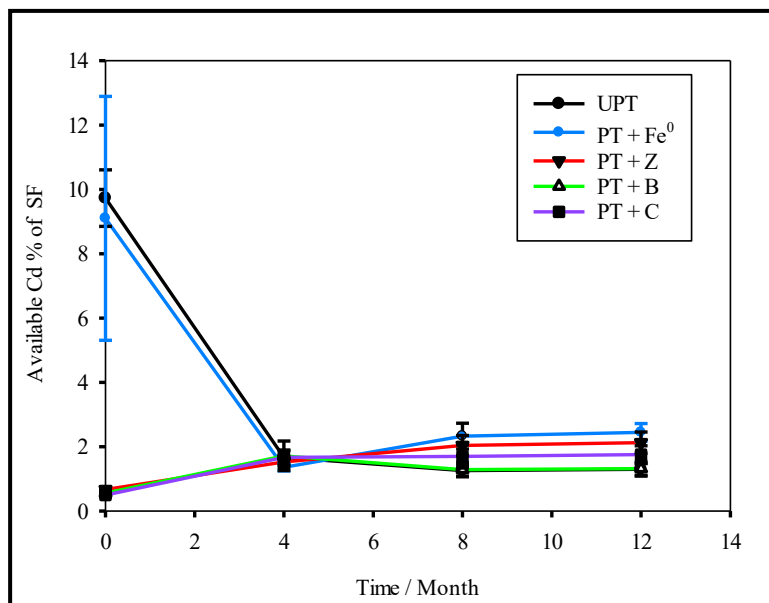


Figure 5. 8 The available % of Cd in all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar, PT+C= Parys tailings amended with compost SF the summation of Cd concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

3. Bioavailability percentage of Zn in Parys tailings

The percentage of available Zn is shown in figure 5.9. It was significantly ($P < 0.05$) lower for the tailings amended with Z, B and C, compared to UPT at the beginning of trial, but Fe⁰ (1.21 ± 0.01 %) did not affect the percentage of Zn availability compared to the control (1.21 ± 0.03 %). The proportion of mobile fractions of Zn in most treatments increased during the period between the first and second sample collection except in PT+Fe⁰ (0.54 ± 0.07 %) which decreased and remained lower than in the control (0.67 ± 0.29 %). After 8 months and to the end of experiment, the proportion of available Zn in all treatments were higher than that in UPT. The available percentage of Zn in PT+Z (1.57 ± 0.17 %) was significantly higher ($P < 0.05$) than all other treatments at 4 months collections and remained the highest up to the end of the trial. At the end of trial, the available proportion of Zn in all treatments were lower than at any other point in the trial.

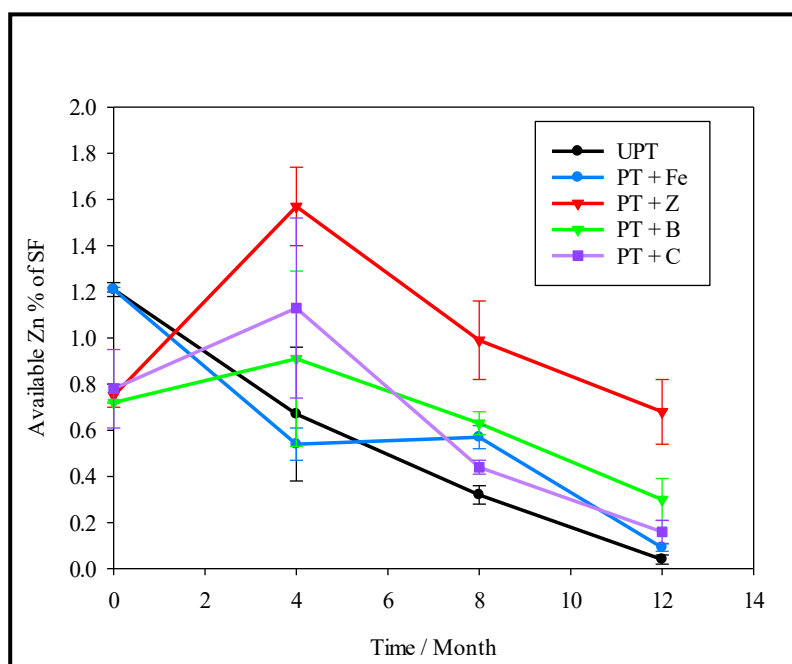


Figure 5. 9 The available % of Zn in all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar, PT+C= Parys tailings amended with compost SF the summation of Zn concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

4. Bioavailability percentage of Pb in Parys tailings

The addition of Fe⁰, Z, B and C separately to PT led to a significant ($P < 0.05$) decreases in the available percentage of Pb (31.77 ± 1.20 %, 29.01 ± 0.45 %, 27.51 ± 0.92 % and 24.23 ± 1.56 %, respectively) compared to UPT (37.31 ± 0.53 %) at the beginning of treatment period (Figure 5.10). After 4 months of incubation, the proportion of available Pb increased significantly ($P < 0.05$) to 37.91 ± 1.17 % in PT+Z and to 36.02 ± 1.00 % in PT+C compared to the percentage of Pb in same treatments collected at the beginning. However, there were no significant change ($P > 0.05$) in the case of UPT, PT+Fe⁰ and PT+B compared to Pb proportion in the same treatments collected at the beginning. The percentage of available Pb in PT+Fe⁰ (28.38 ± 1.20 %) and PT+B (30.28 ± 0.97 %) were significantly lower ($P < 0.05$) than that in UPT (35.55 ± 1.93 %) and remained lower than UPT up to the end of trial. Between 8 and 12 months, the proportion of available Pb declined slightly in all amended samples. After 12 months the percentage of available Pb were lower in all amended treatments than in UPT (28.20 ± 2.72 %). Fe⁰ (22.38 ± 1.34 %) and B (22.40 ± 1.45 %) were the most effective amendments, reducing the percentage of available Pb in F1 and F2 after 12 month significantly ($P < 0.05$) compared to the control.

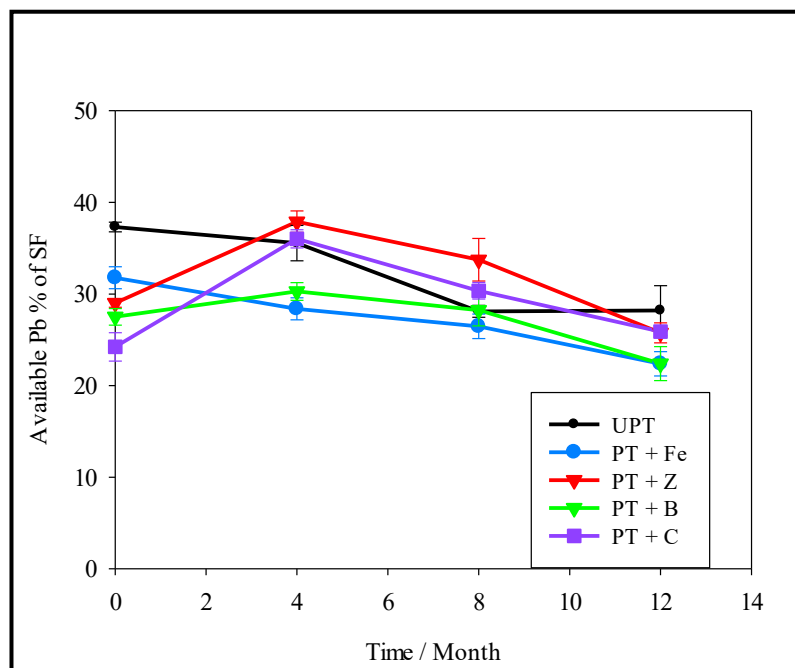


Figure 5. 10 The available % of Pb in all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar, PT+C= Parys tailings amended with compost SF the summation of Pb concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

5.4.4.2 Effects of amendments on the bioavailability of studied metals in Pandora tailings

1. Bioavailability percentage of Cu in Pandora tailings

From figure 5.11 the most effective amendments reduced the percentage of available Cu at the beginning of the trial were Fe⁰ (7.58 ± 0.47 %) and C (24.88 ± 0.65 %). These treatments showed a significantly ($P < 0.01$) lower proportion of available Cu at the beginning of experiment compare to the control (UPaT; 44.72 ± 0.21 %). The reduction of Cu by B (to 40.15 ± 2.49 %) and Z (to 44.00 ± 0.10 %) were not significant ($P > 0.01$). Between 0 and 4 months, the mobile fraction percentage of Cu increased for all treatments, with the most significant ($P < 0.05$) increase recorded for PaT+Fe⁰ (to 56.87 ± 1.63 % at 4 months) and PaT+C (to 48.82 ± 1.92 % at 4 months). At 4 months, the proportion of available Cu was lower for all amended treatments than that in UPaT (52.20 ± 0.46 %) except for PaT+Fe⁰ (56.87 ± 1.63 %) though the difference was not statistically significant ($P > 0.01$). Between 4 and 8 months, the percentage of available Cu declined in all the amended treatments and by 8 months were lower than in UPaT though the difference was only statistically significant ($P < 0.01$) in the case of PaT+Z (35.51 ± 6.05 %). Between 8 and 12 months, the proportion of available Cu decreased significantly in all treatments and the percentage of available Cu in the amended treatments was lower than in UPaT ($25.17 \pm$

4.33 %). This difference was statistically significant ($P < 0.05$) in the case of PaT+Z (16.91 ± 0.17 %) and PaT+C (18.15 ± 1.54 %) in comparison to control (UPaT; 25.17 ± 4.33 %) at the end of trial. Initially, there were no significant ($P < 0.05$) changes in the percentage of available Cu in UPaT over time to 8 months, but a significant decrease was observed between 8 and 12 months.

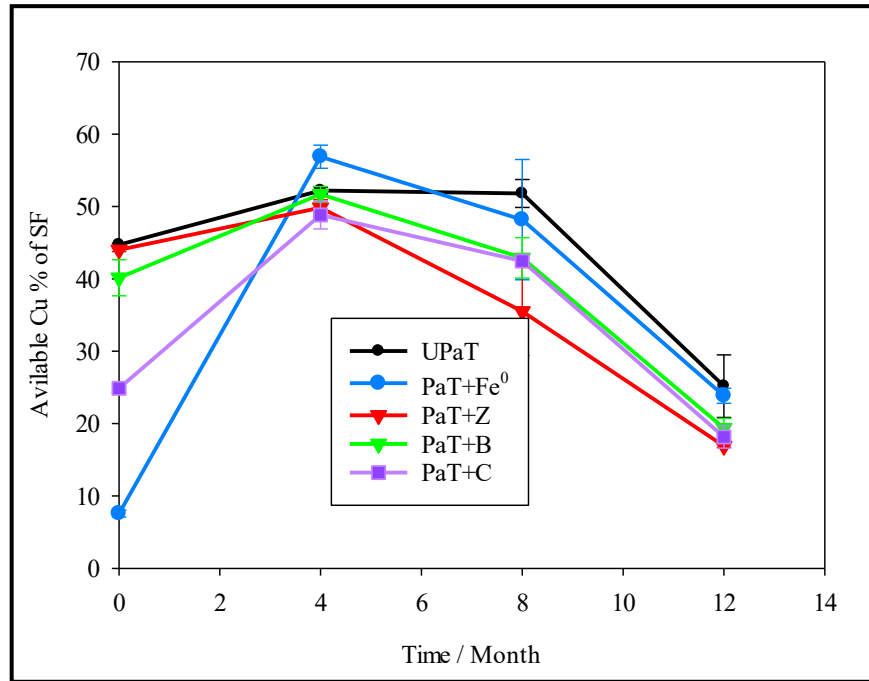


Figure 5. 11 The available % of Cu in all treatments at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar, PaT+C= Pandora tailings amended with compost SF the summation of Cu concentration in all fraction). Measurements presented as mean \pm S.E.M; n = 3.

2. Bioavailability percentage of Cd in Pandora tailings

The percentage of available Cd fluctuated during the study duration in all treatments (Figure 5.12). At the beginning of trial the percentage of available Cd in PaT+Z (58.91 ± 2.35 %), PaT+B (59.74 ± 1.92 %) and PaT+C (59.72 ± 0.74 %) was higher than in UPaT (54.94 ± 4.62 %), but these differences were not statistically significant. By contrast, the percentage of available Cd in the tailings treated with Fe⁰ (48.32 ± 1.16 %) was lower than in UPaT, but again, the difference was not statistically significant. Between 0 and 4 months, the available Cd percentage decreased, in most treatments with a statistically significant reduction ($P < 0.05$) observed in PaT+C and PaT+Z. At 4 months, the proportion of available Cd in UPaT (48.18 ± 16.19 %) was higher than Cd in other treatments and was not significantly different to any of

the amended treatments. Between 4 and 8 months, the percentage of available Cd increased significantly ($P > 0.05$) in PaT treated with Fe^0 , Z and C. However, no significant difference ($P < 0.05$) in the proportion of available Cd was found between the treated tailings and UPaT at 8 months. Between 8 and 12 months, the percentage of available Cd declined in all treatments. Interestingly, at 12 months the proportion of available Cd was similar for all treatments recording $50.77 \pm 0.97 \%$, $51.10 \pm 0.99 \%$, $52.36 \pm 1.20 \%$, $51.77 \pm 5.31 \%$ and $52.28 \pm 3.86 \%$ in UPaT, PaT+ Fe^0 , PaT+Z, PaT+B and PaT+C, respectively. Also, no significant ($P > 0.05$) change in the proportions of available Cd in UPaT was recorded with time.

Overall, the application of 2% of Fe^0 , Z, B and C to reduce the mobility of Cd in PaT was insufficient since at no point during the experiment was a statistically significant ($P > 0.05$) difference observed between the amended treatments and the control.

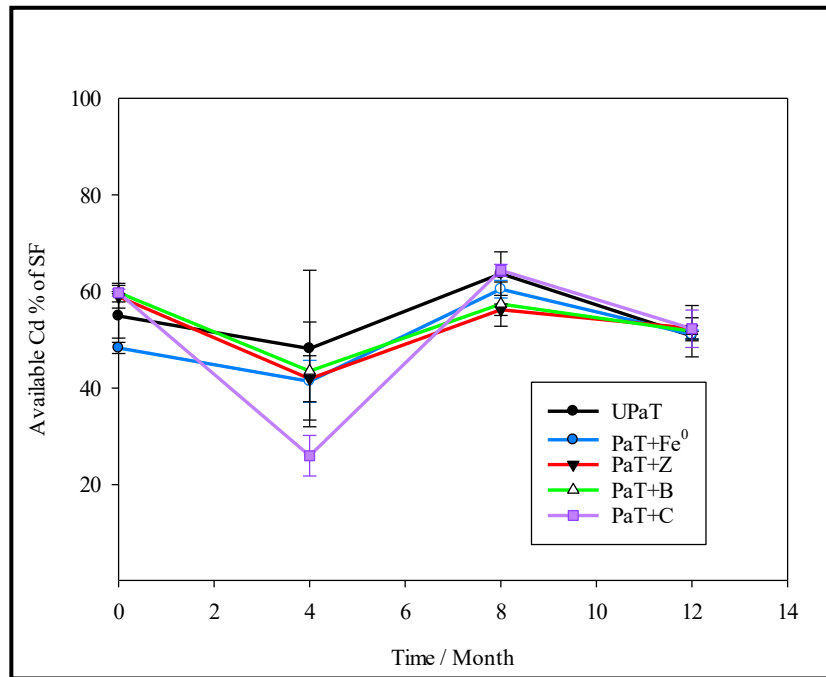


Figure 5.12 The available % of Cd in all treatments at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+ Fe^0 = Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar, PaT+C= Pandora tailings amended with compost SF the summation of Cd concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

3. Bioavailability percentage of Zn in Pandora tailings

Figure 5.13 illustrates the percentage of Zn availability in PaT treatments. At the beginning of the experiment, the incorporation of Fe^0 , Z and C to PaT increased the available proportion of Zn but not significantly ($P > 0.05$). A significant increase ($P < 0.05$) was observed

with the addition of B (59.90 ± 1.66 %) compared to UPaT (50.09 ± 5.12 %). After 4 months, the percentage of available Zn increased significantly ($P < 0.05$) in UPaT at second collection compared to its percentage in the UPaT at the beginning of trial. The percentage of available Zn in 2nd sample collection had increased significantly ($P < 0.05$), by nearly 65% of their initial levels in the same treatments of first collection. At second collection the percentage of available Zn were higher though not significantly ($P > 0.05$), in amended tailings with PaT+Fe⁰ (71.42 ± 1.5 %), PaT+Z (70.82 ± 1.43 %) and PaT+C (71.56 ± 1.89 %) than that in UPaT (68.93 ± 1.35 %). However, the percentage of available Zn was significantly higher ($P < 0.05$) in PaT+B (73.98 ± 1.36 %) compared to Zn proportion in UPaT. At 3rd collection (8 months), all the amended treatments recorded lower proportions of available Zn than in UPaT, but no significant difference ($P > 0.05$) between the percentage of bioavailable Zn in PaT+Fe⁰ (71.32 ± 1.32 %), PaT+B (72.13 ± 1.20 %) and PaT+C (74.18 ± 1.06 %) compared with UPaT (74.32 ± 1.96 %). Z (65.40 ± 0.27 %) did however reduce the percentage of Zn significantly ($P < 0.05$) in PaT samples collected at 8 months. At the end of study, there were no significant changes in the percentage of available Zn in any treatments except PaT+Z, which showed a negligible increase. PaT+B did however, decrease slightly compared to Zn proportion in the same treatment at 3rd collection. The Zn percentage was lower, though not significantly ($P > 0.05$) in PaT+Fe⁰ (71.99 ± 0.54 %), PaT+Z (67.68 ± 1.38 %), PaT+B (69.64 ± 3.38 %) and PaT+C (71.52 ± 1.07 %) than in the control (UPaT; 74.34 ± 0.98 %) in last sample collection. The Scheirer- Ray- Hare test of variance suggested that the interaction between incubation time and treatment had no significant effect on the mobility of Zn in amended PaT. On the other hand, incubation time and treatments separately had a significant ($P < 0.05$) effect on the mobility of Zn in treated and untreated PaT relative to the same treatments at the beginning of trial. The percentage of available Zn was significantly higher in all PaT treatments at the 4th collection compared to its proportion at the beginning of trial.

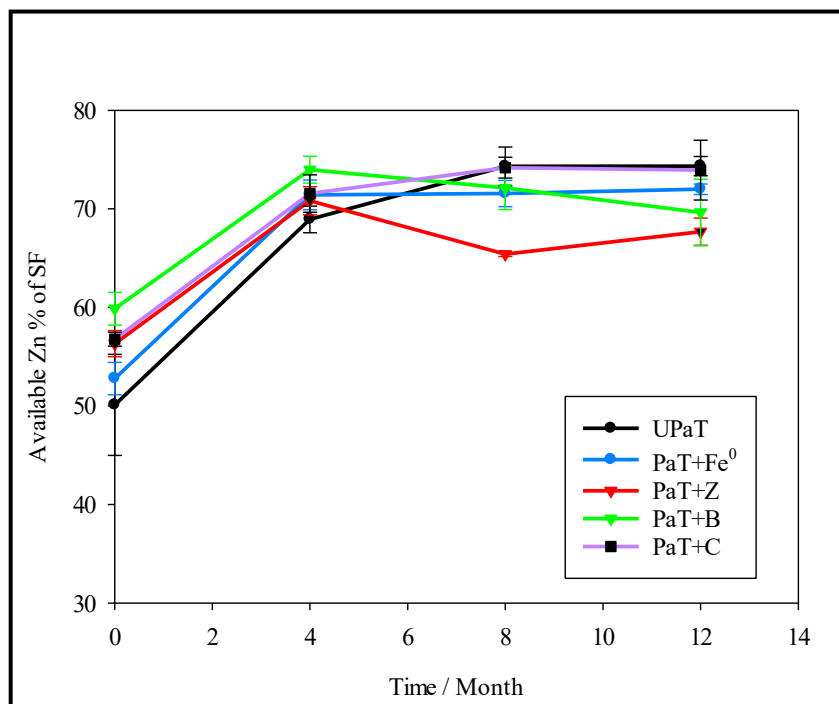


Figure 5. 13 The available % of Zn in all treatments at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar, PaT+C= Pandora tailings amended with compost SF the summation of Zn concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

4. Bioavailability of Pb percentage in Pandora tailings

At the beginning of experiment as figure 5.14 shows, the % of available Pb in PaT+Fe⁰ (83.90 ± 2.29 %), PaT+Z (83.56 ± 0.21 %), PaT+B (83.09 ± 1.22 %) and PaT+C (84.02 ± 0.42 %) were not significantly different ($P > 0.05$) from the control (84.52 ± 0.74 %). After 4 months, the percentage of available Pb decreased in all samples and the reduction was significant ($P < 0.05$) in the case of UPaT and PaT+C. The proportion of available Pb in all the amended tailings was higher than in UPaT but the difference was not statistically significant ($P > 0.05$). At 8 months, there were no significant differences ($P > 0.05$) in the percentage of available Pb between the amended treatments and UPaT. At the end of study, the proportion of available Pb in all the treatments were almost the same as at the 3rd and 4th sample collections. No significant difference ($P > 0.05$) was found between any of the amended treatments and the control by the end of the study. The Scheirer-Ray-Hare test of variance suggested that incubation time and treatment in combination, and treatment alone had no significant effect on the mobility of Pb in Pandora samples but incubation time significantly affected ($P < 0.05$) Pb mobility in all PaT treatments.

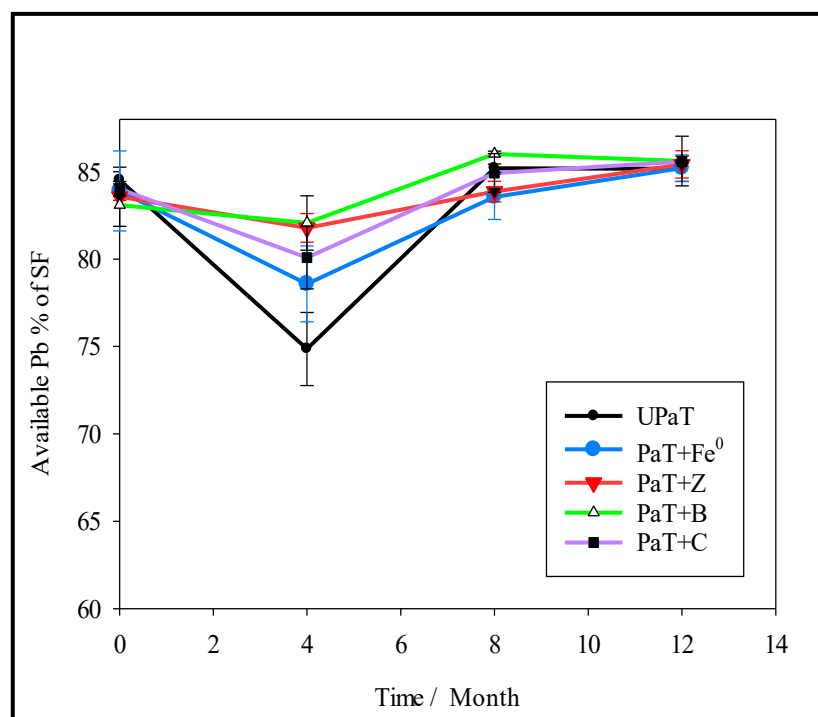


Figure 5. 14 The available % of Pb in all treatments at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar, PaT+C= Pandora tailings amended with compost SF the summation of Zn concentration in all fraction. Measurements presented as mean \pm S.E.M; n = 3.

5.4.5 Effects of amendments on the presence of studied heavy metals in leachate

The leachate collected from column experiments on four occasions were analysed for target metal concentration. All the levels of studied metals in the leachate (assuming the undetected levels of these metals is a half of AAS detection limit) exceeded the threshold levels of these metals in soil pore water, which are $60 \mu\text{g L}^{-1}$ for Zn, $5 \mu\text{g L}^{-1}$ for Pb, $20 \mu\text{g L}^{-1}$ for Cu and $1 \mu\text{g L}^{-1}$ for Cd (Tack *et al.*, 2006). The results are plotted in the figures (5.15-5.18).

5.4.5.1 Heavy metals in UPT and TPT leachate

1. The concentration of Cu in Parys leachate

The concentration of Cu in the leachate of all studied treatments were below the detection limit of AAS (0.23 mg L^{-1}) for the duration of the study.

2. The concentration of Cd in Parys leachate

The level of Cd in the leachate of all samples were not detectable in the collected samples from any of the treatments over the course of the experiment (detection limit of Cd by AAS $\geq 0.042 \text{ mg L}^{-1}$).

3. The concentration of Zn in Parys leachate

The incorporation of amendments to the tailings led to a significant ($P < 0.05$) decrease in Zn levels in the leachate of PaT+Fe⁰ ($0.67 \pm 0.04 \text{ mg L}^{-1}$), PaT+Z ($0.067 \pm 0.02 \text{ mg L}^{-1}$), PaT+B ($1.00 \pm 0.09 \text{ mg L}^{-1}$) and PaT+C ($1.67 \pm 0.40.10 \text{ mg L}^{-1}$) compared to UPT ($4.16 \pm 0.10 \text{ mg L}^{-1}$) at the beginning of trial. The most significant reduction was observed in PT+Z. At the second collection (4 months), the levels of leachable Zn decreased significantly in UPT ($0.73 \pm 0.26 \text{ mg L}^{-1}$) and slightly in PT+C and PT+B, but in PT+Fe⁰ and PT+Z changed little. There was no significant difference ($P > 0.05$) in the concentration of Zn between the amended treatments and the control at the 2nd collection with the exception of PT+Z which was significantly ($P < 0.05$) lower than UPT. After 8 months, the concentration of Zn in PT+Z and UPT were almost as at 4 months. However, the Zn levels increased to $1.36 \pm 0.31 \text{ mg L}^{-1}$ in PT+B and to $1.25 \pm 0.60 \text{ mg L}^{-1}$ in PaT+Fe⁰ though not significantly. A significant increase in PT+C, from $0.86 \pm 0.1 \text{ mg L}^{-1}$ at 4 months to $3.06 \pm 0.52 \text{ mg L}^{-1}$ at 8 months was observed. The concentration of leachable Zn was lower in all the amended tailings than in the control at the end of treatment period. In addition, the Zn levels in PT+ C, PT+Fe⁰ and PT+B decreased between 8 and 12 months and this reduction was significant in the case of PT+C which decreased from $3.06 \pm 0.52 \text{ mg L}^{-1}$ to $0.65 \pm 0.22 \text{ mg L}^{-1}$. Also, the leachable Zn was significantly lower ($P < 0.05$) in PaT+Fe⁰, PaT+Z, PaT+B and PaT+C compared to UPT ($1.74 \pm 0.23 \text{ mg L}^{-1}$). However, the concentration of Zn in the leachate of UPT increased significantly between 8 and 12 months from $0.61 \pm 0.22 \text{ mg L}^{-1}$ to $1.74 \pm 0.23 \text{ mg L}^{-1}$.

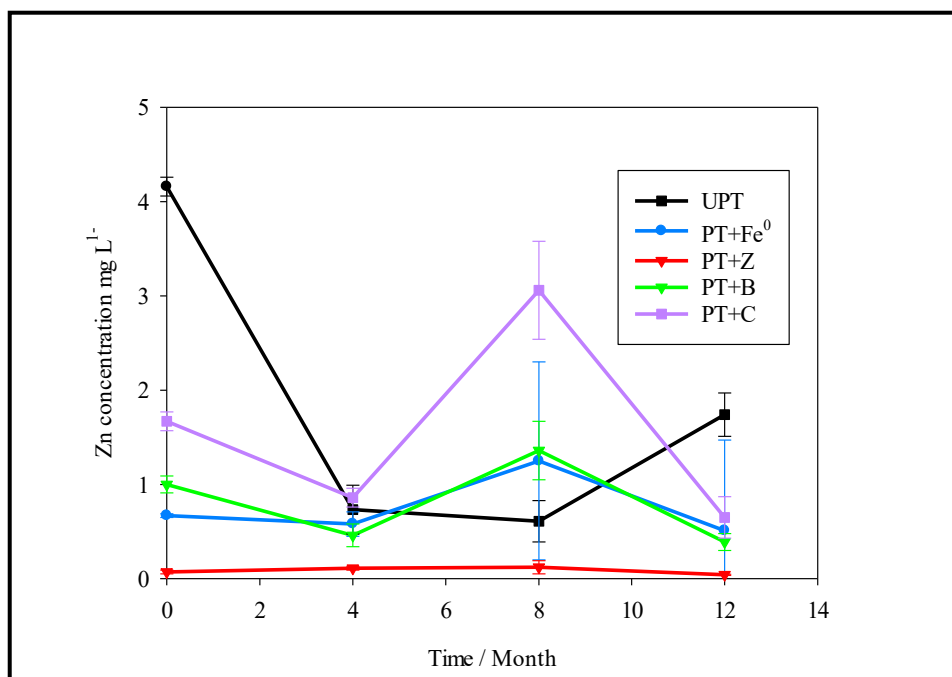


Figure 5. 15 Zn concentration in leachates of all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar and PT+C= Parys tailings amended with compost. Measurements presented as mean \pm S.E.M; n = 3.

4. The concentration of Pb in Parys leachate

At the beginning of experiment all the amendments reduced the concentration of leachable Pb significantly ($P < 0.05$) (Figure 5.16). This reduction were larger for the tailings treated with inorganic amendments (Z and Fe⁰) than for the organic amendments (B and C). After 4 months, the leachate content of Pb increased in all treatments. The increase was negligible for UPT but was greater for PT+B and PT+C, which showed a 145% and 155% increase from initial levels, respectively. The largest increase was observed for PT+Z and PT+Fe⁰ which increased by 836% and 895% of their initial levels, respectively compared to Pb levels in the same treatments at the beginning of trial. Even though, the levels of leachable Pb in PT+Fe⁰ ($5.77 \pm 1.07 \text{ mg L}^{-1}$) and PT+C ($6.90 \pm 0.82 \text{ mg L}^{-1}$) still lower but not significantly ($P > 0.05$) than that in UPT ($9.20 \pm 1.22 \text{ mg L}^{-1}$). However, Pb levels in PT+B ($4.37 \pm 1.05 \text{ mg L}^{-1}$) and PT+Z ($2.81 \pm 1.25 \text{ mg L}^{-1}$) were significantly ($P < 0.05$) lower compared to UPT at 2nd sample collection. Between 4 and 8 months, the concentration of Pb in the leachates decreased significantly ($P < 0.05$) and remained lower than the control in all treated tailings except for PT+C, which showed a slightly higher concentration than the control leachate. There were no significant difference ($P > 0.05$) between the treated tailings and the control after 8 months. At

the end of the experiment the leachable Pb levels were below the limit of detection of AAS for PT+Fe⁰, PT+B and PT+Z, but measured at $1.14 \pm 0.55 \text{ mg L}^{-1}$ in UPT. One exception is the Pb leachable levels in PT+C showed increasing up to $4.42 \pm 2.16 \text{ mg L}^{-1}$ which recorded significant difference ($P < 0.05$) with UPT during the period between 8 to 12 months.

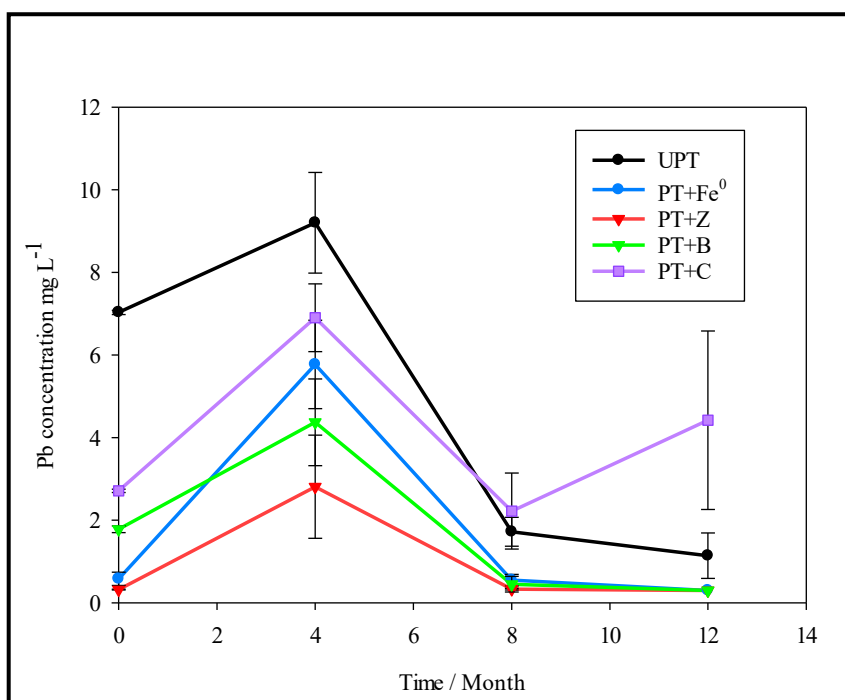


Figure 5. 16 Pb concentration in leachates of all treatments at 4 collection times during trial duration in UPT= untreated Parys tailings, PT+Fe⁰= Parys tailings amended with zero valent iron, PT+Z= Parys tailings amended with zeolite, PT+B= Parys tailings amended with biochar and PT+C= Parys tailings amended with compos. Measurements presented as mean \pm S.E.M; n = 3.

5.4.5.2 Heavy metals in UPaT and TPaT leachate

1. The concentration of Cu in Pandora leachate

The concentration of Cu in the leachate of all treatments of PaT were undetectable during the experiment period (the detection limit of AAS for Cu is $> 0.23 \text{ mg L}^{-1}$).

2. The concentration of Cd in Pandora leachate

Application of all amendments decreased the Cd concentration at the beginning of trial significantly ($P < 0.05$) compared to the control ($0.46 \pm 0.003 \text{ mg L}^{-1}$) (Figure 5.17). After 4 months (2nd sample collection) the levels of leachable Cd incensed in all treatments, but remained significantly lower ($P < 0.05$) than in UPaT ($0.70 \pm 0.02 \text{ mg L}^{-1}$). PaT+Z showed the lowest Cd concentration of all the treatments ($0.20 \pm 0.002 \text{ mg L}^{-1}$). After 8 months, the Cd levels decreased

in comparison with their levels in the same treatments at 4 months. At this time, the leachable Cd levels in PaT+Fe⁰ ($0.35 \pm 0.01 \text{ mg L}^{-1}$), PaT+Z ($0.26 \pm 0.02 \text{ mg L}^{-1}$), PaT+B ($0.52 \pm 0.01 \text{ mg L}^{-1}$) and PaT+C ($0.50 \pm 0.01 \text{ mg L}^{-1}$) remained significantly lower ($P < 0.05$) than in the control ($0.61 \pm 0.01 \text{ mg L}^{-1}$). Leachable Cd in PaT+Fe⁰ fell dramatically between 4 and 8 months, and this decrease was statistically significant ($P < 0.05$). At the end of the treatment period, the most effective ($P < 0.05$) amendments were Z ($0.18 \pm 0.06 \text{ mg L}^{-1}$) followed by Fe⁰ ($0.28 \pm 0.01 \text{ mg L}^{-1}$). However, there was no significant difference ($P > 0.05$) between the levels of Cd in the leachates of PaT+B ($0.47 \pm 0.01 \text{ mg L}^{-1}$) and PaT+C ($0.44 \pm 0.03 \text{ mg L}^{-1}$) and the control.

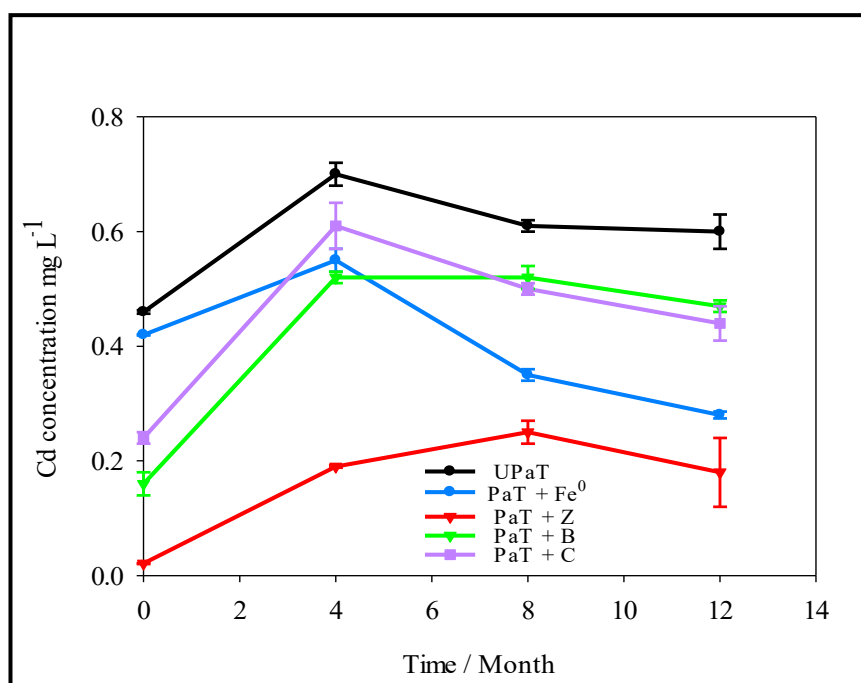


Figure 5. 17 Cd concentration in leachates at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar and PaT+C= Pandora tailings amended with compost. Measurements presented as mean \pm S.E.M; n = 3.

3. The concentration of Zn in Pandora leachate

As figure 5.18 shows, the addition of amendments to PaT reduced the concentration of leachable Zn significantly ($P < 0.05$) in PaT+Fe⁰ ($38.72 \pm 0 \text{ mg L}^{-1}$), PaT+Z ($0.17 \pm 0.02 \text{ mg L}^{-1}$), PaT+B ($16.87 \pm 1.18 \text{ mg L}^{-1}$) and PaT+C ($24.63 \pm 0.41 \text{ mg L}^{-1}$) compared to the control ($44.55 \pm 0.10 \text{ mg L}^{-1}$) initially. However, the level of Zn increased significantly in all treatments between 0 and 4 months. In addition, the levels of Zn in the leachate of most treated tailings was significantly ($P < 0.05$) lower than that in UPaT leachate, with only the Zn concentration in PaT+C leachate ($87.22 \pm 1.49 \text{ mg L}^{-1}$) close to that of UPaT ($89.65 \pm 1.11 \text{ mg L}^{-1}$). Between 4 and 8 months, the leachable levels of Zn decreased in all treatments in comparison with their levels in the same treatments of previous collection. At 8 months, in the samples collected from Pandora tailings treated with organic amendments C ($59.63 \pm 1.69 \text{ mg L}^{-1}$) and B ($55.30 \pm 0.60 \text{ mg L}^{-1}$) were not significantly higher ($P > 0.01$) than in UPaT ($54.67 \pm 2.70 \text{ mg L}^{-1}$). However, leachable levels of Zn were significantly lower ($P < 0.05$) in PaT+Z ($16.58 \pm 0.83 \text{ mg L}^{-1}$) and Pa+Fe⁰ ($18.52 \pm 0.88 \text{ mg L}^{-1}$) than that in UPaT. The reduction in Zn level continued gradually with time up to the end of experiment in all treatments. At the end of the experiment, the inorganic amendments had reduced the Zn levels the most, with Zn levels of $13.69 \pm 1.06 \text{ mg L}^{-1}$ and $6.49 \pm 2.30 \text{ mg L}^{-1}$ recorded for PaT+Fe⁰ and PaT+Z, respectively, compared with $54.27 \pm 3.21 \text{ mg L}^{-1}$ for the control. The levels of Zn in PaT samples amended with organic amendments were also lower than for UPaT, with values of $47.74 \pm 1.14 \text{ mg L}^{-1}$ recorded for B and $48.75 \pm 2.63 \text{ mg L}^{-1}$ for C, though these differences were not statistically significant ($P > 0.05$).

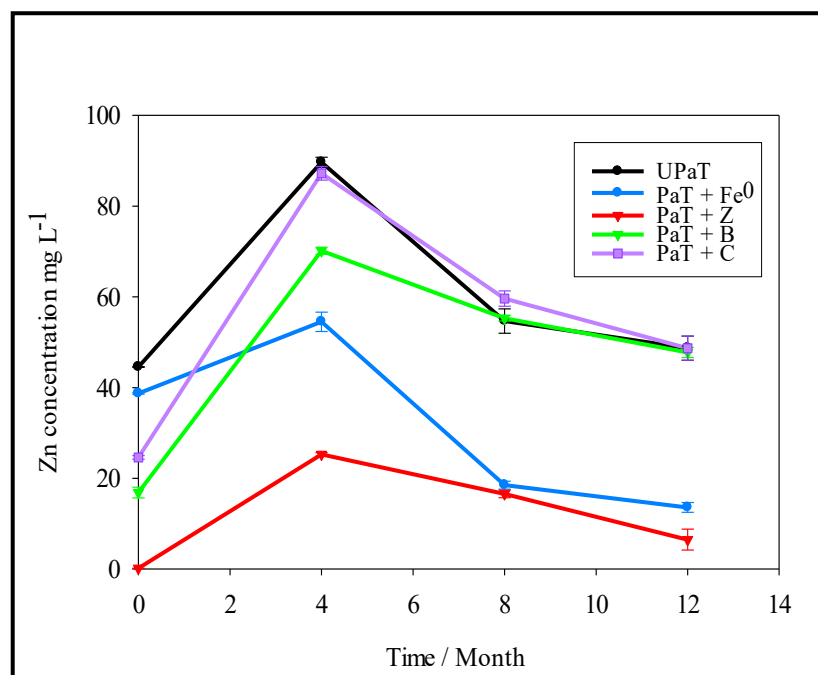


Figure 5. 18 Zn concentration in leachates at 4 collection times during trial duration in UPaT= untreated Pandora tailings, PaT+Fe⁰= Pandora tailings amended with zero valent iron, PaT+Z= Pandora tailings amended with zeolite, PaT+B= Pandora tailings amended with biochar and PaT+C= Pandora tailings amended with compost. Measurements presented as mean \pm S.E.M; n = 3.

4. The concentration of Pb in Pandora leachate

At the beginning of the study the amended tailings recorded lower levels of Pb in the leachate than in the control ($0.31 \pm 0.01 \text{ mg L}^{-1}$) with levels below the limit of detection in all treated tailings except for PaT+Fe⁰ ($0.30 \pm 0.001 \text{ mg L}^{-1}$). During the remainder of the experiment, no Pb was detected in any of the leachates including UPaT (detection limit of AAS for Pb > 0.29 mg L^{-1}) (Figure 5.19).

5.4.6 Effects of amendments on the percentage of target metals in reducible (F3), oxidisable (F4) and residual (F5) fractions

5.4.6.1 Parys tailings

1. Cu percentage

At the beginning of trial, the concentration of Cu increased in F3 in PT+Fe⁰ due to the generation of newly adsorbed sites (Fe oxides) on the surface of Fe⁰ which attracts Cu. In most treatments, a similar pattern of Cu distribution in F3, F4 and F5 was observed over the course of the study, although the Cu percentage in F3 and F4 decreased between 0 and 4 months and between 8 and 12 months. Conversely, the Cu proportion in F5 increased between 0 and 4 months

and between 8 and 12 months. At the end of study, the percentage of Cu in F3, F4 and F5 of UPT was lower than in PT+Z, PT+Fe⁰, PT+B and PT+C, though the difference was only statistically significant ($P < 0.01$).in the case of PT+Z.

2. Cd percentage

The incorporation of amendments altered the percentage of Cd in F3, F4 and F5. In most treatments, a similar decreasing trend in the proportion of Cd in F3 and F4 was observed, reaching below the detection limit of the AAS by the end of the experiment. One exception to this trend was PT+Fe⁰ where the percentage of Cd in F3 and F4 increased initially but later followed the same decreasing pattern as the others. Conversely, the percentage of Cd in F5 showed a significant increase ($P < 0.05$) initially in the case of PT+Z and PT+C. During the remainder of trial, there were no significant differences ($P > 0.05$) between the Cd levels in any treatment compared to UPT for any given sampling time.

3. Zn percentage

Applying Fe⁰ to PT increased the percentage of Zn in the F3 fraction at the first collection but the applications of Z, B and C decreased Zn percentage in F3 (though not significantly; $P > 0.05$) compared to the control. There also appeared to be a negative correlation between the proportion of Zn in the F3 and F4 fractions, and the proportion of Zn in the F5 fraction. For instance, the proportion of Zn in F3 was $3.99 \pm 0.27 \%$ in PT+Fe⁰ at 8 months and decreased to 0.29 ± 0.02 at the end of trial, but in F5 recorded $92.73 \pm 0.31 \%$ at 8 months and increased to $97.73 \pm 0.14 \%$ at the end of study.

4. Pb percentage

Application of all amendments at the beginning of study increased the percentage of Pb in F3 and F5 compared to the control. This increase in F3 was significant ($P < 0.05$) in tailings treated with Fe⁰ and C. As the trial progressed the proportion of Pb in F3, F4 and F5 followed the same pattern as Zn in all treatments. The proportion of Pb in F5 at the end of trial was higher in the amended tailings than in the control but the difference was not statistically significant ($P > 0.05$).

5.4.6.2 Pandora tailings

1. Cu percentage

The incorporation of Fe⁰ and C to PaT at the beginning of study significantly increased ($P < 0.05$) the percentage of Cu in F4 and F5 and decreased the proportion of Cu in F1+F2

significantly ($P < 0.05$) compared to the control. There was also an increase in the proportion of Cu in F3 but this was not statistically significant ($P > 0.05$). This may explain the significant decreased of available Cu percentage (F1+F2) in Fe^0 and C treated PaT and the negligible reduction of Cu availability proportion in PaT amended with Z and B at the beginning of the study. After 4 months, there was a significant decrease in the proportion of Cu in F3 and F5 in all treatments, leading to an increase in the proportion of Cu in the F1 and F2 fractions. The proportion of Cu in the different fractions fluctuated over the course of the study and being at the end of trial above that in the UPaT in each fraction separately, and higher than that in the same fractions in the same treatments but at the beginning of study.

2. Cd percentage

The percentage of Cd in the F3 and F4 fractions was higher in the amended treatments compared to the control at the first sample collection, with the difference statistically significant ($P < 0.01$) in the case of tailings amended with Fe^0 for both fractions. In all fraction the Cd proportion in most treatments were higher than Cd in UPaT at any given collection time.

3. Zn percentage

There were fluctuation in the proportion of Zn in different fractions over time with Zn appearing to show a higher affinity to reducible and residual fractions than the oxidisable fraction, since the proportion of Zn in the F3 and F5 fractions were higher.

4. Pb percentage

At the beginning of the experiment, the application of Z, B and C increased the proportion of Pb in F3 and F4 compared to UPaT, but this difference was not statistically significant. The proportion of Pb changed with time between increase and decrease being at the end of study lower in F3 of amended tailings than Pb in F3 for UPaT, but it was higher in F4 and F5 in treated tailings than in UPaT.

5.5 Discussion

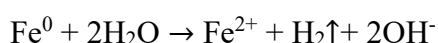
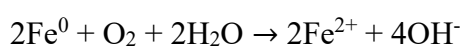
5.5.1 The effect of incorporated amendments on the pH of tailings

Alleviating the acidity of mine soils can inhibit heavy metal mobility. Mohamed *et al.* (2017) report, a 3-fold increase in the soil's adsorption capacity for several heavy metals was observed following an increase in the soil pH by one unit. In this study, the application of amendments to acidic mine tailings increased the pH of treated mine tailings, and was particularly effective in the most acidic mine tailings (PT). Therefore, results of current study

are in line with that obtained by several previous researches including Tandy *et al.* (2009) and Farrell *et al.* (2010). The reduction of mine tailings acidity amended with organic material, Fe⁰ and Z could depend on different factors, which differ in importance according to the amendment. Organic amendments (biochar and compost) may deliver inorganic substances which can modify the pH of tailings by replacing Ca²⁺ from OM surface and binding Al³⁺ and H⁺ on the OM surface to form insoluble complexes (Farrell *et al.*, 2010). Similarly, Rodríguez-Vila *et al.* (2015) reported that a decrease in the acidity of treated mine tailings can be attributed to the presence of basic cations e.g., Ca²⁺, Na⁺ and K⁺ supplied by organic wastes used as amendments. The presence of several functional groups on the surface of amendments e.g., carboxylic and phenolic groups in OM and OH⁻ on the generated Fe oxides and Si-OH and Al-OH groups in the zeolite framework may contribute to a decrease in acidity of acidic tailings. According to Houben and Sonnet (2015) a decrease in the acidity of treated tailings can be attribute to the presence of several deprotonated functional groups e.g., COO⁻ and O⁻ on the surfaces of amendments such as biochar which attract H⁺ ions, thereby reducing the H⁺ concentration in treated tailings solution.

In the present study, the incorporation of zeolite (Figure 5.4 A and B) to both tailings caused a significant increase in pH up to 8.14 ± 0.06 in PaT+Z compared to 6.72 ± 0.02 in the UPaT, and a 2-fold increase from 2.96 ± 0.02 in UPT to 6.17 ± 0.03 in PT+Z both at the beginning of trial. This may be due to the replacement of Na⁺ cations on the zeolite surface by H⁺ from the tailings solution (Edwards *et al.*, 1999). The extreme alkalinity (pH = 12.39) of raw zeolite material can neutralize the acidity of tailings. Querol *et al.* (2006) reported that the incorporation of synthetic zeolite with high alkalinity to acidic multi-metal contaminated soil caused a rise in soil pH from 3.50 to 7.50 after a 1-year remediation period. Indeed, Giannatou *et al.* (2016) concluded that zeolites amendments have a buffering effect due to their high alkalinity which acts to neutralise the extreme acidity of treated sulphide mine tailings.

The ability of Fe⁰ to increase the pH of extremely acidic tailings (from 2.90 ± 0.02 in UPT to 4.47 ± 0.04 in PT+Fe⁰ at the beginning of trial) resulted from the oxidation of Fe⁰ in the tailings solution in the presence of O₂ which releases OH⁻, and leads to a reduction in H⁺ concentration in the tailings solution, thus the pH increased as following reactions illustrate (O'Carroll *et al.*, 2013).



Generating newly Fe oxides may create active sites that can adsorb H^+ from tailings solution. However, the decrease in the pH of PaT+Fe⁰ (6.65 ± 0.06) at the beginning of trial compared to UPaT (6.72 ± 0.02) may be attributed to the corrosion reaction that can take place around natural pHs as a result of adding low rate (2%) of Fe⁰ to tailings. The corrosion reaction (see reaction equation below) is an OH⁻ consumer and H⁺ releaser and will therefore result in a reduction in pH (Hanauer *et al.*, 2011).



The decrease in pH observed in several treatments particularly between the first and second sample collection (in PaT+Z, PaT+B, PaT+C and PT+Fe⁰) may be related to the mineralisation of metal ore that is rich in sulphides. The oxidation of sulphides in mine waste causes the release of H⁺ into the tailings solution, thus decreasing the pH of the mine waste (Simon, 2005).

Generally, the application of amendments increased the pH of amended tailings, and it was most effective in the case of extremely acidic tailings (PT), the most effective amendment being zeolite, which increased the pH of extremely acidic tailings (PT) to a weakly acidic medium.

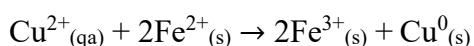
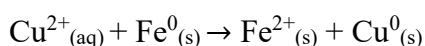
5.5.2 The effect of incorporated amendments on the bioavailable fractions of heavy metals

This study aimed at reducing the bioavailable fractions of Cu, Zn, Cd and Pb through the application of 2% Z, B, C and Fe⁰ separately to mine tailings. The results were variable with several treatments showing a fluctuation in bioavailability over the course of the study period (Figures 5.7 - 5.14). In addition, the mobility of several studied metals in various treated tailings were found to be higher than that in the control (UT). Therefore, the discussion of the current findings seeks to cover both the fluctuation and increased mobility we observed. A number of investigators have highlighted that several amendments may increase metal mobility. For instance, Pérez-de-Mora *et al.* (2006) and Kargar *et al.* (2015) reported that the bioavailability of heavy metals can increase or decrease as a result of treating heavy metal contaminated soils with organic amendments.

At the beginning of the treatment period most amendments showed a reduction in the available fraction of most investigated metals in both sites (Figures 5.7 - 5.14). The magnitude of this reduction differed from one treatment to another. The decreasing mobility of several metals in treated tailings can be attributed to different factors. Firstly, a decrease in metal mobility may have been caused by an increase in pH which was observed for all collection times

except in the PaT+Fe⁰ at first (Figure 5.4 A and B). Another reason is the type of amendment and the mechanisms were used by this amendment to retain target metal. Furthermore, each amendment has specific mechanisms that can involve in the heavy metal immobilization.

The reduction of metal availability in multi-metal contaminated tailings (PaT and PT) by Fe⁰ (e.g., Cu and Pb in PT+Fe⁰ in both collection times 1st and 4th separately (Figures 5.7 and 5.10), and Cu, Cd and Zn in PaT+Fe⁰ (Figures 5.11, 5.12 and 5.13) at the beginning of trial) may be related to the standard redox potential (E^0) of Fe⁰ and that for target element. E^0 of Fe⁰ (- 0.440V) allows Fe⁰ to reduce mobility of Pb and Cu via a reduction reaction because the E^0 of Pb and Cu is more positive than E^0 of Fe⁰ (O'Carroll *et al.*, 2013). The following reactions explain the reduction of Cu in aqueous solution (Pullin *et al.*, 2017).



Other mechanisms may also be involved in the immobilization of several heavy metals using Fe⁰ as a soil amendment. These mechanisms are the precipitation of Pb, and the adsorption, precipitation and complexation of Cu, Cd and Zn on the surface of the shell of Fe⁰ particles, which are shifted to Fe oxides after mixing Fe⁰ with tailings (O'Carroll *et al.*, 2013; Pullin *et al.*, 2017). Fe⁰ reduced the percentage of available Cu and Pb in PT as well as Cu, Cd and Zn in PaT initially. This may be related, as Hanauer *et al.* (2011) reported, to the generation of new iron oxides in treated multi-metal polluted soils creating new adsorptive sites on the surface of Fe⁰ particles allowing to these oxides to attract metal ions, thus reducing the available fraction of majority of metals present during the first hours or days of the trial. However, over time, the adsorption efficiency of Fe oxides is reduced, leading to release of previously adsorbed metals. This may explain the observation of higher availability percentage of Cu in PT+Fe⁰ at 2nd and 3rd sample collections, Zn in PT+Fe⁰ at the 3rd and 4th sample collections, Cu and Pb in PaT+Fe⁰ at 2nd sample collection relative to the controls (Figures 5.7, 5.9, 5.11 and 5.14 respectively). In addition, there are another several possible explanations for this. Firstly, the presence of several anions e.g., PO₄³⁻, NO₃⁻, SO₄²⁻, Cl⁻ and SiO₃²⁻ and cations including some plant macronutrients and other heavy metals in treated tailings may affect the capacity of Fe⁰ to retain target metal (Sun *et al.*, 2016a). For instance, Ou *et al.* (2017) reported that the presence of other heavy metal cations such as Pb had a significant effect on the adsorption of Cd from the solution to the surface of Fe oxides that have been formed after the Fe⁰ was added to the polluted sediments. This leads to a reduction in the ability of Fe⁰ to retain Cd in comparison with Pb retention. The fluctuation

of sample pH, particularly in PaT (Figure 5.4 A and B), and low pHs in PT may also affect the geochemistry of the studied metals. Mustafa *et al.* (2006) reported that, the ability of α -ferrous oxyhydroxide (α -FeOOH) to adsorb heavy metals is low at pH = 6 and decreased with increasing the soil acidity, which could explain the increasing of Cu mobility in PT+ Fe⁰ at 2nd and 3rd collections and in PaT+ Fe⁰ at 2nd collection (Figures 5.7 and 5.11). In the same way, Tiberg *et al.* (2016) concluded that Cu immobilisation by applying Fe⁰ to contaminated soil is pH dependent, and this amendment was not sufficient in reducing Cu mobility in the soils with a pH lower than 6. This may also explain the increase in the percentage of available Cu in PT at 2nd and 3rd collections. The results of our study agreed with that of Calderon and Fullana (2015) who reported that Fe⁰ was an effective adsorbent of Zn, Cu and Cd from polluted water during the first 2 h of the trial and with time the previously adsorbed heavy metal re-released to the treated water. Also, Vítková *et al.* (2017), concluded that Fe⁰ may be an ineffective amendment to immobilize all heavy metals and its capacity to retain these metals depended on the acidity, composition and the adsorption ability of target polluted soil. Also, these results agreed with those recorded by Hanauer *et al.* (2011) who used Fe⁰ to reduce the mobility of Cu, Cd and Zn in agriculture soils and found that the availability of Zn was not affected by Fe⁰ in the field compared to greenhouse trial. At the beginning of the present experiment, the Fe⁰ treatment reduced Cu and Pb in PT+Fe⁰ (Figures 5.7 and 5.10), and Cu, Cd and Zn in PaT+Fe⁰ (Figures 5.11, 5.12 and 5.13). This is in line with Kumpiene *et al.* (2006), who observed that the incorporation of 1% Fe⁰ to soil collected from an industrial area led to a reduction in the levels of Cu, Zn in the leachable and exchangeable fractions.

Organic amendments (compost (C) and biochar (B)) can inhibit the mobility of heavy metals by adsorbing these elements via ion exchange, electronic interaction and complexation reactions. The immobilization of heavy metals in soils using organic material can be attributed to the formation of insoluble complexes with humic substances. Conversely, the formation of soluble compounds with other organic chemicals in OM is expected which can enhance metal mobility (Zeng *et al.*, 2011; Ippolito *et al.*, 2017). The results of current study at the beginning of treatment period showed that the application of organic amendments (B and C) reduced the percentage of studied metals in the available fraction in all amended PT tailings and Cu in treated PaT, but no reduction was observed for Cd, Pb and Zn in PaT+C and PaT+B. The ability of C to minimize metal mobility has been supported by Farrell *et al.* (2010). They conducted a greenhouse trial using mine soil from Parys copper mine and concluded that the application of green waste-derived compost to acidic multi metal-polluted mine soil immobilized the phytoavailable fractions of heavy metals. Furthermore, C and B are likely to add anions that may

reduce the mobility of studied metals by precipitation with inorganic groups and ion exchange process (Zhang *et al.*, 2015). Zhang *et al.* (2005) state that the abundance of organic functional groups in OM leads to adsorb target metal through ion exchange as a main mechanism. In the PaT+C samples collected after 4 months, the available percentage of Cd (25.99 ± 4.21 %) was significantly lower than for UPaT (48.18 ± 16.19 %) (Figure 5.12), and Cd lower in PT+C and PT+B relative to UPT initially (Figures 5.8). In addition, the available proportion of Cu in PT+C and in PT+B at the beginning and the end of trial and Cu in PaT+C at first were lower than Cu percentage in the controls, separately. This reduction may be related to the OM functional groups that contain nitrogen e.g., amino group ($-NH_2$) which have a high affinity to Cd and Cu. Also, delivering anions e.g., CO_3^{2-} and PO_4^{3-} by OM to the treated tailings may reduce the mobility of several heavy metals by forming precipitated metal phosphate and carbonate (Zhang *et al.*, 2015). The formation of insoluble inorganic compounds may explain the reduction in metal mobility in the treated tailings by precipitation process. This may also explain the low available proportion of Pb in PT+C and PT+B at several points of time during the experiment (Figures 5.10). Lu *et al.* (2014) concluded that, the addition of bamboo and rice straw-derived biochars to multi-polluted soils reduced the soil acidity, thereby decreasing the mobility of target elements due to the formation of several insoluble compounds such as $CdCO_3$, $Cu(OH)_2$ and $Pb_5(PO_4)_3OH$. The results of column experiment showed lower levels of studied metals in the leachate, also in F1 and F2 in all tailings treated with B and C initially (e.g., Cu in PT and PaT) and in some cases later in the trial. This may suggest that the reduction in the availability of studied metals may be the result of conversion of these elements to another geochemical fraction as the proportion of target metals showed different behaviour in F3, F4 and F5 with their proportions fluctuating over time. Yuan *et al.* (2011) suggested that the immobilization of heavy metals in contaminated soil using several crop residues-derived biochars might be explained by the conversion of heavy metals to unavailable forms by the formation of metal carbonates, complexation with surface functional groups and co-precipitation with oxyhydroxides in the soil. The concentration of leachable Pb in PT+C at 4th collection and Zn in PT+B and PT+ C at the 3rd sample collection were higher than the proportion of these metals in the controls. This is in agreement with the findings of Kelly *et al.* (2014). They indicated that, the addition of biochars to mine tailings reduced the levels of Cu and Cd in the leachate significantly, but a negligible reduction of leachable Zn was observed at the beginning of incubation period. However, they observed no reduction in the levels of Pb in the leachate solution (this in line with the levels of Pb in the leachate of the PT+C treatment at the end of the present experiment, which showed levels higher than of control). The authors also showed that at the end of their experiment (65 days) the levels

of all studied elements in leachate were higher than their concentrations in control, and this increase was detected for Zn and Cd in the samples collected after 42 days.

Fluctuations in the proportional availability of several metals in PT and PaT with and without amendments may be due to the mineralization of ores during the trial releasing the free metal ions to the tailings solution. In addition, the leaching of metals ions by rainwater (depending on the rain events, Figure 5.19) to the surrounding environment is likely to happen led to decrease metal levels in F1 and F2 (Conesa *et al.*, 2006). Alternatively, the redistribution of these metals to other geochemical fractions, that mainly oxidisable fraction in the case of PT+C and PT+B may be also attributed the metal mobility fluctuation. In the samples collected from PT amended with B the percentage of available Cu was higher than that in UPT at the 3rd sample collection (Figures 5.7). This observation was detected when the application of hardwood-derived biochar and green waste compost were added separately to the polluted soil, which led to increase the available fraction of Cu as a result of the formation of soluble organic complexes with humic acid supplied by the amendments (Beesley *et al.*, 2011; Giannatou *et al.*, 2016). In the PaT samples collected at the end of trial, the proportion of Cu in F4 was higher than F3 and F5. This is likely to be due to the affinity of Cu to organic substance as the OM content recorded higher percentages than of previous collection times in the same treatments of both sites. Kabirinejad *et al.* (2014) reported that Cu has high affinity to organic substances and sulphides. Indeed the studied sites are rich in sulphides such as galena (PbS), sphalerite (ZnS) and chalcopyrite CuFeS₂ (Davies, 1987).

High percentage of available Zn in PT treated with C and B relative to the control at 2nd sample collection and later e.g., Zn proportions in PT+C (0.16 ± 0.05 %) and PT+B (0.30 ± 0.05 %) compared to UPT (0.04 ± 0.02 %) in samples of 4th collection (Figures 5.9). This may be related to the dissociation of several hydrogen bonds in the structure of organic compounds such as humic acid making the shape of the organic molecule planar and leading to the release of Zn stored on these compounds. This process may contribute to increased Zn availability (Kargar *et al.*, 2015). Houben *et al.* (2012) concluded that the ineffectiveness of several organic amendments in reducing Zn or Cd mobility could be attributed to the occurrence of Pb in soils that can form stronger organic complexes with fulvic acid anions than with Cd and Zn. Fulvic acid complexes are soluble in both acidic and alkaline conditions (Guimarães *et al.*, 2013) and this may explain the increased Zn mobility in treated PT at the end of trial specifically in PT+Z and PT+B relative to the control, also Cu mobility in PT+Z and PT+Fe⁰ at the 2nd and 3rd sample collections. This process may also explain the increased Zn availability in PaT+C and PaT+B

and the simultaneous decreased mobility of Pb in PaT+C at the 2nd sample collection (Figures 5.13 and 5.14, respectively).

The degradation of OM over the course of this experiment may explain the increase in the mobility of several heavy metals that had been retained by OM, which were added to the polluted soils as organic amendments (Karaca 2004). The decomposition of organic amendments produces CO₂ which may enhance the formation of PbCO₃ and ZnCO₃ (Pardo *et al.*, 2011). The significant increase at 4 months in the percentage of available Cu in PaT+C and PaT+B compared to its level in the same treatments at the beginning may be due to the formation of soluble organic complexes with Cu in a weak acid solution leading to an increase in Cu mobility in PaT+C and then a decrease with time. In uncontrolled conditions (such as in the current study) the application of several organic amendments to soil contaminated with Cd, Zn, Pb and Cu were ineffective in reducing the EDTA-extractable fraction of studied elements. In addition, the levels of some of these metals in the EDTA-extractable fraction were slightly higher than that in the control at the end of the study (Pérez-de-Mora *et al.*, 2006). Additionally, Kargar *et al.* (2015) stated that some types of biochar were not sufficient in decreasing the mobility of heavy metals in polluted soils. For instance, pine wood-derived biochar was not effective in reducing the leachable levels of Zn, Pb, Cd and Cu in mine tailing containing high levels of heavy metals. Similarly, Kloss *et al.* (2014) stated that the incorporation of biochar made from woodchip was not effective in the reducing the leachability of Pb, Cd and Cu in agriculture soils and they attributed this to an increase in the organic carbon pool.

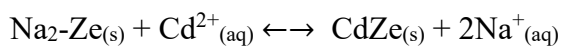
Increased metal availability may be related to the pH of treatments specially that of PaT treated with organic amendments. For example, at 2nd sample collection in the present trial the availability of Pb was higher in PaT+B and PaT+C than in UPaT (Figures 5.14). This supports by the observations of Pardo *et al.* (2011) who stated that the applications of organic amendments (olive mill wastes compost) increased the EDTA-extractable fraction of Pb. They attributed this to a change in pH and the nature of the OM in the used amendment. According to Tiberg *et al.* (2016), at pH > 6-7 (e.g., C and B-treated PaT) the Cu organic complexes may dissolved resulting in the release of Cu adsorbed onto OM and an increase in the mobility of Cu. This may also attribute the fluctuation of metal availability of amended tailings with B and C in several times during experiment. Also, Khan *et al.* (2015) observed that the addition of organic matter to heavy metal polluted soils was not effective in reducing Pb mobility.

The addition of zeolite to the acidic mine tailings resulted in a reduction in metal mobility in most treatments. For example, at the beginning of study, Z reduced the mobility of Cu, Cd, Zn and Pb in PT and Zn in PaT at 3rd sample collection (Figures 5.7, 5.8, 5.9, 5.10 and 5.13

respectively). Similarly, Z reduced Cu and Zn mobility in PaT at the end of experiment (Figures 5.11 and 5.13). The immobilisation of heavy metals using zeolites can be achieved *via* two main mechanisms; firstly by supplying alkalinity to acidic treated soil, with the increased pH promoting the adsorption of target heavy metals, and secondly *via* a cation exchange process in the zeolite framework (Querol *et al.*, 2006; Li *et al.*, 2009). Other mechanisms can also contribute in the immobilization of heavy metals using zeolites including precipitation, co-precipitation of metal with hydroxides and metal adsorption on the zeolite surface (Giannatou *et al.* 2016). In addition, the high CEC of Z (344 ± 4.16 meq/ 100g_{zeolite}) may allow Z to decrease the mobile fraction of heavy metals *via* cation exchange mechanisms. As Wen *et al.* (2016) report, zeolite has a high cation exchange capacity resulting from the replacement of silicon with aluminium creating negative charges on the surface of the zeolite molecule, and encouraging the adsorption of several metal cations.

Abollino *et al.* (2003) attributed the lower capacity of bentonite and montmorillonite as artificial zeolites to retain heavy metals to decreasing the pH of treatments due to the protonation of Si-OH and Al-OH groups. The ability of Z to retain heavy metals may be reduced with decreasing pH (during the trial pH decreased from 7.86 ± 0.02 at the 2nd sample collection to 7.12 ± 0.02 at the end of trial in PT+Z and from 8.18 ± 0.04 at the start of the experiment to 6.93 ± 0.03 at the end of the experiment in PaT+Z). The dosage of Z used (2%) in this study may not have been sufficient to immobilise several metals such as Cu and Zn in PT at the last sample collection. Querol *et al.* (2006) observed that the immobilisation rate of Cu, Cd, Pb and Zn, by synthetic zeolite type Na-P1-TE depended on the amount of Z with higher rates of immobilisation observed when a higher dosage of zeolite was added to the soil.

Competition may play a role in increasing the available proportion of several metals in some treatments which recorded levels in amended treatments were higher than in the controls in both sites. The properties of metals may have a significant effect on metal immobilization by amendments. As Wang and Peng (2010) state in their review, the interaction of heavy metals with different types of zeolites vary from one metal to another. The valences of the metals in PT and PaT may affect the ability of Z to retain them. In natural systems such as mine sites the presence of several metal cations with different valences can affect the ability of the amendments to retain other several metal cations. For example, Edwards *et al.* (1999) reported that zeolites have a higher selectivity for divalent cations than monovalent cations and the highest charged cations have the strongest interaction within the zeolite lattice *via* the ion exchange process as following equation illustrates:



The size of the metal ion may play an important role in the retention selectivity of the metal on the exchange sites of zeolite molecules because the metals have to move through the channels and micro pores of the zeolite lattices to replace exchangeable ions. Pb appeared to have highest adsorption rate than others and was present at extremely high levels in both explored sites ($74533 \pm 1572 \mu\text{g g}^{-1}$ and $15360 \pm 324 \mu\text{g g}^{-1}$ in PaT and PT tailings respectively) and Zn ($37476 \pm 797 \mu\text{g g}^{-1}$) in PaT. The very high levels of Pb and Zn with low application rate of amendment may cause increased metal mobility as stated by Giannatou *et al.* (2016). Who observed that the application of different zeolites to heavily contaminated soils ($42000 \mu\text{g g}^{-1}$ Pb and $29800 \mu\text{g g}^{-1}$ Zn) increased the levels of bioavailable of Pb and Zn in treated mine soils compared to the untreated soil. The high levels of Pb may affect the ability of Z to retain other heavy metals. According to Motsi *et al.* (2009) the variation of the ability of natural zeolite to retain heavy metal can be attributed to various features of the elements. For example, the hydrated radius of studied metals are; $\text{Pb}^{2+} = 0.401 \text{ nm}$, $\text{Cu}^{2+} = 0.419 \text{ nm}$, $\text{Zn}^{2+} = 0.430 \text{ nm}$ and $\text{Cd}^{2+} = 0.426 \text{ nm}$ and in heavily polluted soils the smallest hydrated cation (Pb^{2+}) may diffused inside the framework of zeolites easier and faster than other cations with a larger hydrated radius. Another factor that may explain the differences in the adsorption rate of different metal cations by zeolite is hydration energy which is $-2010 \text{ kJ mol}^{-1}$ for Cu^{2+} , $-1955 \text{ kJ mol}^{-1}$ for Zn^{2+} , $-1755 \text{ kJ mol}^{-1}$ for Cd^{2+} and $-1425 \text{ kJ mol}^{-1}$ for Pb^{2+} (Marcus, 1993). Metals with a smaller hydrated radius and higher hydration energy are preferentially adsorbed by amendments (Chaturvedi *et al.*, 2006; Motsi *et al.*, 2009). This may have reduced the adsorption of other metals and/or increased re-release them into the treated tailing solution.

Additionally, studies by Chaturvedi *et al.* (2006) and Shaheen *et al.* (2012) showed that, in multi-metal polluted soils, a competition phenomenon between elements to occupy active sites on the surface of amendments and bind with organic legends or metal oxides may occur. Another explanation for the difference in the affinity of metals to the organic amendments active sites relates to the Lewis acid-bases forces. For example, Pb^{2+} can form organic complexes with organic matrixes faster than Cd^{2+} and Cu^{2+} in the soil due to the great affinity of Pb^{2+} to the organic functional groups, particularly carboxylic and phenolic groups. These organic groups are classified as hard Lewis bases, preferentially binding strongly with hard or borderline hard Lewis acids such as Pb^{2+} . By contrast, Cd^{2+} and Cu^{2+} are classed as soft Lewis acids. In addition, metal with larger electronegativity may participate in a complexation reaction on the surface of amendment faster than others with lower electronegativity (Pearson, 1963; Alloway, 1990a).

Competition resulting from the factors described, in addition to variation in weather conditions may explain the variability in results obtained from the present study. Nwachukwu and Pulford (2008) state that, in multi-metal contaminated environments, Pb and Cu have a higher ability to be retained on the amendments adsorption sites than Zn, and Cu and Pb can displace adsorbed Zn resulting in increased Zn bioavailability.

The fluctuation of the availability of several metals over time in some treatments in comparison to the control may be also related to changes in environmental conditions since this study was conducted in uncontrolled conditions. The results showed a decrease in the proportion of Cu in the bioavailable fractions in all samples of PaT by the end of study linked to a seasonal change in weather conditions from drier to wetter conditions as figure 5.19 illustrates. Hanauer *et al.* (2011) attributed the variability of available Cu in the control treatment to natural seasonal variations in the mobility of Cu, which was lower in spring and higher in autumn. In addition, Shen and Alessi (2018) stated that long term remediation period of heavy metal polluted soil using different amendments may be effected by several environmental factors e.g., annual climate cycle, plant presence and microbial activities. For example, in the present study, 4 months after amendments were added to PaT, the % of available Zn increased significantly ($P < 0.05$) by nearly 65% compared to initial levels in all treatments and was higher in all the amended PaT treatments than in UPaT. This major increase can be attributed to the mineralization of ZnS ore in the presence of water and O_2 during the first four months of the experiment (Dec.2015 to Apr. 2016). High levels of Zn (e.g., $40645 \pm 1334 \mu\text{g g}^{-1}$ Zn in UPaT) and changing weather conditions may have caused the release of free Zn, making it available to plants and soil biota. O'Connor *et al.* (2018) stated that high rainfall and seasonal temperature variation could affect the solid-liquid equilibrium of heavy metals in soils.

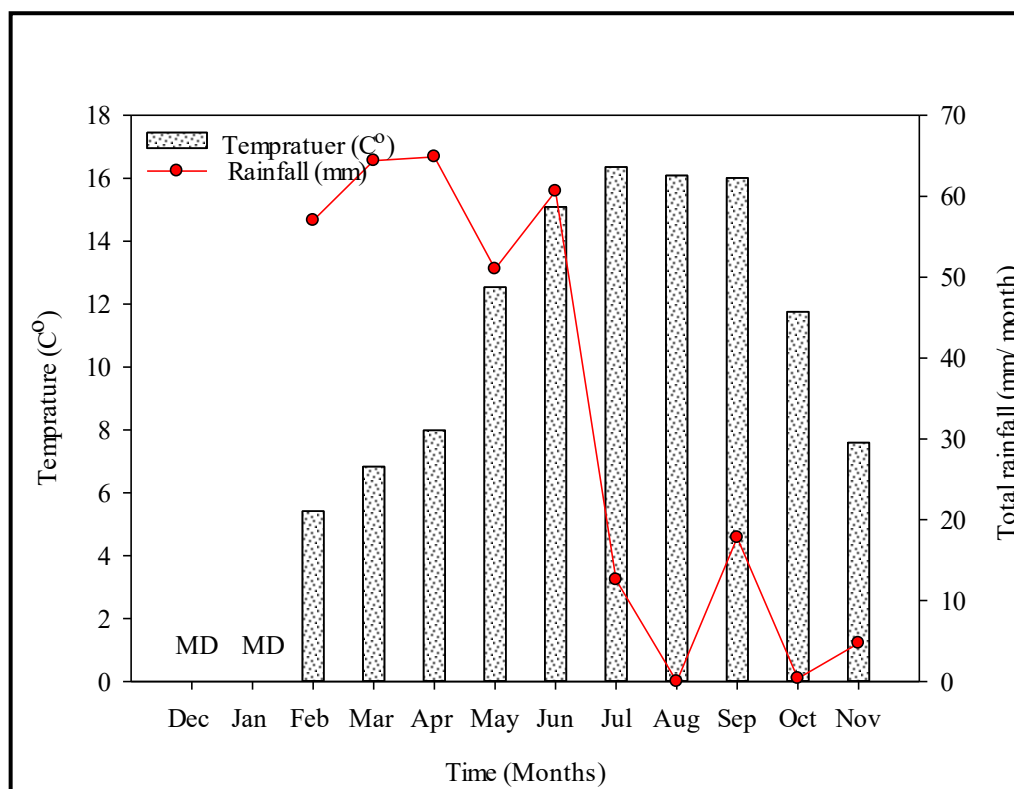


Figure 5. 19 Total rainfall in millimetres and mean air temperature in Celsius recorded at Henfaes research centre during the study duration from Dec. 2015 to Nov. 2016. MD = missing data (Source; Henfaes research station archives).

5.6 Conclusion

In this experiment, we demonstrated the effect of green waste compost, green waste biochar, zeolite and (Fe^0) on the availability of heavy metals in multi-contaminated mine tailings from Parys Mountain and Pandora mine. Results showed that the addition of amendments increased the pH of all samples at the beginning of treatment period compared to untreated tailings and remained broadly higher than the pH in untreated tailings in both sites at the end of the trial. The levels of organic matter in the samples treated with biochar and compost increased. The concentrations of explored elements in the bioavailable fractions (water-soluble and exchangeable fractions) were lower than that in untreated tailings in both sites initially and then fluctuated with time. At the end of trial, the levels of several studied metals in the bioavailable fractions were generally lower than in the unamended tailings. However, at the end of treatment period, bioavailability of Zn in all treated Parys tailings and Cu in Parys tailings treated with zeolite were higher than in unamended tailings and the levels of available Pb and Cd in amended Pandora tailings were not significantly different to the untreated tailings. We concluded that the

application of organic and inorganic amendments have diverse effects on the bioavailability of investigated elements, and the doses of amendments used were not sufficient to reduce the mobility of several heavy metal significantly. Further studies are required by applying higher dosage of organic and inorganic amendments and/or using them simultaneously with plants to investigate their effect on the bioavailability and uptake of heavy metals by plants in controlled conditions and in the field.

6 USING LEAD ISOTOPES TO IDENTIFY GEOCHEMICAL ROUTES OF LEAD CONTAMINATION IN MINE TAILINGS REMEDIATED WITH RYEGRASS AND UNCONTAMINATED SOIL

6.1 Introduction

Metallurgical processes released large amounts of potentially waste into surrounding areas (Rodríguez-Vila *et al.*, 2015b). The hazardous contents of mine tailings are reported to cause harm to flora and fauna and the wider ecosystem (Anawar, 2015). Water leaching from mine tailings with high levels of heavy metals, particularly after rainfall, has caused the degradation of the surrounding agronomic land and leaches down into groundwater where it will be present for hundreds of years (Mackay *et al.*, 2013). Consequently, these dangerous components are being transported through the food chain and can enter the human body (Bird, 2011). Due to all of these impacts on ecosystems, revegetation of mine sites have considered as a potential remediation strategy (Menzies *et al.*, 2007; Asensio *et al.*, 2013; Banerjee *et al.*, 2016).

Phytoremediation is based on establishment of several heavy metal-tolerant plants with high biomass yield. This approach is reported to be a relatively simple, suitable for energy production, cost effective and environmentally friendly remediation strategy (Chirakkara *et al.*, 2016; Anh *et al.*, 2017; Fang *et al.*, 2017; Bhatti *et al.*, 2018). Also, establishing plants in mine sites can improve some physical and chemical properties of mine tailings e.g., elevating the pH, OM content and the concentrations of several nutrients and activating several biological cycles (Arienzo *et al.*, 2004). More than 400 plants are classified as heavy metal accumulators. The *Brassicaceae* family includes a large number of accumulator species, which can tolerate high concentrations of heavy metals. Mahar *et al.* (2016) noted that, hyper-accumulators are plants that can accumulate heavy metals in their tissues to a level from 50 to 500 times of that in other plants e.g., *Lamiaceae*, *Fabaceae* and *Asteraceae* families. They can also store 100 times higher concentrations in the above ground biomass without any effect on their growth. Several hyper-accumulators appear to accumulate more than 10,000 mg Zn and Cu, >1000 mg Pb and >100 mg Cd per kilogram of dry plant shoots in heavily polluted soil (Bini *et al.*, 2012). These plants can tolerate high levels of heavy metals such as in mine tailings soil using different techniques such as rhizofiltration, rhizodegradation, phytostabilisation and phytoextraction (Sun *et al.*, 2016b). Ryegrass has also been used in phytoremediation technology due to its ability to tolerate elevated levels of heavy metal in heavily multi-metal polluted soil (Cui *et al.*, 2016b). The stabilisation of heavy metals in plant roots grown in heavily polluted soil even with lower levels accumulated in the roots of that was expected is likely promised technology because of the establishing of

plants can accumulate high levels of heavy metals in their roots still far away (Zhang *et al.*, 2018). The remains of plants used in phytoremediation can be used in generating bioenergy, as a raw material for pyrolysis and in wood production (Shaheen and Rinklebe, 2015; Kovacs and Szemmelveisz, 2017; Touceda-González *et al.*, 2017). Khalid *et al.* (2017) treated mine sites using various fractions of fertile uncontaminated soil to replace the tailings, thus improving the mixture of tailing-soil fertility and biological functions. Similarly, Douay *et al.* (2008) used the technique of adding uncontaminated soil to dilute contaminated garden soils. The garden soils contain $3300 \mu\text{g g}^{-1}$ Pb and $24 \mu\text{g g}^{-1}$ Cd resulting from smelting of Pb and Cd ores, consequently target soil functionality and vegetable biomass production were improved.

The levels of Pb are very high in the areas investigated in the present study. The regions where the Pb mines and smelters are located have been found with contaminated water, soil and flora (Nakata *et al.*, 2016). Humans started mining for Pb thousands of years ago. Globally, 1Mt of Pb has been produced since discovering the Pb industry (Komárek *et al.*, 2008). Lead sources can be classified as either un-indigenous or indigenous sources. Anthropogenic sources vary and include leaded gasoline, mining and smelting lead ores, whilst geogenic sources are due to the weathering of the parent rocks. As reported by Brewer *et al.* (2016) human activities are considered as the major sources of Pb pollution. It was estimated that 95% of Pb contamination is because of anthropogenic sources. Soil and plants are the primary receivers of Pb during the weathering period of rocks. Uptake by plant root systems is considered as the main entry route of lead into plant tissues (Miller *et al.*, 2007; Brewer *et al.*, 2016). The use of total and fraction concentrations of trace metals is not sufficient to determine the original sources and geochemical routes of heavy metals (Ettler *et al.*, 2006).

To classify Pb pollution sources, different geochemical tracers have been used. The choice of technique for investigating contamination sources is dependent on the source of pollution and underlying bedrock heterogeneity. For example, to distinguish between different sources of pollution, and identify the origin and the geological routes of lead, its isotopes have been commonly used to distinguish between the pollution sources in aquatic and terrestrial ecosystem (Miller *et al.*, 2007; Bird *et al.*, 2010; Bird, 2011; Luo *et al.*, 2014; Brewer *et al.*, 2016). Pb within the natural environment is found in four main isotopes. ^{206}Pb , ^{207}Pb , ^{208}Pb are the dominant isotopes with 98% of all lead isotopes occurring at this mass (Nakata *et al.*, 2016). These isotopes have long half-lives and are produced because of the decay of ^{238}U , ^{235}U and ^{232}Th , respectively. The half-life time of lead is 1.4×10^{17} years (Shotyk *et al.*, 2015). The fourth isotope ^{204}Pb is the stable isotope and rare (1.4%) in abundance (Reimann *et al.*, 2012). Due to the relatively high abundance of ^{206}Pb , ^{207}Pb , ^{208}Pb and the ability to determine them with reasonable accuracy the

composition of Pb isotopes was widely used in the tracer of Pb sources in environment (Das *et al.*, 2018). This isotopic composition can provide beneficial information about Pb fingerprinting and valuable indicators for Pb pollution routes classification (Region *et al.*, 2018). To distinguish between different Pb pollution sources the $^{206}\text{Pb}/^{207}\text{Pb}$ composition ratio was used along with other isotopic ratios, for example when $^{206}\text{Pb}/^{207}\text{Pb}$ ratios are ranging from 1.18 to 1.19 reflecting indigenous Pb pollution routes such as Pb ores and Pb naturally occurring. In addition, moderate ratios of these isotopes ranging from 1.14 to 1.16 indicating both indigenous and non-indigenous Pb fingerprinting sources such as waste incineration and Pb secondary smelting of them, however, low $^{206}\text{Pb}/^{207}\text{Pb}$ ratios ranging from 1.11 to 1.13 may be attributed to the anthropogenic sources of Pb, such as leaded gasoline (Bellis *et al.*, 2004; Shotyk *et al.*, 2015). High isotopic ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ recording values more than 1.18 were found, reflecting a high radiogenic content of the Pb isotope (^{206}Pb). This was due to the involvement of Pb isotopes in the weathering of soil parent materials over time, as the levels of ^{207}Pb did not show significant change because the decay of ^{235}U was completed, but because the decay of ^{238}U is still in process, as it still has a high level of occurrence on Earth. However, lower $^{206}\text{Pb}/^{207}\text{Pb}$ ratios (1.06-1.10) reflecting lower levels of radiogenic isotopes (^{206}Pb) as both ^{235}U and ^{238}U are still in the process of decay (Hansmann and Köppel, 2000; Komárek *et al.*, 2008).

6.2 Objectives

This study is aimed at:

- 1- Evaluating the translocation of Cu, Zn, Cd and Pb from tailings to ryegrass grown in controlled conditions in tailings collected from the Parys Mountain and Pandora sites in order to examine the efficacy of using this type of grass in phytoremediation strategy. This will involve calculating the transfer degree (TD), bioaccumulation factor (BF) and Biological Accumulation coefficients (BAC).
- 2- Characterizing the sources of Pb in mine tailings by measuring lead isotope concentrations (^{206}Pb , ^{207}Pb and ^{208}Pb) in mine tailings.
- 3- Evaluating the Pb isotopic signature of translocated Pb and to evaluate the dominant sources of Pb in plants.

By achieving these aims, the study seeks to answer the following questions:

- To what extent is Cu, Zn, Cd and Pb translocated through the roots to the shoots of plant systems?
- Is Pb pollution generated from anthropogenic or geogenic sources?
- Does Pb present within plants originate from a dominant source?

6.3 Methodology

6.3.1 Study Sites and sample collection and preparation

Soil and mine tailings was collected randomly in January 2017 from Parys copper mine (PT) and Pandora lead mine (PaT) (approximately 60.0 kg of wet material from each site) (chapter 3 section 3.3.1 and 3.3.2). Uncontaminated sandy soil (US) was collected randomly from an agricultural field situated at Henfaes research station, Abergwyngregyn, Gwynedd, UK (53° 14' N, 4° 01' W) (approximately 60.0 kg of wet soil). The collected samples were air-dried in a glass house for 14 days then sieved to < 2mm and stored in polyethylene bags at 4 °C to perform target trials.

6.3.2 Experimental design

Dry mine tailings (PT and PaT; section 6.3.1) were mixed thoroughly by hand in percentage of 100, 75, 50, 25, 0 with dry US soil. Each mixture was divided between three 0.5 L pots, with each pot containing 300 g of mixture. The pots were watered with deionised water up to 70% of the water holding capacity (WHC) of tailings, and incubated in a greenhouse under controlled condition for a week. 0.6 g (approximately 202 ± 4.4 seeds) of type Aber HSG 2 ryegrass seeds (IGER, 2005) (from Henfaes Seeds Bank) were then sown into each pot and irrigated with commercial diluted nutrient solution as plant fertilizer (2 g L^{-1} of N:P:K=28:18:16) as required for 6 weeks, all the treatments received the same quantity of nutrients solution. Which is recommended to establishing grass cover on mine sites (Chaney and Baklanov, 2017). During the trial, the pots were kept in a greenhouse under controlled conditions (18-22°C in natural light, photoperiod approx. 15 h). The ryegrass was harvested 45 days after germination. Roots and shoots were separated and washed gently with tap water and put in a 0.05 % triton solution for 10 min. The grass material was then rinsed with deionised water before being dried at 80°C for 3 days and weighed (Perkins *et al.*, 2016). The shoot samples were milled using a FOSS CT 193 Cyclotec™ mill and the root samples ground using a RETSCH MM 200 grinder. Milled and ground samples were stored in polyethylene plastic bags until further analysis.

6.3.3 Soil and mine tailings analysis

pH and EC were measured in tailings suspensions of 1:2.5 tailings: water (w/v) ratio. Moisture and organic matter content were evaluated gravimetrically by the loss-on-ignition method. H_2O -extractable NO_3^- -N and H_2O -extractable NH_4^+ -N were measured according to the procedure described in section 4.2.3.2 (Mulvaney, 1996). The total content of investigated heavy metals was measured by acid digestion and analysed using atomic absorption spectroscopy following the protocol described in section 4.3.3.2.8.

6.3.4 NH₄OH-extractable levels (phytoavailable fraction) of Cu, Zn, Cd and Pb in mine tailings (T) and uncontaminated soil (US) mixtures

The levels of the extractable fraction of target metals was measured using 1M of ammonium acetate (Edwards *et al.*, 1999; Bolan *et al.*, 2003; Menzies *et al.*, 2007). Organic salts are designed to act similar to the root excretion of low molecular weight organic acids in the rhizosphere (Vargas *et al.*, 2016). Also, ammonium acetate can extract the phytoavailable fraction of heavy metals from the soil medium effectively without any effect on the other fractions (reducible, oxidisable and residual fractions) of heavy metals (Yu *et al.*, 2004). The extraction followed the second step of the sequential extraction procedure described in section 4.3.3.2.9.

6.3.5 Plant analysis

The sub-samples of roots and shoots were digested separately using the following procedure: 0.3 g of ground shoot sample and 0.25 g of ground root sample were placed in a 100 mL borosilicate digestion tubes followed by 10 mL of 70% nitric acid (≥ 99.999 % trace metal basis). Each tube was covered and left overnight to digest in the fume cupboard before being heated on a digestive block at 100 °C for 3 h in the fume cupboard. The samples were left to cool to room temperature before being filtered through Whatman 42 filter paper. The remains of the sample was washed three times and the solution being made up to a total volume of 20 mL. Solutions were stored at 4°C before analysis of total Pb, Zn, Cd and Cu by atomic absorption spectroscopy (Khan and Jones, 2009). A Magnetic Sector ICP-MS was used to measure lead isotopes in selected samples (Perkins *et al.*, 2016).

6.3.6 The efficiency of metal accumulation

According to Malik *et al.* (2010) and Bini *et al.* (2012) the efficiency of metal accumulation in plants can be estimated by calculating the BF, TD and BAC as following equations show:

BF = Concentration of metal in roots / Concentration of metal in tailings

TD = Concentration of metal in shoots / Concentration of metal in roots

BAC = Concentration of metal in shoots / Concentration of metal in tailings

BF and BAC values greater than 1, suggest that the investigated plant is suitable for use in the phytoextraction of heavy metals from polluted soils. Alternatively, if the TD value is less than 1 and the BF value is greater than 1 the studied plant may be used in the phytostabilisation of toxic metals. To be considered the examined plant a hyper-accumulator, one or both of the BF and TD values should be greater than 1.

6.3.7 Statistical analysis

The data were analysed statistically using SPSS v 22.0 software with SigmaPlot v 12.5 used to present the data graphically. The results are presented as mean \pm SEM ($n = 3$) with the analysed soil, tailings and plant tissue samples treated as dry material. A Shapiro-Wilk test was used to check the normality of data. To investigate the effect of the addition of US to tailings on various physical and chemical properties of the mixtures, as well as the accumulation of investigated metals in ryegrass roots and their translocation within plant tissues, a one-way ANOVA with a Tukey HSD post hoc test ($P < 0.05$ and 0.01) was used. The results measured below the detection limit of AAS are reported as BD (below detection limit), but were assumed to be half of AAS (USEPA, 2000) detection limit for calculating the BAC, TD and BF factors from the row data, and presenting the data graphically. Linear regression was performed to examine the relationships between several physiochemical properties of tailings–soil mixtures and plant parameters.

6.4 Results and discussion

6.4.1 Physio-chemical characteristics of mine tailings (T) and uncontaminated soil (US) mixtures

The tested parameters for both tailings and their mixtures with uncontaminated soil (US) are presented in tables 6.1 and 6.2. The pH of Parys mine tailings (PT) increased significantly ($P < 0.05$) as the proportion of uncontaminated soil (US) increased; the pH recorded 3.62 ± 0.02 when the mixture contained 75% PT+25% US and recorded 4.73 ± 0.02 in the mixture containing 25% PT+75% US: (Table 6.1). The reduction in the acidity of different mixtures of PT and US can be ascribed to the dilution effect of US which recorded pH 5.86 ± 0.02 and/or the ability of US to attract H^+ from the tailings solution and exchange them with other cations occupying the exchangeable sites on US (Kabata-Pendias and Pendias, 2001). This process makes the exchanged cations more available to enhance plant growth. Electrical conductivity (EC) did not show a significant change up to the mixture of 25% PT+75% US, but was significantly reduced relative to the mixture containing 100% PT. The OM content decreased significantly ($P < 0.05$) with increasing percentage of US, measuring $6.68 \pm 0.07\%$ in 100% PT, and $3.22 \pm 0.02\%$ in 100% US due to the dilution effect and probably the effect of OM mineralization via several soil biota supplied to the treatments by US.

The concentration of H_2O -extractable NO_3^- -N increased significantly ($P < 0.05$) as the US fraction increased. This may attributed to the increasing the pH of PT+US mixtures and the decreasing of total metal content with increasing the fraction of US enhancing the rate of

nitrification (Jeschke *et al.*, 2013) or to the dilution effect of US, as the available NO_3^- -N was the highest ($7.02 \pm 0.22 \mu\text{g g}^{-1}$) in the 100% US samples. The H_2O -extractable NH_4^+ -N levels was higher in 75% PT+25% US ($2.90 \pm 0.10 \mu\text{g g}^{-1}$) compared to 100% PT ($2.43 \pm 0.48 \mu\text{g g}^{-1}$) and then declined as the proportion of US in the mixtures increased, though this reduction was not significant ($P > 0.05$). The increase observed may be attributed to the same factors described above which may have contributed to the rise in NO_3^- -N levels.

The addition of US to PaT resulted in an insignificant ($P > 0.05$) change in the pH from 6.84 ± 0.02 in 25% PaT+75% US to 6.88 ± 0.01 in 50% PaT+50% US, 6.85 ± 0.01 in 75% PaT+25% US and 6.82 ± 0.01 in 100% PaT (Table 6.2). The EC of the 25% PaT+75% US mixture was significantly lower than the control, but the reduction in the other mixtures was insignificant. The addition of US to PaT resulted in a similar pattern of decreasing OM levels with increasing proportions of US, with $7.26 \pm 0.04 \%$ recorded for 100% PaT, and $4.14 \pm 0.10\%$ for 25% PaT+75% US. The levels of H_2O -extractable NO_3^- -N in the PaT treatments increased significantly ($P < 0.05$) as the proportion of US increased. H_2O -extractable NO_3^- -N by 55.76% in 75% PaT+25% US, 112% in 50%PaT+50%US and 172% in 25% PaT+75% US related to the control 100% PaT, which may attributed to the reduction of the pseudo-total levels of target metals (Jeschke *et al.*, 2013). Also, there was a significant increase in the concentration of H_2O -extractable NH_4^+ -N in 50% PaT+50% US ($1.94 \pm 0.09 \mu\text{g g}^{-1}$) compared to 100% PaT ($1.52 \pm 0.09 \mu\text{g g}^{-1}$), but for 25% PaT+75% US only a minor increase was observed compared with 100% PaT. H_2O -extractable NH_4^+ -N in 75% PaT+25% US was the same as for 100%PaT.

Table 6. 1 Physical and chemical properties of Parys tailings (PT) and PT mixtures with uncontaminated soil (US) (values presented as mean \pm S.E.M; n = 3).

Parameter	100% PT	75% PT + 25% US	50% PT + 50% US	25% PT + 75% US	100% US
pH	3.23 \pm 0.02 ^a	3.62 \pm 0.02 ^b	4.11 \pm 0.01 ^c	4.73 \pm 0.02 ^d	5.86 \pm 0.03 ^e
EC <i>mS/cm</i>	0.52 \pm 0.003 ^a	0.46 \pm 0.01 ^{a, b}	0.47 \pm 0.01 ^{a, b}	0.44 \pm 0.02 ^b	0.26 \pm 0.01 ^c
O. M %	6.68 \pm 0.07 ^a	5.73 \pm 0.06 ^b	4.91 \pm 0.04 ^c	4.05 \pm 0.04 ^d	3.22 \pm 0.02 ^e
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	0.19 \pm 0.01 ^a	1.15 \pm 0.07 ^b	3.56 \pm 0.13 ^c	5.57 \pm 0.09 ^d	7.02 \pm 0.22 ^e
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	2.43 \pm 0.48 ^{a, b}	2.90 \pm 0.10 ^b	2.51 \pm 0.29 ^{a, b}	2.11 \pm 0.10 ^a	1.74 \pm 0.14 ^a
Extractable metals (μ g g ⁻¹)					
Cu	16.51 \pm 1.12 ^a	13.17 \pm 0.72 ^a	13.77 \pm 2.12 ^a	5.08 \pm 1.06 ^b	BD
Zn	37.07 \pm 5.73 ^a	23.54 \pm 2.48 ^{a, b}	18.34 \pm 0.59 ^{b, c}	14.69 \pm 0.72 ^{b, c}	7.22 \pm 0.22 ^e
Cd	2.33 \pm 0.64 ^a	2.20 \pm 0.09 ^a	1.73 \pm 0.70 ^a	1.54 \pm 0.28 ^a	BD
Pb	340.0 \pm 10.41 ^a	285.8 \pm 25.87 ^a	184.2 \pm 23.93 ^b	61.80 \pm 5.28 ^c	BD
Total metals (μ g g ⁻¹)					
Cu	949.7 \pm 24.20 ^a	633.4 \pm 2.69 ^b	396.9 \pm 5.22 ^c	221.7 \pm 9.54 ^d	BD
Zn	13140 \pm 572 ^a	7085 \pm 192 ^b	5575 \pm 15.53 ^c	3880 \pm 827 ^d	18.67 \pm 0.22 ^e
Cd	39.93 \pm 0.77 ^a	26.90 \pm 0.45 ^b	16.40 \pm 0.61 ^c	7.93 \pm 0.18 ^d	BD
Pb	6570 \pm 187 ^a	5320 \pm 573 ^a	3170 \pm 144 ^b	1916 \pm 94 ^b	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 6. 2 Physical and chemical properties of Pandora tailings (PaT) and PaT mixtures with uncontaminated soil (US) (values presented as mean \pm S.E.M; n = 3).

Parameter	100% PaT	75% PaT + 25% US	50% PaT + 50% US	25% PaT + 75% US	100% US
pH	6.82 \pm 0.01 ^a	6.85 \pm 0.01 ^a	6.88 \pm 0.01 ^a	6.84 \pm 0.02 ^a	5.86 \pm 0.03 ^b
EC <i>mS/cm</i>	0.45 \pm 0.02 ^a	0.45 \pm 0.01 ^a	0.42 \pm 0.01 ^a	0.35 \pm 0.02 ^b	0.26 \pm 0.01 ^c
O. M %	7.26 \pm 0.04 ^a	6.01 \pm 0.1 ^b	5.14 \pm 0.08 ^c	4.14 \pm 0.1 ^d	3.22 \pm 0.02 ^e
H ₂ O-extractable NO ₃ ⁻ -N $\mu\text{g g}^{-1}$	2.17 \pm 0.03 ^a	3.38 \pm 0.10 ^b	4.61 \pm 0.36 ^c	5.90 \pm 0.19 ^d	7.02 \pm 0.22 ^e
H ₂ O-extractable NH ₄ ⁺ -N $\mu\text{g g}^{-1}$	1.52 \pm 0.09 ^a	1.52 \pm 0.06 ^a	1.94 \pm 0.09 ^b	1.76 \pm 0.2 ^{a, b}	1.74 \pm 0.14 ^{a, b}
Extractable metals ($\mu\text{g g}^{-1}$)					
Cu	71.33 \pm 3.68 ^a	61.14 \pm 1.16 ^a	42.75 \pm 6.11 ^b	29.57 \pm 3.52 ^b	BD
Zn	25463 \pm 732 ^a	20077 \pm 390 ^b	14838 \pm 428 ^c	7029 \pm 186 ^d	7.22 \pm 0.22 ^e
Cd	32.42 \pm 0.60 ^a	28.17 \pm 0.82 ^b	21.44 \pm 0.35 ^c	10.32 \pm 0.34 ^d	BD
Pb	90187 \pm 2870 ^a	73227 \pm 1950 ^b	44853 \pm 387 ^c	17733 \pm 970 ^d	BD
Total metals ($\mu\text{g g}^{-1}$)					
Cu	192.3 \pm 3.70 ^a	137.3 \pm 7.47 ^b	81.60 \pm 0.61 ^c	35.07 \pm 3.70 ^d	BD
Zn	30145 \pm 1170 ^a	22679 \pm 457 ^b	17841 \pm 720 ^c	8865 \pm 274 ^d	18.67 \pm 0.23 ^e
Cd	91.47 \pm 7.13 ^a	68.93 \pm 7.87 ^a	37.47 \pm 2.19 ^b	19.07 \pm 1.04 ^b	BD
Pb	100160 \pm 2518 ^a	73840 \pm 2010 ^b	52240 \pm 2474 ^c	24427 \pm 1793 ^d	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. BD= below detection limit.

6.4.2 Ryegrass biomass

The grass grew well in the pots containing US only, as figures 6.1 – 6.4 show, but the discussion will not be focussed on these pots because of they do not contain tailings and the behaviour of plant growth was as expected in this agricultural soil. Figures 6.1 and 6.2 show the ryegrass grown in different mixtures of PT + US, control (100% PT) and 100% US. The addition of US appeared to stimulate the growth of ryegrass as the weight of dry shoots increased significantly ($P < 0.05$) by 20-fold from $0.11 \pm 0.02 \text{ g pot}^{-1}$ in 100% PT to $2.2 \pm 0.06 \text{ g pot}^{-1}$ in the mixture of 25% PT+75% US. Similarly, the length of shoots increased significantly ($P < 0.05$) by approximately 4 times from $5.67 \pm 0.34 \text{ cm}$ in the 100% PT mixture to $22.00 \pm 0.29 \text{ cm}$ in the 25% PT + 75% US mixture. The same pattern was observed in the root dry matter weight which generally showed a significant increase from very low weight (unmeasurable) in 100% PT to $0.82 \pm 0.04 \text{ g pot}^{-1}$ in the 25% PT+75% US treatment (Figure 6.1). This increase in the biomass of ryegrass may be due to a decrease in mixture acidity as the ryegrass growth parameters correlated significantly ($P < 0.05$) and positively with the pH of PT mixtures. The increase in ryegrass growth rate with increasing the US proportion may also related to increasing levels of nutrients delivered by US such as NO_3^- - N and pH alteration. It appeared that the ryegrass grown in 25 % PT +75 % US and 50% PT + 50 % US produced a high biomass and developed good cover within 45 days.

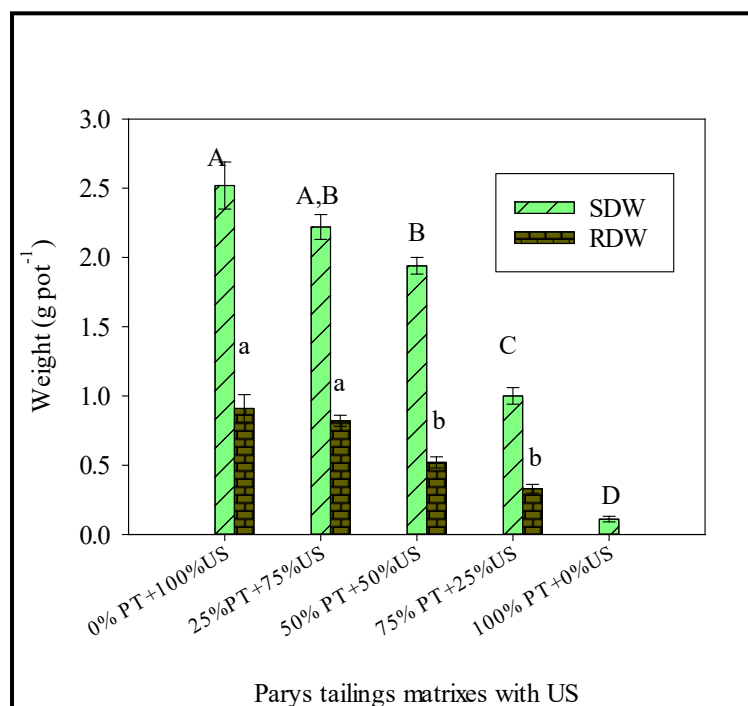


Figure 6. 1 Dry weight (g pot^{-1}) of shoots (SDW) and roots (RDW) of ryegrass after 45 days of growth in different mixtures of Parys tailings (PT) and uncontaminated soil (US). Same upper case letters (A, B, C and D) show no statistic significant difference among the SDW, but same lower case letters (a and b) illustrate no statistic significant difference between RDW of grass, roots of 100%PT+0%US was unmeasurable. Results are presented as mean \pm SEM, $n = 3$.

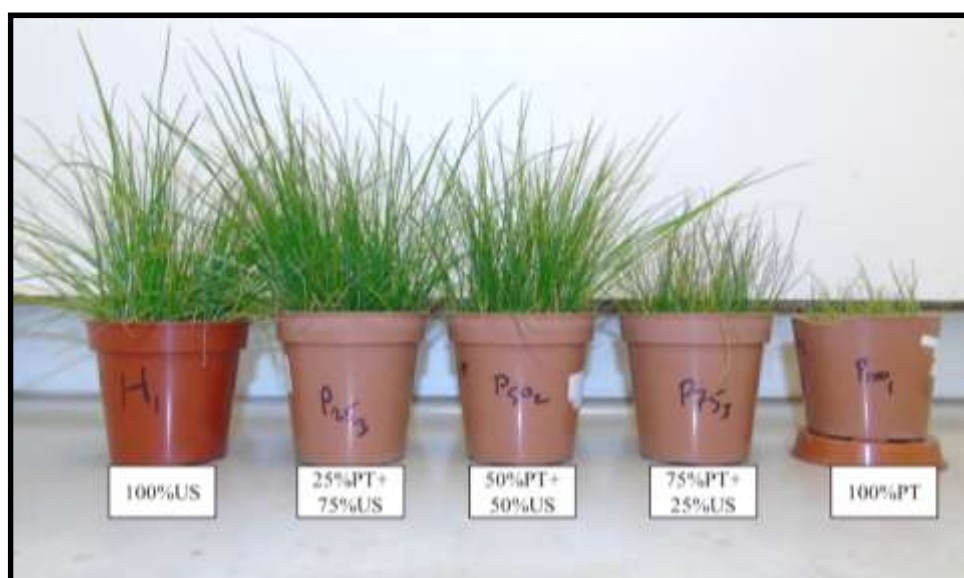


Figure 6. 2 Ryegrass growth in different mixtures 45 days after germination, from left to right: 100% uncontaminated soil (US), 25 % PT + 75 % of US, 50 % of PT + 50 % of US, 75 % of PT + 25 % of US and 100% of PT.

Ryegrass grown in 100%PaT and its mixtures combined with US (Figures 6.3 and 6.4), generally did not show a significant difference ($P > 0.05$) in the shoot length and biomass in comparison to the control (100% PaT) with the exception of the 25% PaT+75% US treatment which showed significantly higher ($P < 0.05$) shoot length compared to the control (100%PaT). In most cases, the dry weight of shoot material in the combined treatments also showed no significant difference compared to control (100% PaT). One exception to this was shoot dry weight for in 25% PaT+75% US, which was significantly higher ($P < 0.05$) than the control (100% PaT). Roots dry weight increased, though not significantly ($P < 0.05$) with increasing proportion of US. Figure 6.4 shows a view of the exterior appearance of the grass in all the mixtures of PaT+US and in 100% PaT the ryegrass growth stunted and chlorosis occurred. The grass grown in the mixture of 25% PaT+75% US appeared to be healthier than that grown in the other PaT and US mixtures. However, in terms of plant production, all of PaT and US mixtures produced similar biomass to 100% PaT, which may suggest that phytoremediation of PaT can be conducted without any addition of US. Because the pH of PaT and its mixtures with US did not show significant change, therefor the amelioration of nutrients levels e.g., NO_3^- -N levels increased with increasing US proportion may stimulate the insignificant grass development in PaT mixtures rather than the pH.

As tables 6.1 and 6.2 show, the mixtures containing mine tailings either with or without US, showed high levels of Pb, Zn, Cd and Cu, which exceeded the limitation levels of these metals in European agricultural soils. These are set at $200 \mu\text{g g}^{-1}$ for Zn, $60 \mu\text{g g}^{-1}$ for Pb, $1 \mu\text{g g}^{-1}$ for Cd and $100 \mu\text{g g}^{-1}$ for Cu (Tóth *et al.*, 2016). Thus, the negative effect of these metals on plant growth is expected. Yet, Cu and Zn are macronutrients, as the plants need them at lower levels, Cu can act as an enzyme catalyst and Zn is involved in the structure of several proteins. However, at high levels, which are more than $200 \mu\text{g g}^{-1}$ of Cu and $400 \mu\text{g g}^{-1}$ of Zn, these metals become phytotoxic (Grotz and Guerinot, 2006). For example, the high levels of Cu in soils may cause oxidative stress leads to disturb the metabolic process and macromolecules damaging (Ambika *et al.*, 2016), which may be attributed the poor developing rate of ryegrass. In the case of PaT, the high levels of Pb and Zn (Table 6.2) appeared to inhibit ryegrass growth in the samples of both tailings without US and in the 75% PT+25% US treatment and all the mixtures of PaT. According to Kabata-Pendias and Mukherjee (2007) indications of Zn toxicity is expected at levels above $400 \mu\text{g g}^{-1}$ in plants. This is supported in the present study by the significant negative correlation between total and extractable levels of studied metals, and plant growth parameters in the PaT mixtures ($P < 0.05$). It appears that the high acidity and heavy

metals in the investigated tailings adversely affected the development of ryegrass roots. As Mohamed *et al.* (2017) and Fang *et al.* (2017) state, the main indicator of Pb toxicity is a reduced rate of root development resulting from reduced cell division. Pb can also affect the availability of K^+ and Ca^{2+} to plants. Ca^{2+} and K^+ are essential for plant as macronutrients, K^+ acts to control the pH in cell cytoplasm and the extension of plant cells (Malkowski *et al.*, 2002) and Ca^{2+} is necessary for plant growth as it plays a role in several plant cell functions (Li *et al.*, 2007). The reduced root growth rate is likely to be related to the direct exposure of roots to the tailings content of toxic metals. The addition of US enhanced the establishment of grass in both sites but was more effective in PT because of pH amelioration. Also, increasing the pool of NO_3^- -N by increasing the fraction of US may have enhanced the plant development in both sites, which supported statistically by significant positive relationships between plant parameters and treatment pH and extractable NO_3^- -N in PT treatment and plant parameters with extractable NO_3^- -N in PaT. Similar symptoms of root growth reduction can be linked to the occurrence of Cu at toxic levels above $20 \mu g g^{-1}$ in tested soils (Marschner, 2011). Karami *et al.* (2011) also observed that the ryegrass grown in unamended mine tailings suffered from chlorosis and low biomass productivity due to the presence of Cu and Pb at total levels of $603 \pm 23 \mu g g^{-1}$, $20703 \pm 2501 \mu g g^{-1}$, respectively.

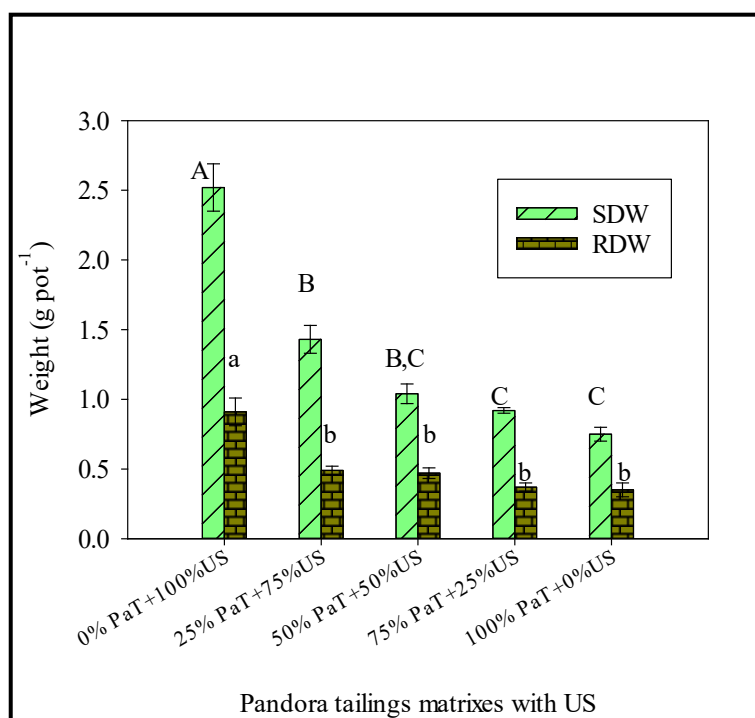


Figure 6. 3 Dry weight (g pot⁻¹) of shoots (SDW) and roots (RDW) of ryegrass after 45 days of growth in different mixtures of Pandora tailings (PaT) and uncontaminated soil (US). Same upper case letters (A, B, C and D) show no statistic significant difference among the SDW, but same lower case letters (a and b) illustrate no statistic significant difference between RDW of grass, roots of 100%PT+0%US was unmeasurable. Results are presented as mean \pm SEM, n = 3.



Figure 6. 4 Ryegrass growth in different mixtures 45 days of germination, from left to right; 100% uncontaminated soil (US), 25 % of Pandora mine tailings (PaT) + 75 % of US, 50 % of PaT + 50 % (w) of US, 75 % of PaT + 25 % of US and 100% of PaT.

Table 6. 3 Several parameters of Ryegrass shoots grown in Parys tailings (PT) and PT mixtures with uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% PT	75% PT +25% US	50% PT +50% US	25% PT+75% US	100% US
Shoots length (cm)	5.67 \pm 0.34 ^a	13.0 \pm 0.58 ^b	19.50 \pm 0.29 ^c	22.00 \pm 0.29 ^d	21.50 \pm 0. 29 ^d
Shoots dry weight (g pot ⁻¹)	0.11 \pm 0.02 ^a	1.00 \pm 0.06 ^b	1.94 \pm 0.02 ^c	2.22 \pm 0.06 ^d	2.52 \pm 0.17 ^e
Total elements ($\mu\text{g g}^{-1}$)					
Cu	41.67 \pm 1.17 ^a	32.80 \pm 0.35 ^b	22.11 \pm 0.43 ^c	17.92 \pm 1.59 ^c	BD
Zn	1121 \pm 103 ^a	172 \pm 8.39 ^b	117 \pm 4.16 ^b	51.59 \pm 0.15 ^b	19.51 \pm 3.68 ^b
Cd	6.32 \pm 1.24 ^a	1.37 \pm 0.13 ^b	1.30 \pm 0.15 ^b	0.84 \pm 0.21 ^b	0.61 \pm 0.40 ^b
Pb	BD	BD	BD	BD	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. BD = undetectable levels.

Table 6. 4 Several parameters of Ryegrass roots grown in Parys tailings (PT) and PT mixtures with uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% PT	75% PT +25% US	50% PT +50% US	25% PT+75% US	100% US
Roots dry weight (g pot ⁻¹)	DM	0.33 \pm 0.03 ^a	0.52 \pm 0.02 ^a	0.82 \pm 0.04 ^b	0.91 \pm 0.10 ^b
Total elements ($\mu\text{g g}^{-1}$)					
Cu	DM	137.1 \pm 12.76 ^{a, b}	90.84 \pm 9.01 ^a	166.40 \pm 22.6 ^b	5.00 \pm 0.1 ^c
Zn	DM	596 \pm 30.0 ^a	546 \pm 22.40 ^a	356.6 \pm 13.0 ^c	180.6 \pm 47.10 ^d
Cd	DM	5.50 \pm 0.79 ^a	2.93 \pm 0.41 ^b	2.53 \pm 0.08 ^b	1.2 \pm 0.08 ^b
Pb	DM	232.96 \pm 16.6 ^a	76.44 \pm 3.88 ^b	60.44 \pm 6.17 ^c	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. DM = did not measured, and BD= below detection limit.

Table 6. 5 Several parameters of Ryegrass shoots grown in Pandora tailings (PaT) and PaT mixtures with uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% PaT	75% PaT +25% US	50% PaT +50% US	25% PaT+75% US	100% US
Shoots length (cm)	8.17 \pm 0.18 ^a	8.67 \pm 0.44 ^a	9.82 \pm 0.17 ^a	12.50 \pm 0.58 ^b	20.50 \pm 0.29 ^c
Shoots dry weight (g pot ⁻¹)	0.75 \pm 0.05 ^a	0.92 \pm 0.02 ^a	1.04 \pm 0.07 ^{a,b}	1.43 \pm 0.10 ^b	2.52 \pm 0.17 ^c
Total elements ($\mu\text{g g}^{-1}$)					
Cu	26.04 \pm 2.17 ^a	30.04 \pm 1.62 ^a	29.33 \pm 1.52 ^a	13.92 \pm 1.65 ^b	BD
Zn	1767 \pm 84 ^a	1178 \pm 48.20 ^b	923 \pm 30.75 ^c	705.50 \pm 66.72 ^c	19.51 \pm 3.68 ^d
Cd	5.95 \pm 0.62 ^a	5.12 \pm 0.43 ^a	2.67 \pm 0.73 ^b	0.84 \pm 0.40 ^{b,c}	0.61 \pm 0.40 ^c
Pb	122.67 \pm 12.22 ^a	70.22 \pm 48.14 ^b	76.95 \pm 8.02 ^b	74.48 \pm 6.78 ^b	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 6. 6 Several parameters of Ryegrass roots grown in Pandora tailings (PaT) and PaT mixtures with uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% PaT	75% PaT +25% US	50% PaT +50% US	25% PaT+75% US	100% US
Roots dry weight (g pot ⁻¹)	0.35 \pm 0.05 ^a	0.37 \pm 0.03 ^a	0.47 \pm 0.038 ^a	0.49 \pm 0.03 ^a	0.91 \pm 0.10 ^b
Total elements ($\mu\text{g g}^{-1}$)					
Cu	377.9 \pm 8.26 ^a	424.1 \pm 10.45 ^a	162.4 \pm 7.71 ^b	124.1 \pm 11.09 ^b	5.00 \pm 0.10 ^c
Zn	1657 \pm 31.90 ^a	1521 \pm 141 ^a	1364 \pm 48.71 ^a	944 \pm 60.76 ^b	180.60 \pm 47.10 ^c
Cd	70.31 \pm 0.88 ^a	74.76 \pm 0.57 ^a	65.29 \pm 3.32 ^a	29.50 \pm 0.49 ^b	1.2 \pm 0.08 ^c
Pb	10265 \pm 587 ^a	10377 \pm 538 ^a	11076 \pm 145 ^a	10876 \pm 309 ^a	BD

Different lower case letters within the same row indicate a significant difference according to Tukey's HSD test at P < 0.05. BD= below detection limit.

6.4.3 The effect of adding US to PT and PaT tailings on the NH₄OH-extractable levels (phytoavailable fraction) of Cu, Zn, Cd and Pb

The application of uncontaminated soil to mine tailings led to a reduction in the phytoavailable fraction of explored metals. The NH₄OH-extractable fraction of Cu decreased but not significantly ($P > 0.05$) in the mixtures of 75% PT + 25% US by 20% and in 50% PT+50% US by 17% and declined significantly in 25% PT+75% US by 69% compared to the control (100% PT). The addition of US decreased NH₄OH-extractable Zn significantly ($P < 0.05$) in the mixtures of 50% PT+50% US by 52% and in 25% PT+75% US by 61% in comparison to the control (100% UPT). A reduction was also observed in the 75% PT+25% US treatment compared to the control, though the difference was not statistically significant. NH₄OH-extractable Cd in all mixtures showed no significant difference compared with the control (100% PT). NH₄OH-extractable Pb decreased significantly ($P < 0.05$) with increasing the proportions of US by 16% in 75% PT+25% US, 46% in 50% PT+50% US and 82% in 25% PT+75% US relative to 100% PT.

In the mixtures of PaT (Table 6.2), the addition of US significantly decreased ($P < 0.05$) the extractable levels of target metals in most mixtures compared to 100% PaT. The phytoavailable Cu fraction was significantly reduced ($P < 0.05$) with the addition of US by 40% for 50% PaT+50% US and by 58% for 25% PaT+75% US compared to the control (100% PaT). However, the reduction was not significant in 75% PaT+ 25% US compared to 100% PaT. Zn showed very high levels in the phytoavailable fraction and decreased significantly with increasing the proportion of US. The concentration of NH₄OH-extractable Cd decreased significantly ($P < 0.05$) in the following order 100% PaT > 75% PaT+25% US > 50% PaT+50% US > 25% PaT+75% US. The extreme levels of NH₄OH-extractable Pb in 100% PaT ($90187 \pm 2870 \mu\text{g g}^{-1}$) decreased significantly ($P < 0.05$) with the addition of US and this reduction was proportional to the decrease in the percentage of PaT. Extractable Pb decreased by 19% in 75% PaT + 25% US, 50% in 50% PaT + 50% US, and 80% in 25% PaT+75% US. The phytoavailable fractions of studied metals in both tailings and their mixtures correlated positively and significantly with total levels of these metals.

The reduction in the NH₄OH-extractable fraction of investigated elements following the addition of US can be attributed to a number of factors. Firstly, this may have been caused by a dilution effect as the proportion of US in the mixtures increased resulting in a decrease of the pseudo-total concentration of metals as the proportions of tailings in the mixtures decreased. Secondly, the effect may be linked to the ability of US to adsorb part of the available fraction of target metals when added to tailings through ion exchange, complexation or co-

precipitation with Fe and Mn oxides and other soil compounds. This reduction in the extractable fraction of Cu, Cd, Zn and Pb will diminish the negative effects of heavy metals on soil biota, and reduce the translocation of these toxic metals to plants (Kabata-Pendias and Pendias, 2001).

6.4.4 Distribution of studied heavy metals in ryegrass tissues and transfer degree (TD), bioaccumulation factor (BF) and biological accumulation coefficients (BAC)

6.4.4.1 Ryegrass grown in Parys tailings mixtures

Tables 6.3 and 6.4 and figure 6.5 illustrate the shoots and roots content of studied metals and table 6.7 shows several factors calculated to study the feasibility of using ryegrass as a phytoremediation technique. The pseudo-total levels of target metals decreased significantly ($P < 0.05$) as the proportion of US increased. Cu, Cd and Pb were undetectable in US but Zn recorded $18.67 \pm 0.22 \mu\text{g g}^{-1}$. The highest heavy metal concentration found in 100% PT was for Zn ($13140 \pm 572 \mu\text{g g}^{-1}$). Zn remained the most abundant heavy metal in all the PT mixtures (Table 6.1).

The levels of investigated metals in ryegrass shoots decreased as the proportion of US increased except in the case of Pb, which was undetectable in all plant shoots grown in the mixtures of PT, and Cu, which was undetectable in the shoots of 100% US. Significantly lower ($P < 0.05$) concentrations of Cu, Zn and Cd were observed in 75% PT+25% US compared to the control. For example, Cu was reduced by 21% in shoots of 75% PT+25% US compared to 100% PT. The concentration of investigated metals were significantly higher in roots than in shoots (roots of ryegrass grown in 100% PT were very short and unmeasurable). For example, the level of Pb in plant roots of 75% PT+25% US was $232.96 \pm 16.62 \mu\text{g g}^{-1}$, but in the shoots was undetectable.

Cu concentration in roots was higher than in shoots for all mixtures. For example, in the roots of the ryegrass grown in 75% PT+25% US, the Cu level was higher 4.2 times than Cu levels in the shoots of same mixture. This difference may related to the low translocation of Cu within plant tissues resulting from several mechanisms such as the binding of Cu to plant root cell walls, storage of Cu in the vacuole and the chelation of Cu by organic acids in the cytoplasm (Li *et al.*, 2009; Marschner, 2011; Chu *et al.*, 2018). According to Kozlov *et al.* (2000) the lower translocation rate of Cu to foliar parts of plant has been observed by several researchers but not in all flower species.

The levels of Cd in the shoots of PT and US mixtures were lower significantly ($P < 0.05$) than of 100%PT recording reduction by 78%, 79% and 87% in 75% PT+25% US, 50%

PT+50% US and 25% PT+75% US, respectively relative to that in shoots of control (100%PT). Roots content of Cd measured $2.93 \pm 0.41 \mu\text{g g}^{-1}$ in 50% PT+50% US and $2.53 \pm 0.08 \mu\text{g g}^{-1}$ in 25% PT+75% US lower significantly ($P < 0.05$) than of 75% PT+25% US ($5.50 \pm 0.79 \mu\text{g g}^{-1}$). Cd levels in ryegrass tissues exceeded its normal levels in grass tissue ($0.05 - 0.32 \mu\text{g g}^{-1}$) (Kabata-Pendias and Mukherjee, 2007) and much lower than the phytotoxic threshold of Cd in plant tissues ($100 \mu\text{g g}^{-1}$; Healy *et al.*, 2016).

The concentration of Zn in ryegrass shoots of control (100% PT) was higher 6.5 times than Zn levels in 75% PT+25% US and decreased insignificantly with increasing US fraction. The addition of US therefore led to a reduction in Zn concentration in grass shoots to below phytotoxic levels ($400 \mu\text{g g}^{-1}$; Healy *et al.*, 2016). In the roots of grass developed in 50% PT+50% US, Zn levels were recorded at $546.0 \pm 22.40 \mu\text{g g}^{-1}$ and in 75% PT+25% US, recorded at $596.0 \pm 30.0 \mu\text{g g}^{-1}$, which is higher than phytotoxicity levels of Zn in plants.

Pb concentrations in plant shoots grown in 100% PT and its mixtures were undetectable, but were high in roots, measuring $232.96 \pm 16.62 \mu\text{g g}^{-1}$ in 75% PT+25% US, $76.44 \pm 3.88 \mu\text{g g}^{-1}$ in 50% PT+50% US and $60.44 \pm 16.17 \mu\text{g g}^{-1}$ in 25% PT+75% US. Pb findings agreed with the results of several previous studies reporting that the translocation of Pb within plant tissue is very low (Liu *et al.*, 2003; Yoon *et al.*, 2006; Liang *et al.*, 2017b). Even though, the levels of Pb in tailings is high, the uptake of Pb by roots was low relative to its pseudo-total levels in tailings, and its translocation among plant tissues was restricted, thus the high adsorbed fraction of Pb was accumulated in grass roots (Kabata-Pendias and Mukherjee, 2007; Peralta-Videa *et al.*, 2009). It has been reported that Pb can associate with root tissue through the ion exchange process on the cell wall and precipitate in the form of PbCO_3 in the cell wall, preventing the transfer of Pb to plant shoots (Jarvis and Leung, 2001). Additionally, removing the tailings completely from ryegrass roots by washing process is impossible which may contribute to increase the levels of Pb and other metals in digested root solution (Farrell and Jones, 2010).

Metal levels in roots harvested from PT+US treatments were higher than the threshold levels of studied metals except Cd. The threshold are, $20 \mu\text{g g}^{-1}$ for Pb (Boussen *et al.*, 2013), $100 \mu\text{g g}^{-1}$ for Cd, $20 \mu\text{g g}^{-1}$ for Cu and $400 \mu\text{g g}^{-1}$ for Zn (Healy *et al.*, 2016). Also, the levels of Zn in the shoots of plant grown in 100% PT and the levels of Cu in shoots of plants grown in 100% PT, 75% PT+25% US and 50% PT+50% US exceeded their threshold of phytotoxicity. Therefore, the harvested shoots cannot be used for animal feeding due to its Cu and Zn content exceeding the levels permissible in animal feed (Mcgrath and Fleming, 2007).

Normal Cu and Cd levels in grass range between $2\text{--}10\ \mu\text{g g}^{-1}$ and $0.05\text{--}0.320\ \mu\text{g g}^{-1}$, respectively (Kabata-Pendias and Mukherjee, 2007). It should be noted however that the phytotoxicity levels of several heavy metals are reported to vary both within and between plant species (Levizou *et al.*, 2016).

Biological Accumulation coefficients (BAC) values varied between PT treatments and among metals (Table 6.7) and being in 100% PT and 100% PT+US mixtures lower than 1. In the case of 100% PT mixtures, the BAC for Cu and Cd was 0.16 ± 0.01 and 0.16 ± 0.03 , respectively, indicating that the ryegrass used is a moderate accumulator for Cu and Cd. This may suggest that the ryegrass used is suitable for the phytoextraction of Cu and Cd. However, this grass is classified as a low accumulator for Zn and non-accumulator for Pb in the cause of PT, because of their BAC factors were very low. The difference in the BAC values between elements may relate to variations in the total concentration of elements. For example, in 100% PT levels of Cu and Cd were lower than those of Pb and Zn, which may be affected the BAC calculation.

The values of the transfer degree (TD) were lower than 1 for all studied metals in all PT treatments except TD in 100% PT wasn't measured as the roots of the plants in this treatment were very short and non-harvestable. The TD findings suggested that the translocation of investigated metals from the roots to the above ground biomass was low. The lower metal translocation from roots to shoots may categorized one of several strategies of phytoremediation technology (Verkleij *et al.*, 1990). This category is named phytostabilisation technique which refers to plants with performances to minimise the translocation of hazard elements from roots to above-ground parts resulting to reduces the pollution risk of surrounding environments and food chain (Castaldi *et al.*, 2018). Thus, in the case of Pb successful phytostabilisation was achieved since the shoot content were undetectable while that Pb level in the roots varied from undetectable levels in 100% US to $232.96 \pm 16.62\ \mu\text{g g}^{-1}$ in 75% PT+25% US.

The BF values of all elements in all treatments were lower than 1 except for Zn in the 100% US treatment which was 9.64 ± 2.41 indicating that ryegrass may use as a good accumulator for Zn at low levels of Zn in target soils like US here ($18.67 \pm 0.22\ \mu\text{g g}^{-1}$ Zn). Additionally, to classify a plant as a hyper-accumulator, their content of heavy metals must be over $100\ \mu\text{g g}^{-1}$ for Cd, $1000\ \mu\text{g g}^{-1}$ for Cu and Pb and $10000\ \mu\text{g g}^{-1}$ for Zn (Kabata-Pendias and Mukherjee, 2007; Wuana and Okieimen, 2011). Therefore, the used grass cannot be categorized as a hyper- accumulator plant to treat heavily polluted soils with heavy metals, but

it may use in the phytoimmobilization technology of these metals in mine sites (Lam *et al.*, 2017; Yoon *et al.*, 2006).

Generally, the proportion of metals transported from roots to shoots was mostly lower than 33% of the root content except for Cd in 50% PT+50% US as the content of studied metals in ryegrass shoots was lower than in their roots, suggesting that used grass is a suitable plant for heavy metal phytostabilisation in this site. Thus, based on the results obtained, it is likely that in Parys mine tailings, consecutive seasons of ryegrass cultivation may be effective in the remediation of heavy metal pollution.

Table 6. 7 The values of accumulation coefficients (BAC), transfer degree (TD) and bioaccumulation factor (BF) in ryegrass tissues grown in Parys tailings (PT) and the mixtures of PT + uncontaminated soil (US). Values presented as mean \pm S.E.M, n = 3. DC = did not calculate, because plant roots of 100%PT were too short and unmeasurable.

Factor		100% US	25% PT+75% US	50% PT +50% US	75% PT+25% US	100% PT
BAC	Cu	1	0.08 \pm 0.001	0.07 \pm 0.001	0.700 \pm 0.001	0.16 \pm 0.01
	Zn	1.04 \pm 0.19	0.014 \pm 0.003	0.02 \pm 0.001	0.02 \pm 0.001	0.09 \pm 0.01
	Cd	3.19 \pm 1.23	0.11 \pm 0.03	0.08 \pm 0.1	0.05 \pm 0.01	0.16 \pm 0.03
	Pb	1	\cong 0	\cong 0	\cong 0	\cong 0
TD	Cu	1	0.11 \pm 0.02	0.33 \pm 0.04	0.33 \pm 0.04	DC
	Zn	0.13 \pm 0.05	0.15 \pm 0.01	0.22 \pm 0.03	0.29 \pm 0.03	DC
	Cd	1	0.33 \pm 0.08	0.45 \pm 0.02	0.27 \pm 0.06	DC
	Pb	1	\cong 0	\cong 0	\cong 0	DC
BF	Cu	1	0.75 \pm 0.08	0.23 \pm 0.02	0.22 $0 \pm .02$	DC
	Zn	9.64 \pm 2.41	0.10 \pm 0.02	0.1 \pm 0.004	0.08 \pm 0.004	DC
	Cd	1	0.32 \pm 0.01	0.18 \pm 0.03	0.20 \pm 0.03	DC
	Pb	1	0.03 \pm 0.01	0.02 \pm 0.001	0.04 \pm 0.005	DC

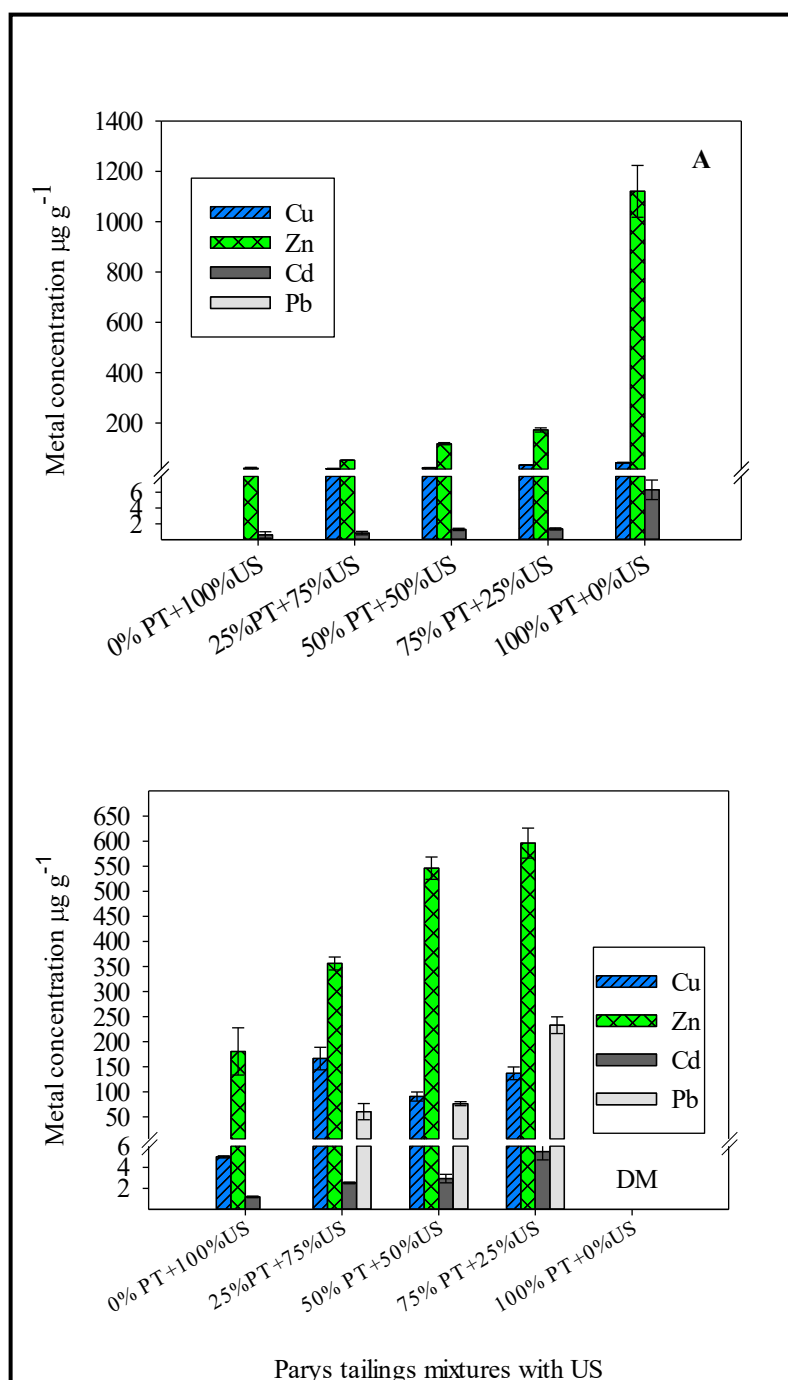


Figure 6. 5 Concentration of studied metals ($\mu\text{g g}^{-1}$) in (A) shoots and (B) roots of ryegrass after 45 days of growth in Parys tailings and uncontaminated soil (US) mixtures. Data presented as mean \pm SEM, n = 3. DM= did not measured.

6.4.4.2 Ryegrass grown in Pandora tailings mixtures

Tables 6.5, 6.6 and figure 6.6 show the levels of target metals in the shoots and roots of ryegrass grown in PaT mixtures. The concentration of Cu, Cd and Pb were significantly higher in the roots than in the shoots of ryegrass grown in the same mixtures. For example, Cu was $377.9 \pm 8.26 \mu\text{g g}^{-1}$ in roots but in shoots measured $26.04 \pm 2.71 \mu\text{g g}^{-1}$ for ryegrass grown in 100% PaT. One exception was the levels of Zn in most mixtures, which were high in both shoots and roots and in the shoots harvested from 100% PaT was significantly higher ($P < 0.05$) than Zn in the shoots grown in other mixtures. In 100%PaT, Zn concentration was $1657 \pm 31.90 \mu\text{g g}^{-1}$ in the roots but in the shoots was $1767 \pm 84 \mu\text{g g}^{-1}$. Also, in 75% PaT+25% US, Zn levels measured $1521 \pm 141 \mu\text{g g}^{-1}$ in roots and $1178 \pm 48.2 \mu\text{g g}^{-1}$ in shoots. The Zn results in the present study in agreement with KieKens (1990), who stated that Zn can translocate easily from plant roots to above-ground biomass.

Cu showed lower levels (significantly ($P < 0.05$)) in shoots than in roots within the same mixtures as the translocation proportion of Cu was 7%, 8%, 18% and 11% in 100% PaT, 75% PaT+25% US, 50% PaT+50% US and 25% PaT+75% US, respectively. There were no significant difference between the levels of Cu accumulated in the shoots harvested from 100% PaT, 75% PaT+25% US, 50% PaT+50% US, however shoots of 25% PaT+75% US stored significant ($P < 0.05$) lower levels of Cu than others. Plant roots taken up different levels of Cu and these levels decreased with increasing the proportion of US and this reduction was significant ($P < 0.05$) in the roots harvested from 50% PaT+50% US and 25% PaT+75% US comparative to the control.

The translocation of Cd within grass tissue can be assumed to be low since the Cd levels in roots were significantly higher than in shoots. The Cd levels recorded $5.95 \pm 0.62 \mu\text{g g}^{-1}$ and $5.12 \pm 0.43 \mu\text{g g}^{-1}$ in grass shoots harvested from 100% PaT and 75% PaT+25% US, and were higher significantly than of 50% PaT+50% US ($2.67 \pm 0.73 \mu\text{g g}^{-1}$) and 25% PaT+75% US ($0.48 \pm 0.40 \mu\text{g g}^{-1}$). Ryegrass roots accumulated concentrations of Cd insignificantly differ in the case of 100% PaT, 75% PaT+25% US, 50% PaT+50% US, but that in roots collected from 25% PaT+75% US was lower significantly than of other treatments.

Plant shoots stored much lower levels of Pb than that accumulated in its roots and in both roots and shoots were higher than the phytotoxicity threshold of Pb in ryegrass tissues, which is $20 \mu\text{g g}^{-1}$ for Pb (Boussen *et al.*, 2013). The shoot content of Pb in PaT + US mixtures were lower significantly than of control. Extremely levels of Pb accumulated in the roots harvested from all PaT treatments without any significant difference among them, which may

be attributed to the high NH_4OH -extractable levels of Pb in PaT tailings (Table 6.2). It was impossible to determine if all tailings were removed from roots and this has led to increased metal levels in analysed solution through acid digested process of grass roots.

The BAC values for Cu in all PaT mixtures ranged from 0.14 ± 0.0 to 0.40 ± 0.04 except for 100% US, suggesting the ryegrass used is a moderate accumulator of Cu in PaT. BAC values for Zn and Cd (except BAC of Cd in 100% US) ranged between 0.05 and 0.08. Since the values tended to remain below 1, the ryegrass can be considered a low accumulator for Zn and Cd.

The translocation factor (TD) of all studied metals from roots to shoots of ryegrass was low except in the case of Zn in 100% PaT which measured 1.07 ± 0.05 . This suggests that ryegrass may be suitable for Zn phytoextraction in heavily polluted soils with Zn as was the case with the Pandora. The translocation percentages of Zn were higher, ranging from 68% to 107% of its contents in the roots which, along with the TD value (1.07 ± 0.05) suggests that the studied grass is an appropriate plant for the phytoextraction of Zn in soils heavily polluted with Zn and for the conditions found at the Pandora site. However, the translocation percentages of Cu, Cd and Pb were lower than 20% of the root content of these metals, supporting the use of ryegrass in the phytostabilisation of Cu, Cd and Pb in the Pandora site. Karami *et al.* (2011) reported that plants with a low rate of translocation of heavy metals within their tissues and appropriate biomass productivity represent suitable candidates for use in phytostabilisation technology.

The BF values of Zn in all treatments were lower than 1 (except for Zn in the 100% US treatment which was 9.64 ± 2.41) which may indicate lower uptake rate by ryegrass roots. The BF values were higher than 1 for Cu and Cd in all PaT mixtures suggesting that the grass roots grown in several mixtures of PaT accumulated levels of Cu and Cd higher than total levels of these metals in PaT and US mixtures (Tables 6.2 and 6.6). This agrees with Kim *et al.* (2003) who stated that plants are able to accumulate heavy metals in their tissues with concentrations greater than pseudo-total levels of these metals in surrounding environments.

The BF values of Pb are also lower than one, though grass roots accumulated very high levels of Pb that may assume that the BF factor cannot be used here in the classification of grass to be hyper-accumulated. However, the metal content of plant tissues was directly used here as a cause of Pb, to classify the ryegrass to be a hyper-accumulator plant of Pb. This is because this grass accumulated more than $1000 \mu\text{g g}^{-1}$ of Pb in its roots (Bini *et al.*, 2012; Fernández *et al.*, 2017; Reeves *et al.*, 2018).

According to Bini *et al.* (2012) and Malik *et al.* (2010), when the TD value is less than one and the BF value is more than 1, the studied plant is proper for use in the phytostabilisation of toxic metals. These conditions were met in the case of Cu and Cd (except the BF value of Cd in 100% PaT, which was < 1) suggesting that the ryegrass used in the present study is suitable for use in the phytostabilisation of Cu and Cd at the Pandora site. Yoon *et al.* (2006) reported that the high stability of heavy metals in root material and with limit re-release of them to surrounding terrestrial environment may supported the use of plant as phytostabilisation tool of heavy metal in mine sites.

Table 6. 8 The values of accumulation coefficients (BAC), transfer degree (TD) and bioaccumulation factor (BF) in ryegrass tissues grown in Pandora tailings (PaT) and mixtures of PaT + uncontaminated soil (US). Values presented as mean \pm S.E.M, n = 3.

Factor		100% US	25% PaT+75%US	50% PaT +50% US	75% PTa+25%US	100% PaT
BAC	Cu	1	0.40 ± 0.04	0.36 ± 0.02	0.22 ± 0.02	0.14 ± 0.01
	Zn	1.04 ± 0.19	0.08 ± 0.01	0.05 ± 0.004	0.05 ± 0.004	0.06 ± 0.004
	Cd	3.19 ± 1.23	0.05 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.01
	Pb	1	0.002 ± 0.001	0.001 ± 0.00	0.0014 ± 0.00	0.001 ± 0.00
TD	Cu	1	0.11 ± 0.01	0.19 ± 0.03	0.07 ± 0.01	0.07 ± 0.01
	Zn	0.13 ± 0.05	0.75 ± 0.01	0.68 ± 0.03	0.79 ± 0.07	1.07 ± 0.05
	Cd	1	0.03 ± 0.01	0.04 ± 0.01	0.70 ± 0.01	0.09 ± 0.01
	Pb	1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.001
BF	Cu	1	3.55 ± 0.10	1.99 ± 0.33	3.11 ± 0.18	1.97 ± 0.05
	Zn	9.64 ± 2.41	0.11 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.001
	Cd	1	1.56 ± 0.1	1.74 ± 0.03	1.13 ± 0.20	0.79 ± 0.06
	Pb	1	0.42 ± 0.02	0.20 ± 0.01	0.15 ± 0.01	0.12 ± 0.01

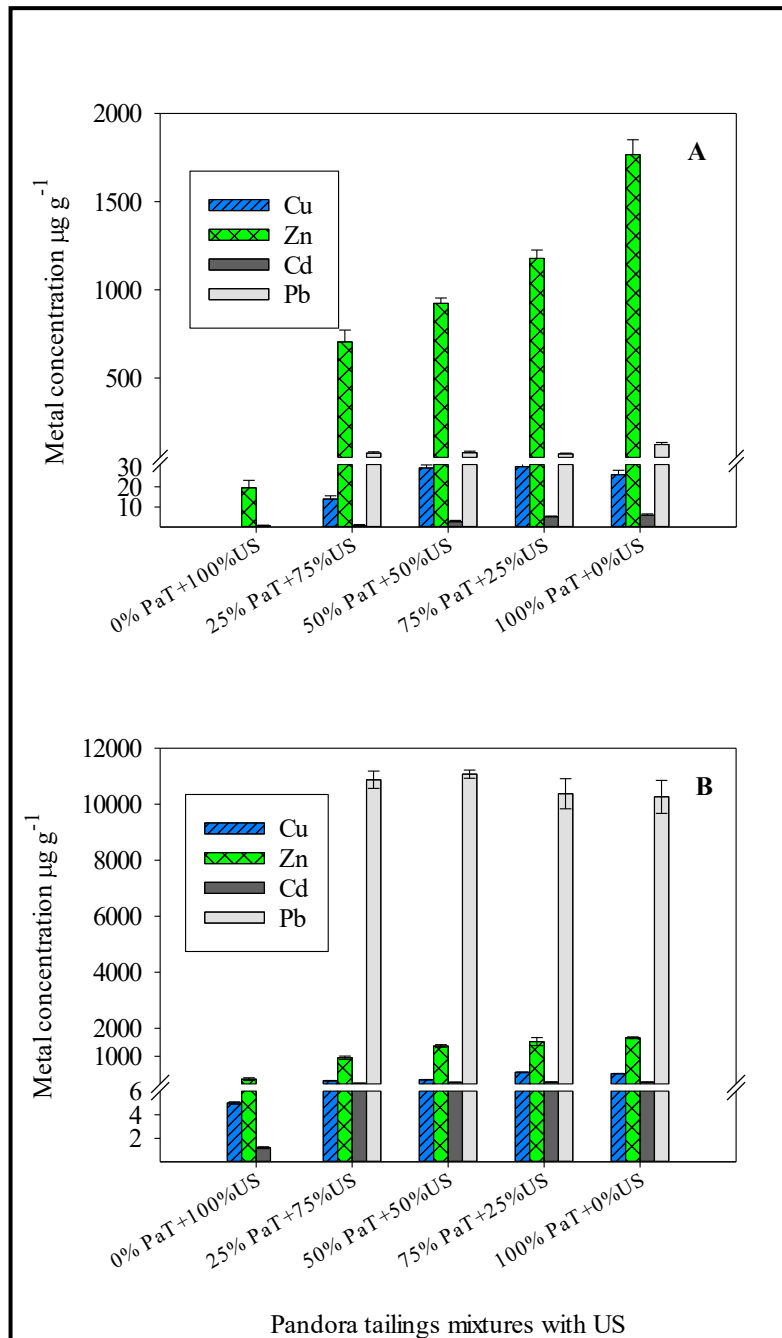


Figure 6. 6 The concentration of studied metals ($\mu\text{g g}^{-1}$) in (A) shoots and (B) roots of ryegrass after 45 days of growth in Pandora tailings and uncontaminated soil (US) mixtures. Results are presented as mean \pm SEM, $n = 3$.

Generally, the findings of this study concluded that the heavy metal content of roots was higher than in shoots, which agrees with the results of several previous studies according to Chu *et al.* (2018). In multi-metal contaminated sites, the uptake and translocation of several metals within plant tissue may be abstracted due to the presence of other metal cations.

For example, the extremely high levels of Zn in both tailings may affect the translocation and accumulation of Cd in plant tissues, because the chemical behaviour of both of them is similar allow to them to compete for bonding on the exchange sites of root surface and to the carrier ligands within plant tissue (Kabata-Pendias and Mukherjee, 2007; Erdem *et al.*, 2012). Our findings are also in agreement with Santibáñez *et al.* (2008) who concluded that ryegrass has the ability to reduce the translocation of heavy metals to above-ground biomass via accumulation in the roots. The uptake of heavy metals by plants is affected by several factors. As Broadley *et al.* (2007) and Kabata-Pendias and Mukherjee (2007) report, the translocation of Zn within plant tissues varies depending on the plant species, metal geochemistry and soil properties. In addition, this study's results in consistent with previous observation saying that the plants were used to amend heavy metal polluted sites, their content of heavy metals were much lower than the levels of these metals in the target contaminated soil (Chaney and Baklanov, 2017). Also, it may applicable applying the phytoremediation of contaminated sites by aim that decreasing or/and removing the phytoavailable fraction rather than reducing the pseudo-total levels of target metals (Liu *et al.*, 2018).

Noticeably, there is apparent contradiction in the Pb translocation within ryegrass tissues as Pb recorded un-detectable levels in the shoots of ryegrass grown in PT and their mixtures with US (Table 6.3), whereas Pb recorded tangible levels in grass shoots grown in the mixtures containing the same proportions of US and PaT (Table 6.5). One possible explanation for this is that the pseudo-total levels of Pb is extremely high in PaT with very high levels of NH₄OH-extractable Pb comparative to those in PT. The grass grown to different extent in the extremely high concentrations of several heavy metals in the studied sites, thus repeated harvesting of above-ground ryegrass biomass may be necessary to reduces the degree of pollution by promoting the phytoextraction of target metals (Wuana and Okieimen, 2011). This supports the use of this type of ryegrass in the phytostabilisation of heavy metals in Parys and Pandora mines. According to Mugica-Alvarez *et al.* (2015), hyper-accumulators are plants can accumulate several heavy metals at the same time are rare and cannot tolerate different environmental conditions, therefore considering other plant species for extracting heavy metals rather than plants classified as hyper-accumulators is an alternative choice. Consequently, the results of this study illustrated that the alternative plant used have the ability to grow fast in extremely polluted mine tailings with low levels of nutrients and its growth was stimulated by the addition of US. Additionally, the roots of ryegrass take up moderate levels of target metals with generally low translocation of metals within plant tissue and may produce proper vegetative cover to minimise the distribution of mine tailings dust. The repetition of

phytoremediation of studied mine tailings using the studied grass can be led to a reduction in the phytoavailable fraction of target metals with time as illustrated by Li *et al.* (2018) who found that the available levels of Zn and Cd in contaminated soil decreased due to the repetition of phytoremediation of polluted soils. The ryegrass must be harvested opportunely before the death to avoid re-release the previous accumulated heavy metals to the terrestrial environment and dispose them using several routes including pyrolysis, composting and compaction (Kovacs and Szemmelveisz, 2017).

6.4.5 Pb fingerprinting in mine tailings, uncontaminated soil and ryegrass tissues

To identify the Pb pollution route and investigate the influence of different Pb pollution sources the two isotopes plots utilizing the ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ are widely employed (Ettler *et al.*, 2004; Komárek *et al.*, 2008; Sun *et al.*, 2011; Jiang : Sun, 2014). Several researchers have found a linear relationship in the fingerprinting signature of Pb isotopes in soils and sediments using the three isotopes plots (Bird, 2011). The end-members of the linear trend are defined by potential sources. Pb isotopic composition was analysed in the application of 75% (PT or PaT) + 25%US ($^w/w$), because the applications of 100% UPT and there treatments did not produce harvestable roots and inability to analyse high number of samples. The results are presented to the 4th decimal which is common in Pb isotopes studies (Varrica *et al.*, 2018). Figure 6.7 (A) shows a linear trend of Pb isotope signatures for PT, US and plant tissues ($r^2 = 0.45$), however in the case of PaT the trend was not found to be linear, and the error bars tended to overlap (Figure 6.7 (B)).

The Pb isotopes in the samples collected from Parys Mountain were presented in table 6.9. The mean ratio of measured Pb isotopes in PT and plant tissues harvested from it were in the range of those obtained by several previous studies of mine tailings according to Bird (2011). The ratio of $^{206}\text{Pb}/^{207}\text{Pb}$ in 100%PT was higher and more radiogenic than in PaT. Lead isotope signatures for tissues grown in soils containing Parys tailings (Figure 6.7-A) generally plotted between the signatures for tailings and soil. The $^{206}\text{Pb}/^{207}\text{Pb}$ $^{208}\text{Pb}/^{206}\text{Pb}$ ratios in ryegrass tissues suggest an influence of both PT and US. To calculate the proportion of Pb isotope sources in the samples, a bivariate mixing model can be applied (Ettler *et al.*, 2004; Komárek *et al.*, 2008 : Bird *et al.*, 2010):

$$\%_T = \frac{\left(\frac{^{206}\text{Pb}}{^{207}\text{Pb}}\right)_S - \left(\frac{^{206}\text{Pb}}{^{207}\text{Pb}}\right)_{\text{US}}}{\left(\frac{^{206}\text{Pb}}{^{207}\text{Pb}}\right)_T - \left(\frac{^{206}\text{Pb}}{^{207}\text{Pb}}\right)_{\text{US}}}$$

Here % T is the contribution proportion of tailings Pb in the investigated sample (S), $(^{206}\text{Pb}/^{207}\text{Pb})_s$ is the Pb isotopic ratio in the shoots or roots, $(^{206}\text{Pb}/^{207}\text{Pb})_{\text{US}}$ is the Pb isotopic ratio in uncontaminated soil and $(^{206}\text{Pb}/^{207}\text{Pb})_{\text{T}}$ is the Pb isotopic ratio in tailings. The model was also performed using $^{208}\text{Pb}/^{206}\text{Pb}$ ratios and an average contribution calculated.

For plants grown in Parys tailings (PT), the mixing model calculation for $^{206}\text{Pb}/^{207}\text{Pb}$ suggests that PT contributes 30% of Pb in plant shoots, and in plant roots contributes 8%. That of $^{208}\text{Pb}/^{206}\text{Pb}$ suggests that 67% and 54% of Pb in shoots and roots respectively, was contributed by PT. Pb fingerprinting in PT seemed to be derived from geogenic sources.

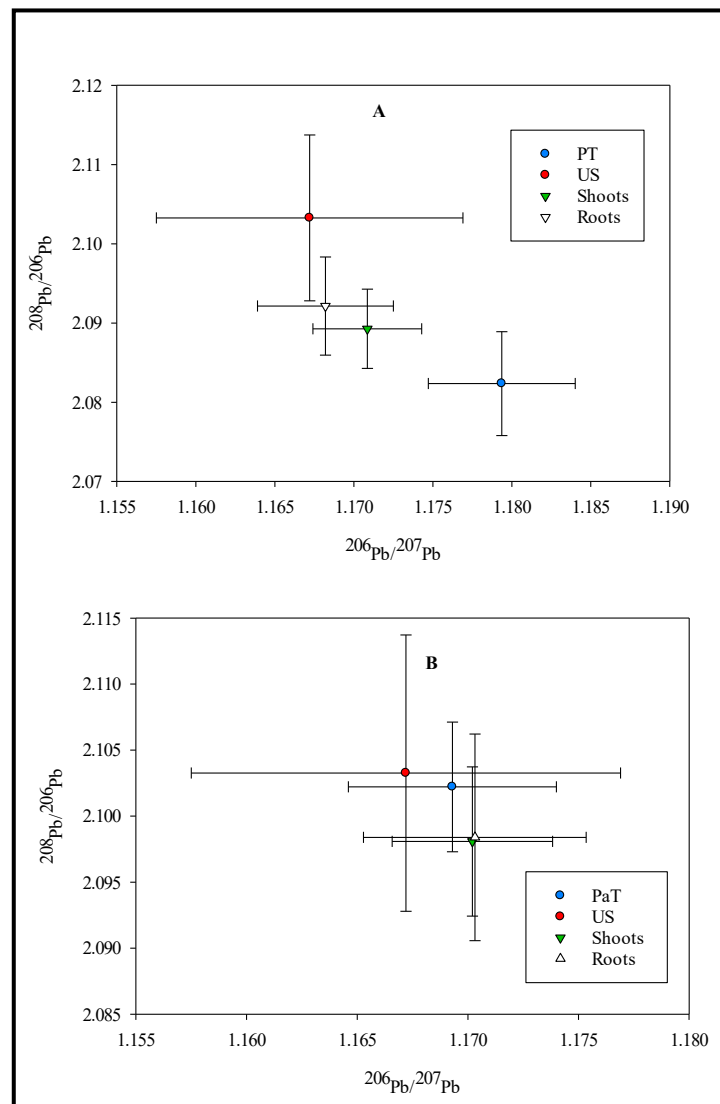


Figure 6. 7 $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ isotopic ratios measured in PT (A) and PaT (B), untreated soil (US) and in plant tissues grown in the treatments of 75% PT+25% US and 75% PaT+25% US, separately.

Table 6. 9 Pb isotopic composition for PT, US and ryegrass tissues harvested from matrices of 75% PT+25% US.

Sample	N	$^{208}\text{Pb}/^{206}\text{Pb}$		$^{206}\text{Pb}/^{207}\text{Pb}$		$^{208}\text{Pb}/^{207}\text{Pb}$	
		Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD
Parys tailings (PT)	1		2.0824 ± 0.007		1.1794 ± 0.005		2.4540 ± 0.008
Soil	1		2.1033 ± 0.012		1.1672 ± 0.010		2.4531 ± 0.018
Shoots	3	2.0853- 2.0929	2.0893 ± 0.005	1.699-1.1724	1.1709 ± 0.003	2.4432- 2.4456	2.4441 ± 0.006
Roots	3	2.0902- 2.0935	2.0921 ± 0.006	1.1646-1.1703	1.1682 ± 0.004	2.4338- 2.4479	2.4426 ± 0.007

Table 6. 10 Pb isotopic composition for PT, US and ryegrass tissues harvested from matrices of 75% PaT+25% US.

Sample	N	$^{208}\text{Pb}/^{206}\text{Pb}$		$^{206}\text{Pb}/^{207}\text{Pb}$		$^{208}\text{Pb}/^{207}\text{Pb}$	
		Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD
Pandora tailings (PaT)	1		2.1022 ± 0.005		1.1693 ± 0.004		2.4563 ± 0.005
Soil	1		2.1033 ± 0.012		1.1672 ± 0.010		2.4531 ± 0.018
Shoots	3	2.0966- 2.0997	2.0981 ± 0.006	1.1694-1.1717	1.1702 ± 0.004	2.4506- 2.4559	2.4533 ± 0.007
Roots	3	2.0952- 2.1010	2.0984 ± 0.006	1.1698-1.1711	1.1703 ± 0.005	2.4489- 2.4553	2.4532 ± 0.009

The Pb isotopic composition data of PaT, US and plant tissues harvested from the matrices of 75% PaT+25% US were within the analytical errors of each other (Figure 6.7 (B)). The Pb isotopic ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ in US were in the range of those obtained by Reimann *et al.* (2012) in their survey of Northern Europe agricultural soils. These authors reported values of 1.143 to 1.727 for $^{206}\text{Pb}/^{207}\text{Pb}$ and 1.477 to 2.702 for $^{208}\text{Pb}/^{206}\text{Pb}$. The binary model did not clearly indicate for the probable contribution of different Pb sources in ryegrass tissues, therefore to identify the Pb fingerprinting in plant tissue, the Pb isotopic ratios in 75% PaT+25% US (Table 6. 10) were used directly. The $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio in plant tissues (1.1702 ± 0.004 in shoots and 1.1702 ± 0.004 in roots) were closer to those of PaT (1.1693 ± 0.004) than of US (1.1672 ± 0.010). In addition, the $^{208}\text{Pb}/^{206}\text{Pb}$ ratios of plant shoots and roots were closer to those of PaT than of US. These results suggested that PaT is the main source of Pb in plant tissues which also explains by the very high levels of Pb in the mixture of 75% PaT+25% US ($73840 \pm 2010 \mu\text{g g}^{-1}$) and the undetected levels of Pb in the 100% US treatment. The similarity in the Pb isotopic ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ in PaT and US may be attributed to contamination of US with Pb of PaT during the initial preparation for remediation trial.

According to Jiang and Sun (2014) and Sun *et al.* (2011) the anthropogenic sources of Pb are indicated by low ratio of $^{206}\text{Pb}/^{207}\text{Pb}$ (lower than 1.2), and a higher ratio of $^{206}\text{Pb}/^{207}\text{Pb}$ (around 1.2) indicates geogenic sources of Pb pollution. Accordingly, the $^{206}\text{Pb}/^{207}\text{Pb}$ composition ratio calculated here, which ranged from 1.17 to 1.18 likely indicates an anthropogenic sources of Pb pollution in the investigated sites e.g., mining activities in Pandora mine (ore processing waste) (Sun *et al.*, 2011). The obtained results in the range of $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in UK soils reflects the natural and indigenous (Pb ores) Pb isotopic signature ranging from 1.17 to 1.19 (Bellis *et al.*, 2004).

6.5 Conclusion

A glasshouse experiment was conducted over a period of 6 weeks to investigate the effect of adding different proportions of uncontaminated soil to mine tailings collected from two abandoned mines distributed in Wales, UK. The study also examined the potential for the use of British ryegrass in the phytoremediation process. The application of US was most effective in reducing the acidity of Parys tailings than of Pandora tailings. The addition of US enhanced the production of H_2O -extractable $\text{NO}_3\text{-N}$, and reduced the phytoavailable fraction of studied metals and the OM content in both tailings. The plants grown in the Parys tailings

mixtures appeared healthier plants than those grown in the Pandora mixtures. The symptoms of heavy metals toxicity decreased as the proportion of US in the mixtures as did the plant development parameters. In terms of heavy metal translocation from tailings to and within plant tissues, the roots accumulated higher levels of heavy metals than shoots confirming the suitability of ryegrass for the phytostabilisation of heavy metals in the studied areas also ryegrass may be used as a hyper-accumulator of Pb in Pandora site. However, BF and BAC cannot be used to categorise plants to be hyper-accumulators for heavy metals in tailings/ soils contain extreme levels of these metals. The harvested grass cannot be used for animal feed or in compost production due to its high content of heavy metals. Nevertheless, it may be utilized in the energy production sector with the remains treated rather than disposed of directly into the ecosystem. The Pb fingerprinting study suggested that the Pb sourced from geogenic path in Parys mine, but in Pandora site may result from anthropogenic sources. Parys mine tailings were sampled from areas away from the direct processing area but Pandora tailings from sites close to processing areas. This may explain the differences in ratios observed and highlights a need to fully understand the geographical locations of processing activities in historic mine sites such as these. There is a need for more long-term studies in both controlled and uncontrolled conditions to examine the ability and efficacy of used ryegrass as appropriate plant in the phytostabilisation and probably phytoextraction technologies to restore mine sites. In addition, examining the ability of combining ryegrass and other amendments to treat heavy metal contaminated Parys and Pandora tailings is required technique.

7 FEASIBILITY OF USING ZERO VALENT IRON AND GREEN WASTE COMPOST COMBINED WITH RYEGRASS IN THE PHYTOREMEDIATION OF MINE TAILINGS

7.1 Introduction

The potential hazardous elements in mine waste can be immobilized by adding several amendments with the aim of reducing their available fraction to soil biota and plants. Several amendments have been documented to have a significant effect on the soil retention of elements and their uptake by plants. Chemical immobilization, excavation and capping are considered expensive technologies in the treatment of mine spoils. For example, the estimated cost of using these methods in the USA ranged between \$ 130,000 to \$ 1,600,000 /ha. By contrast, the cost of phytoremediation ranges from \$ 60,000 to \$ 300,000 /ha (Mendez and Maier, 2008). Several plants can survive and tolerate polluted environments and are useful bio-indicators for assessing the effect of organic and inorganic material in the immobilization of the available fraction of heavy metals (Bini *et al.*, 2012). According to Karaca *et al.* (2018) the addition of several types of amendments is required to modify several physiochemical properties of target tailings promoting optimum plant growth in treated mine tailings.

Co-amending extremely multi-metal contaminated soil by the combination of two technologies may be more effective than using them separately. Using the amendments e.g., composts, lime and fertilizer in combination with plants might be more effective to clean up polluted soils (Smolinska, 2015; Gil-Loaiza *et al.*, 2016). Green waste compost was found to be effective in reducing the translocation of Cu from former mine tailings to sunflower (*Helianthus annuus L.*) (Jones *et al.*, 2016). The separate applications of lime, Fe oxide (goethite), Fe⁰ and iron sulphates + lime to soils polluted with arsenic (As) resulted in the successful growth of *Lolium perenne var. Elka* particularly in soils treated with goethite (Hartley and Lepp, 2008). Incorporation of Fe⁰ to contaminated soils which generates new Fe oxides may reduce the phytotoxicity of heavy metal and metalloids (Oustriere *et al.*, 2017). Additionally, El-Temsah and Joner (2012) proposed that a lower dosage of Fe⁰ may be combined with plants in the remediation of polluted mine lands. Qiao *et al.* (2018) found that application of Fe⁰ resulted in a minor reduction in the concentration of Cd in the roots and aerial parts of rice. Farrell and Jones (2010) used *Agrostis capillaris L.* to assess the effect of two types of compost on the mobility and dynamics of several trace elements in extremely acidic heavily polluted soil. However, the application of green waste compost (C) to highly

acidic mine tailings had non-significant effect in the immobilisation of Cu, Pb and Zn and the shoot length of ryegrass (*Lolium perenne*), though it did adjust the pH and organic matter (OM) content (Alvarenga *et al.*, 2009). The application of compost has been found to modify several physical and chemical properties of degraded soils. For example, a modifying of pH and a reduction in the extractability of heavy metals in soils has been observed, because of the high levels of active functional groups e.g., COOH, amino, phenolic and quinone contained in OM, which have large negative charges and therefore attract H⁺ and heavy metal cations (Manzano *et al.*, 2016; Ohsowski *et al.*, 2017). Also, compost can stimulate soil biota activities by supplying OM to the treated tailings (Karaca *et al.*, 2018). Applications of 15-20% compost were sufficient to promote plant growth and the production of healthier above ground vegetation after 41 months of compost addition. This healthier vegetation is similar to that seen growing in unpolluted sites (Gil-Loaiza *et al.*, 2016). Applying organic amendments to mine soils may increase the phytoavailability of several heavy metals to vegetation. This results in increased metal uptake by plants, which is a favourable phenomenon for cleaning mine soils (Karaca *et al.*, 2018)

For efficient revegetation of mine tailings, the utilized plant species must have suitable properties e.g., high toleration for heavy metals, and the ability to accumulate and translocate toxic metals, allowing them to survive in extremely multi-metal polluted soils (Anning and Akoto, 2018). The growth rate of different grass species may also affect their appropriateness for phytoremediation purposes (Mingorance *et al.*, 2017). In phytoremediation technology ryegrass is widely used due to its ability to tolerate a range of environmental conditions and its high rate of biomass production (Mugica-Alvarez *et al.*, 2015; Liang *et al.*, 2017). The application of organic or inorganic amendments is thought to enhance the efficacy of plants in the remediation of multi-metal contaminated soils (Beesley *et al.* 2011; Li *et al.*, 2012; Mohamed *et al.*, 2017). In the UK, ryegrass (*Lolium perenne* L. var. *Cadix*) was used to examine the ability of biochar and green waste compost to reduce the transfer of Cu and Cd to plants from mine soils (Karami *et al.*, 2011). *Lolium perenne* L. var *Cadix* was used as a bio-indicator by Beesley *et al.* (2010) to assess the ability of green waste compost and biochar separately to reduce of the bioavailable fraction of Cd, Zn, Cu, Pb and As in contaminated soils. As a result, the applications of amendments enhanced the growth rate of aboveground parts of *Lolium perenne* L. var *Cadix*. Several factors should be considered which may affect the efficiency of the phytoremediation of mine sites including tailings acidity, high pseudo-total levels of heavy metals, a low rate of metal translocation within plant tissues and shallow root development in several plant species. The root system of several plants ranged between 20 cm to 2 m,

consequently these plants (grasses and trees) cannot reach and clean-up deep layers of tailings (Karaca *et al.*, 2018). As the sources of Pb pollution into the ecosystem are varied, the combustion ratios of Pb isotopes (^{206}Pb , ^{207}Pb , ^{208}Pb and ^{204}Pb) have been used in order to trace and distinguish between different Pb pollution routes (Kong *et al.*, 2018). The Pb isotopic signature in plant tissues depends upon the source of Pb pollution, when the plant takes up Pb from the soil. Therefore, the isotopic fingerprints of Pb are similar in plant tissue and soil, however, if additional sources of Pb are involved within the growing medium, a significant difference should occur in the Pb isotopic ratios in plants (Li *et al.*, 2012a). Bellis *et al.* (2004) pointed out that the isotopic ratio of ^{206}Pb and ^{207}Pb decreased from 1.17 in the 1800s to 1.11-1.13 in the 1980s in stored grass and peat from the UK, suggesting a change in the effect initially by geogenic sources and then later due to the exposure of non- indigenous sources of Pb.

7.2 Objectives

To achieve the overall objectives of research, this experiment aimed to:

- Examine the effect of green-waste compost (C) and zero valent iron (Fe^0) on the phytoavailability, uptake and translocation of Cu, Zn, Cd and Pb.
- Explore the effect of used amendments on Pb isotopic ratios (^{206}Pb , ^{207}Pb and ^{208}Pb) in ryegrass tissues.
- Evaluate the efficacy of the combination of amendments and ryegrass as a phytoremediation tool in clean up the multi-metal mine tailings.

To achieve these objectives the following question is posed:

To what extent does the addition of amendments affect the uptake and translocation of target metals and Pb isotopes to ryegrass from amended mine tailings.

7.3 Methodology

7.3.1 Study Sites

The samples of tailings were collected from two locations that had been affected by mining operations for prolonged periods. These two locations are Parys copper mine, and Pandora lead mine (chapter 3 section 3.3).

7.3.2 Experimental design

The residual part of air-dried uncontaminated soil and tailings collected and prepared in experiment 3 (chapter 6 section 3.1) was used in the present experiment. Uncontaminated soil (US) was mixed with mine tailings in proportions of 0%, 25%, 50%, 75% and 100 % (w/w)

separately to make 5 mixtures for one location. Each of these mixtures was separated to four parts and treated with two rates of C and Fe⁰, the dosages of these amendments have chosen followed the recommendations of several researchers, in the use of Fe⁰ as an amendment to treat heavy metal polluted soils and water. They pointed out that applications of 1 and 2% of Fe⁰ were commonly utilized in heavy metal polluted soil and water treatments, in addition 5% of Fe⁰ was added for the same target (Komárek *et al.*, 2013) . Lefevre *et al.* (2015) also pointed out that dosage of 50 g L⁻¹ of Fe⁰ were used to treat polluted soils and water. Similarly, it was recommended that Fe⁰ be applied to contaminated media in low doses to avoid the aggregation of its particles, resulting in the reduction of Fe⁰ reactivity and surface area and consequently its ability to adsorb pollutants. High doses of Fe⁰ are also reported to be toxic to soil biota (Tang and Lo, 2013). Accordingly, C was applied at the same rate of Fe⁰, these four parts were amended as follows (Tables 7.1 and 7.2):

- First part was amended with 2% Fe⁰ (8.64 t ha⁻¹) in triplicate for each mixture (15 pots in total).
- Second part was amended with 5% Fe⁰ (21.60 t ha⁻¹) in triplicate for each mixture (15 pots in total).
- Third part was amended with 2% C (8.64 t ha⁻¹) in triplicate for each mixture (15 pots in total).
- Fourth part was amended with 5% C (21.60 t ha⁻¹) in triplicate for each mixture (15 pots in total).

This made a total of 120 pots from the 2 locations, each pot contained 300 g in total. The pots were watered with deionised water up to 70% of the water holding capacity (WHC) of tailings or US, and incubated in the greenhouse under controlled condition for a week. After that, 20g was taken from each pot and its physical and chemical properties analysed. Then, 0.6 g of ryegrass seeds (approximately 202 ± 4.4 seeds) type Aber HSG 2 (from Henfaes Research Farm Seeds Bank) was sown in every pot and irrigated with a diluted nutrient solution (2g L⁻¹ of N:P:K = 24:8:16) as required for 6 weeks, all the treatments received the same quantity of nutrients solution.

Treatments were assigned to pots according to a randomized design. During the trial, pots were maintained at 18–22 °C in natural light (photo period approx.15 h). The ryegrass was harvested 45 days after germination. Roots and shoots were separated and washed gently with tap water and put in a 0.05% Triton solution for 10 min before being rinsed with deionised water and then dried at 80°C for 3 days and weighted as dry matter (Perkins *et al.*, 2016). The

plant samples were ground separately using mill type FOSS CT 193 Cyclotec™ for shoots and a RETSCH MM 200 mill for roots. All milled samples were kept in polyethylene plastic bags until further analysis.

Table 7. 1 The amount of 2% (w/w) amendments added to the mixtures of tailings and uncontaminated soil.

	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
Tailings	100 % (882 g)	75% (662 g)	50% (441 g)	25% (220 g)	0%
Soil	0%	25% (220 g)	50% (441 g)	75% (662 g)	100 % (882 g)
2% Fe ⁰ (8.64 t/ha)	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*
2% C (8.64 t/ha)	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*	18g** + 882 g*

*Weight of Mixture of soil and tailings. C = compost. Numbers with (**) refer to amendment weigh.

Table 7. 2 The amount of 5% (w/w) amendments added to the mixtures of tailings and uncontaminated soil.

	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
Tailings	100 % (855 g)	75% (641 g)	50% (428 g)	25% (214 g)	0%
Soil	0%	25% (214 g)	50% (428 g)	75% (641 g)	100 % (855 g)
5% Fe ⁰ (21.60 t/ha)	45g** + 855g*	45g** + 855g*	45g** + 855g*	45g** + 855g*	45g** + 855g*
5% C (21.60 t/ha)	45g** + 855g*	45g** + 855g*	45g** + 855g*	45g** + 855g*	45g** + 855g*

*Weight of Mixture of soil and tailings. C = compost. Numbers with (**) refer to amendment weigh.

7.3.3 Tailings analysis

Selected tailings properties was analysed following the protocol mentioned in chapter 4 section 4.3.3.

7.3.4 Plant analysis

The sub-samples of roots and shoots were digested using 10 mL of concentrated HNO₃ trace metal grade following the procedure mentioned in chapter 6 section 6.3.5.

7.3.5 Statistical analysis

All the analyses were based on the dry weight of samples except the OM content of treatments, which were measured using samples before air-drying, and run in triplicate. The statistical analysis was carried out using SPSS v.24 and data presented graphically using Sigma Plot v.13 as mean \pm SEM. The mean of measured parameters of grass tissue and tailings were

compared using a one-way ANOVA test after being first tested for normality using Shapiro-Wilk test. A Tukey's Honestly Significant Difference (HSD) post hoc test was run to identify differences between means of treatment properties and plant parameters at ($P < 0.05$) and ($P < 0.01$). Independent-T tests at $P < 0.05$ were conducted to compare the mean of several parameters measured for the ryegrass roots grown in C-amended 100% PT. The results measured below the detection limit of Atomic Absorption Spectrometer (AAS) are reported as BD (below detection limit), but were assumed to be half of AAS detection limit (USEPA, 2000) when presenting the data graphically.

7.4 Results and discussion

7.4.1 Overview

In the wider context, the results of amending the studied tailings by applying two rates of Fe^0 and C individually to different mixtures of tailings + US and to controls showed that the effect of amendments on the physio-chemical characteristics of tailings and US mixtures displayed a similar pattern of that in previous trial (Chapter 6). For example, in both studies the pH increased significantly as the proportions of US applied to PT+ (Fe^0 or C) increased, and the affect was negligible on the pH of PaT (Appendix 10.3). Also, the extractable levels of target metals decreased as the proportion of US increased. Similarly, ryegrass grew better in the treatments with a higher percentage of US, which is similar to the previous experiment's results (Chapter 6). All of the measured parameters of plant and tailings mixtures are included in the appendix 10.3 at the end of this thesis. In order to avoid repeating the discussion of similar results pattern, the current section will focus on the physiochemical characteristics and plant parameters of amended tailings without US and compare them to those of the controls (tailings without any additive material; 100% UPaT and 100% UPT). Tables 7.3 and 7.4 summarise the selected properties of chosen treatments for discussion.

Table 7. 3 Physical and chemical properties of Parys tailings and its treatments with two rates of C and Fe⁰ addition (values presented as mean ± S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% C	100% PT + 5% C	100% PT + 2% Fe ⁰	100% PT + 5% Fe ⁰
pH	3.23 ± 0.02 ^a	3.84 ± 0.03 ^b	4.26 ± 0.03 ^c	3.66 ± 0.02 ^d	3.72 ± 0.01 ^d
EC <i>mS/cm</i>	0.52 ± 0.003 ^a	0.70 ± 0.04 ^b	1.14 ± 0.07 ^c	0.30 ± 0.01 ^d	0.38 ± 0.01 ^{a,d}
O. M %	6.68 ± 0.06 ^a	5.74 ± 0.03 ^b	6.74 ± 0.14 ^a	5.68 ± 0.06 ^b	7.01 ± 0.07 ^a
H ₂ O-extractable NO ₃ ⁻ -N $\mu\text{g g}^{-1}$	1.45 ± 0.21 ^a	6.23 ± 4.11 ^{a,b}	11.82 ± 1.16 ^b	1.01 ± 0.32 ^a	0.58 ± 0.23 ^a
H ₂ O-extractable NH ₄ ⁺ -N $\mu\text{g g}^{-1}$	8.47 ± 0.37 ^a	12.46 ± 0.24 ^{a,b}	15.38 ± 1.79 ^b	6.80 ± 0.62 ^a	5.32 ± 0.22 ^a
Extractable metals ($\mu\text{g g}^{-1}$)					
Cu	16.51 ± 1.20 ^a	19.62 ± 0.51 ^{a,b}	15.85 ± 1.35 ^a	25.43 ± 1.53 ^b	25.55 ± 1.55 ^b
Zn	37.07 ± 5.57 ^a	21.28 ± 1.70 ^b	17.38 ± 1.65 ^b	16.59 ± 3.96 ^b	10.52 ± 1.75 ^b
Cd	2.33 ± 0.64 ^a	0.28 ± 0.15 ^b	0.52 ± 0.1 ^b	0.56 ± 0.18 ^b	0.44 ± 0.11 ^b
Pb	340 ± 10.41 ^a	341 ± 10.93 ^a	321 ± 10.47 ^a	323 ± 7.0 ^a	330 ± 6.64 ^a
Total metals ($\mu\text{g g}^{-1}$)					
Cu	950 ± 24.20 ^a	861 ± 13.40 ^b	854 ± 17.39 ^b	920 ± 4.23 ^{a,b}	863 ± 5.09 ^b
Zn	12460 ± 370 ^a	11579 ± 551 ^a	12030 ± 353 ^a	12629 ± 214 ^a	11367 ± 366 ^a
Cd	39.93 ± 0.77 ^a	40.10 ± 0.76 ^a	40.65 ± 0.87 ^a	37.60 ± 0.31 ^a	33.67 ± 0.55 ^b
Pb	6570 ± 187 ^a	7160 ± 122 ^{a,b}	6947 ± 157 ^a	7053 ± 81 ^a	7720 ± 61 ^b

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 7. 4 Physical and chemical properties of Pandora tailings and its treatments with two rates of C and Fe⁰ addition (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	100% PaT + 5% C	100% PaT + 2% Fe ⁰	100% PaT + 5% Fe ⁰
pH	6.82 \pm 0.01 ^{a,b}	6.83 \pm 0.07 ^{a,b}	6.99 \pm 0.03 ^b	6.67 \pm 0.04 ^a	6.69 \pm 0.04 ^a
EC <i>mS/cm</i>	0.45 \pm 0.02 ^a	0.70 \pm 0.04 ^b	0.95 \pm 0.04 ^c	0.43 \pm 0.03 ^a	0.49 \pm 0.004 ^a
O. M %	7.26 \pm 0.04 ^a	8.07 \pm 0.21 ^b	9.05 \pm 0.17 ^c	6.86 \pm 0.08 ^a	7.10 \pm 0.10 ^a
H ₂ O-extractable NO ₃ ⁻ -N $\mu\text{g g}^{-1}$	2.39 \pm 0.18 ^a	19.80 \pm 2.03 ^b	34.69 \pm 4.65 ^c	2.00 \pm 0.20 ^a	1.54 \pm 0.28 ^a
H ₂ O-extractable NH ₄ ⁺ -N $\mu\text{g g}^{-1}$	9.07 \pm 0.51 ^a	13.15 \pm 0.73 ^b	15.69 \pm 0.26 ^c	9.24 \pm 0.15 ^a	10.55 \pm 0.28 ^a
Extractable metals					
Cu ($\mu\text{g g}^{-1}$)	71.33 \pm 3.68 ^a	76.34 \pm 0.71 ^a	65.15 \pm 1.83 ^a	97.60 \pm 4.39 ^b	105 \pm 2.52 ^b
Zn (mg g ⁻¹)	25.46 \pm 0.73 ^a	24.94 \pm 0.73 ^{a,b}	23.15 \pm 0.59 ^{a,b}	23.41 \pm 0.43 ^{a,b}	22.50 \pm 0.24 ^b
Cd ($\mu\text{g g}^{-1}$)	32.42 \pm 0.60 ^a	39.69 \pm 1.66 ^b	38.31 \pm 1.67 ^b	36.85 \pm 0.55 ^{a,b}	35.46 \pm 0.40 ^{a,b}
Pb (mg g ⁻¹)	90.19 \pm 2.87 ^a	76.14 \pm 0.10 ^b	69.55 \pm 0.32 ^b	75.20 \pm 1.71 ^b	69.07 \pm 3.47 ^b
Total metals ($\mu\text{g g}^{-1}$)					
Cu	192 \pm 3.70 ^a	120 \pm 25.84 ^{b,c}	107 \pm 5.06 ^c	154 \pm 2.22 ^{a,b,c}	173 \pm 3.62 ^{a,b}
Zn	30145 \pm 1170 ^a	29968 \pm 1197 ^a	38075 \pm 1416 ^{b,c}	39536 \pm 809 ^c	33845 \pm 366 ^{a,b}
Cd	91.47 \pm 7.13 ^a	83.20 \pm 14.22 ^a	92.80 \pm 9.61 ^a	94.40 \pm 12.32 ^a	79.00 \pm 4.65 ^a
Pb	101600 \pm 2518 ^a	85867 \pm 1087 ^{b,d}	91680 \pm 333 ^{b,c}	96933 \pm 1709 ^{a,c}	79093 \pm 1419 ^d

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

7.4.2 Effects of Fe⁰ and compost on the characteristics of amended tailings

Applying the amendments to Parys tailings (100% PT) increased the pH significantly ($P < 0.05$) as table 7.3 and figure 7.1 show. The most significant increase occurred with the addition of 5% C where pH increased to 4.26 ± 0.03 compared to 3.23 ± 0.02 in the control (100% PT). The applications of Fe⁰ significantly increased ($P < 0.05$) the pH to 3.66 ± 0.02 in 100% PT + 2%Fe⁰ and 3.72 ± 0.01 in 100% PT+5% Fe⁰, but there were no significant difference ($P > 0.05$) between 2 and 5% treatments. The increase in pH appeared to be in proportion to the amount of C added as the application of 5% C (4.26 ± 0.03) was more effective in the improvement of the pH of 100% PT than 2% C (3.84 ± 0.03). The incorporation of both rates of the Fe⁰ and 2% C did not affect the pH of the treated 100%PaT (Table 7.4 and Figure 7.1).

The increasing of the pH due to the addition of compost may related to the functional groups contained in the OM which are effective in attracting the H⁺ from the treatment solution by protonation process (Medina *et al.*, 2015; Liang *et al.*, 2017a; Touceda-González *et al.*, 2017). Also, the application of OM may have supply basic cations and produce NH₃ during the further decomposition of OM resulting in reduced tailings acidity (Walker *et al.*, 2004). The incorporation of Fe⁰ to 100% PT reduced the acidity of PT. This is likely to be due to the Fe⁰ oxidising in moist conditions, generating Fe oxides and providing newly adsorptive sites for H⁺ as a form of H₃O⁺, which can compete with other presented cations to occupy active sites on the oxidised surface of Fe⁰ particles (Komárek *et al.*, 2013). Also, the fact that the application of Fe⁰ increased the pH of highly acidic tailings (100% PT) more effectively than that of weakly acidic tailings (100% PaT) can be attributed to the high alkalinity of the Fe⁰ suspension (9.50 ± 0.12) (Table 5.3). This observation is similar to that of Gil-Díaz *et al.*, (2017).

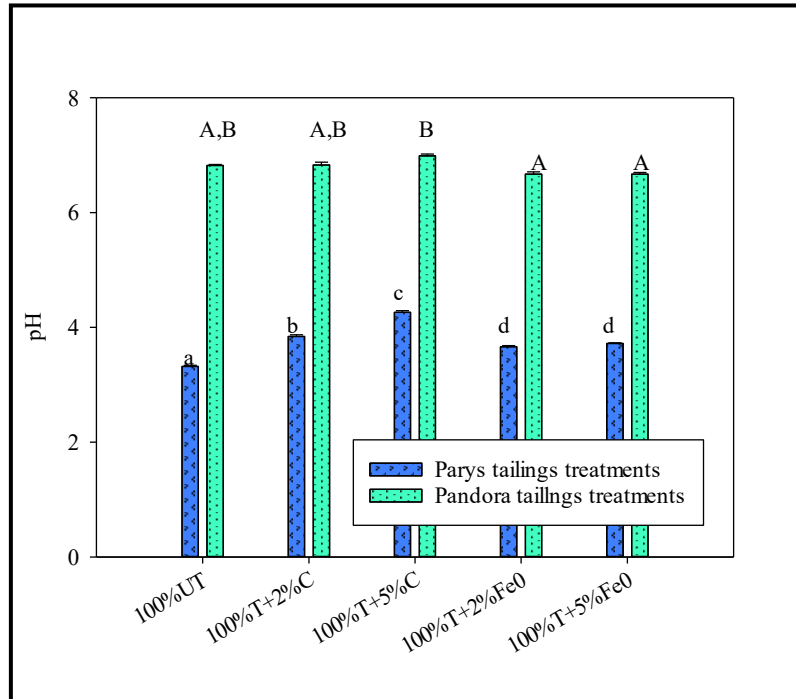


Figure 7. 1 The pH in Parys tailings (100% PT) and Pandora tailings (100% PaT) and tailings treated with 2% and 5% Fe^0 and 2%C and 5% C. The same upper case (A, B) letters indicated no statistical difference in the pH of 100% PaT treatments, and the same lower case (a, b, c, d) letters indicated no statistical difference in the pH of 100% PT treatments. Measurements are presented as mean \pm S.E.M; $n = 3$. Error bars are too small to be fully visible in some cases.

The addition of C to both target tailings increased the electro conductivity (EC) significantly ($P < 0.05$) compared to the control in both sites due to the delivering of several nutrient ions by C to the treated tailings solution (Tables 7.3 and 7.4). However, Fe^0 did not appear to have a significant effect on the EC of treated 100% PaT. The reduction in the EC of 2% Fe^0 -amended 100% PT may have been the result of the adsorption of several anions by the newly formed Fe oxides (Sun *et al.*, 2016a). The same observation were found by Kumpiene *et al.* (2011) who reported that the EC of Cu-contaminated soil reduced due to the application of 2% Fe^0 . Noticeably, in the present study, 2% Fe^0 reduced the EC more than the 5% Fe^0 in 100% PT treatment, which can be attributed to the aggregation of Fe^0 in higher rate treatment, reducing the surface area of Fe^0 and consequently diminishing the adsorption rate of several cations and anions.

The OM content in 100% PT amended with the lower rate (2%) of both compounds decreased significantly ($P < 0.05$) compared to the control (100% UPT). However, treatments with the higher dosage (5%) of Fe^0 and C showed no change in the proportions of OM compared to 100% UPT. OM % in 100% PaT increased significantly ($P < 0.05$) as the

proportion of compost added to increased. OM % was 9.05 ± 0.17 % in 100% PaT+5% C, significantly higher than that in 100% PaT+2% C (8.07 ± 0.21 %) and both of these were higher significantly than the OM % in 100% UPaT (7.26 ± 0.04 %). However, the application of Fe^0 had no effect on the OM content of 100% PaT. Fe^0 does not supply additional OM, which is reflected in our collected data.

Applications of C increased the H_2O -extractable pool of NO_3^- -N and NH_4^+ -N in treated 100% PT and the higher dosage of C was significantly effective than lower rate. However, the addition of Fe^0 to 100% PT had no significant effect on H_2O -extractable levels of NO_3^- -N and NH_4^+ -N, in treatments of Fe^0 -amended 100% PaT, no significant effect was observed. However, incorporation of C to 100% PaT led to significant increase of H_2O -extractable pool of NO_3^- -N and NH_4^+ -N.

7.4.3 Effects of Fe^0 and C on the $\text{NH}_4\text{-OH}$ -extractability of target metals

The phytoavailability of heavy metals is directly proportional to the concentration of the bio-available metal fraction rather than the pseudo-total levels of elements in soils (Menzies *et al.*, 2007). The use of 1 M $\text{NH}_4\text{-OH}$ to extract the most mobile fraction has been reported by several researches (Edwards *et al.*, 1999; Bolan *et al.*, 2003; Menzies *et al.*, 2007). The $\text{NH}_4\text{-OH}$ -extractability of target metals in both studied sites are given in tables 7.3 and 7.4 and plotted on figures 7.2 and 7.3.

Copper

Incorporation the both rates of C to 100% PT had no effect on the $\text{NH}_4\text{-OH}$ -extractable fraction of Cu compared to the control (100% UPT), which is in apparent contraction with several studies including Hattab *et al.* (2014) and Forján *et al.* (2018), who noted that the incorporation of compost to contaminated soil reduced the phytoavailable fraction of Cu. The applications of Fe^0 increased the $\text{NH}_4\text{-OH}$ -extractable fraction of Cu significantly ($P < 0.01$) by 54% in 100% PT+2% Fe^0 and by 55% in 100% PT+5% Fe^0 comparative to the control (100% UPT) as figure 7.2 and table 7.3 show, though $\text{NH}_4\text{-OH}$ -extractable Cu in raw Fe^0 was undetectable

Both applications of C-treated 100%PaT did not affect the extractable levels of Cu compared to the control, for both rates of Fe^0 -treated 100%PaT, a significant ($P < 0.05$) increase in the $\text{NH}_4\text{-OH}$ -extractable Cu fraction was observed by 37% in 100% PaT+2% Fe^0 and by 47% in 100% PaT+5% Fe^0 relative to the control (100% UPaT) as figure 7.3 and table 7.4 show. The increase in the Cu extractability in the Fe^0 -treated PaT and PT may be attributed to the conversion of Cu form that was detected as an initial content in the used Fe^0 (675 ± 11.44

$\mu\text{g g}^{-1}$; Table 7.9) to more soluble fraction during the equilibrium period of trial due to the high acidic conditions of treated PT. Consequently, releasing Cu from the surface of Fe^0 particles to the solution in form of Cu^{2+} contributing to increase the pool of extractable Cu in treatments solution.

Cadmium

$\text{NH}_4\text{-OH}$ -extractable Cd decreased in 100% PT significantly ($P < 0.05$) after the addition of amendments, the most effective amendment (but not significantly relative to other applications) being 2% C, which decreased the concentration of Cd in the phytoavailable phase from by 88% relative to 100%UPT as figure 7.2 and table 7.3 illustrate. In contrast, the applications of both rates of C increased significantly ($P < 0.05$) the concentration of $\text{NH}_4\text{-OH}$ -extractable Cd in 100% PaT treatments (Figure 7.3 and Table 7.4). The increase in Cd phytoavailability in the 100% PaT treated with C may be related to the occurrence of Cd in the exchangeable fraction of PaT at moderate levels (Chapter 4) which may be discharged to the tailings solution due to the replacement of Cd from the exchangeable sites by the cations that were delivered to the treated tailings medium by added amendments. However, 2 % and 5% of Fe^0 did not effected the Cd $\text{NH}_4\text{-OH}$ -extractability in 100%PaT. Gil-Díaz *et al.* (2017) stated that Cd availability did not affect due to the incorporation of 10% Fe^0 to an acidic multi-metal contaminated soil.

Zinc

All additive amendments to 100% PT significantly reduced ($P < 0.05$) the $\text{NH}_4\text{-OH}$ -extractable levels of Zn. The lowest extractable levels of Zn was observed in 100% PT treated with 5% Fe^0 ($10.52 \pm 1.75 \mu\text{g g}^{-1}$) relative to control (100% PT; $37.10 \pm 5.70 \mu\text{g g}^{-1}$) as figure 7.2 and table 7.3 illustrate.

The concentration of Zn in the phytoavailable fraction of treated 100% PaT did not effected by the addition of 2% Fe^0 and 2%, 5% of C which agreed with Forján *et al.* (2018) who found that the application of OM (composted manure) had no effect on Zn phytoavailabilty in amended Cu-mine tailings (Figure 7.3 and Table 7.4). A significant reduction was observed with the addition of 5% Fe^0 where Zn decreased by 12% compared to the control (100% PaT). The higher rate of Fe^0 (5%) was more effective in decreasing the extractable Zn than the lower rate, which agrees with the findings of Gil-Díaz *et al.* (2017). Fe^0 was more effective than C in reducing the phytoavailable fraction of Zn in amended 100% PaT. This is because Fe^0 and its oxides have a higher capacity and selectivity to adsorb Zn (Liang *et al.*, 2014).

Lead

Incorporation of the amendments to 100% PT did not affect Pb phytoavailability as figure 7.2 and table 7.3 show, because of the change was not statistically significant, for example, 2% C-treated 100%PT caused a slight increase in NH₄-OH-extractable Pb by 0.30% compared to in the control (100% PT). Fe⁰ dosages were not effective in reducing Pb NH₄-OH-extractability, which may be attributed to the aggregation of Fe⁰ particles at high dosage was observed in current study. Calderon and Fullan (2015) reported that the aggregations of Fe⁰ particles may limit the efficacy of Fe⁰ for remediation purposes.

The application of all amendments reduced the concentration of Pb in the NH₄-OH-extractable fraction significantly ($P < 0.05$) in the treatments of 100% PaT (Figure 7.3 and Table 7.4). Also, Fe⁰ was only slightly efficient than C in reducing Pb phytoavailability in 100% PaT treatments. Gil-Díaz *et al.* (2017) noted that Fe⁰ is an effective amendment in immobilising Pb in weak acidity tailings like that of PaT, this agrees with the results of Pb availability in PaT which decreased significantly relative to control due to applying both dosages Fe⁰ (Table 7.4).

Generally, inactivation of Zn and Cd in all treatments of 100% PT, and Cu in 5% Fe⁰-treated 100% PT may be related to increasing the pH in the extremely acidic tailings (100% PT) and delivering organic material to the tailings solution by added C (Forj'an *et al.*, 2017). The reduction in the phytoavailable phase of the studied heavy metals could be due to the effect of a combination of altering the pH and the added organic substances (humic acid compounds) that can form insoluble organic complexes with target metals (Van Herwijnen *et al.*, 2007b). Touceda-González *et al.* (2017) also attributed the ability of organic amendments to reduce the mobility of several heavy metals may be related to their ability to alter the pH and the capacity of OM to adsorb heavy metals. The low efficiency of Fe⁰ in mitigating the levels of several extractable heavy metals may be linked to the high acidity of tailings inhibiting the formation of the inner sphere component between heavy and generated Fe oxides (PT in this study) (Mench *et al.*, 2003). This may explain the minor reduction in extractable fraction of several target metals and the fact that this fraction was only found to increase in a small number of cases in the current study. Walker *et al.* (2004) reported that the phytoavailability of several heavy metals and particularly Cu in soils may be controlled by pH, which agree with our results as the pH of amended 100% PaT did not change and Cu availability in C treatments did not altered. However, the reduction in extractability of Pb in all amended 100% PaT and Zn in Fe⁰-amended 100% PaT may related to other process such as ion exchange, reduction reaction, complexation and precipitation rather than pH changing (O'Carroll *et al.*, 2013; Zhang *et al.*,

2015). However, the increase in Cd phytoavailability in the applications of C-treated 100% PaT may results from the chelating of Cd by dissolved organic substances e.g., fulvic acid delivered by OM (Bradl, 2004; Beesley and Dickinson, 2010).

The competition between heavy metals and several ions such as humates delivered by C may affect the retention or displacement of target metals on Fe-Mn oxides fraction in amended tailings (Tandy *et al.*, 2009). Likewise, Gil-Díaz *et al.* (2017) concluded that the competition between different metals in multi-metal contaminated soils may affect the ability of Fe^0 to retain several heavy metals. Also, in acidic soils H^+ cations have the ability to compete with heavy metals to occupy active sites on the amendments surfaces led to increase heavy metals cations levels in the solution, specifically Pb^{2+} (Zhai *et al.*, 2018). The applications of Fe^0 reduced the phytoavailable fraction of Pb in treated 100% PaT and Cu, Zn and Cd in amended 100% PT, which may attributed chemical reduction of metals that have a standard potential (E°) more positive than Fe^0 e.g., Pb^{2+} and Cu^{2+} which may reduce electrochemically to Pb^0 and Cu^0 (Mar Gil-Díaz *et al.*, 2014). Compost was not effective in reducing the phytoavailable fraction of Cu and Zn in 100%PaT and Cu and Pb in 100% PT. This may be attributed to (1) a low quantity of C being added. Moreover, (2) increasing the EC (treatments salinity) with the addition of C led to an increase in the pool of several cations that can replace heavy metals via the exchangeable process on the tailings surface, an increase in the OM solubility and the creation of soluble metal substances e. g., Chlorides, resulting in an increase of metal mobility (Farrell *et al.*, 2010). Furthermore, it was noted that the mobility of heavy metal may increase or decrease by the incorporation of compost to polluted soils, which is related to the nature of compost raw material (van Herwijnen *et al.*, 2007).

Furthermore, the richness of studied tailings in sulphides and with regular oxidation of these compounds resulting in altering the acidity of target tailings and therefore increasing metal mobility (Lindsay *et al.*, 2015; Pardo *et al.*, 2016). This fact may be an alternative explanation for the increase in Cd and Cu availability and their uptake by ryegrass observed in some treatments (Tables 7.3, 7.4 and 7.5-7.8).

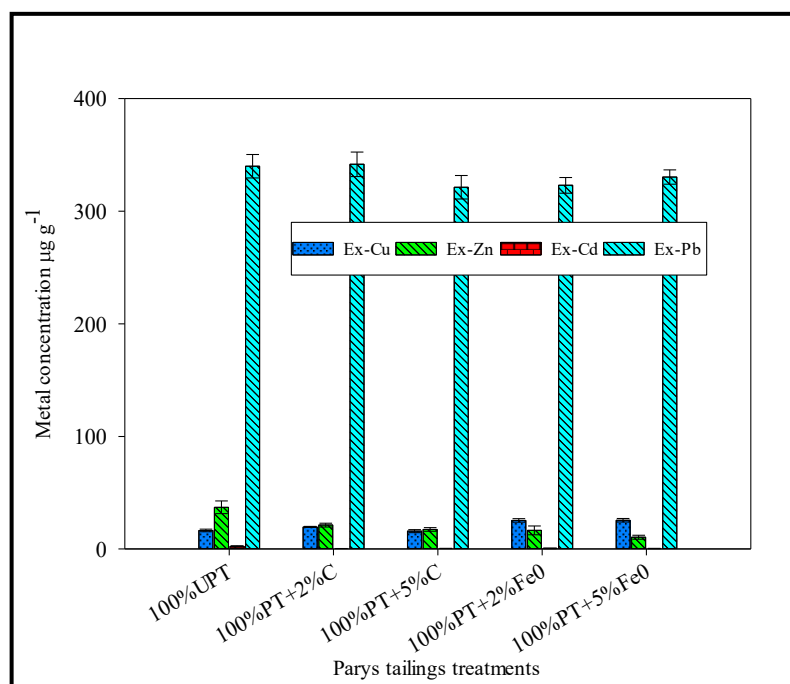


Figure 7. 2 The concentration of $\text{NH}_4\text{-OH}$ -extractable target metals in untreated Parys tailings (100% UPT) and 100% PT treated with 2% and 5% of C and Fe^0 . Measurements are presented as mean \pm S.E.M; $n = 3$ and Cd levels are low too be visible in come causes.

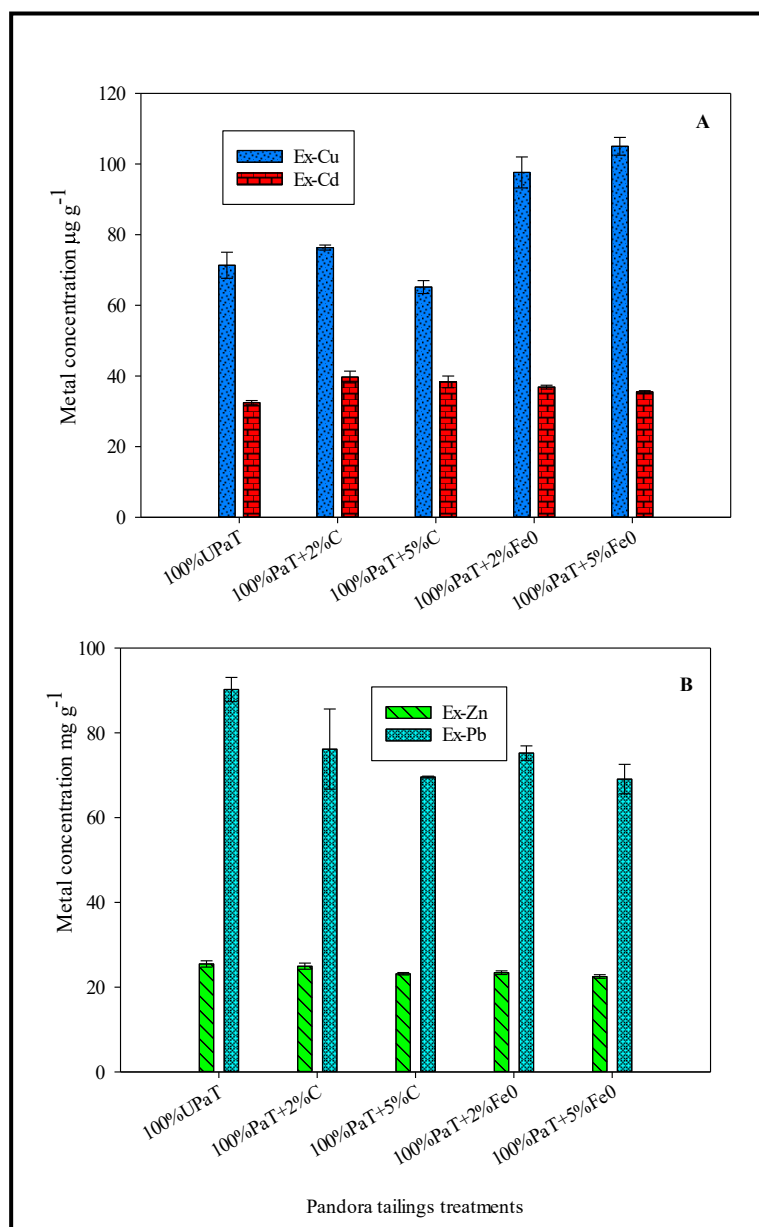


Figure 7. 3 The concentration of $\text{NH}_4\text{-OH}$ -extractable target metals in untreated Pandora tailings (100% UPaT) and 100% PaT treated with 2% and 5% of C and Fe^0 . Measurements are presented as mean \pm S.E.M; n = 3. Because y axis values have a wide range, results have been split into panel A (levels of Cu and Cd in $\mu\text{g g}^{-1}$) and panel B (levels of Zn and Pb in mg g^{-1}).

7.4.4 Effects of Fe^0 and compost on the ryegrass growth

Extreme environmental conditions resulting from high levels of heavy metals, low pH, lack of nutrients and low OM content in the mining area led to the inhibition of plant growth in these sites (Mingorance *et al.*, 2017). High levels of essential (Cu and Zn) and non-essential (Pb and Cd) heavy metals in the studied areas can affect the plant growth, due to the inhibition of chlorophyll synthesis, photosynthesis processes, several enzyme activates and a reduction

in the uptake of several nutrients by the plant. For example, the exposure to Pb and Cd may cause a decrease in the plant content of chlorophyll due to inhabiting the activity of several enzymes e.g., δ -aminolevulinic acid dehydratase that plays a role in the synthesis of chlorophyll (John *et al.*, 2009). In addition, Cu with levels over the requirements of flora posing harm threatens to plants by affecting photosynthesis, several metabolic processes and a reduction in plant growth (Shaheen and Rinklebe, 2015). Therefore, applying the amendments to the acidic multi-metal contaminated soils stimulated plant growth by altering pH, delivering essential nutrients and reducing metal phytoavailability (van Herwijnen *et al.*, 2007; Tandy *et al.*, 2009). Farrell and Jones (2009) and Tandy *et al.* (2009) also reported that applying composts to infertile multi-metal contaminated acidic soils stimulate plant growth. Extreme levels of Cu, Pb, Zn as in mine tailings are known to inhibit plant growth (Pardo *et al.*, 2016). Surprisingly therefore, in this study, the ryegrass seeds germinated after just one week of cultivation in all treatments except in 100% UPT and 100% PaT treated with both dosages of Fe^0 and in 100% UPaT, which germinated after two weeks of cultivation. The motivation for seeds germinating may be related to the addition of compost to heavy metal polluted soils, which leads to a stimulation of microbial activities and a pH adjustment (Khalid *et al.*, 2017). However, the delay in the seeds germinating in several treatments may be related to the simultaneous effect of extreme levels of extractable Pb in both PaT and Fe^0 particles, that may be diffused to the growth medium inside the seed through the seed hull pores, which leads to a direct effect on the germination process (Wierzbicka and Obidzińska, 1998; Lee *et al.*, 2010). Rede *et al.*, (2016) reported a similar result, with Fe^0 inhibiting the germination of lettuce seeds and decreased their root length. Also, El-Temsah and Joner (2012) found that seeds germination and ryegrass growth were inhibited by 60% after the application of $500 \text{ mg L}^{-1} \text{ Fe}^0$ solution. This led to reduce the roots and shoots growth and the seeds germination reduced by 100% when dose of 1000 mg L^{-1} was added.

In the present study, the grass grew to different extents depending on the type and dosage of amendment. Incorporation of Fe^0 to 100% PT increased the shoot length but not significantly ($P > 0.05$), however both dosages of C significantly increased ($P < 0.05$) the above ground length of ryegrass grown in 100% PT, with the increase being proportional to the dose of C. The aerial parts biomass dry weight showed a significant ($P < 0.05$) 21-fold increase in 100% PT+5% C, a 13-fold increase in 100% PT+2% C, a 7-fold increase in 100% PT+5% Fe^0 and a 6-fold increase in 100% PT+2% Fe^0 in comparison to the control (100% UPT) (Figure 7.5 and Tables 7.5 and 7.6).

The shortness of the ryegrass shoots in 100% UPT and tailings treated with Fe^0 can be attributed to the lack of nutrients (Hartley and Lepp, 2008) and tailings acidity (Caterino *et al.*, 2017). Also, the poor development of plant roots and shoots could be related to the combined toxicity of presented heavy metals (Yang *et al.*, 2017). According to Zipper *et al.* (2011) the developing of grass roots in mine tailings were shallow and grown only in the zone just under the surface of tailings. The grass in tailings amended with both rates of C (Figures 7.4 and 7.5) produced more healthy shoots than the grass planted in PT treated with Fe^0 . Root production was promoted by adding C and increased with increasing rate of C addition. By contrast, in Fe^0 -treated 100% PT and in the control the, root growth was stunted (unmeasurable). Nwachukwu and Pulford (2009) observed that C is effective particularly at higher rates of addition in promoting ryegrass biomass production. The addition of Fe^0 led to the generation of iron oxides in moist condition resulting in a reduced supply of several nutrients e.g., P and N, consequently reducing the biomass production of ryegrass (Hartley and Lepp, 2008). The results of the present study also agree with Luisa (2013) who concluded that the application of Fe^0 to As-contaminated soil had an insignificant effect on plant biomass productivity.

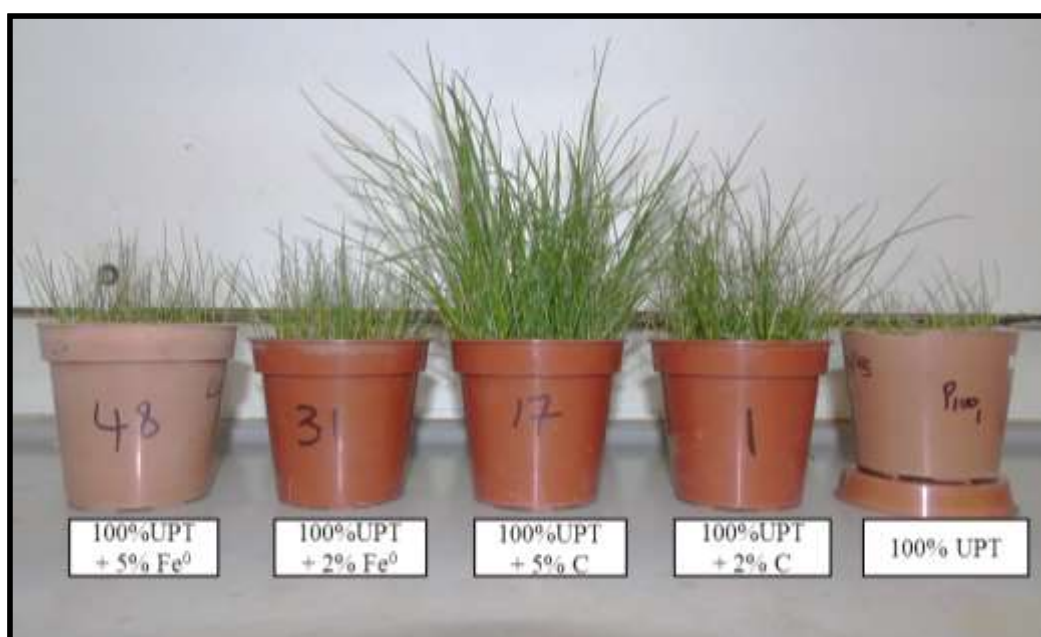


Figure 7. 4 Ryegrass growth in treated 100% PT 45 days after germination in, from left to right; 100% PT+ 5% Fe^0 , 100% PT+ 2% Fe^0 , 100% PT+ 2% C, 100% PT+ 2% C and 100% PT.

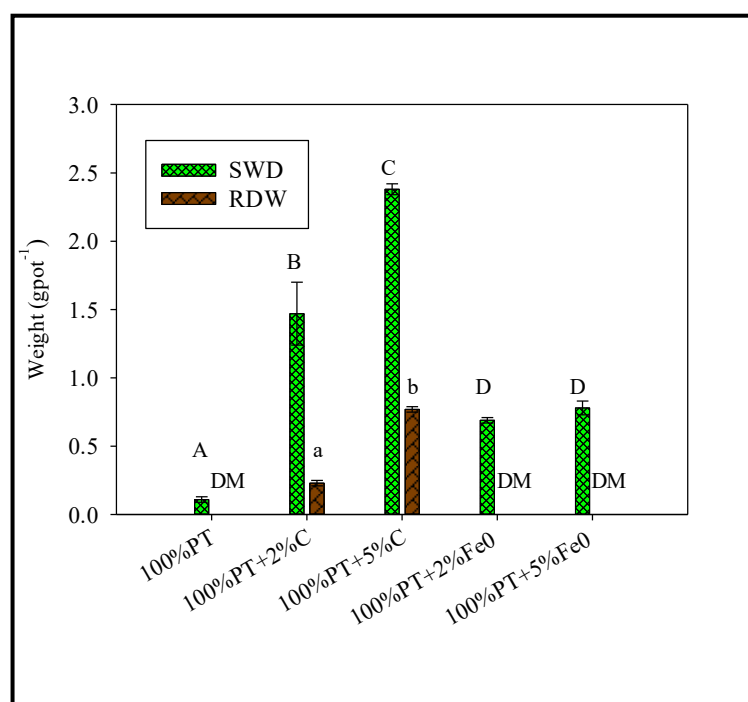


Figure 7. 5 Dry weight (g pot⁻¹) of shoots (SDW) and roots (RDW) of ryegrass after 45 days of growth in untreated Parys tailings (100% UPT) and 100% PT treated with 2% and 5% of C and Fe⁰, respectively. The same upper case (A, B, C and D) letters indicate no statistical difference between the SDW of 100%PT treatments, and the same lower case (a, b and c) letters indicated no statistical difference between the RDW of 100% PT treatments. Measurements presented as mean \pm S.E.M; n = 3. DM = roots were very short and their weight did not measured.

Applying the amendments to 100% PaT stimulated ryegrass growth, but this stimulation depended on the type and quantity of added amendment. As figure 7.6 shows, in terms of morphology, the plants grown in treated 100% PaT seemed healthier than those grown in 100% UPaT. Treatments with 5% C produced healthier plants than the others as the chlorosis of shoot appears to be much less than that in 100% UPaT. Nevertheless, indications of heavy metal toxicity in the form of chlorosis appeared to some extent in 100% UPaT treated with 2% C, 2% Fe⁰ and 5% Fe⁰. The shoot lengths were not affected by the addition of both rates of Fe⁰ relative to those cultivated in 100% PaT (Figure 7.7 and Tables 7.7 and 7.8). The above ground parts of the ryegrass developed in all amended 100% PaT showed significant greater dry weight than those developed in 100% UPaT. C was more effective in stimulation the shoot growth; shoots length increased by 31% in 100% PaT+2% C and by 52 % in 100% PaT+5% C with both found to be significantly higher ($P < 0.05$) than 100% UPaT. The most productive treatment was 100% PaT+5% C that recorded increase by 212% of shoots dry weight compared to the control (100% PaT). Fe⁰ increased the shoot dry weight significantly by 128% and 135%

when grown in 100%PaT+ 2% Fe⁰, 100% PaT+5% Fe⁰, respectively. This is in agreement with Kumpiene *et al.* (2006), who observed that the addition of 1% Fe⁰ to acidic soil affected by the deposition of industrial wastes increased aerial biomass growth up to 2-fold compared to that grown in untreated soil and caused a minor increase in plant root biomass.

The root development in the amended tailings with 5% C was significantly better than in the control (100%UPaT) with dry weight increased by 129%. However, the applications of 100%PaT+ 2% Fe⁰, 100% PaT+5% Fe⁰ and 100% PaT+ 2% C did not affect the grass root growth (the differences were not statistically significant, $P > 0.05$) relative to the control. This might be attribute to the significant amelioration of several physiochemical properties of 5% C-treated 100% PaT relative to other treatments (Table 7.4). The same observation of inhibiting the growth rate of ryegrass due to the application Fe⁰ to contaminated soil of former copper smelter was obtained by Goecke *et al.* (2011).

The incorporation of amendments to 100% PaT increased the biomass (roots + shoots) of ryegrass, and the increased were in proportion with the amendment dose. It was also observed that 5% C-amended 100% PaT was more effective than other amendments in terms of plant growth, as the grass looked the healthier plant, since it was greener and showed fewer symptoms of heavy metal toxicity, such as chlorosis. Consequently, this treatment produced the highest biomass among others recorded, with an increase of 212% and 129% respectively in the weight of the shoots and roots, relative to the control. The indicators of heavy metal toxicity appeared as yellow leaves in grasses grown in treatments of 2% C, 2% and 5% Fe⁰, however, this was much less than the control (Figure 7.6). It should be noted however, that the degradation of C over time may lead to rerelease of previously immobilized heavy metals into the tailings solution resulting in an increase in uptake by plant roots and inhibiting ryegrass growth (Cui *et al.*, 2016b).

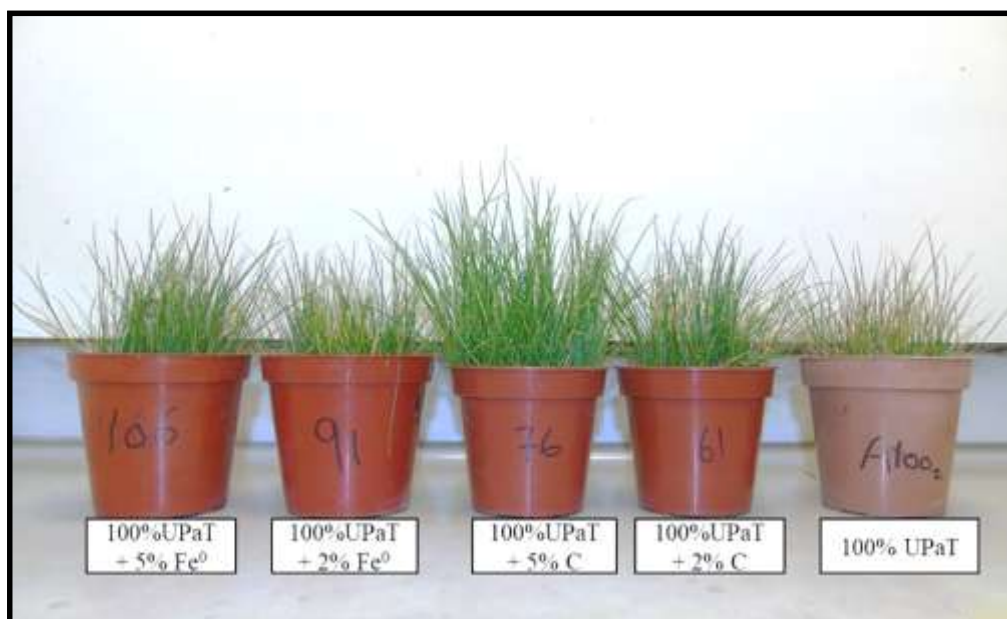


Figure 7. 6 The ryegrass grown in treated 100%PaT after 45 days of germination, from left to right; 100%PaT+ 5% Fe⁰, 100%PaT+ 2% Fe⁰, 100%PaT+ 2% C, 100%PaT+ 2% C and 100% PaT.

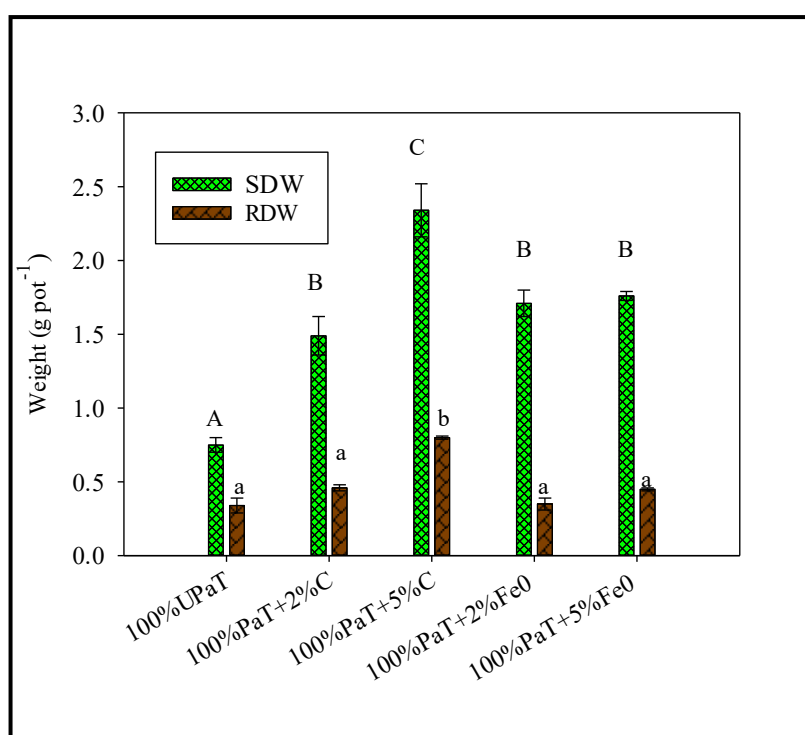


Figure 7. 7 Dry weight (g pot⁻¹) of shoots (SDW) and roots (RDW) of ryegrass after 45 days of growth in untreated Pandora tailings (100% UPaT) and 100% PaT treated with 2% and 5% of C and Fe⁰, respectively. The same upper case letters (A, B and C) indicate no statistical difference between the SDW of 100% PaT treatments, and the same lower case letters (a and b) indicate no statistical difference between the RDW of 100% PaT treatments. Measurements presented as mean \pm S.E.M; n = 3.

Our study results agree with the findings of Farrell *et al.* (2010) who observed that as the application rate of C to extremely acidic mine tailings from Parys Mountain increased, accordingly the *Agrostis capillaris* L. growth rate increased compared with the control. As mentioned above, in several treatments and in the untreated tailings, the cultivated grass was affected by chlorosis and the biomass production was inhibited, indicating Pb, Zn and Cu toxicity (Karami *et al.*, 2011). Also, they found that C enhanced ryegrass (*Lolium perenne* L. var. *Cadix*) growth and its biomass productivity. Applying C led to an increase in the pool of OM and encouraged biological processes, which promote ryegrass growth in both sites. Tang *et al.* (2015) also found that straw-derived manure increased the content of OM and stimulated plant development because of reducing Pb phytoavailable fraction, which was observed in the cause of PaT (Table 7.4) and delivering several essential nutrients for plant development. In addition, Touceda-González *et al.* (2017) observed a significant increase in the productivity of *A. tenuis* grown in pyrite mine tailing amended with C. The modification of the pH by C in 100% PT, which was recorded the highest pH (4.26 ± 0.03) in 100% PT+5% C among the 100% PT treatments, may be another factor which promoted the increasing the ryegrass biomass productivity along with the delivery of more nutrients. The results of this study showed that higher rate of C modified tailings acidity and promoted plant growth which in agreement with van Herwijnen *et al.* (2007).

Increasing the yield of ryegrass in amended tailings compared to controls after applying amendments particularly C may be explained by; (1) delivering essential nutrients for plant development, (2) Modifying several physical and chemical characteristic e.g., pH, nitrogen availability and OM, (3) enhancing nutrient cycle, and (4) reducing the phytoavailable form of heavy metals (Walker *et al.*, 2004; Tandy *et al.*, 2009). Brown spots on the shoots of ryegrass developed in both tailing amended with Fe^0 specially at high dosage may due to the generation of new Fe oxides that may effected the plant growth by effecting photosynthesis process (Aslani *et al.*, 2014). In both studied tailings, C was more effective than Fe^0 at enhancing plant growth in this study the same observation was obtained by Hartley and Lepp (2008). They found that C was more effective to increase the ryegrass biomass than goethite ($\alpha\text{-FeOOH}$) after incorporating both of them separately to soil from landfill of industrial wastes but iron grit had various efficacy in grass growth. Also, they mentioned that Fe and its oxides may obstructed plant growth.

Table 7. 5 Several parameters of Ryegrass shoots grown in Parys tailings and its treatments with C and Fe⁰ (values presented as mean ± SEM; n = 3).

Parameters	100% UPT	100% PT + 2% C	100% PT + 5% C	100% PT + 2% Fe ⁰	100% PT +5% Fe ⁰
Shoots length (cm)	5.67 ± 0.34 ^a	15.83 ± 0.72 ^b	18.5 ± 1.15 ^b	8.17 ± 0.60 ^a	8.20 ± 0.15 ^a
Shoots dry weight (g pot ⁻¹)	0.11 ± 0.02 ^a	1.47 ± 0.23 ^b	2.38 ± 0.04 ^c	0.69 ± 0.02 ^d	0.78 ± 0.05 ^d
Total elements (µg g ⁻¹)					
Cu	41.67 ± 1.17 ^a	24.23 ± 0.84 ^b	27.71 ± 1.44 ^b	60.00 ± 3.86 ^c	67.14 ± 47 ^c
Zn	1121 ± 103 ^a	114.21 ± 9.08 ^b	69.72 ± 3.29 ^b	86.81 ± 5.60 ^b	70.67 ± 4.00 ^b
Cd	6.32 ± 1.24 ^a	1.73 ± 0.35 ^b	4.67 ± 0.58 ^{a,b}	6.33 ± 0.24 ^a	6.80 ± 0.12 ^a
Pb	BD	BD	BD	35.33 ± 4.37 ^{a*}	67.33 ± 7.51 ^{b*}

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. Values with different lower case letters marked with * indicate a significant difference according to independent-T test at P < 0.05.

Table 7. 6 Several parameters of Ryegrass roots grown in Parys tailings and its treatments with C and Fe⁰ (values presented as mean ± SEM; n = 3).

Parameters	100% UPT	100% PT + 2% C	100% PT + 5% C	100% PT + 2% Fe ⁰	100% PT +5% Fe ⁰
Roots dry weight (g pot ⁻¹)	DM	0.28 ± 0.02 ^a	0.77 ± 0.02 ^b	DM	DM
Total elements (µg g ⁻¹)					
Cu	DM	177.1 ± 4.68 ^a	142.3 ± 4.92 ^b	DM	DM
Zn	DM	471.5 ± 24.86 ^a	260.2 ± 8.44 ^b	DM	DM
Cd	DM	16.12 ± 0.76 ^a	14.00 ± 0.84 ^a	DM	DM
Pb	DM	256.6 ± 18.92 ^a	209.1 ± 6.49 ^a	DM	DM

Different lower case letters within the same row indicate a significant difference according to independent-T test at P < 0.05, DM= did not measured.

Table 7. 7 Several parameters of Ryegrass shoots grown in Pandora tailings and its treatments with C and Fe⁰ (values presented as mean ± SEM; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	100% PaT + 5% C	100% PaT + 2% Fe ⁰	100% PaT +5% Fe ⁰
Shoots length (cm)	8.20 ± 0.18 ^a	10.73 ± 0.37 ^b	12.50 ± 0.29 ^b	8.50 ± 0.58 ^a	8.83 ± 0.44 ^a
Shoots dry weight (g pot ⁻¹)	0.75 ± 0.05 ^a	1.49 ± 0.13 ^b	2.34 ± 0.18 ^c	1.71 ± 0.09 ^b	1.76 ± 0.03 ^b
Total elements (µg g ⁻¹)					
Cu	26.04 ± 2.71 ^a	69.89 ± 1.70 ^b	97.33 ± 3.71 ^c	78.67 ± 2.49 ^b	75.07 ± 1.84 ^b
Zn	1767 ± 84.0 ^a	808 ± 40.58 ^b	573 ± 17.12 ^c	1159 ± 25.40 ^d	701 ± 21.37 ^{b,c}
Cd	5.96 ± 0.62 ^{a,c}	5.47 ± 0.18 ^{a,b}	4.33 ± 0.18 ^b	7.07 ± 0.14 ^c	6.33 ± 0.26 ^{a,c}
Pb	122.67 ± 12.22 ^a	85.50 ± 4.75 ^b	97.33 ± 3.71 ^{a,b}	124.0 ± 6.11 ^a	124.67 ± 1.76 ^a

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 7. 8 Several parameters of Ryegrass roots grown in Pandora tailings and its treatments C and Fe⁰ (values presented as mean ± SEM; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	100% PaT + 5% C	100% PaT + 2% Fe ⁰	100% PaT +5% Fe ⁰
Roots dry weight (g pot ⁻¹)	0.35 ± 0.05 ^a	0.46 ± 0.02 ^a	0.80 ± 0.01 ^b	0.35 ± 0.04 ^a	0.45 ± 0.02 ^a
Total elements (µg g ⁻¹)					
Cu	377.9 ± 8.26 ^{a,c}	371.6 ± 11.75 ^{a,c}	309.1 ± 2.82 ^b	321.4 ± 9.35 ^{a,b}	401.5 ± 20.92 ^c
Zn	1657 ± 31.90 ^a	2667 ± 56.71 ^b	2452 ± 250 ^b	2535 ± 180 ^b	3012 ± 169 ^b
Cd	70.31 ± 0.88 ^a	179.4 ± 11.53 ^b	150.6 ± 9.06 ^{b,c}	152.4 ± 10.49 ^{b,c}	114.6 ± 2.59 ^c
Pb	11787 ± 895 ^a	8896 ± 161 ^{b,c}	7424 ± 231 ^b	8272 ± 782 ^{b,c}	10312 ± 353 ^{a,c}

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

7.4.5 Effects of Fe⁰ and compost on the uptake and transfer of studied metals within ryegrass

The pseudo-total levels of studied metals were measured in roots, shoots, and the results are presented in tables 7.5 to 7.8 and plotted in figures 7.8 -7.11.

7.4.5.1 Pseudo-total concentration of heavy metals in different treatments

The total levels of explored heavy metals did not show significant difference relative to the control in the treatments of 100% PT except for Cu in all treated 100% PT, Cd and Pb in 100%PT+5% Fe⁰. However, there were generally significant differences between the concentrations of studied metals comparative to the 100% UPaT excluding Cd in all treatments of 100%PaT, Zn in 100% PaT+2% C, Cu and Zn in 100% PaT+5% Fe⁰ and Pb in 100% PaT+2% Fe⁰. Non-homogeneity of total metal distribution in mine tailings can be caused by extremely high levels of heavy metals (Herreweghe *et al.*, 2003) which may explain the variation in heavy metal concentrations recorded in our study (Tables 7.3 and 7.4). In addition, the heavy metal content of the initial amendments (Table 7.9) may alter the pseudo-total concentration of several investigated metals in the amended tailings.

Table 7. 9 The concentration of target metals ($\mu\text{g g}^{-1}$) in the amendments used (values presented as mean \pm SEM; n = 3).

Amendment	Cu	Cd	Zn	Pb
Fe ⁰	675 \pm 11.4	6.00 \pm 1.13	290.7 \pm 15.1	163.5 \pm 4.82
C	76.20 \pm 1.58	BD	238.3 \pm 8.53	135.5 \pm 3.51

BD = below the detection limit.

7.4.5.2 Heavy meal concentration in ryegrass roots

As mentioned earlier the ryegrass roots which developed in 100% UPT, and those treated with both doses of Fe⁰ were too short to harvest, but those grown in 100% PT treated with both rates of C were harvestable. Therefore, the comparison will discuss only the treatments of 100% PT amended with both rates of C. Plant material was also harvestable in all treatments of 100% PaT. Figures 7.8 and 7.9, tables 7.6 and 7.8 show the concentrations of studied metals accumulated in ryegrass roots developed in treated and untreated 100% PT and 100% PaT.

Copper

The accumulation of Cu was significantly lower ($P < 0.05$) in plant roots grown in 100% PT amended with the higher dosage of C (5%) than in the roots of grass grown in the 100% PT+2% C treatment.

Compost dosed at a rate of 5% was significantly reduced ($P < 0.05$) the Cu concentration in ryegrass roots harvested from 100% PaT+5% C by 18% comparative to the control (100% UPaT). However, 2% C, 2% Fe⁰ and 5% Fe⁰ did not show significant change in Cu levels in the roots of ryegrass.

Zinc

The higher C dosage (5%) was more effective ($P < 0.05$) by in reducing the uptake of Zn by ryegrass roots than the lower dosage as the levels of Zn reduced by 20% in 100% PT+5% C relative to 2% C-treated 100%PT.

The applied amendments significantly increased ($P < 0.05$) the uptake of Zn by the grass roots by 61%, 48%, 53% and 81% grown in 100% PaT+2% C, 100% PaT+5% C, 100% PaT+2% Fe⁰ and 100% PaT+5% Fe⁰, respectively relative to control (100% PaT).

Cadmium

The concentration of Cd in the ryegrass roots harvested from 100% PT treated with both rates of C were not differ significantly from each other. A significant increase ($P < 0.05$) observed in Cd levels in the ryegrass roots grown in all amended 100% PaT relative to the control, 2%C-treated 100%PaT recorded the highest levels of Cd in ryegrass roots among other treatments and control as the increase was by 155 % relative to control. In addition, the increase was by 114%, 117 % and 63% in 100% PaT+5% C, 100% PaT+2% Fe⁰ and 100% PaT+5%Fe⁰, respectively relative to control. A reduction in Cd uptake by plants from polluted soils amended with OM is generally assumed, though not for all plant species because in several cases the root uptake and translocation of Cd within plant tissues has been found to increase in the Cd-polluted soils treated with OM (Khan *et al.*, 2017).

Lead

Pb accumulation in the roots of ryegrass developed in 100% PT treated with 5% C was not significantly different ($P > 0.05$) from Pb levels in roots harvested from 100% PT+2%C amended with 2% C, which may suggest increasing C application rate did not affect the uptake of Pb by ryegrass roots. The applications of most amendments to 100% PaT decreased the levels of Pb in plant roots. For 2% and 5% C and 2% Fe⁰ this reduction was significant

($P < 0.05$) but 5% Fe^0 -amended 100% PT did not show significant reduction in Pb levels in grass roots relative to the control. Noticeably, the grass roots adsorbed high concentration of Pb ranging from $7424 \mu\text{g g}^{-1}$ to $11787 \mu\text{g g}^{-1}$ in plant roots grown in amended and unamended 100% PaT. This may be related to the formation of soluble Pb-organic complexes leading to an increased uptake of Pb by roots from the surrounding polluted soil (Huang *et al.*, 1997). Additionally, high levels of Pb in grass roots were harvested from 100% PaT treatments may reflect the extreme high concentration of Pb in the phytoavailable fraction of PaT. The ability of studied grass to accumulate higher levels of Pb than $1000 \mu\text{g g}^{-1}$ (Reeves *et al.*, 2018) in their roots may suggest using this grass as a hyper-accumulator plant for Pb in Pandora site.

Generally, the application of amendments to PaT increased the uptake of Zn and Cd, but 5% C- amended 100% PaT was effective in reducing the uptake of Cu and Pb by ryegrass. 5% Fe^0 treated 100%PaT enhanced the uptake of Cu and Zn. The application 5%C may be considered a valuable amendment for the remediation of PaT given the enhancement in ryegrass growth with high levels of heavy metals accumulated in the grass roots. A study conducted by Mugica-Alvarez *et al.* (2015) concluded that the applications of OM to mine tailings promoted the extraction of Zn and Pb more effectively than Cu within Italian *Lolium Multiflorum* tissues. It is likely that the reduction in the acidity of C-amended 100% PT affected the uptake of heavy metals by ryegrass roots (Brandão *et al.*, 2018).

The grass roots developed in 100% PaT treated with both doses of C accumulated more heavy metals than roots grown in C-amended 100% PT. This difference may be attributed to the higher concentration of target metals in the $\text{NH}_4\text{-OH}$ -extractable fraction in 100% PaT and its C treatments than in 100% PT and its C-treatments. This is in agreement with the results of Chapters 4 and 5, which found that the presence of target metals with high proportions were in exchangeable fraction in 100% PaT, but in 100% PT most heavy metals were associated with residual fraction. Plant tissue heavy metal content was not affected by the pseudo-total levels of these elements (Murray *et al.*, 2000) but depended on the pH of the soil and metal levels in the most mobile fractions (Smith, 2009).

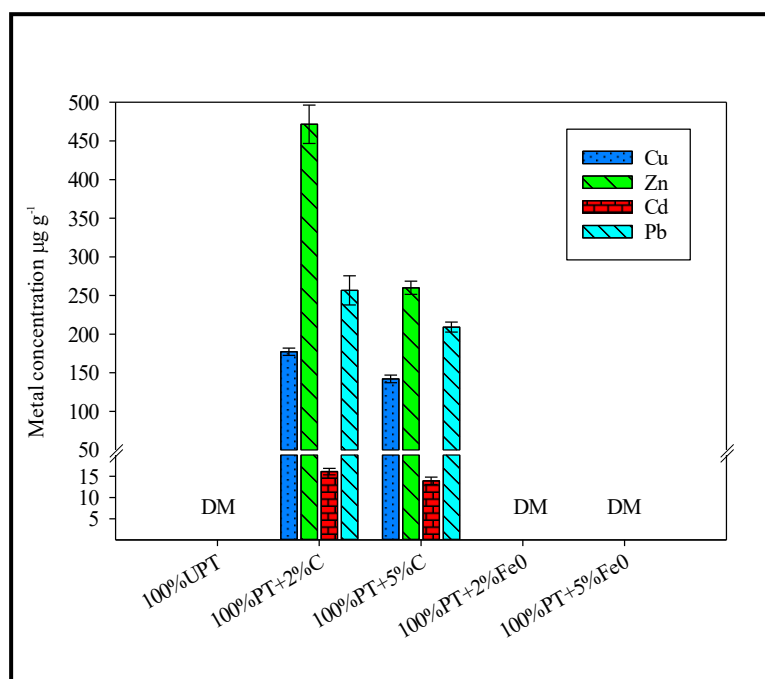


Figure 7. 8 Heavy metals accumulation in the roots of ryegrass grown in unamended Parys tailings (100% PT) and 100% PT amended with 2% and 5% Fe⁰, and 2% and 5% C. Measurements are presented as mean \pm S.E.M; n = 3, DM = did not measure because the roots of grass grown in treatments tagged with DM were not harvestable.

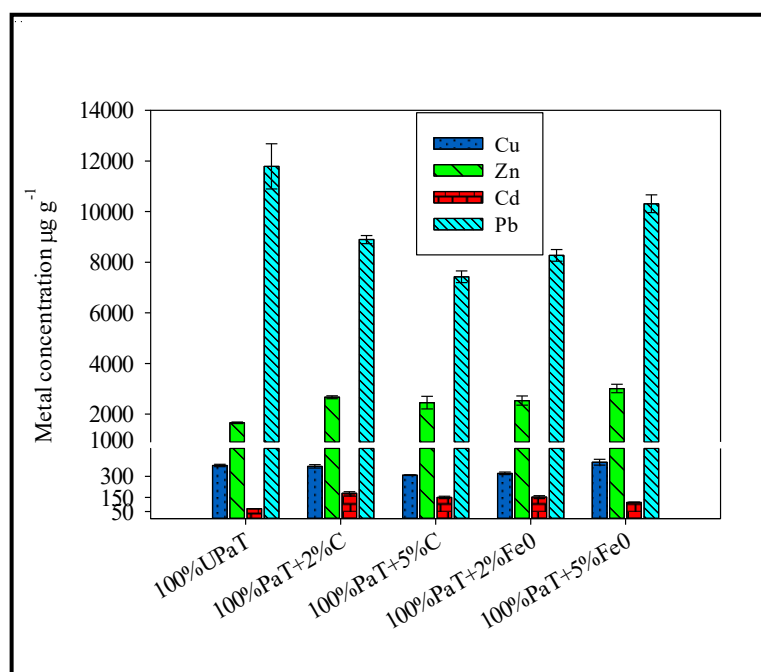


Figure 7. 9 Heavy metal accumulation in the roots of ryegrass grown in unamended Pandora tailings (100% PaT) and 100% PaT amended with 2% and 5% Fe⁰, and 2% and 5% C. Measurements are presented as mean \pm S.E.M; n = 3.

7.4.5.3 Heavy metal concentration in ryegrass shoots

Tables 7.5 and 7.7, and figures 7.10 and 7.11 show the concentrations of studied metals in ryegrass shoots grown in treated and untreated 100% PT and 100% PaT.

Copper

The addition of amendments affected the accumulation of Cu in ryegrass shoots to different extents. Addition of Fe^0 increased the concentration of Cu significantly ($P < 0.05$) in the shoots by 44% and 61% in 100% PT treated with 2% and 5% Fe^0 respectively, compared to the control (100% PT). This may be related to increasing the Cu extractability in Fe^0 -treated PT (Table 7.3). By contrast, the applications of C to 100% PT reduced the Cu content in grass shoots significantly ($P < 0.05$) by 42% and 34%, respectively relative to the control. Karami *et al.* (2011) reported that amending copper mine tailings with C (30% v/v) reduced effectively the translocation of Cu within ryegrass shoots (*L. preenne L. var. Cadix*).

In the case of 100% PaT, the addition of amendments significantly increased the translocation of Cu within grass tissue as it recorded significant ($P < 0.05$) higher levels in 100%PaT treated with both of amendments relative to control (100% PaT). The 5% C dosage was more effective significantly ($P < 0.05$) than others of C and Fe^0 in increasing Cu translocation from roots to the aerial parts of the plant as the Cu level recorded rising by 274% compared to the control (100% UPaT).

Zinc

The applications of both amendments caused a significant ($P < 0.05$) reduction in the concentration of Zn in ryegrass shoots. The Zn levels reduced by 90%, 94%, 92% and 94% in grass grown in 100% PT amended with 2%, 5% C, 2% and 5% Fe^0 , respectively relative to control.

In the case of the 100% PaT treatments, incorporation of amendments reduced the levels of Zn in grass shoots significantly ($P < 0.05$) the most effective treatment was 5% C-amended 100% PaT reduced Zn level in ryegrass shoots by 68% relative to the control (100% PaT). Compost especially at the higher dosage (5%) was more effective than either rate of 2% of C and Fe^0 doses in reducing the translocation of Zn from roots to above-ground parts of grass but the difference was not significant with that of 5% Fe^0 treatment.

The grass shoots grown in both tailings and their treatments accumulated high levels of Zn which is in agreement with the observation of Kashem *et al.* (2010). Who found that high levels

of Zn ($24000 \mu\text{g g}^{-1}$) accumulated in the vegetative parts of plant grown in artificially polluted soil with $1000 \mu\text{g Zn}$, they indicated that Zn can be easily translocate within plant tissues.

Cadmium

Application of Fe^0 did not alter the Cd content of plant shoots developed in treated 100% PT relative to control. By contrast, the application of 2% C reduced the Cd levels in the shoots and the reduction was significant ($P < 0.05$) by 73% in the plant shoots developed in 100% PT+2%C relative to control (100% PT).

In treated 100% PaT, shoots developed in tailings amended with lower dose of Fe^0 containing higher levels of Cd as it increased by 19% comparative to the control. The green parts of the grass grown in the 100% PaT treated with 5% C dosage resulting in reduction of shoot concentration of Cd significantly ($P < 0.05$) by 27% relative to the control (100% PaT). Applying 2% of C and Fe^0 treated 100% PaT did not affect the shoot content of Cd.

Lead

The accumulated levels of Pb were undetectable in the control and in 100% PT treated with both rates of C and control, however the applications of Fe^0 increased the shoot content of Pb. The higher dosage of Fe^0 showed significantly higher levels of Pb in plant shoots approximately 2 times ($67.33 \pm 7.51 \mu\text{g g}^{-1}$) compared to the lower dosage ($35.33 \pm 4.37 \mu\text{g g}^{-1}$).

In the 100% PaT treatments, applications of Fe^0 and 5% C did not alter the Pb shoot content relative to the control. However, 2% C application was more effective at reducing the concentration of Pb in ryegrass shoots by 30% relative to that in the shoots of plant grown in the control (100% UPaT) ($P < 0.05$). The levels of Pb in the grass roots that developed in all 100% PaT treatments were considerably higher by approximately, 104, 76, 67 and 83 times than that in shoots grown in the same treatments. Therefore, the translocation of Pb from roots to shoots was very low, which agrees with Huang *et al.* (1997) and Ho *et al.* (2008), who stated that the lower levels of Pb in plant shoots may be related to the lower ability of Pb to transfer to above-ground parts of plants.

Kumpiene *et al.* (2006) found that the concentration of Cu and Zn increased in grass shoots developed in acidic soil ($\text{pH} = 4.9$) affected by the deposition of industrial wastes amended with 1% Fe^0 . They attributed this to the enhancing of root growth caused by Fe^0 increasing the adsorption of Zn and Cu by roots and then translocating them to shoot, which

agree with our results showed that the levels of Zn increased in ryegrass roots of PaT treatments with increasing the rate of Fe⁰-treated 100% PaT.

Generally, the results of current study, in the case of higher rate of C, agree with those of Nwachukwu (2007), who found that different rates of C were effective in reducing the adsorption of Pb and Cu by ryegrass roots and their translocation within plant tissue and the higher dosage of C was more effective than lower dosage. Nwachukwu and Pulford (2009) found that various doses (1%, 10% and 20%) of C to a former Zn-Pb mine tailings reduced the ryegrass aerial parts content of Pb and Zn, but that 20% C did not affect the Cu concentration in the shoots. Regarding the shoots, the results indicated that C reduced the shoot content of Cu, Zn and to some extent Cd, however, there was no effect on shoot content of Pb in PT treatments. This is not in total agreement with Tandy *et al.* (2009), who found that the application of OM to mine tailings collected from Parys Mountain reduced the shoot content of Cu, however it had no effect on the translocation of Zn within wheat (*Triticum aestivum* L. cv. "Atlas") tissues. The difference between both results may be related to the plant species and their response and tolerance of heavy metals (Quezada-Hinojosa *et al.*, 2015; Mahdavian *et al.*, 2017).

The concentrations of several studied metals in the ryegrass shoots cultivated in 100% PT and its treatments were in the range of the recommended phytotoxic levels of heavy metals in leaf tissue ranging between 3-5 $\mu\text{g g}^{-1}$ for Cd, 20-100 $\mu\text{g g}^{-1}$ for Cu, 30-100 $\mu\text{g g}^{-1}$ for Pb and 100-400 $\mu\text{g g}^{-1}$ for Zn (Gabarrón *et al.*, 2018). Several exceptions to this were the Cd levels in 100% PT and Fe⁰-amended 100% PT that were recorded slightly higher than the phytotoxic levels of Cd in plant leaves, also Zn in the shoots of grass of 100% PT were higher than the critical levels of Zn in plant leaves. In 100% PaT and its treatments heavy metals recorded levels in the range or higher than phytotoxic levels of these metals in plant leaves. Also, higher than phytotoxic levels in plant roots in all 100%PaT treatments. For example, Cd, Cu, Pb and Zn in roots of grass planted in C-treated 100% PaT were much higher than the soil-plant phytotoxic limit which is 3 $\mu\text{g g}^{-1}$ for Cd, 200 $\mu\text{g g}^{-1}$ for Cu, 100- 500 $\mu\text{g g}^{-1}$ for Pb and 400 $\mu\text{g g}^{-1}$ Zn (Gabarrón *et al.*, 2018). Also, the use of grass were harvested from mine sites for animal feeding is banned (Liu *et al.*, 2018). The levels of several heavy metals in the shoots of ryegrass grown in 100% PaT+5% C were high, though the plants showed good biomass productivity. Utmazian *et al.* (2007) found that high levels of Cd (315 $\mu\text{g g}^{-1}$) and Zn (3180 $\mu\text{g g}^{-1}$) accumulated in plant leaves in artificial polluted soil with a single dosage of target metals, though the biomass productivity was high. On the other hand, high levels of studied metals in

tailings, especially Cu, Pb and Zn may influence the efficacy of the phytoremediation of studied sites as a result of the competition effect among heavy metals and also with other nutrients reducing their adsorption by plant roots (Quezada-Hinojosa *et al.*, 2015; Kovacs and Szemmelveisz, 2017). For example, Cu absorption by plant roots is limited by the presence of NH_4^+ , K, Ca and Zn (Alloway, 1990b).

Phytostabilisation technology refers to plants with high biomass productivity and low heavy metal translocation rates from roots to shoots (Alvarenga *et al.*, 2008). In this study, compost stimulated the development and productivity of ryegrass biomass and lowered the translocation of target metals except Cu in PaT amended with both dosages of C, relative to control. Therefore, the combination of ryegrass and compost appears to be an effective option in the phytostabilisation of investigated mines. Additionally, the use of C and Fe^0 with ryegrass led, in general, to an increase in the levels of Cu in above ground biomass compared control suggesting that this combination may be effective as a phytoextraction technology of Cu in Pandora sites. The decrease of several heavy metal shoot content relative to that of control of PaT observed in the current study is a favourable outcome, but more comprehensive analysis is required combining the measurement of concentration of heavy metals in ryegrass tissue and the value of biomass available to primary consumer (Karami *et al.*, 2011). The disposal of grass harvested during the phytoremediation of mine sites should be follow a specific procedure e.g., burning of dried grass biomass then extracting the accumulated heavy metals from the resultant ash, and for use in energy production, for example Fang *et al.* (2017) stated that Italian ryegrass showed an ability to use for energy production. These disposal procedures should be carried out to avoid re-contamination of the environment.

Repeated ryegrass cultivation in C-amended mine tailings for a long period may be sufficient to clean-up the mine sites. Pardo *et al.* (2014) reported that the heavy metal content of *Lolium perenne L.* tissues increased with growth time and significantly exceeded the threshold limits of heavy metal toxicity in plant tissue that had been planted in mine tailings amended with separate dosage compost and animal manure. Also, frequent revegetation of mine sites recharges additional organic substances compensating the degraded OM and balancing the pool of OM in mine tailings, consequently minimizing the concern of re-release the previously restricted heavy metals resulting from the decay of added organic amendments (Tandy *et al.*, 2009).

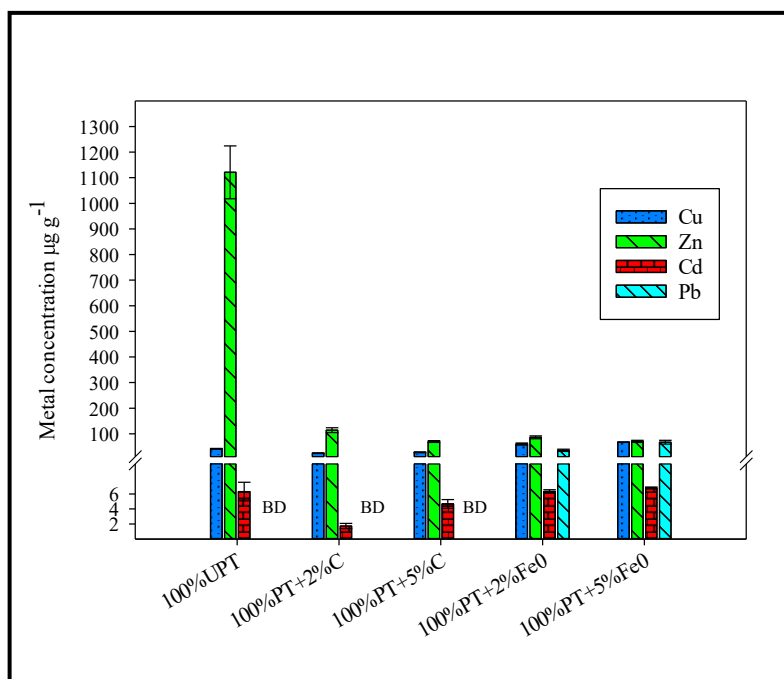


Figure 7. 10 Heavy metals accumulation in the shoots of ryegrass grown in unamended Parys tailings (100% PT) and 100% PT amended with 2% and 5% Fe⁰, and 2% and 5% C, (BD)= Pb levels blow AAS limit detection. Measurements are presented as mean \pm S.E.M; n = 3.

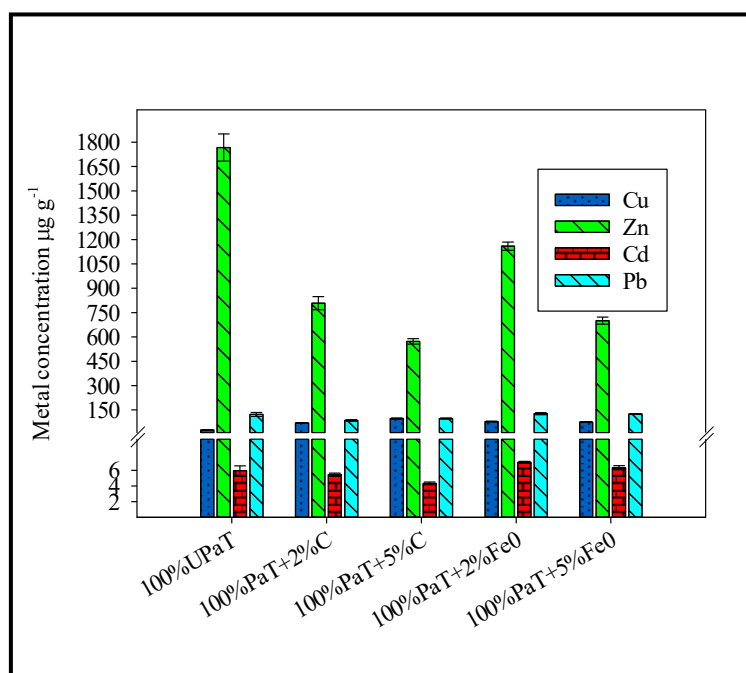


Figure 7. 11 Heavy metals accumulation in the shoots of ryegrass grown in unamended Pandora tailings (100% PaT) and 100% PaT amended with 2% and 5%Fe⁰, and 2% and 5% C. Measurements are presented as mean \pm S.E.M; n = 3.

7.4.6 Effects of Fe⁰ and compost in the Pb fingerprinting in plant tissues

In environmental studies, the ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ determined for samples have been used to identify the Pb pollution sources (Varrica *et al.*, 2018). Therefore, the $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ relationship (Figures 7.12 and 7.13) and Pb isotopic composition of $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ (Tables 7.10 and 7.11) was used to distinguish between Pb sources (Hansmann and Köppel, 2000). Pb isotopic composition were analysed in the application of 75% (PT or PaT) + 25% US (^{w/w}), because the applications of 100%UPT and their treatments with both dosages of Fe⁰ did not produce harvestable roots and inability to analyse high number of samples. The results are presented to the 4th decimal which is common in Pb isotopes studies (Varrica *et al.*, 2018). Also, the analyses did not determine the ^{204}Pb due to its negligible abundance relative to ^{206}Pb , ^{207}Pb and ^{208}Pb (Miller *et al.*, 2007).

7.4.6.1 Ryegrass grown in treatments of 75% PT+25% US+ amendments

Figure 7.12 shows the relationship between the Pb isotopic composition ratios in ryegrass tissues harvested from PT amended with dosages of Fe⁰ and C. A generally linear relationship ($r^2 = 0.48$) was observed between the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios. The Pb isotopic signature for Pb in shoots and roots were generally more similar to that of PT than US and much closer than that of Fe⁰ and C. This confirms that most of Pb in ryegrass tissues is sourced from tailing's Pb rather than from any geogenic Pb within US, or present as an impurity in C and Fe⁰.

The $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in shoots of grass grown in 75% PT+ Fe⁰ (1.1735 ± 0.004 in shoots of 75% PT+ 2% Fe⁰ and 1.1733 ± 0.004 in shoots of 75% PT+ 5% Fe⁰) are closer to that of PT (1.1794 ± 0.005) than of US (1.1672 ± 0.010) (Table 7.10). However, the Pb isotopic composition ($^{206}\text{Pb}/^{207}\text{Pb}$) in shoots harvested from 75% PT+2% C (1.1705 ± 0.004) and in 75% PT+5% C (1.1720 ± 0.004) seemed to be closer to that in US than in PT. The Pb signature in the roots appears to be related to that of PT excluding ryegrass roots harvested from 75% PT+5% Fe⁰ (1.1697 ± 0.004) rather than that of US and may be affected by the Pb fingerprinting in Fe⁰ (1.1350 ± 0.003). The ratios of $^{208}\text{Pb}/^{206}\text{Pb}$ composition of all ryegrass tissues is lower and closer to that of PT than that of US, C and Fe⁰. A lower $^{208}\text{Pb}/^{206}\text{Pb}$ ratio indicates geogenic Pb routes (Jiang and Sun, 2014), suggesting the Pb pollution of PT and plant tissues relate to geological processes rather than anthropogenic activities. $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratios in roots of 75% PT+25% US treated with 2% and 5% C are more similar to that of PT than of shoots harvested from the same treatments. However, the $^{206}\text{Pb}/^{207}\text{Pb}$ ratio in the shoots of 75% PT+25%U S treated with 2% and 5% of Fe⁰ are more similar to that of PT than of roots

harvested from the same treatments. This may be attributed to the lower $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio in Fe^0 (1.1305 ± 0.003) relative to C (1.1502 ± 0.003). Mostly the same trend was observed in the data of $^{208}\text{Pb}/^{206}\text{Pb}$.

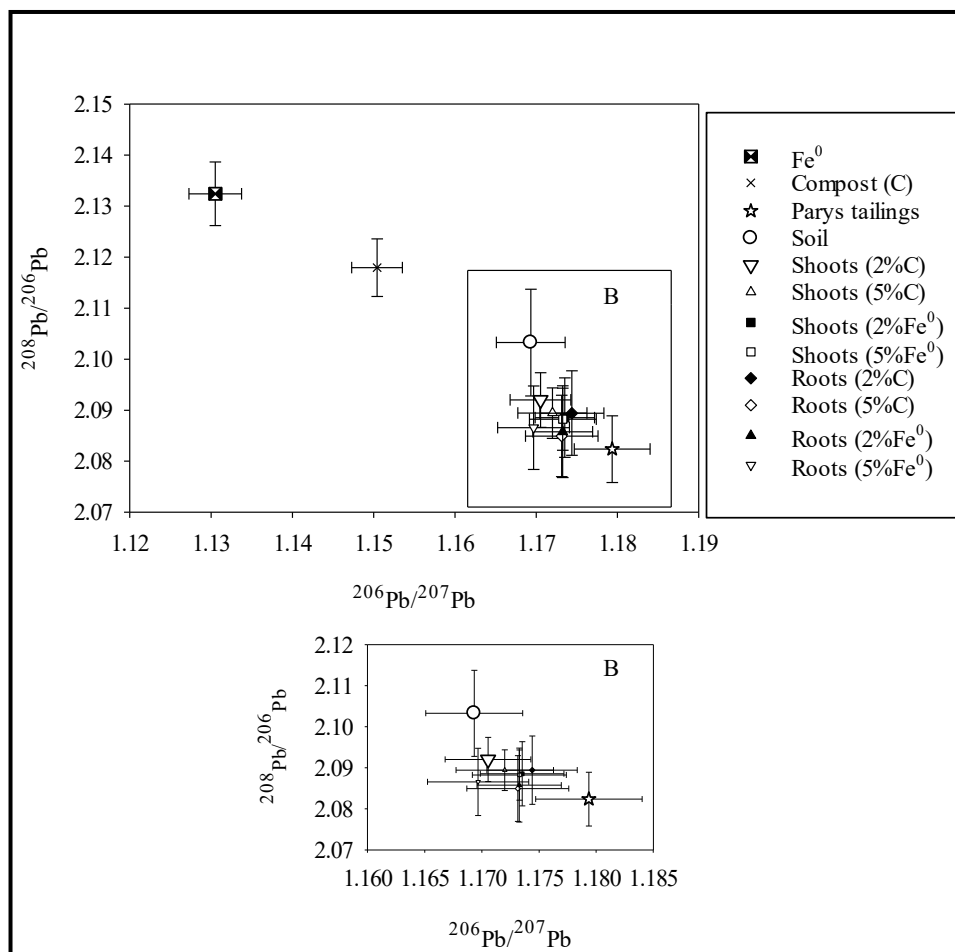


Figure 7. 12 Relationship between $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios in Parys tailings, untreated soil, Fe^0 , compost and ryegrass tissues harvested from different 75% PT+25% US treatments amended with Fe^0 and compost separately. Panel B is shown in an expanded format to further show detail.

Table 7. 10 Lead isotopic composition in PT, US, Fe⁰, compost and ryegrass tissues harvested from different 75% PT+25% US treatments amended with Fe⁰ and compost.

Sample	N	²⁰⁸ Pb/ ²⁰⁶ Pb		²⁰⁶ Pb/ ²⁰⁷ Pb		²⁰⁸ Pb/ ²⁰⁷ Pb	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Parys tailings (PT)	1		2.0824 ± 0.007		1.1794 ± 0.005		2.4540 ± 0.008
Soil	1		2.1033 ± 0.012		1.1672 ± 0.010		2.4531 ± 0.018
Fe ⁰	1		2.1324 ± 0.006		1.1305 ± 0.003		2.4102 ± 0.007
Compost	1		2.1179 ± 0.006		1.1502 ± 0.003		2.4354 ± 0.006
Shoots(2%C)	2	2.0906- 2.0934	2.0920 ± 0.005	1.1701-1.1710	1.1705 ± 0.004	2.4465- 2.4478	2.4472 ± 0.007
Shoots(5%C)	2	2.0885- 2.0903	2.0894 ± 0.005	1.1715-1.1725	1.1720 ± 0.004	2.4450- 2.4490	2.4470 ± 0.007
Shoots (2%Fe ⁰)	2	2.0884- 2.0886	2.0885 ± 0.008	1.1725-1.1745	1.1735 ± 0.004	2.4470- 2.4508	2.4489 ± 0.008
Shoots (5%Fe ⁰)	2	2.0864- 2.0900	2.0882 ± 0.006	1.1722-1.1743	1.1733 ± 0.004	2.4481- 2.4483	2.4482 ± 0.006
Roots (2%C)	2	2.0886- 2.0902	2.0894 ± 0.008	1.1742-1.1746	1.1744 ± 0.004	2.4506- 2.4534	2.4520 ± 0.007
Roots (5%C)	2	2.0825- 2.0873	2.0849 ± 0.008	1.1722-1.1741	1.1731 ± 0.005	2.4432- 2.4446	2.4439 ± 0.008
Roots (2%Fe ⁰)	2	2.0836- 2.079	2.0858 ± 0.009	1.1721-1.1745	1.1733 ± 0.004	2.4449- 2.4451	2.4450 ± 0.003
Roots (5%Fe ⁰)	2	2.0841- 2.0890	2.0866 ± 0.008	1.1672-1.1722	1.1697 ± 0.004	2.4364- 2.4412	2.4388 ± 0.009

7.4.6.2 Ryegrass grown in treatments of 75% PaT+25% US+ amendments

The Pb isotopic composition relationship illustrated in figure 7.13-A shows a linear trend ($r^2 = 0.49$) and indicates that Pb in the amendments is sourced from different origins and did not influence the Pb fingerprinting of treatments and harvested plant tissues. Table 7.11 presents the Pb isotopic ratios in different clusters of PaT treatments. The $^{208}\text{Pb}/^{206}\text{Pb}$ fingerprint in plant samples, PaT and US overlaps and not overlapping with that of the amendment (Figure 7.13) suggesting indigenous routes of Pb in ryegrass tissues reflecting that of PaT and less likely US. The lower possibility of Pb signature in grass tissues reflects that of US may be due to very low levels of pseud-total Pb in this soil. According to Galušková *et al.* (2014) the wide range of the relationship between Pb isotopic composition ratios ($^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$) in tailings indicates several Pb pollution routes with a long period of exposure to the Pb inputs. Consequently, the Pb contamination signature is likely derived from mining of metallic ores and/or metal extracting from ores that has taken place at the site over a considerable time period (Ettler *et al.*, 2006). In addition, it is likely that PaT and US (US collected from Henfaes Research farm, Abergwynnregyn, Gwynedd, UK (53° 14' N, 4° 01' W)) have the same Pb source as the samples collected from sites located not far from each other rather than Parys copper mine which is far from those.

Generally, the Pb isotopic ratios in the plant tissues of all treatments suggest that Pb accumulated in grass shoots and roots are derived from the mine tailings of Parys, because the $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratios in most plant samples were similar to those of PT. However, in the case of PaT, because the $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratios of plant tissues, PaT and US were within the same range of statistical errors as each other (Figure 7.13), it is difficult to determine the origin of the Pb in the plant tissues planted in PaT treatments.

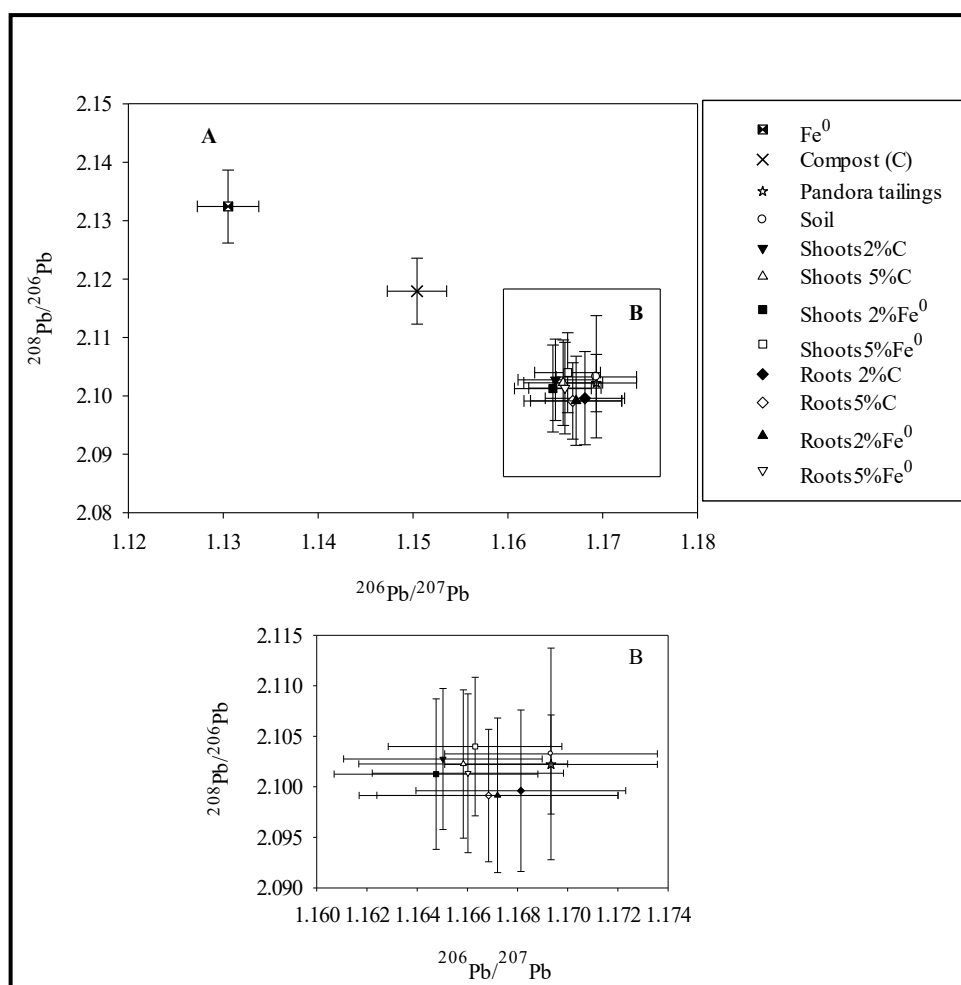


Figure 7. 13 Relationship between $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios in Pandora tailings, untreated soil, Fe^0 , compost and ryegrass tissues harvested from different 75% PT+25% US treatments amended with Fe^0 and compost separately. Panel B is shown in an expanded format to further show detail.

Table 7. 11 Lead isotopic composition in PaT, US, Fe⁰, compost and ryegrass tissues harvested from different 75% PaT+25% US treatments amended with Fe⁰ and compost.

Sample	N	²⁰⁸ Pb/ ²⁰⁶ Pb		²⁰⁶ Pb/ ²⁰⁷ Pb		²⁰⁸ Pb/ ²⁰⁷ Pb	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Pandora tailings (PaT)	1		2.1022 ± 0.005		1.1693 ± 0.004		2.4563 ± 0.005
Soil	1		2.1033 ± 0.012		1.1672 ± 0.010		2.4531 ± 0.018
Fe ⁰	1		2.1324 ± 0.006		1.1305 ± 0.003		2.4102 ± 0.007
Compost	1		2.1179 ± 0.006		1.1502 ± 0.003		2.4354 ± 0.006
Shoots(2%C)	2	2.1014- 2.1041	2.1028 ± 0.007	1.1642-1.1658	1.1650 ± 0.004	2.4484- 2.4487	2.4486 ± 0.008
Shoots(5%C)	2	2.1032- 2.1013	2.1023 ± 0.007	1.1655-1.1662	1.1658 ± 0.004	2.4493- 2.4501	2.4497 ± 0.007
Shoots (2%Fe ⁰)	2	2.1000- 2.1026	2.1013 ± 0.007	1.1647-1.1648	1.1648 ± 0.004	2.4486- 2.4480	2.4463 ± 0.009
Shoots (5%Fe ⁰)	2	2.0994- 2.1086	2.1040 ± 0.007	1.1629-1.1697	1.1663 ± 0.004	2.4500- 2.4536	2.4518 ± 0.010
Roots (2%C)	2	2.0980- 2.1012	2.0996 ± 0.008	1.1659-1.1703	1.1681 ± 0.004	2.4482- 2.4551	2.4517 ± 0.008
Roots (5%C)	2	2.0985- 2.0997	2.0991 ± 0.007	1.1661-1.1676	1.1668 ± 0.005	2.4453- 2.4500	2.4476 ± 0.008
Roots (2%Fe ⁰)	2	2.0962- 2.1021	2.1013 ± 0.008	1.1643-1.1701	1.1672 ± 0.005	2.4464- 2.4518	2.4491 ± 0.007
Roots (5%Fe ⁰)	2	2.1000- 2.1027	2.1013 ± 0.008	1.1631-1.189	1.1660 ± 0.004	2.4445- 2.4530	2.4487 ± 0.006

7.5 Conclusion

The efficacy of two rates of organic and inorganic amendments in combination with ryegrass in decreasing the bioavailability of Cu, Cd, Zn and Pb in acidic mine tailings and uptake these metals by plants was assessed. The pH of highly acidic tailings increased in proportion with the dosage of the amendments, the most effective treatment that used was 5% C for both mine tailings. However, the amendments were not effective in altering the pH of weakly acidic tailings. The amendments was used in the present study affected the phytoavailability of studied metals to different extents depending on the tailings, amendment properties, and the total and phytoavailable concentrations of metals and their characteristics. Ryegrass growth was enhanced by the application of C but applying Fe^0 did not affect biomass productivity relative to unamended tailings. The applications of Fe^0 and C increased the content of several heavy metals in the ryegrass roots especially Zn and Cd by grass roots supporting the usage of them in combination with ryegrass to extract these metals and practically Cd and Zn from PaT tailings. However, both rates of C reduced the uptake and therefore translocation of Pb in both tailings promoting the applying them in the immobilising of heavy metal mobility in mine tailings. The applications of amendments did not result in a significant change in the Pb fingerprint of plant tissues. We concluded that C might be a suitable option to immobilising of several heavy metals in multi-metal contaminated mine tailings and promoting ryegrass growth in the mine sites. A long-term field experiment is required to confirm the findings of the present experiment.

8 GENERAL DISCUSSION

8.1 Introduction

Serious environmental issues (e.g., heavy metal pollution and increased soil acidity) have resulted from the presence of large amounts of mine waste in areas previously used for metal extraction. Recently, it has been estimated that more than 20 million hectares of land are effected by heavy metal contamination globally (Liu *et al.*, 2018). The remediation of heavy metal polluted environments to reduce the risk of these pollutants to the ecosystem using various amendments has been a priority in several countries (Wu *et al.*, 2018). Several organic and inorganic amendments have been utilised in mine sites with the aim of removing these metals and/or reducing the metal mobility and bioavailability to soil biota and plants. These amendments have been applied either on their own or in combination with plants that are able to take up harmful metals and survive in mine sites (Song *et al.*, 2017).

The current study aimed to examine the ability of two technologies separately and together, to reduce the mobility and bioavailability of Cu, Zn, Cd and Pb in mine tailings. The approaches used were phytoremediation using ryegrass as a bio-indicator, and chemical and organic amendments including inorganic (Fe^0 and zeolite) and organic (green waste biochar and green waste compost) amendments. The research included a determination of the geochemistry of target metals in studied sites and their total levels, with a focus on several physical and chemical properties of studied tailings that are known to influence metal chemistry. Tailings were treated using a single method in uncontrolled climatic conditions (one-year outdoor trial) to investigate the effect of time and amendment type (chemical and organic amendments) on the bioavailability of target metals. Building on the results of this investigation, greenhouse experiments were then conducted using different fractions of uncontaminated soil and ryegrass as a bio-indicator for phytoremediation of heavy metals. Ultimately, the ability of Fe^0 and green waste compost to reduce the phytoavailable fractions of heavy metals using two dosage rates was examined under controlled conditions simultaneously with ryegrass to indicate the ability of both approaches to clean up the heavy metal-contaminated mine tailings. Lead isotopes were measured to identify the origin of lead pollution.

This chapter seeks to conclude and combine the result of the work outlined above by responding to the initial hypotheses set out at the beginning of this study (section 1.6), which were:

1. Heavy metals distribution and their chemical speciation are similar in all studied tailings.
2. The applications of a single 2% dosage of organic and inorganic amendments into acidic tailings will:
 - a. reduce the bioavailability of target heavy metals and
 - b. the level of reduction will depend on the type of amendment.
3. Increasing the added proportion of uncontaminated soil to mine tailings will
 - a. reduce the phytoavailable fraction of target heavy metals,
 - b. reduce uptake of studied metals by ryegrass, and
 - c. enhance the ryegrass biomass productivity.
4. The phytoavailable fraction, uptake and translocation of studied metals within ryegrass tissues will decrease with increasing the application rate and depend on the type of amendment.
5. Pb distribution in mine tailings and plant tissues reflects that of parent material.

8.2 Metal chemistry in mine tailings

Exploring the geochemistry of target metals in different studied sites indicated approximately similar chemical behaviour of studied metals in Parys and Britannia tailings with high proportions accumulated in the residual fraction. Conversely, the dominant fraction of heavy metals in Pandora tailings was that associated with the exchangeable fraction (Chapter 4 and 5). The pH and CEC seemed to have a large influence on the dynamics of the studied metals in these locations (Chapter 4). The concentrations of target metals in most mobile fractions in tailings collected from Parys (Chapters 4 and 5) and Britannia mines (Chapter 4) were very low and the pollution risk recorded low values. However, the high pseudo-total levels of several heavy metals and changing weather conditions are likely to influence metal chemistry and make the heavy metals more available, posing a threat to the surrounding terrestrial and aquatic environments (Damrongsiri, 2018). Therefore, reducing the phytoavailability of heavy metals using different approaches is an option to minimise their utilisation and translocation within the ecosystem. The developed scheme of sequential extraction, which assessed the chemical behaviour of studied metals in target tailings, was effective in recovering a satisfactory proportion of extracted metals. The recovery percentages generally ranged from 74 ± 7.27 % to 114 ± 5.46 for Cu, Cd and Zn, but for Pb was higher, ranging from 138 ± 13.31 % to 169 ± 7.02 %. This reflects the high extraction efficiency for Pb using the sequential extraction protocol.

8.3 Single approach for mine tailings remediation

The concern about the risks associated with heavy metal contamination for plants, soil biota and humans varies depending on the levels associated with the bioavailable fractions. The water soluble and exchangeable fractions are more bioavailable forms of heavy metals than other fractions (Liu *et al.*, 2018). Thus, reducing the levels of metals in these fractions by using several immobilising materials is a remediation technology can mitigate against the harm caused by heavy metals. Green waste compost and biochar, zeolite and Fe^0 were applied to amend Parys and Pandora tailings (Chapter 5).

The amendments used had a range of effects on the tailings e.g., modifying the pH, especially that of highly acidic tailings. The modification of tailings pH depended on the amendment type and remediation period. The OM content increased due to the application of compost and biochar. At the end of trial, the occurrence of investigated metals in most mobile fractions (water soluble and exchangeable) depended on the type of amendment and metal characteristics. The levels of several studied metals in the bioavailable fractions were lower than that in untreated tailings. This was the case for Cu in Parys tailings (PT) treated with C, B and Fe^0 , Pb in Fe^0 + PT and B + PT treatments, Cu in Pandora tailings (PaT) amended with Z, B and C and Zn in PaT treated with Z and B. On the other hand, also at the end of study period, bioavailability of Zn in all treated Parys tailings and Cu in Parys tailings treated with zeolite were higher than the levels of these metals in unamended tailings. However, the differences in the metal availability proportions (compared with the controls) were not significant in some cases. Re-release of heavy metals from the adsorptive sites of amendments may occur in response to changes to the physiochemical properties of the amended tailings (Cui *et al.*, 2016). The application of amendments were ineffective in significantly reducing the concentrations of available Pb and Cd in PaT as the phytoavailable proportion of these metals were not different to those in the unamended Pandora tailings.

On several occasions during the experiment, the lower mobile fractions (reducible, oxidisable fractions) and stable form (residual fraction) retained higher levels of heavy metals than the control and the metal levels fluctuated between these fractions throughout the trial. The metal redistribution among different geochemical fractions depended on several factors e.g., amendment type and changes in season (Damrongsiri, 2018). The fluctuation in heavy metals dynamics in the mobile fractions and other fractions during the study period can be explained by several factors. Firstly, several metal cations may compete with investigated metals to occupy the adsorptive sites on /in the amendment, inhibiting the efficiency of amendments to hold target metals. Secondly, the high levels of some target

metals especially associated with exchangeable fraction in Pandora tailings may have led to make the adsorbed levels of these metals by amendments intangible. Thirdly, the dose of amendments used were insufficient to significantly reduce the mobility of several heavy metals. Fourth, there are issues associated with the use of pots for these types of experiment, according to O'Connor *et al.* (2018) the variation of the results of the field remediation experiments decreased with increasing the area size although the results obtained from very large plot studies can also be affected by tailings heterogeneity. Therefore, the variability in results in this study may be related to the small size of the pots used (0.5 L) and using a small quantity of tailings (245 g) which may have increased the competition between heavy metals for active sites in the amendments used as well as affecting the chemical interaction of these metals with the tailings. Fifth, sampling before and after the period of equilibration may produce differing results (Cui *et al.*, 2016a). Finally, study period is an important factor as the biogeochemical properties of tailings are affected by weather conditions which can vary during the study period. For example, different weather conditions may affect the chemistry of metals in studied the tailings and the chemical behaviour of amendments used. Sampling was repeated on several occasions and was performed seasonally for 1 year in order to explore the effects the climate factors (Chapter 5). According to O'Connor *et al.* (2018) several investigators reported that short time field studies (0-6 months) with average of 1 month were insufficient to produces valuable results. Several of the studied metals in the mobile fractions showed similar behaviour during the study period e.g., Cd and Zn in both sites (PT and PaT) during the first 4 months of the trial. However, Cu and Pb showed contrasting chemical behaviour. This may be attributed to the different response of metal chemistry to the changing weather conditions (O'Connor *et al.*, 2018).

8.4 Phytoremediation of mine tailings

Phytoremediation is an alternative remediation strategy that can be used to clean up polluted mine sites by using several plants that can tolerate the toxic levels of heavy metals present (Brandão *et al.*, 2018; Palutoglu *et al.*, 2018). Uncontaminated soil was used in combination with target tailings in different proportions. The application of US to Parys tailings (PT) modified several physical and chemical properties of the tailings, diminished the phytoavailability of studied metals and enhanced the development of ryegrass. Overall, it was concluded that ryegrass has the potential for use in cleaning up Parys mine tailings (PT) *via* phytostabilisation. In the case of Pandora tailings (PaT), there was a negligible change in the pH of the matrices of PaT and US and a significant reduction in OM content

and extractable levels of target metals was recorded as the proportion of US was increased. The development of ryegrass shoots and roots did not show significant differences between the matrices of PaT + US. This study aimed at using adopted grass as a phytoremediation tool. It appears that the remediation of PT may be the result of the contribution of both US and ryegrass; plant growth increased with increasing US fraction due to increased production of biomass resulting in greater uptake of heavy metals. However, the incorporation of different quantities of US to PaT did not stimulate grass development, consequently the phytoremediation of PaT may be carried out without any application of US.

The study findings suggest that, in general, ryegrass could not be considered a hyperaccumulator for target metals in the sites under investigation, except in the case of Pb at the Pandora mine. The inability of the grass used to accumulate high levels of several heavy metals may related to several factors according to van der Ent *et al.* (2013). They reported that several factors can control the ability of plants to be hyperaccumulator and therefore be used as a phytoremediation tool in polluted soils, including: (1) plant genotype (2) the pseudo-total levels and chemical fractions of target metal (3) physicochemical characteristics of the target soil and (4) local environmental factors e.g., rainfall, temperature and seasonal changes.

8.5 Mine tailings remediation via a combination of phytoremediation and amendments

1- The combination of phytoremediation and green waste compost (C):

- I. Showed potential for amending Parys mine tailings as it increased pH and the supply of several nutrients (EC increased), decreasing the extractable levels of Zn, Pb and Cd and the translocation of heavy metals to grass shoots and enhanced the growth of ryegrass biomass.
- II. Showed a similar performance in Pandora mine tailings to that in Parys tailings in terms of modifying several physiochemical characteristics except the pH did not affect by the added amendments.

2- The combination of phytoremediation and Fe⁰:

- I. Showed a poor performance in treating Parys mine tailings since grass growth did not enhanced and the shoot content of heavy metals increased, except in the case of Zn. However, in terms of phytoremediation, these applications may be more effective than C in stimulating the uptake and translocate of target metals within plant tissue. Indeed, as the results presented in Chapter 7 showed, higher levels of Cu, Pb and to some extent Cd were found in plant shoots grown in Fe⁰-treated Parys tailings

than in C-amended Parys tailings and control. Phytoremediation is enhanced by using agents (amendments) with the ability to increase the mobility of target metals and to be more available for adsorption by plant roots. As the heavy metal content of the roots increases, the chance of metals translocating to harvestable parts is improved (Huang *et al.*, 1997).

- II. Showed a significant ability to reduce the extractable fraction of Zn and Pb in Pandora mine tailings, although Cu extractable levels were found to increase. In addition, no significant effect was observed on plant growth and treatments pH. The plant roots accumulated higher levels of Cu, Zn, Pb and Cd, and generally, Cu, Zn and Cd concentrations in ryegrass roots were higher than in the control. Cu translocation in plant tissues increased supporting the use of Fe⁰ in combination with ryegrass in phytoextraction of Cu.

8.6 Lead pollution routes

The target metals found at the different sites exhibited contrasting geochemical behaviour as evidenced by a differing relative abundance of fractions (chapter 4 and 5). This may suggest different sources (anthropogenic or geogenic) of heavy metals. Additionally, Pb levels in both sites recorded extremely high levels ($6570 \pm 187 \mu\text{g g}^{-1}$ in Parys tailings and $101600 \pm 2518 \mu\text{g g}^{-1}$ in Pandora tailings; Chapter 6 and 7) and were the highest recorded level among the elements measured in these sites. Therefore, lead isotopes were measured in order to determine the source of Pb pollution in the mine tailings. The results obtained suggested that the Pb in the Parys mine could be categorised as an indigenous Pb resulted from the weathering of parent material. On the other hand, the Pb isotopic signature in the Pandora tailings was similar to that of uncontaminated soil, which may suggest the Pb in both sites (Pandora site and Henfaes farm) originated from same source, possibly a secondary mining process where the Pb is extracted from its ores. The Pb isotopic signature in the roots and foliar parts of ryegrass reflect that in both sources (tailings and uncontaminated soil).

8.7 Research contribution

Heavy metals were distributed within different fractions of tailings with high pseudo-total levels. The mineralisation of mine spoil under certain weather conditions may lead to the release of heavy metals over a long period, and necessitate several approaches, either separately or together, to treat these sites. The efficacy of several mine tailings remediation

strategies, including chemical stabilisation, tailings replacement and phytoremediation was assessed. Chemical stabilisation of heavy metals in mine tailings was carried out by applying binding materials (compost, biochar, zeolite and Fe^0) at low doses to the sites. Application of immobilised materials is ineffective since the contaminants remain in the target tailings and the immobilised metals may become available again. Remobilisation of heavy metals can take place because added amendments tend to dissolve or/ and degrade over time and because continued ore mineralisation will generate a highly acidic medium, which may facilitate the amendments degradation. Replacement of tailings with uncontaminated soil enhanced grass growth and led to the establishment of proper vegetative cover on degraded mine sites, thereby increasing the capacity of the ryegrass to take up heavy metals and diminishing tailing erosion by wind and rainwater.

Using organic amendments (green waste compost) at the appropriate dosage and on a regular basis in combination with ryegrass may be an effective technique for mine tailings remediation. In addition, the establishment of plants with a high tolerance for heavy metal toxicity and the ability to produce large amounts of biomass within a short space of time is likely to promote successful phytoremediation in the investigated mine sites. The application of compost to mine tailings increases the organic matter content of mine tailings and encourages the binding of heavy metals. Consequently, metal mobility is reduced as well as metal bioavailability resulting from organic matter decomposition. For phytoremediation of mine tailings, the choice of plant species is critical, with those associated with limited metal translocation within their tissues decreasing the risk of heavy metal accumulation in herbivores grazing on the above ground biomass. Immobilisation and phytostabilisation approaches for treating heavy metal-polluted sites can be associated with significant challenges. These can include environmental factors, sustainability, financial cost and public acceptance, all of which should be considered if successful remediation of heavy metals in mine tailings is to be achieved. Pb isotopic composition studies produced useful information about Pb pollution sources. Given the scale of the problem, with approximately 5 million sites having been contaminated with heavy metals worldwide, heavy metal pollution has been categorised as a universal issue (Khalid *et al.*, 2017). Amongst the various sources of contamination, the pollution associated with mining activities represents a particular challenge, having caused significant harm to flora, fauna and human health. The present research therefore has important implications for this persistent and far reaching issue, highlighting the potential of chemical and natural stabilisers, and phytoremediation technologies for reducing heavy metal mobility in contaminated sites.

8.8 Specific recommendations

- Prior to applying any amendment to the site, the physiochemical properties of the amendments (especially heavy metal content) should be analysed.
- Adding H₂O₂ (10 ml) when conducting the oxidisable fraction step of the sequential extraction procedure to extracted heavy metals associated with the OM and sulphides fraction should be systematic (0.25 ml every 5 min) to avoid effervescence and dispersal of the suspension.
- In pot experiments conducted in the field, soil samples should be taken from the trial area before and after setting up the pot experiment to determine whether there is metal leaching from the treated tailings (pots) to the nearby ecosystem. Also, the effect of applying Fe⁰ and zeolites on the biological properties of tailing medium should be considered.

8.9 Future work

The results of this study support the use of the studied remediation approaches for in-situ remediation of mine sites. Therefore, in terms of future work, it is suggested that:

- 1- Remediation studies should be carried out in controlled and uncontrolled conditions to derive more information on the impacts of the treatment technologies used on the bioavailability, transportation, inactivation, uptake and translocation of heavy metals from mine tailings in plants. Further investigations are needed to explore the effect of higher dosage rates and amendment particle sizes on metal chemistry in mine tailings.
- 2- The geochemical behaviour of target metal in studied sites should be explored using different sequential extraction procedures which can yield more reliable results about metal distribution in mine tailings than those obtained using the protocol applied this study.
- 3- For an improved understanding of the impacts of organic and inorganic amendments on the availability of nutrients in amended tailings, various forms of nitrogen and phosphate in tailings should be monitored during the remediation period.
- 4- Due to the fluctuation in target metal mobility in treated tailings, a competitive isotherm study should be carried out to investigate the competition between metals for adsorption onto the active sites of applied amendments. Also, the mine sites is composited of metal sulphides and other minerals with low concentrations (Farrell *et al.*, 2010; Kossoff *et al.*, 2014), studying the occurrence of several anions e.g.,

SO_4^{2-} , PO_4^{3-} and Cl^- in mine tailings solution is beneficial, because of these anions likely effect the amendments capacity to hold heavy metals.

- 5- For phytoremediation purposes, several native plants (grasses and trees) should be examined in terms of their ability to accumulate and tolerate high levels of heavy metals. Also, more studies (both in greenhouses and in the field) are necessary to evaluate the effectiveness, capabilities and limitations of the use of ryegrass in the phytoremediation of mine sites. In addition, the combined effect of organic and inorganic amendments on phytoremediation in mine sites in the field should be explored.
- 6- A long-term study should be carried out to explore the fate of the retained heavy metals in the added organic amendments when the organic material is degraded.
- 7- More intensive investigations are required to evaluate the effect of the distribution of mine tailings airborne particles by dust and rain water on human and environment health around the studied sites.
- 8- There is a need for more studies to help understand the mechanisms involved in heavy metal uptake by plants in mine tailings and thereby improve phytoremediation processes in contaminated mine sites

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10 Appendices

Appendix 10.1 (Additional results related to chapter 4).

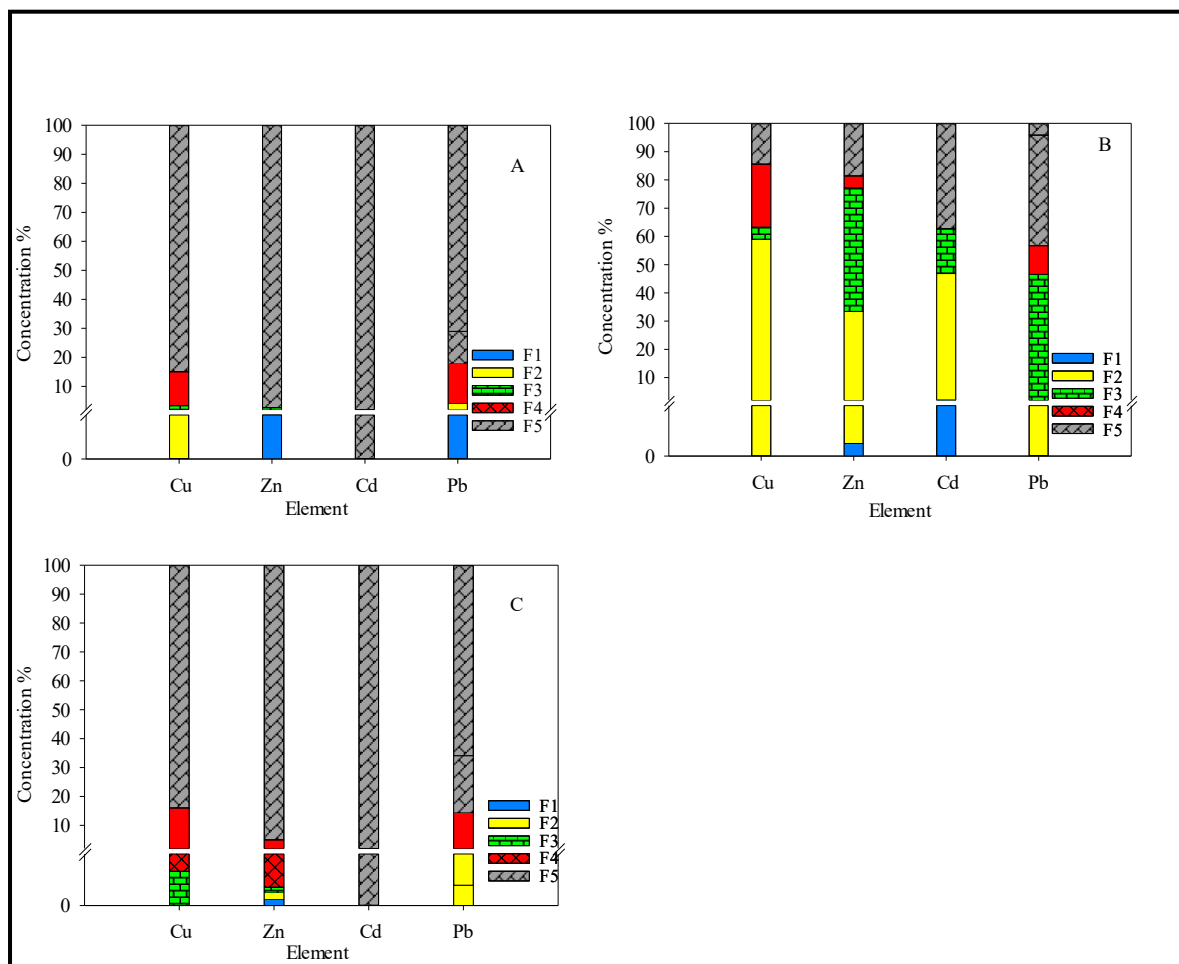


Figure 1 The distribution of Cu, Zn, Cd and Pb in different tailings fractions of A= Britannia mine, B= Pandora mine, C= Parys mine

Appendix 10.2 (Additional results related to chapter 5).

Table 1 Recovery % of Cu, Zn, Cd and Pb determined in treated and untreated mine tailings of Pandora and Parys mines in 4 collection times.

Location	Collection time/ month	Elements			
		Cu %	Zn%	Cd%	Pb%
Parys	0	90 – 115	95 – 106	87 – 111	94 – 109
	4	79 – 102	81 – 112	61 – 120	89 – 112
	8	97 - 123	82 - 105	58 – 110	108 – 131
	12	85 – 111	70 – 110	64 - 125	80 - 114
Pandora	0	80 – 108	83 – 104	91 - 108	117 - 129
	4	74 – 88	104 – 119	40 – 88	94 – 124
	8	70 – 120	81 – 108	60 - 115	116 – 140
	12	40 - 63	101 - 140	75 - 115	70 - 85

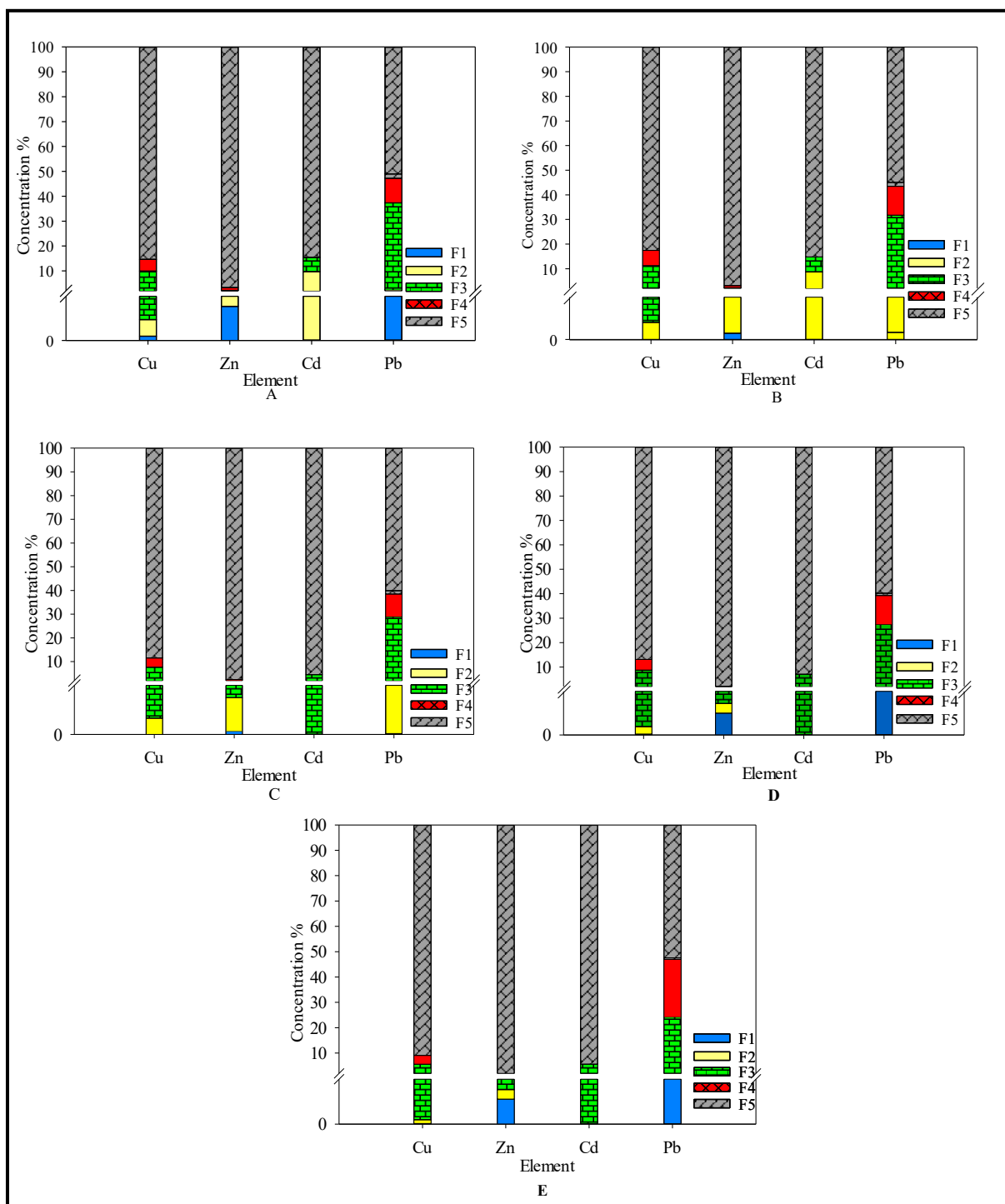


Figure 1. The distribution of Cu, Zn, Cd and Pb in Parys samples at the beginning of treatment period; A) untreated tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compost.

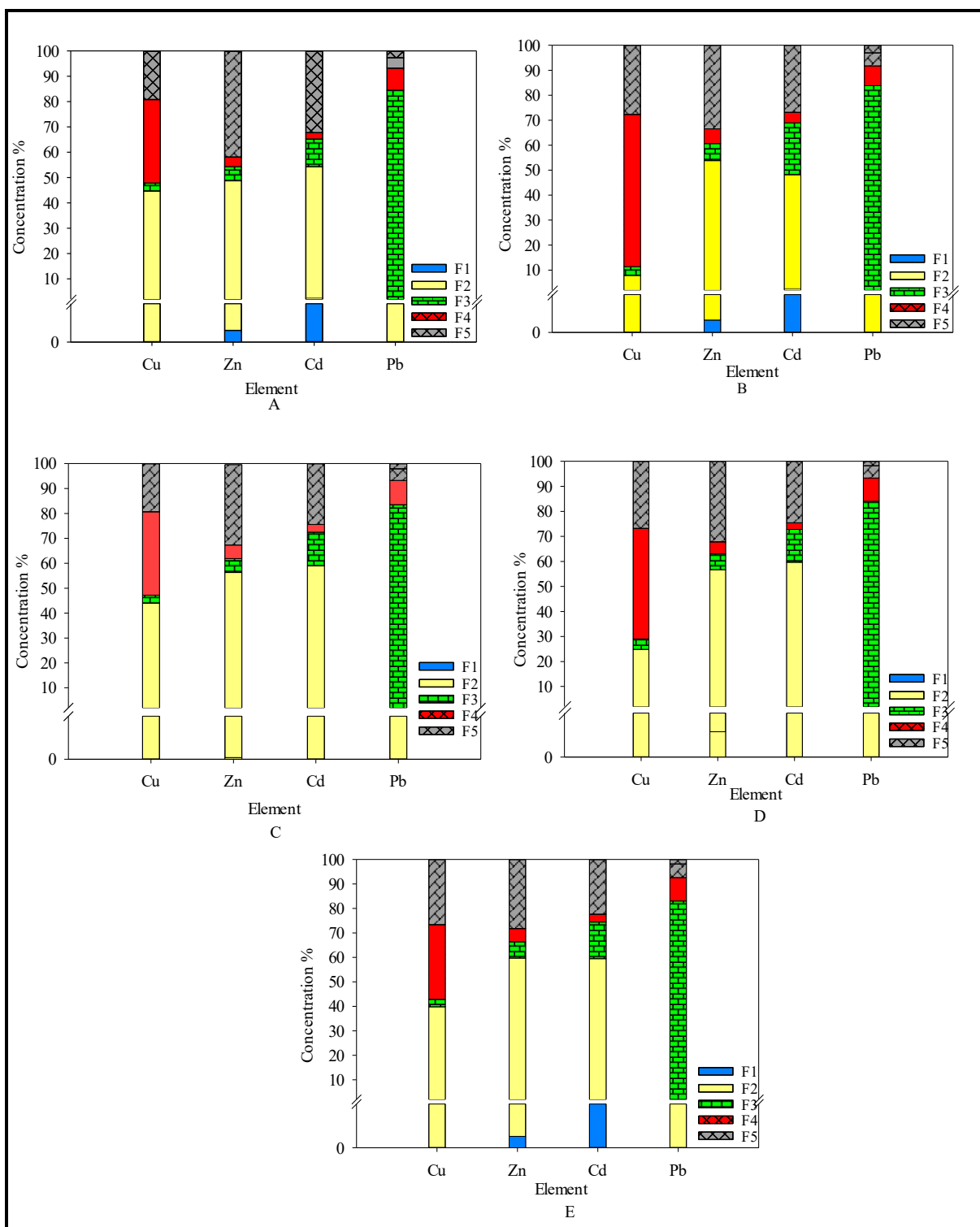


Figure 2. The distribution of Cu, Zn, Cd and Pb in Pandora samples at the beginning of treatment period; A) untreated tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compost.

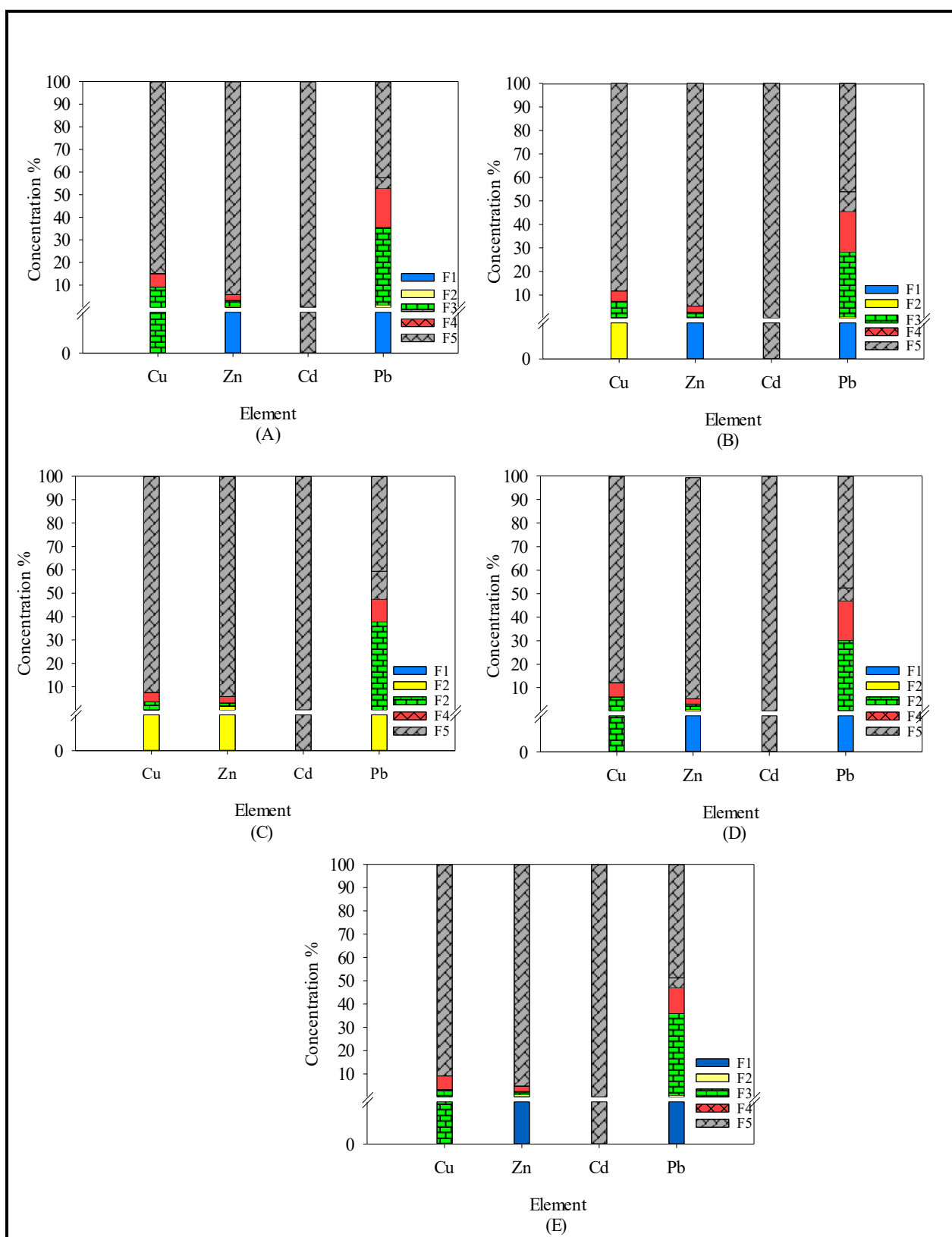


Figure 3. The distribution of Cu, Zn, Cd and Pb after 4 months incubation in: A) untreated Parys mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated wit

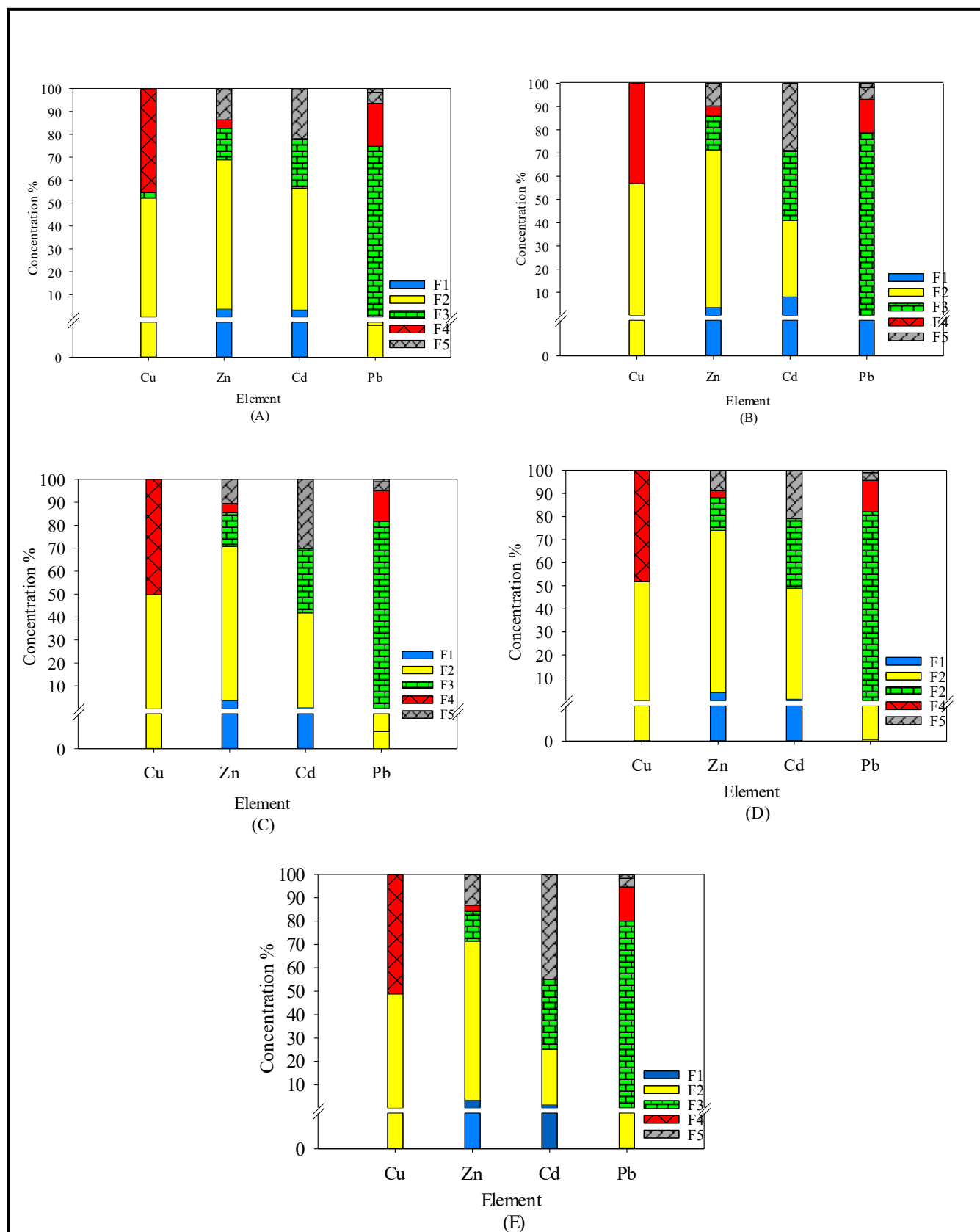


Figure 4. The distribution of Cu, Zn, Cd and Pb after 4 months incubation in: A) untreated Pandora mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, (E) tailings treated with compost.

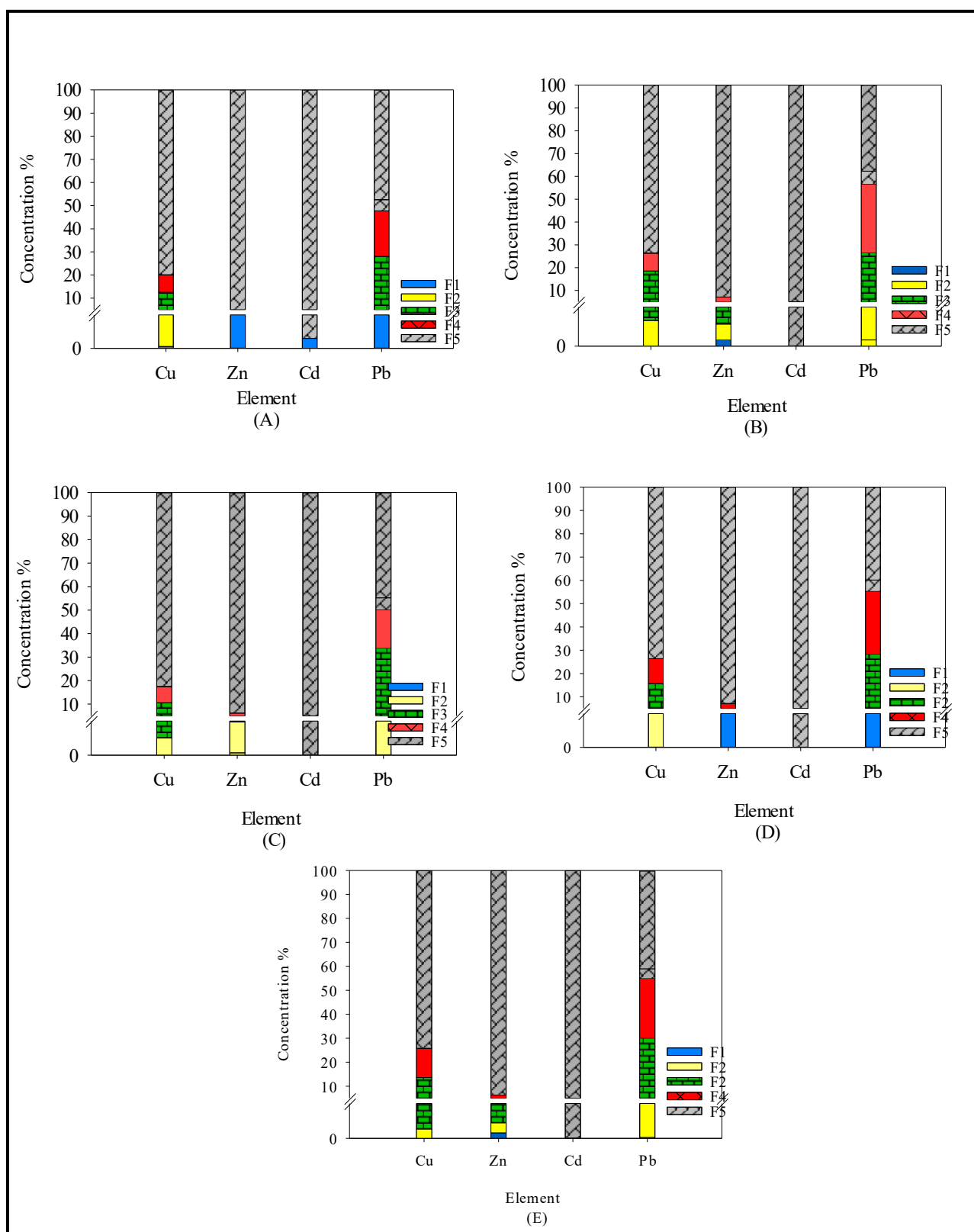


Figure 1. The distribution of Cu, Zn, Cd and Pb after 8 months incubation in: A) untreated Parys mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compost.

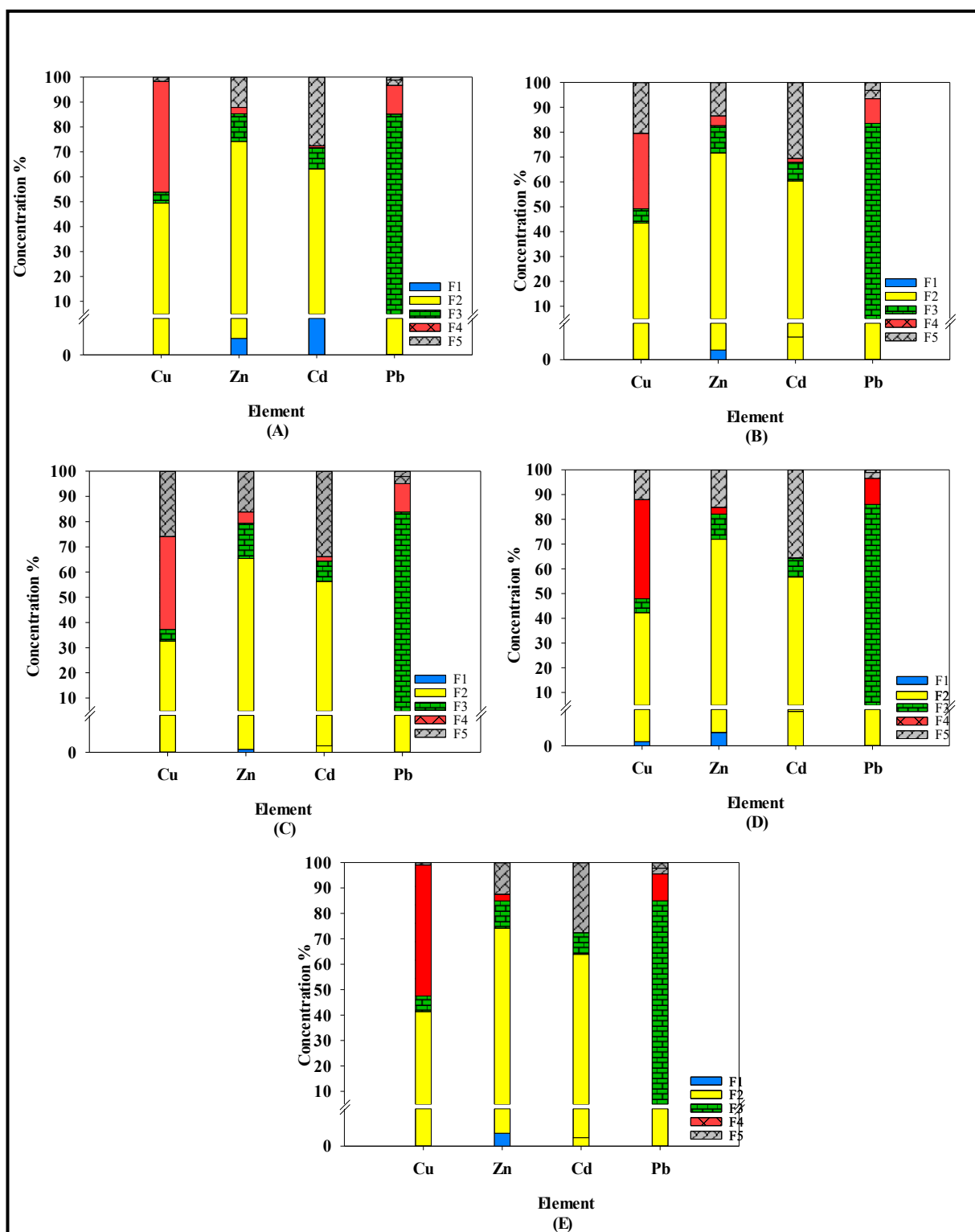


Figure 6. The distribution of Cu, Zn, Cd and Pb after 8 months incubation in: A) untreated Pandora mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compos.

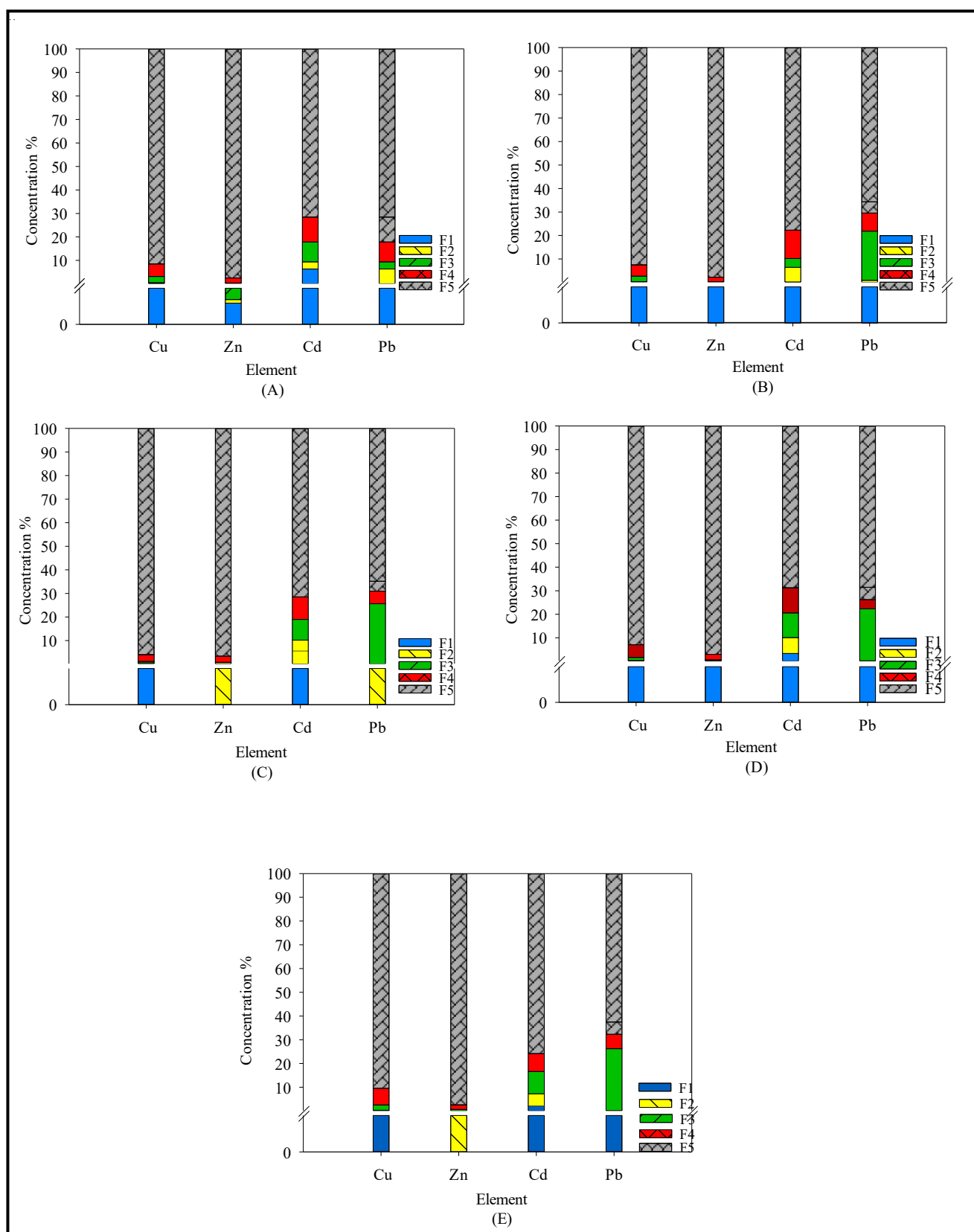


Figure 7. The distribution of Cu, Zn, Cd and Pb after 12 months incubation in: A) untreated Parys mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compost.

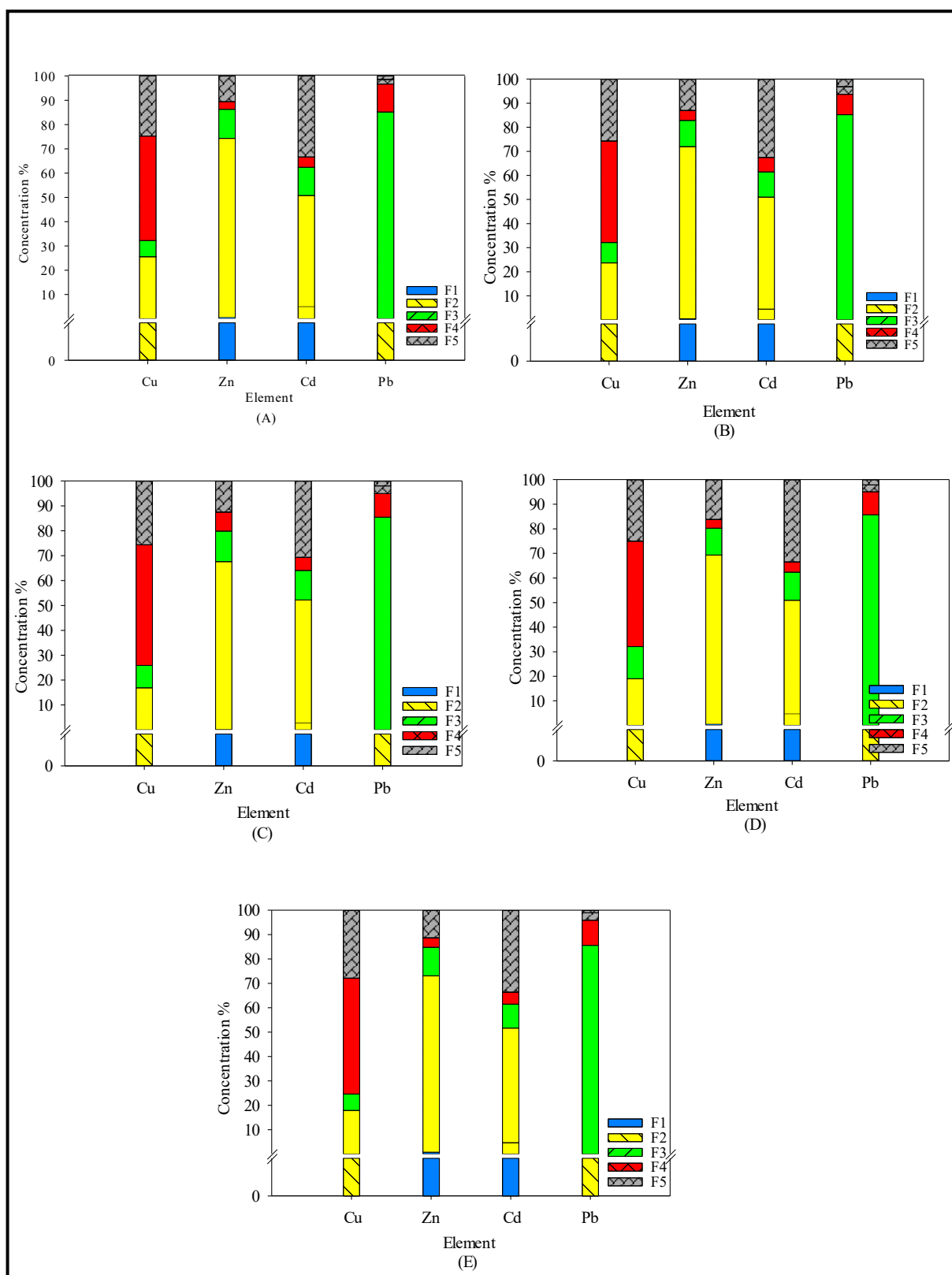


Figure 8. The distribution of Cu, Zn, Cd and Pb after 12 months incubation in: A) untreated Pandora mine tailings, B) tailings treated with Fe^0 , C) tailings treated with zeolite, D) tailings treated with biochar, E) tailings treated with compos.

Table 2 Characteristics of treated Parys Mine tailings at the beginning of treatment period (measurements presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	2.96 \pm 0.02 ^a	4.47 \pm 0.04 ^b	6.17 \pm 0.03 ^c	4.12 \pm 0.05 ^d	3.61 \pm 0.02 ^e
EC <i>mS/cm</i>	0.44 \pm 0.01 ^a	0.50 \pm 0.02 ^b	1.10 \pm 0.02 ^c	1.08 \pm 0.04 ^c	0.63 \pm 0.01 ^e
O. M %	4.65 \pm 0.17 ^a	4.46 \pm 0.12 ^a	5.35 \pm 0.05 ^b	6.81 \pm 0.04 ^c	5.91 \pm 0.04 ^d
TC g kg ⁻¹	13.57 \pm 1.29 ^a	13.83 \pm 0.64 ^a	15.97 \pm 0.42 ^a	26.90 \pm 4.90 ^b	17.40 \pm 0.66 ^a
TN g kg ⁻¹	0.97 \pm 0.04 ^{a, d}	0.82 \pm 0.056 ^b	0.96 \pm 0.04 ^{a, b}	1.22 \pm 0.08 ^c	1.11 \pm 0.003 ^{d, c}
C/N	14: 1 ^a	17: 1 ^{a, b}	16.7 : 1 ^a	22: 1 ^b	16 :1 ^a
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	1.30 \pm 0.37 ^a	1.90 \pm 0.18 ^a	0.82 \pm 0.08 ^a	1.78 \pm 0.45 ^a	5.61 \pm 4.40 ^a
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	11.54 \pm 3.21 ^a	7.24 \pm 0.16 ^a	5.78 \pm 0.37 ^b	12.47 \pm 0.62 ^a	12.04 \pm 0.85 ^a
Sand %	65.9				
Clay %	7.5				
Silt %	26.7				
Total elements					
Cu μ g g ⁻¹	1516 \pm 56.52 ^a	1522 \pm 6.07 ^a	1320 \pm 104 ^b	1468 \pm 5.63 ^a	1543 \pm 16.31 ^a
Zn μ g g ⁻¹	2065 \pm 96 ^a	2108 \pm 40.20 ^a	1697 \pm 56.32 ^b	2024 \pm 78.15 ^{a, b}	2209 \pm 90.77 ^a
Cd μ g g ⁻¹	7.60 \pm 0.35 ^a	8.93 \pm 0.44 ^a	5.93 \pm 0.13 ^b	7.87 \pm 5.35 ^a	7.67 \pm 0.33 ^a
Pb μ g g ⁻¹	15360 \pm 324 ^{a, b}	15533 \pm 181 ^{a, b}	13207 \pm 248 ^b	15143 \pm 28.4 ^{a, b}	17860 \pm 1401 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05.

Table 3 Characteristics of treated Parys mine tailings leachates at the beginning of treatment period (measurements presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	2.99 \pm 0.02 ^a	4.60 \pm 0.09 ^b	6.33 \pm 0.05 ^c	4.68 \pm 0.04 ^b	3.99 \pm 0.04 ^d
EC mS/cm	0.37 \pm 0.02 ^a	0.79 \pm 0.02 ^b	0.52 \pm 0.01 ^c	0.26 \pm 0.01 ^d	0.24 \pm 0.01 ^d
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	BD	0.38 \pm 0.03 ^b	BD	0.29 \pm 0.07 ^b	4.66 \pm 0.03 ^c
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	1.46 \pm 0.22 ^a	1.79 \pm 0.07 ^a	0.32 \pm 0.02 ^b	1.57 \pm 0.26 ^a	1.60 \pm 0.15 ^a
Cu mg L ⁻¹	1.06 \pm 0.02 ^a	BD	BD	0.02 \pm 0.001 ^b	0.03 \pm 0.02 ^b
Zn mg L ⁻¹	4.16 \pm 0.1 ^a	0.67 \pm 0.03 ^b	0.06 \pm 0.02 ^c	0.99 \pm 0.10 ^b	1.67 \pm 0.10 ^c
Cd mg L ⁻¹	BD	BD	BD	BD	BD
Pb mg L ⁻¹	7.03 \pm 0.06 ^a	0.58 \pm 0.16 ^b	0.07 \pm 0.01 ^c	1.78 \pm 0.08 ^d	2.71 \pm 0.04 ^c

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 4 Characteristics of treated Pandora Mine tailings at the beginning of treatment period (measurements presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.72 \pm 0.02 ^a	6.65 \pm 0.06 ^a	8.14 \pm 0.04 ^b	7.15 \pm 0.04 ^c	6.81 \pm 0.04 ^a
EC <i>mS/cm</i>	0.42 \pm 0.02 ^a	0.49 \pm 0.03 ^a	1.68 \pm 0.08 ^b	1.08 \pm 0.01 ^c	0.72 \pm 0.01 ^d
O. M %	7.67 \pm 0.1 ^{a, c}	7.34 \pm 0.12 ^a	7.47 \pm 0.11 ^a	8.69 \pm 0.18 ^b	8.01 \pm 0.03 ^c
TC g kg ⁻¹	51.17 \pm 1.26 ^a	52.17 \pm 0.63 ^a	51.60 \pm 0.46 ^a	66.30 \pm 3.58 ^b	57.67 \pm 0.35 ^a
TN g kg ⁻¹	1.27 \pm 0.03 ^{a, b}	1.05 \pm 0.14 ^b	1.33 \pm 0.07 ^{a, b}	1.68 \pm 0.13 ^a	1.58 \pm 0.08 ^a
C/N	40: 1 ^{a, b}	51: 1 ^b	39: 1 ^{a, b}	40:1 ^{a, b}	36.7: 1 ^a
H2O-extractable NO3--N μ g g ⁻¹	2.45 \pm 0.07 ^a	3.55 \pm 0.18 ^a	7.22 \pm 0.41 ^b	3.57 \pm 0.03 ^a	21.82 \pm 1.19 ^c
H2O-extractable NH4+-N μ g g ⁻¹	9.04 \pm 0.21 ^a	9.47 \pm 0.26 ^{a, b}	9.62 \pm 0.9 ^{a, b}	10.87 \pm 0.89 ^{a, b}	12.64 \pm 1.06 ^b
Sand %	59.7				
Clay %	4.5				
Silt %	35.8				
Total elements					
Cu μ g g ⁻¹	207.5 \pm 6.31 ^{a, b}	214.8 \pm 3.06 ^a	183.7 \pm 6.24 ^b	200 \pm 5.94 ^{a, b}	192.7 \pm 5.6 ^{a, b}
Zn μ g g ⁻¹	37476 \pm 797 ^a	39149 \pm 907 ^a	35564 \pm 930 ^a	38555 \pm 779 ^a	36584 \pm 1449 ^a
Cd μ g g ⁻¹	102.67 \pm 3.35 ^a	106.27 \pm 5.10 ^a	86.4 \pm 0.92 ^a	96.80 \pm 5.45 ^a	91.87 \pm 5.35 ^a
Pb μ g g ⁻¹	74533 \pm 1572 ^a	76933 \pm 1733 ^a	55867 \pm 3014 ^b	74400 \pm 1222 ^a	72933 \pm 4846 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test P < 0.05.

Table 5 Characteristics of treated Pandora Mine tailings leachates at the beginning of treatment period (measurements presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.88 \pm 0.03 ^a	7.00 \pm 0.06 ^{a, d}	7.99 \pm 0.02 ^b	7.15 \pm 0.01 ^d	7.12 \pm 0.03 ^d
EC mS/cm	0.49 \pm 0.02 ^a	0.51 \pm 0.002 ^a	1.70 \pm 0.3 ^b	1.05 \pm 0.04 ^{a, b}	0.72 \pm 0.01 ^a
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	0.47 \pm 0.02 ^{a, b}	0.80 \pm 0.04 ^{a, c}	0.45 \pm 0.02 ^b	0.88 \pm 0.07 ^c	6.01 \pm 0.06 ^d
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	0.23 \pm 0.02 ^a	1.61 \pm 1.25 ^a	0.14 \pm 0.01 ^a	0.38 \pm 0.05 ^a	0.32 \pm 0.06 ^a
Cu mg L ⁻¹	BD	BD	BD	BD	BD
Zn mg L ⁻¹	44.55 \pm 0.08 ^a	38.72 \pm 0.19 ^b	0.17 \pm 0.03 ^c	16.87 \pm 1.18 ^d	24.63 \pm 0.41 ^e
Cd mg L ⁻¹	0.46 \pm 0.003 ^a	0.42 \pm 0.001 ^b	BD	0.16 \pm 0.02 ^d	0.24 \pm 0.003 ^e
Pb mg L ⁻¹	0.31 \pm 0.01 ^a	0.23 \pm 0.04 ^a	0.01 \pm 0.01 ^b	0.20 \pm 0.02 ^a	0.21 \pm 0.02 ^a

• Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 16 Physical and chemical properties of Parys tailings after 4 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	3.55 \pm 0.04 ^a	3.99 \pm 0.06 ^b	7.86 \pm 0.02 ^c	4.11 \pm 0.01 ^{b, c}	3.67 \pm 0.02 ^a
EC <i>mS/cm</i>	0.15 \pm 0.01 ^a	0.1 \pm 0.001 ^b	0.07 \pm 0.003 ^c	0.09 \pm 0.004 ^b	0.12 \pm 0.007 ^{a, b}
O. M %	3.98 \pm 0.03 ^a	3.99 \pm 0.03 ^a	3.87 \pm 0.03 ^a	5.51 \pm 0.07 ^b	4.45 \pm 0.09 ^c
TC g kg ⁻¹	15.1 \pm 0.1 ^a	14.2 \pm 1.1 ^a	14.0 \pm 0.50 ^a	26.2 \pm 1.2 ^b	21.0 \pm 0.7 ^c
TN g kg ⁻¹	0.87 \pm 0.03 ^a	0.81 \pm 0.03 ^a	0.80 \pm 0.01 ^a	1.23 \pm 0.07 ^b	1.11 \pm 0.03 ^b
C/N	17 : 1 ^a	17.5 : 1 ^{a, b}	17.6 : 1 ^{a, b}	22 : 1 ^b	19 : 1 ^{a, b}
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	0.36 \pm 0.32 ^a	0.14 \pm 0.07 ^a	1.16 \pm 0.32 ^a	0.23 \pm 0.11 ^a	0.19 \pm 0.19 ^a
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	4.32 \pm 1.18 ^a	5.49 \pm 1.08 ^a	6.07 \pm 0.97 ^a	3.93 \pm 0.51 ^a	4.32 \pm 1.36 ^a
Total elements					
Cu μ g g ⁻¹	1602 \pm 42.1 ^a	1634 \pm 24.4 ^a	1568 \pm 31.9 ^a	1591 \pm 12.9 ^a	1667 \pm 31.5 ^a
Zn μ g g ⁻¹	1888 \pm 63.2 ^a	2013 \pm 113 ^a	1927 \pm 53.6 ^a	1977 \pm 156.6 ^a	2003 \pm 14.3 ^a
Cd μ g g ⁻¹	1.67 \pm 0.17 ^a	2.3 \pm 0.3 ^a	2.53 \pm 0.58 ^a	3.6 \pm 0.83 ^a	3.0 \pm 0.23 ^a
Pb μ g g ⁻¹	12800 \pm 1034 ^a	13053 \pm 152 ^a	13800 \pm 600 ^a	12867 \pm 29 ^a	13467 \pm 351 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05.

Table 7 Physical and chemical properties of Parys tailings leachates after 4 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	3.50 \pm 0.04 ^a	4.26 \pm 0.31 ^a	7.39 \pm 0.2 ^b	4.45 \pm 0.27 ^a	6.81 \pm 0.04 ^b
EC mS/cm	0.18 \pm 0.02 ^{a, b}	0.11 \pm 0.01 ^a	0.15 \pm 0.003 ^{a, b}	0.14 \pm 0.03 ^{a, b}	0.20 \pm 0.01 ^b
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	1.66 \pm 0.52 ^{a, b}	1.66 \pm 0.52 ^{a, b}	2.68 \pm 0.30 ^b	0.56 \pm 0.19 ^a	BD ^a
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	1.59 \pm 0.06 ^a	1.87 \pm 0.12 ^{a, b}	2.67 \pm 0.40 ^b	1.52 \pm 0.12 ^a	1.24 \pm 0.03 ^a
Cu mg L ⁻¹	0.01 \pm 0.001 ^a	0.03 \pm 0.01 ^a	0.21 \pm 0.11 ^a	BD	0.02 \pm 0.002 ^a
Zn mg L ⁻¹	0.73 \pm 0.26 ^{a, b}	0.58 \pm 0.14 ^{a, b}	0.11 \pm 0.02 ^b	0.46 \pm 0.12 ^{a, b}	0.86 \pm 0.01 ^a
Cd mg L ⁻¹	BD	BD	BD	BD	BD
Pb mg L ⁻¹	9.2 \pm 1.2 ^a	5.8 \pm 1.1 ^{a, b}	2.81 \pm 1.25 ^b	4.37 \pm 1.1 ^{a, b}	6.9 \pm 0.82 ^{a, b}

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 8 Physical and chemical properties of Pandora tailings after 4 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.60 \pm 0.02 ^a	6.63 \pm 0.01 ^a	6.74 \pm 0.03 ^b	6.62 \pm 0.02 ^a	6.55 \pm 0.01 ^a
EC <i>mS/cm</i>	0.23 \pm 0.003 ^a	0.25 \pm 0.01 ^a	0.16 \pm 0.02 ^b	0.21 \pm 0.004 ^{a, b}	0.26 \pm 0.02 ^a
O. M %	7.06 \pm 0.01 ^a	7.01 \pm 0.22 ^a	6.87 \pm 0.20 ^a	8.93 \pm 0.06 ^b	8.10 \pm 0.21 ^b
TC g kg ⁻¹	52.4 \pm 1.1 ^a	51.1 \pm 0.10 ^a	52.9 \pm 0.52 ^{a, c}	61.2 \pm 1.5 ^{b, c}	57.3 \pm 0.3 ^c
TN g kg ⁻¹	1.2 \pm 0.04 ^a	1.17 \pm 0.05 ^a	1.26 \pm 0.03 ^{a, c}	1.7 \pm 0.08 ^{b, c}	1.50 \pm 0.1 ^c
C/N	45 : 1 ^a	44 : 1 ^{a, b}	42 : 1 ^{a, b}	39 :1 ^b	38 :1 ^b
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	0.86 \pm 0.01 ^a	0.38 \pm 0.07 ^a	0.89 \pm 0.38 ^a	0.96 \pm 0.14 ^a	1.26 \pm 0.32 ^a
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	4.32 \pm 1.08 ^a	6.65 \pm 0.51 ^a	16.8 \pm 2.61 ^b	6.45 \pm 1.16 ^a	5.29 \pm 1.54 ^a
Total elements					
Cu μ g g ⁻¹	149.1 \pm 3.98 ^a	172.67 \pm 1.3 ^b	150.27 \pm 5.3 ^a	172.53 \pm 0.67 ^b	204 \pm 8.0 ^c
Zn μ g g ⁻¹	40645 \pm 1334 ^a	41925 \pm 696 ^a	44277 \pm 1874 ^a	421357 \pm 594 ^a	44363 \pm 1767 ^a
Cd μ g g ⁻¹	80.0 \pm 1.6 ^a	80.53 \pm 1.8 ^a	83.07 \pm 0.81 ^a	81.47 \pm 4.1 ^a	96.53 \pm 8.2 ^a
Pb μ g g ⁻¹	81947 \pm 419 ^{a, b}	85573 \pm 2158 ^{a, c}	72880 \pm 3395 ^b	93627 \pm 1910 ^c	84933 \pm 2175

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05.

Table 9 Physical and chemical properties of Pandora tailings leachates after 4 months incubation with amendment (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	7.04 \pm 0.01 ^a	7.24 \pm 0.03 ^b	7.23 \pm 0.03 ^b	7.23 \pm 0.026 ^b	7.06 \pm 0.03 ^a
EC mS/cm	0.67 \pm 0.01 ^a	0.73 \pm 0.01 ^{a, b}	0.62 \pm 0.01 ^a	0.62 \pm 0.02 ^a	0.88 \pm 0.08 ^b
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	1.89 \pm 0.26 ^{a, b}	0.70 \pm 0.13 ^b	4.42 \pm 1.60 ^a	1.69 \pm 0.15 ^{a, b}	2.13 \pm 0.60 ^{a, b}
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	0.48 \pm 0.03 ^a	2.20 \pm 0.15 ^b	0.44 \pm 0.03 ^a	0.43 \pm 0.02 ^a	0.66 \pm 0.21 ^a
Cu mg L ⁻¹	BD	BD	BD	BD	BD
Zn mg L ⁻¹	89.65 \pm 1.11 ^a	54.51 \pm 2.14 ^b	25.28 \pm 0.39 ^c	70.17 \pm 0.79 ^d	87.22 \pm 1.48 ^a
Cd mg L ⁻¹	0.68 \pm 0.02 ^a	0.55 \pm 0.04 ^b	0.20 \pm 0.002 ^d	0.52 \pm 0.01 ^b	0.61 \pm 0.04 ^{a, b}
Pb mg L ⁻¹	BD	BD	BD	BD	BD

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 10 Physical and chemical properties of Parys tailings after 8 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH	3.44 \pm 0.03 ^a	3.77 \pm 0.02 ^{b,d}	7.42 \pm 0.07 ^c	4.01 \pm 0.1 ^d	3.55 \pm 0.02 ^{a,b}
EC <i>mS/cm</i>	0.15 \pm 0.003 ^a	0.10 \pm 0.006 ^b	0.08 \pm 0.007 ^b	0.1 \pm 0.004 ^b	0.13 \pm 0.003 ^a
O. M %	3.81 \pm 0.04 ^a	3.96 \pm 0.08 ^a	4.10 \pm 0.04 ^a	5.43 \pm 0.15 ^b	4.75 \pm 0.12 ^c
TC g kg ⁻¹	16.13 \pm 0.9 ^a	16.67 \pm 1.6 ^a	16.43 \pm 1.24 ^a	29.63 \pm 0.56 ^b	18.4 \pm 0.69 ^a
TN g kg ⁻¹	0.74 \pm 0.05 ^a	0.80 \pm 0.06 ^a	0.87 \pm 0.03 ^{a,c}	1.21 \pm 0.1 ^b	1.13 \pm 0.05 ^{c,b}
C/N	22 : 1 ^a	21 : 1 ^a	19 : 1 ^a	25 : 1 ^a	16 : 1 ^a
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	BD	BD	BD	BD	BD
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	2.98 \pm 1.41 ^a	3.65 \pm 1.11 ^a	2.09 \pm 0.93 ^a	0.89 \pm 0.77 ^a	2.73 \pm 1.18 ^a
Total elements					
Cu μ g g ⁻¹	728.1 \pm 8.10 ^a	712.6 \pm 7.72 ^a	734.8 \pm 4.80 ^a	745.6 \pm 4.35 ^a	742.5 \pm 12.90 ^a
Zn μ g g ⁻¹	2113 \pm 161 ^a	2154 \pm 71 ^a	2248 \pm 39 ^a	2360 \pm 19 ^a	2192 \pm 197 ^a
Cd μ g g ⁻¹	3.10 \pm 0.42 ^a	3.03 \pm 0.19 ^a	2.8 \pm 0.12 ^a	3.47 \pm 0.18 ^a	2.70 \pm 0.74 ^a
Pb μ g g ⁻¹	13740 \pm 163 ^{a,c}	12640 \pm 185 ^b	14280 \pm 144 ^a	13267 \pm 353 ^{a,b,c}	13146 \pm 202 ^{b,c}

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05.

Table 11 Physical and chemical properties of Parys tailings leachates after 8 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.95 \pm 0.29 ^a	6.57 \pm 0.2 ^a	7.77 \pm 0.09 ^b	7.05 \pm 0.13 ^a	6.61 \pm 0.2 ^a
EC mS/cm	0.17 \pm 0.02 ^a	0.13 \pm 0.01 ^a	0.28 \pm 0.02 ^b	0.19 \pm 0.01 ^a	0.14 \pm 0.01 ^a
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	BD ^a	BD ^a	0.024 \pm 0.02 ^a	0.019 \pm 0.02 ^a	BD ^a
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	1.21 \pm 0.17 ^a	1.74 \pm 0.52 ^a	0.97 \pm 0.11 ^a	1.07 \pm 0.13 ^a	0.73 \pm 0.11 ^a
Cu mg L ⁻¹	0.013 \pm 0.01 ^{a,b}	0.003 \pm 0.002 ^b	0.03 \pm 0.001 ^c	BD ^{d,b}	BD ^{d, b}
Zn mg L ⁻¹	0.61 \pm 0.22 ^a	1.25 \pm 0.61 ^{a,b}	0.12 \pm 0.07 ^a	1.36 \pm 0.31 ^{a,b}	3.10 \pm 0.52 ^b
Cd mg L ⁻¹	BD	BD	BD	BD	BD
Pb mg L ⁻¹	1.72 \pm 0.35 ^a	0.51 \pm 0.18 ^a	0.31 \pm 0.043 ^a	0.45 \pm 0.20 ^a	2.22 \pm 0.92 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 12 Physical and chemical properties of Pandora tailings after 8 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH	6.65 \pm 0.05 ^a	6.98 \pm 0.02 ^b	6.93 \pm 0.03 ^{b,c}	6.80 \pm 0.01 ^{c,d}	6.79 \pm 0.03 ^d
EC <i>mS/cm</i>	0.28 \pm 0.02 ^{a,c}	0.29 \pm 0.01 ^a	0.15 \pm 0.01 ^b	0.23 \pm 0.003 ^{c, d}	0.22 \pm 0.01 ^d
O. M %	7.73 \pm 0.09 ^a	7.37 \pm 0.04 ^a	7.41 \pm 0.02 ^a	8.92 \pm 0.06 ^b	7.97 \pm 0.1 ^c
TC g kg ⁻¹	53.87 \pm 0.83 ^a	53.10 \pm 0.25 ^a	54.67 \pm 0.66 ^a	63.97 \pm 1.10 ^b	57.2 \pm 2.20 ^a
TN g kg ⁻¹	1.21 \pm 0.012 ^a	1.06 \pm 0.06 ^a	1.22 \pm 0.05 ^{a,b}	1.48 \pm 0.04 ^b	1.33 \pm 0.13 ^{a,b}
C/N	45 : 1 ^a	50 : 1 ^a	45 : 1 ^a	43 : 1 ^a	43 : 1 ^a
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	BD	BD	BD	BD	BD
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	3.08 \pm 0.19 ^a	2.11 \pm 0.15 ^{a, b}	2.05 \pm 0.14 ^{a, b}	1.21 \pm 0.58 ^b	3.29 \pm 0.20 ^a
Total elements					
Cu μ g g ⁻¹	91.7 \pm 4.19 ^{a,b}	111.9 \pm 12.6 ^b	72.1 \pm 3.68 ^a	98.1 \pm 8.44 ^{a, b}	84.7 \pm 1.9 ^{a,b}
Zn μ g g ⁻¹	45872 \pm 1968 ^a	47813 \pm 3301 ^a	45571 \pm 1231 ^a	53146 \pm 9199 ^a	45893 \pm 2163 ^a
Cd μ g g ⁻¹	101.2 \pm 9.70 ^a	102.8 \pm 17.6 ^a	93.1 \pm 2.18 ^a	96.1 \pm 4.1 ^a	102.1 \pm 4.56 ^a
Pb μ g g ⁻¹	72773 \pm 1274 ^a	72320 \pm 1162 ^a	58653 \pm 1421 ^b	67680 \pm 675 ^a	69547 \pm 2063 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05 .

Table 13 Physical and chemical properties of Pandora tailings leachates after 8 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.85 \pm 0.06 ^a	7.09 \pm 0.04 ^b	7.11 \pm 0.03 ^b	6.97 \pm 0.02 ^{a,b}	6.98 \pm 0.04 ^{a,b}
EC mS/cm	0.86 \pm 0.01 ^a	1.37 \pm 0.07 ^b	0.64 \pm 0.02 ^c	0.89 \pm 0.06 ^a	0.87 \pm 0.01 ^a
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	BD	BD	BD	BD	BD
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	0.97 \pm 0.10 ^{a,b}	0.95 \pm 0.16 ^{a,b}	1.11 \pm 0.22 ^a	0.94 \pm 0.02 ^{a,b}	0.42 \pm 0.12 ^b
Cu mg L ⁻¹	BD	BD	BD	BD	BD
Zn mg L ⁻¹	54.67 \pm 2.70 ^a	18.51 \pm 0.88 ^b	16.58 \pm 0.83 ^b	55.30 \pm 0.60 ^a	59.63 \pm 1.69 ^a
Cd mg L ⁻¹	0.61 \pm 0.01 ^a	0.35 \pm 0.013 ^b	0.26 \pm 0.02 ^c	0.52 \pm 0.02 ^d	0.50 \pm 0.01 ^d
Pb mg L ⁻¹	0.19 \pm 0.02 ^a	0.23 \pm 0.02 ^a	0.07 \pm 0.01 ^a	0.18 \pm 0.05 ^a	0.22 \pm 0.06 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Table 14 Physical and chemical properties of Parys tailings after 12 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH	3.45 \pm 0.02 ^a	3.71 \pm 0.01 ^b	7.12 \pm 0.02 ^c	3.84 \pm 0.02 ^d	3.55 \pm 0.02 ^c
EC <i>mS/cm</i>	0.15 \pm 0.003 ^a	0.11 \pm 0.003 ^b	0.09 \pm 0.006 ^c	0.10 \pm 0.001 ^{b,c}	0.13 \pm 0.003 ^d
O. M %	5.76 \pm 0.09 ^a	5.69 \pm 0.11 ^a	5.56 \pm 0.06 ^a	7.28 \pm 0.14 ^b	6.48 \pm 0.09 ^c
TC g kg ⁻¹	16.37 \pm 1.05 ^a	15.00 \pm 0.1 ^a	15.93 \pm 0.24 ^a	27.1 \pm 0.95 ^b	18.53 \pm 0.09 ^a
TN g kg ⁻¹	0.67 \pm 0.01 ^{a,c}	0.45 \pm 0.06 ^{b,c}	0.55 \pm 0.05 ^c	0.86 \pm 0.06 ^a	0.75 \pm 0.05 ^{a,c}
C/N	25 : 1 ^a	34: 1 ^a	30 : 1 ^a	32: 1 ^a	25 : 1 ^a
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	1.20 \pm 0.15 ^a	1.01 \pm 0.05 ^a	2.31 \pm 0.86 ^a	0.96 \pm 0.29 ^a	1.04 \pm .07 ^a
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	6.68 \pm 0.19	6.53 \pm 0.05	6.27 \pm 0.57	5.19 \pm 0.34	5.75 \pm 0.59
Total elements					
Cu μ g g ⁻¹	1433 \pm 40.84 ^a	1327 \pm 33.50 ^a	1400 \pm 7.11 ^a	1394 \pm 14.67 ^a	1377 \pm 21.56 ^a
Zn μ g g ⁻¹	2348 \pm 201 ^a	2141 \pm 36.10 ^a	2361 \pm 81.57 ^a	2311 \pm 43.44 ^a	2274 \pm 75.78 ^a
Cd μ g g ⁻¹	17.60 \pm 1.74 ^a	17.80 \pm 0.53 ^a	19.47 \pm 1.73 ^a	16.0 \pm 1.63 ^a	17.27 \pm 0.41 ^a
Pb μ g g ⁻¹	15773 \pm 1328 ^a	13600 \pm 1190 ^a	16547 \pm 426 ^a	14107 \pm 678 ^a	13893 \pm 578 ^a

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05.

Table 15 Physical and chemical properties of Pandora tailings after 12 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH	6.61 \pm 0.04 ^a	6.85 \pm 0.01 ^b	6.93 \pm 0.01 ^b	6.72 \pm 0.01 ^c	6.70 \pm 0.01 ^{a,c}
EC <i>mS/cm</i>	0.23 \pm 0.002 ^a	0.25 \pm 0.01 ^a	0.14 \pm 0.004 ^b	0.23 \pm 0.004 ^a	0.23 \pm 0.01 ^a
O. M %	8.20 \pm 0.07 ^a	8.13 \pm 0.07 ^a	8.25 \pm 0.03 ^a	9.78 \pm 0.05 ^b	8.88 \pm 0.07 ^c
TC g kg ⁻¹	51.27 \pm 1.27 ^a	51.03 \pm 0.32 ^a	51.10 \pm 0.69 ^a	62.83 \pm 1.98 ^b	56.47 \pm 1.05 ^a
TN g kg ⁻¹	0.63 \pm 0.03	0.60 \pm 0.06	0.70 \pm 0.06	0.97 \pm 0.03	0.83 \pm 0.09
C/N	82 : 1 ^a	86 : 1 ^a	77 : 1 ^a	65 : 1 ^a	68 : 1 ^a
H ₂ O-extractable NO ₃ ⁻ -N μ g g ⁻¹	2.52 \pm 0.33 ^a	1.92 \pm 0.18 ^a	1.42 \pm 0.13 ^a	1.12 \pm 0.13 ^a	1.43 \pm 0.46 ^a
H ₂ O-extractable NH ₄ ⁺ -N μ g g ⁻¹	7.62 \pm 0.52 ^a	7.13 \pm 0.14 ^{a, b, c}	5.51 \pm 0.45 ^{b, c}	6.38 \pm 0.57 ^{a, b, c}	5.55 \pm 0.25 ^c
Total elements					
Cu μ g g ⁻¹	290.30 \pm 15.45 ^a	265.3 \pm 15.90 ^a	268.360 \pm 13.67 ^a	269.10 \pm 19.85 ^a	229.7 \pm 1.76 ^a
Zn μ g g ⁻¹	33893 \pm 1606 ^{a, b, c}	37475 \pm 857 ^b	37899 \pm 562 ^b	34992 \pm 864 ^{a, b, c}	32203 \pm 1373 ^c
Cd μ g g ⁻¹	107.47 \pm 6.09 ^{a, b}	123.2 \pm 3.78 ^{a, b}	135.30 \pm 4.98 ^a	115.87 \pm 6.49 ^{a, b}	93.60 \pm 9.65 ^b
Pb μ g g ⁻¹	89413 \pm 734 ^a	92800 \pm 1276 ^{a, c}	86773 \pm 803 ^a	99093 \pm 3858 ^c	77547 \pm 796 ^d

Measurements with the same letter within the same row do not differ significantly according to the Tukey's HSD test at P < 0.05

Table 16 Physical and chemical properties of Parys tailings leachates after 12 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.96 \pm 0.05 ^{a*}	7.48 \pm 0.14 ^{a, b*}	7.84 \pm 0.03 ^{b*}	7.29 \pm 0.04 ^{a, b*}	6.83 \pm 0.22 ^{a*}
EC mS/cm	0.19 \pm 0.01 ^{a*}	0.23 \pm 0.01 ^{a*}	0.30 \pm 0.01 ^{b*}	0.21 \pm 0.02 ^{a*}	0.20 \pm 0.05 ^{a*}
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	0.003 \pm 0.01	0.001 \pm 0.001	0.26 \pm 0.06	0.04 \pm 0.02	0.041 \pm 0.004
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	0.38 \pm 0.03	0.51 \pm 0.06	0.13 \pm 0.003	0.31 \pm 0.03	0.44 \pm 0.08
Cu mg L ⁻¹	0.014 \pm 0.01	0.085 \pm 0.02	0.085 0.04	0.05 \pm 0.02	0.054 0.004
Zn mg L ⁻¹	1.74 \pm 0.23 ^a	0.51 \pm 0.1 ^b	BD ^b	0.39 \pm 0.09 ^b	0.65 \pm 0.22 ^b
Cd mg L ⁻¹	0.02 \pm 0.01 ^a	0.04 \pm 0.004 ^a	0.02 \pm 0.01 ^a	0.01 \pm 0.002 ^a	0.02 \pm 0.01 ^a
Pb mg L ⁻¹	1.14 \pm 0.95 ^a	BD	BD	BD	2.54 \pm 2.54 ^b

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05 and P* < 0.01, BD = below limit of detection.

Table 17 Physical and chemical properties of Pandora tailings leachates after 12 months incubation with amendments (values presented as mean \pm S.E.M; n = 3).

	Untreated Tailings	Tailings+ Fe ⁰	Tailings+ Zeolite	Tailings+ Biochar	Tailings+ Compost
pH _{H2O}	6.67 \pm 0.1 ^a	7.22 \pm 0.07 ^b	7.43 \pm 0.05 ^b	7.12 \pm 0.03 ^b	7.17 \pm 0.02 ^b
EC mS/cm	0.85 \pm 0.02 ^a	1.25 \pm 0.02 ^b	0.62 \pm 0.01 ^c	0.98 \pm 0.06 ^a	0.91 \pm 0.03 ^a
H ₂ O-extractable NO ₃ ⁻ -N mg L ⁻¹	1.78 \pm 0.35	2.30 \pm 0.28	2.23 \pm 0.21	0.83 \pm 0.37	1.02 \pm 0.16
H ₂ O-extractable NH ₄ ⁺ -N mg L ⁻¹	1.11 \pm 0.15	1.30 \pm 0.19	1.02 \pm 0.11 ^a	1.30 \pm 0.09	1.28 \pm 0.1
Cu mg L ⁻¹	BD	BD	BD	BD	BD
Zn mg L ⁻¹	54.27 \pm 3.21 ^a	13.59 \pm 1.06 ^b	6.49 \pm 2.30 ^b	47.74 \pm 1.14 ^a	48.69 \pm 2.66 ^a
Cd mg L ⁻¹	0.60 \pm 0.03 ^{a, c}	0.28 \pm 0.01 ^{b, d}	0.18 \pm 0.06 ^d	0.48 \pm 0.01 ^{c, b*}	0.44 \pm 0.03 ^{a, b, c, d}
Pb mg L ⁻¹	BD	BD	BD	BD	BD

Measurements with the same letter within the same row don not differ significantly according to the Tukey's HSD test at P < 0.05, BD = below limit of detection.

Appendix 10.3 (Additional results related to chapter 7).

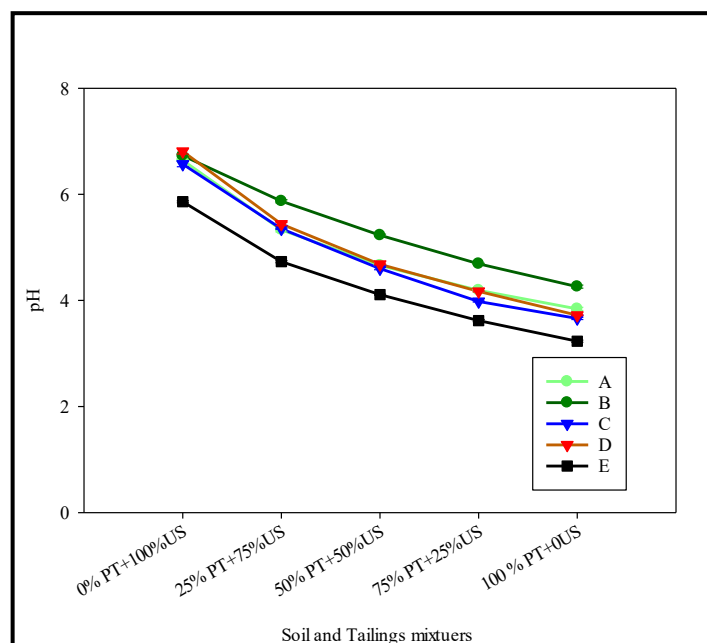


Figure 1 Treatments pH in different mixtures of Parys tailings (PT) without (100%PT) and with uncontaminated soil (US) (E) and the same mixtures (PT+US) amended with 2%C (A), 5%C (B), 5% Fe⁰ (C) and 2% Fe⁰ (D) (Measurements presented as mean \pm S.E.M; n = 3).

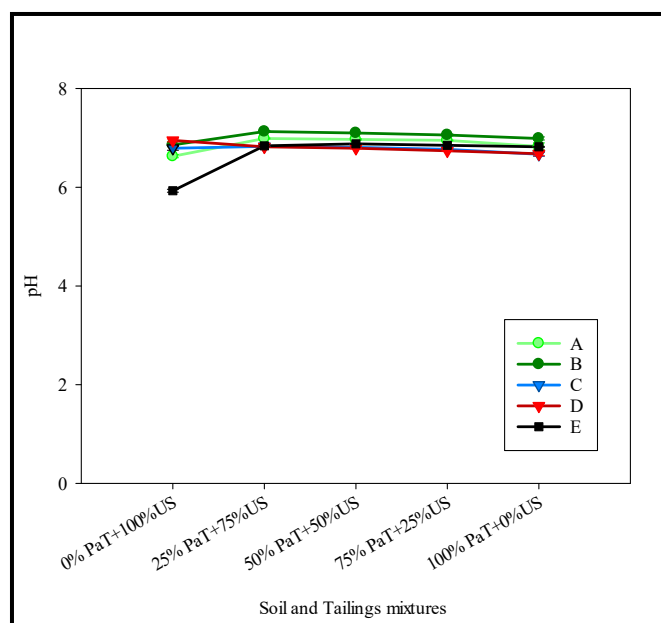


Figure 2 Treatments pH in different mixtures of Parys tailings (PT) without (100%PT) and with uncontaminated soil (US) (E) and the same mixtures (PT+US) amended with 2%C (A), 5%C (B), 5% Fe⁰ (C) and 2% Fe⁰ (D) (Measurements presented as mean \pm S.E.M; n = 3).

Table 1 Physical and chemical properties of treatments of 2% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% C	75% PT + 25% US + 2% C	50% PT + 50% US + 2% C	25%PT +75% US + 2% C	100% US + 2% C
pH	3.23 \pm 0.02 ^a	3.84 \pm 0.03 ^b	4.19 \pm 0.03 ^c	4.66 \pm 0.01 ^d	5.33 \pm 0.04 ^e	6.64 \pm 0.02 ^f
EC <i>mS/cm</i>	0.52 \pm 0.003 ^{a,c}	0.70 \pm 0.04 ^a	0.74 \pm 0.02 ^b	0.71 \pm 0.07 ^{a,b}	0.66 \pm 0.07 ^a	0.42 \pm 0.02 ^c
O. M %	6.67 \pm 0.06 ^a	5.74 \pm 0.03 ^b	5.18 \pm 0.07 ^c	4.81 \pm 0.04 ^d	4.28 \pm 0.1 ^e	3.75 \pm 0.05 ^f
Extractable metals ($\mu\text{g g}^{-1}$)						
Cu	16.51 \pm 1.20 ^{a,b}	19.62 \pm 0.51 ^a	10.61 \pm 2.73 ^{b,c}	8.57 \pm 1.69 ^c	5.89 \pm 1.84 ^c	4.12 \pm 0.93 ^c
Zn	37.07 \pm 5.57 ^a	21.28 \pm 1.70 ^b	20.11 \pm 0.90 ^b	14.47 \pm 0.74 ^{c,b}	8.15 \pm 0.16 ^c	4.59 \pm 0.82 ^c
Cd	2.33 \pm 0.64 ^a	0.28 \pm 0.15 ^b	0.45 \pm 0.11 ^b	0.60 \pm 0.17 ^b	0.34 \pm 0.23 ^b	0.20 \pm 0.07 ^b
Pb	340 \pm 10.41 ^a	342 \pm 10.93 ^a	282.20 \pm 6.0 ^b	177 \pm 21 ^c	67.93 \pm 2.50 ^d	0.05 \pm 0.03 ^e
Total metals ($\mu\text{g g}^{-1}$)						
Cu	949.67 \pm 24.20 ^a	861.20 \pm 13.40 ^a	601 \pm 8.13 ^c	375 \pm 17.50 ^d	189.67 \pm 3.93 ^e	1.61 \pm 0.98 ^f
Zn	12460 \pm 370 ^a	11579 \pm 551 ^a	7875 \pm 485 ^b	5622 \pm 250 ^c	3376 \pm 299 ^d	48.79 \pm 10.11 ^e
Cd	39.93 \pm 0.77 ^a	40.10 \pm 0.76 ^a	28.40 \pm 0.50 ^b	17.15 \pm 1.17 ^c	7.55 \pm 0.41 ^d	BD
Pb	6570 \pm 187 ^a	7160 \pm 122 ^b	5227 \pm 109 ^c	3535 \pm 82 ^d	1712 \pm 43.86 ^e	80 \pm 20.10 ^f

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 2 Several parameters of Ryegrass shoots grown in treatments of 2% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% C	75% PT + 25% US + 2% C	50% PT + 50% US + 2% C	25%PT +75% US + 2% C	100% US + 2% C
Shoots length (cm)	5.67 \pm 0.34 ^a	15.83 \pm 0.73 ^b	17.42 \pm 0.65 ^{b, c}	17.83 \pm 0.44 ^{b, c}	19.83 \pm 1.42 ^c	19.83 \pm 0.73 ^c
Shoots dry weight (g)	0.11 \pm 0.02 ^a	1.47 \pm 0.23 ^b	2.33 \pm 0.14 ^c	2.52 \pm 0.11 ^c	2.73 \pm 0.08 ^{c, d}	3.30 \pm 0.07 ^d
Total elements ($\mu\text{g g}^{-1}$)						
Cu	41.67 \pm 1.17 ^a	24.23 \pm 0.84 ^b	22.86 \pm 1.34 ^b	28.11 \pm 1.36 ^b	25.54 \pm 1.10 ^b	24.80 \pm 2.82 ^b
Zn	1121 \pm 103 ^a	114.21 \pm 9.08 ^b	81.83 \pm 1.55 ^b	63.97 \pm 3.17 ^b	35.84 \pm 0.96 ^b	26.82 \pm 0.39 ^b
Cd	6.32 \pm 1.24 ^a	1.73 \pm 0.35 ^b	2.0 \pm 0.23 ^b	2.74 \pm 0.26 ^b	2.34 \pm 0.30 ^b	2.86 \pm 0.25 ^b
Pb	BD	BD	BD	BD	BD	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 3 Several parameters of Ryegrass roots grown in treatments of 2% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% C	75% PT + 25% US + 2% C	50% PT + 50% US + 2% C	25%PT +75% US + 2% C	100% US + 2% C
Roots dry weight (g)	DM	0.28 \pm 0.02 ^a	0.54 \pm 0.06 ^{a, b}	0.56 \pm 0.03 ^{a, b}	0.81 \pm 0.14 ^b	1.34 \pm 0.14 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	DM	177.13 \pm 4.68 ^a	163.19 \pm 6.28 ^a	194.13 \pm 17.78 ^a	187.40 \pm 4.70 ^a	192.98 \pm 21.71 ^a
Zn	DM	471.49 \pm 24.86 ^a	376.43 \pm 18.71 ^b	202.89 \pm 13.24 ^c	142.72 \pm 1.84 ^{c, d}	113.92 \pm 1.95 ^d
Cd	DM	16.12 \pm 0.76 ^a	12.48 \pm 0.49 ^b	9.81 \pm 0.64 ^b	10.04 \pm 0.73 ^{b, c}	9.57 \pm 0.25 ^c
Pb	DM	256.60 \pm 18.92 ^a	249.60 \pm 19.98 ^a	138.67 \pm 12.02 ^b	142.22 \pm 1.78 ^b	16.89 \pm 10.92 ^c

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. DM= did not measured.

Table 4 Physical and chemical properties of treatments of 5% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 5% C	75% PT + 25% US + 5% C	50% PT + 50% US + 5% C	25%PT +75% US + 5% C	100% US + 5% C
pH _{H2O}	3.23 \pm 0.02 ^a	4.26 \pm 0.03 ^b	4.69 \pm 0.01 ^c	5.23 \pm 0.02 ^d	5.87 \pm 0.03 ^e	6.73 \pm 0.02 ^f
EC <i>mS/cm</i>	0.52 \pm 0.003 ^a	1.14 \pm 0.07 ^{b, c}	1.25 \pm 0.07 ^b	1.11 \pm 0.06 ^{b, c}	0.96 \pm 0.05 ^c	0.68 \pm 0.03 ^a
O. M %	6.68 \pm 0.06 ^{a, b}	6.74 \pm 0.14 ^a	6.12 \pm 0.08 ^{b, c}	5.54 \pm 0.11 ^c	4.76 \pm 0.13 ^d	4.54 \pm 0.19 ^d
Extractable metals ($\mu\text{g g}^{-1}$)						
Cu	16.51 \pm 1.20 ^a	15.85 \pm 1.35 ^{a, b}	11.08 \pm 3.04 ^{a, b}	9.36 \pm 0.40 ^{a, b}	13.83 \pm 2.04 ^{a, b}	7.49 \pm 1.49 ^b
Zn	37.07 \pm 5.57 ^a	17.38 \pm 1.65 ^b	12.75 \pm 2.48 ^{b, c}	11.54 \pm 0.09 ^{b, c}	7.73 \pm 0.01 ^{b, c}	3.60 \pm 0.07 ^c
Cd	2.33 \pm 0.64 ^a	0.52 \pm 0.1 ^b	0.34 \pm 0.14 ^b	0.48 \pm 0.19 ^b	0.41 \pm 0.09 ^b	0.31 \pm 0.05 ^b
Pb	340 \pm 10.41 ^a	321 \pm 10.47 ^a	270.27 \pm 1.62 ^b	150 \pm 2.1 ^c	61.20 \pm 2.50 ^d	BD
Total metals ($\mu\text{g g}^{-1}$)						
Cu	949.67 \pm 24.20 ^a	853.60 \pm 17.39 ^b	569.10 \pm 7.40 ^c	358.73 \pm 11.94 ^d	173.33 \pm 6.64 ^e	2.87 \pm 0.79 ^f
Zn	12460 \pm 370 ^a	12030 \pm 353 ^a	7194 \pm 64.80 ^b	5721 \pm 146 ^c	2692 \pm 79.99 ^d	33.64 \pm 1.08 ^e
Cd	39.93 \pm 0.77 ^a	40.65 \pm 0.87 ^a	27.65 \pm 0.65 ^b	17.55 \pm 6.90 ^c	7.05 \pm 0.57 ^d	BD
Pb	6570 \pm 187 ^a	6947 \pm 157 ^a	4800 \pm 120 ^b	3096 \pm 92.26 ^c	1499 \pm 60.23 ^d	45.30 \pm 0.67 ^e

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 5 Several parameters of Ryegrass shoots grown in treatments of 5% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3)

Parameters	100% UPT	100% PT + 5% C	75% PT + 25% US + 5% C	50% PT + 50% US + 5% C	25%PT +75% US + 5% C	100% US + 5% C
Shoots length (cm)	5.67 \pm 0.34 ^a	18.5 \pm 1.15 ^b	18.33 \pm 0.06 ^b	18.00 \pm 0.29 ^b	19.00 \pm 1.15 ^b	18.5 \pm 0.29 ^b
Shoots dry weight (g)	0.11 \pm 0.02 ^a	2.38 \pm 0.04 ^b	2.33 \pm 0.11 ^b	2.88 \pm 0.06 ^c	2.93 \pm 0.17 ^c	3.10 \pm 0.07 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	41.67 \pm 1.17 ^a	27.71 \pm 1.44 ^{b,c}	40.23 \pm 5.18 ^{a,c}	31.43 \pm 1.40 ^{a,b,c}	31.43 \pm 1.38 ^{a,b,c}	29.37 \pm 1.39 ^c
Zn	1121 \pm 103 ^a	69.72 \pm 3.29 ^b	55.77 \pm 1.99 ^b	43.0 \pm 1.73 ^b	30.42 \pm 1.78 ^b	21.73 \pm 1.23 ^b
Cd	6.32 \pm 1.24 ^a	4.67 \pm 0.58 ^a	4.27 \pm 0.07 ^a	4.34 \pm 0.41 ^a	3.43 \pm 0.43 ^a	4.40 \pm 0.50 ^a
Pb	BD	BD	BD	BD	BD	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 6 Several parameters of Ryegrass roots grown in treatments of 5% compost was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 5% C	75% PT + 25% US + 5% C	50% PT + 50% US + 5% C	25%PT +75% US + 5% C	100% US + 5% C
Roots dry weight (g)	DM	0.77 \pm 0.02 ^a	0.83 \pm 0.02 ^a	1.00 \pm 0.02 ^b	0.79 \pm 0.05 ^a	1.13 \pm 0.05 ^b
Total elements ($\mu\text{g g}^{-1}$)						
Cu	DM	142.29 \pm 4.92 ^b	155.31 \pm 8.53 ^a	147.38 \pm 8.01 ^a	113.87 \pm 3.29 ^a	149.93 \pm 15.62 ^a
Zn	DM	260.16 \pm 8.44 ^a	198.29 \pm 7.18 ^b	149.27 \pm 2.27 ^c	107.73 \pm 4.54 ^d	79.42 \pm 4.66 ^c
Cd	DM	14.00 \pm 0.84 ^a	13.26 \pm 0.23 ^a	10.49 \pm 0.79 ^b	10.72 \pm 0.16 ^b	1.10 \pm 0.39 ^c
Pb	DM	209.07 \pm 6.49 ^a	189.87 \pm 16.76 ^a	183.47 \pm 22.58 ^a	107.73 \pm 15.49 ^b	26.67 \pm 9.11 ^c

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. DM=did not measured.

Table 7 Physical and chemical properties of treatments of 2% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% Fe ⁰	75% PT + 25% US + 2% Fe ⁰	50% PT + 50% US + 2% Fe ⁰	25%PT +75% US + 2% Fe ⁰	100% US + 2% Fe ⁰
pH	3.23 \pm 0.02 ^a	3.66 \pm 0.02 ^b	3.98 \pm 0.01 ^c	4.60 \pm 0.02 ^d	5.35 \pm 0.01 ^e	6.57 \pm 0.05 ^f
EC <i>mS/cm</i>	0.52 \pm 0.003 ^a	0.30 \pm 0.01 ^b	0.37 \pm 0.01 ^c	0.40 \pm 0.02 ^c	0.35 \pm 0.01 ^c	0.14 \pm 0.004 ^d
O. M %	6.68 \pm 0.06 ^a	5.68 \pm 0.06 ^b	5.05 \pm 0.02 ^c	4.40 \pm 0.01 ^c	3.87 \pm 0.01 ^c	3.54 \pm 0.06 ^f
Extractable metals ($\mu\text{g g}^{-1}$)						
Cu	16.51 \pm 1.20 ^{a,b}	25.43 \pm 1.53 ^b	17.72 \pm 1.56 ^{a,b}	14.88 \pm 2.22 ^{a,c}	15.72 \pm 1.55 ^{a,c}	16.46 \pm 3.02 ^{a,b}
Zn	37.07 \pm 5.57 ^a	16.59 \pm 3.96 ^b	11.52 \pm 2.50 ^b	6.10 \pm 0.65 ^b	6.08 \pm 0.31 ^b	7.41 \pm 0.79 ^b
Cd	2.33 \pm 0.64 ^a	0.56 \pm 0.18 ^b	0.44 \pm 0.14 ^b	0.56 \pm 0.06 ^b	0.23 \pm 0.11 ^b	0.49 \pm 0.09 ^b
Pb	340 \pm 10.41 ^a	323 \pm 7.0 ^a	248.27 \pm 9.51 ^b	165.50 \pm 14.88 ^c	58.80 \pm 1.47 ^d	BD
Total metals ($\mu\text{g g}^{-1}$)						
Cu	949.67 \pm 24.20 ^a	919.60 \pm 4.23 ^a	655 \pm 8.40 ^b	441 \pm 15.09 ^c	237.20 \pm 10.67 ^d	49.33 \pm 3.15 ^e
Zn	12460 \pm 370 ^a	12629 \pm 214 ^a	8640 \pm 289 ^b	5629 \pm 186 ^c	2869 \pm 116 ^d	258 \pm 44 ^e
Cd	39.93 \pm 0.77 ^a	37.60 \pm 0.31 ^a	26.27 \pm 0.70 ^b	17.07 \pm 0.70 ^c	8.47 \pm 0.59 ^d	0.61 \pm 0.40 ^e
Pb	6570 \pm 187 ^a	7053 \pm 81 ^b	5387 \pm 58 ^c	3573 \pm 96 ^d	1679 \pm 70 ^e	62.67 \pm 1.76 ^f

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 8 Several parameters of Ryegrass shoots grown in treatments of 2% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100%.UPT	100% PT + 2% Fe ⁰	75% PT + 25% US + 2% Fe ⁰	50% PT + 50% US + 2% Fe ⁰	25%PT +75% US + 2% Fe ⁰	100% US +2% Fe ⁰
Shoots length (cm)	5.67 \pm 0.34 ^a	8.17 \pm 0.60 ^b	11.20 \pm 0.15 ^c	13.43 \pm 0.60 ^{c,d}	12.15 \pm 0.32 ^{c,d}	13.50 \pm 0.58 ^d
Shoots dry weight (g)	0.11 \pm 0.02 ^a	0.69 \pm 0.02 ^b	1.28 \pm 0.12 ^c	1.32 \pm 0.16 ^c	1.60 \pm 0.06 ^{c, d}	1.83 \pm 0.14 ^d
Total elements ($\mu\text{g g}^{-1}$)						
Cu	41.67 \pm 1.17 ^a	60.00 \pm 3.86 ^b	42.69 \pm 2.47 ^a	44.57 \pm 2.85 ^a	40.91 \pm 1.06 ^a	42.0 \pm 1.49 ^a
Zn	1121 \pm 103 ^a	86.81 \pm 5.60 ^b	46.36 \pm 3.51 ^b	48.02 \pm 1.92 ^b	36.06 \pm 1.89 ^b	26.71 \pm 1.86 ^b
Cd	6.32 \pm 1.24 ^a	6.33 \pm 0.25 ^a	6.00 \pm 0.42 ^a	4.23 \pm 0.15 ^a	5.63 \pm 0.10 ^a	4.81 \pm 0.35 ^a
Pb	BD	35.33 \pm 4.37 ^{a*}	4.68 \pm 1.34 ^{b*}	BD	BD	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. And, that with the same letter within the same row with (*) don not differ significantly according to independent-T test at $P < 0.05$. BD= below detection limit.

Table 9 Several parameters of Ryegrass roots grown in treatments of 2% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 2% Fe ⁰	75% PT + 25% US + 2% Fe ⁰	50% PT + 50% US + 2% Fe ⁰	25%PT +75% US + 2% Fe ⁰	100% US + 2% Fe ⁰
Roots dry weight (g)	DM	DM	0.29 \pm 0.03 ^a	0.47 \pm 0.02 ^{a,b}	0.63 \pm 0.07 ^{b,c}	0.91 \pm 0.11 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	DM	DM	212.08 \pm 3.24 ^a	161.07 \pm 4.22 ^b	145.78 \pm 3.03 ^c	115.82 \pm 5.01 ^d
Zn	DM	DM	316.29 \pm 18.32 ^a	145.10 \pm 13.24 ^b	115.78 \pm 9.15 ^b	55.50 \pm 3.36 ^c
Cd	DM	DM	15.07 \pm 1.49 ^a	11.20 \pm 1.34 ^{a,b}	10.33 \pm 0.29 ^b	11.02 \pm 0.86 ^{a,b}
Pb	DM	DM	290.64 \pm 13.14 ^a	176.89 \pm 13.97 ^b	174.22 \pm 20.20 ^b	14.22 \pm 6.41 ^c

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. DM= did not measured.

Table 10 Physical and chemical properties of treatments of 5% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean ± S.E.M; n = 3).

Parameters	100% UPT	100% PT + 5% Fe ⁰	75% PT + 25% US + 5% Fe ⁰	50% PT + 50% US + 5% Fe ⁰	25%PT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
pH	3.23 ± 0.02 ^a	3.72 ± 0.01 ^b	4.17 ± 0.01 ^c	4.68 ± 0.01 ^d	5.44 ± 0.03 ^e	6.81 ± 0.05 ^f
EC <i>mS/cm</i>	0.52 ± 0.003 ^a	0.38 ± 0.01 ^b	0.41 ± 0.02 ^b	0.52 ± 0.04 ^a	0.44 ± 0.01 ^{a,b}	0.17 ± 0.01 ^c
O. M %	6.68 ± 0.06 ^{a,b}	7.01 ± 0.07 ^a	5.93 ± 0.18 ^{b, c}	5.61 ± 0.014 ^c	4.82 ± 0.08 ^d	3.97 ± 0.09 ^e
Extractable metals (µg g ⁻¹)						
Cu	16.51 ± 1.20 ^a	25.55 ± 1.55 ^b	22.81 ± 0.83 ^{a,b}	25.96 ± 1.55 ^b	22.96 ± 0.25 ^{b,a}	25.40 ± 2.16 ^b
Zn	37.07 ± 5.57 ^a	10.52 ± 1.75 ^b	7.72 ± 1.76 ^b	7.86 ± 0.07 ^b	7.86 ± 1.03 ^b	10.74 ± 0.39 ^b
Cd	2.33 ± 0.64 ^a	0.44 ± 0.11 ^b	0.16 ± 0.02 ^b	0.46 ± 0.11 ^b	0.11 ± 0.03 ^b	0.54 ± 0.21 ^b
Pb	340 ± 10.41 ^a	330 ± 6.64 ^a	214.93 ± 5.25 ^b	111.10 ± 4.40 ^c	47.33 ± 3.57 ^d	BD
Total metals (µg g ⁻¹)						
Cu	949.67 ± 24.20 ^a	863.07 ± 5.09 ^b	693.10 ± 5.09 ^c	488.87 ± 10.97 ^d	271.67 ± 10.67 ^e	77.47 ± 8.41 ^f
Zn	12460 ± 370 ^a	11367 ± 366 ^a	8356 ± 289156 ^b	5401 ± 373 ^c	2487 ± 119 ^d	56.49 ± 5.34 ^e
Cd	39.93 ± 0.77 ^a	33.67 ± 0.55 ^b	26.20 ± 0.70 ^c	16.13 ± 1.14 ^d	8.13 ± 0.75 ^e	0.47 ± 0.18 ^f
Pb	6570 ± 187 ^a	7720 ± 61 ^b	5280 ± 61 ^c	3320 ± 129 ^d	1754 ± 34 ^e	170 ± 24.85 ^f

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 11 Several parameters of Ryegrass shoots grown in treatments of 5% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 5% Fe ⁰	75% PT + 25% US + 5% Fe ⁰	50% PT + 50% US + 5% Fe ⁰	25%PT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
Shoots length (cm)	5.67 \pm 0.34 ^a	8.2 \pm 0.15 ^b	11.83 \pm 0.60 ^{c,d}	13.67 \pm 0.44 ^c	12.87 \pm 0.19 ^{c,d}	11.00 \pm 0.58 ^d
Shoots dry weight (g)	0.11 \pm 0.02 ^a	0.78 \pm 0.50 ^b	1.29 \pm 0.11 ^c	1.98 \pm 0.07 ^d	1.89 \pm 0.12 ^d	1.95 \pm 0.07 ^d
Cu	41.67 \pm 1.17 ^a	67.14 \pm 47 ^b	46.51 \pm 0.41 ^a	47.49 \pm 2.14 ^a	42.97 \pm 3.21 ^a	39.87 \pm 1.32 ^a
Zn	1121 \pm 103 ^a	70.67 \pm 4.00 ^b	34.99 \pm 1.24 ^b	31.14 \pm 0.17 ^b	32.23 \pm 1.12 ^b	22.19 \pm 0.39 ^b
Cd	6.32 \pm 1.24 ^a	6.80 \pm 0.12 ^a	6.60 \pm 0.12 ^a	5.43 \pm 0.25 ^b	5.14 \pm 0.43 ^b	4.80 \pm 0.20 ^b
Pb	BD	67.33 \pm 7.51 ^{a*}	20.67 \pm 3.71 ^{b*}	BD	BD	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 12 Several parameters of Ryegrass roots grown in treatments of 5% Fe⁰ was added to the mixtures of Parys tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPT	100% PT + 5% Fe ⁰	75% PT + 25% US + 5% Fe ⁰	50% PT + 50% US + 5% Fe ⁰	25%PT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
Roots dry weight (g)	DM	DM	0.26 \pm 0.02 ^a	0.51 \pm 0.03 ^b	0.60 \pm 0.04 ^{b, c}	0.67 \pm 0.04 ^c
Total elements (μ g g ⁻¹)						
Cu	DM	DM	271.41 \pm 12.90 ^a	196.80 \pm 10.97 ^b	170.13 \pm 4.54 ^{b,c}	131.11 \pm 7.42 ^c
Zn	DM	DM	206.22 \pm 12.04 ^a	134.08 \pm 0.62 ^b	80.30 \pm 4.44 ^c	52.70 \pm 1.34 ^c
Cd	DM	DM	16.79 \pm 0.31 ^a	10.93 \pm 0.25 ^{a,b}	10.84 \pm 0.24 ^b	14.22 \pm 2.55 ^{a,b}
Pb	DM	DM	259.97 \pm 10.80 ^a	182.44 \pm 13.90 ^b	109.33 \pm 5.55 ^c	14.22 \pm 7.75 ^d

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. DM= did not measured.

Table 13 Physical and chemical properties of treatments of 2% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	75% PaT + 25% US + 2% C	50% PaT + 50% US + 2% C	25%PaT +75% US + 2% C	100% US + 2% C
pH	6.82 \pm 0.01 ^a	6.83 \pm 0.07 ^{a,b}	6.95 \pm 0.01 ^{a,c}	6.97 \pm 0.01 ^{b,c}	7.00 \pm 0.01 ^c	6.63 \pm 0.03 ^d
EC <i>mS/cm</i>	0.45 \pm 0.02 ^{a,c}	0.70 \pm 0.04 ^b	0.70 \pm 0.02 ^b	0.70 \pm 0.07 ^b	0.62 \pm 0.01 ^b	0.44 \pm 0.04 ^a
O. M %	7.26 \pm 0.04 ^a	8.07 \pm 0.2 ^a	7.50 \pm 0.33 ^a	6.62 \pm 0.08 ^b	5.08 \pm 0.2 ^c	4.25 \pm 0.34 ^c
Extractable metals ($\mu\text{g g}^{-1}$)						
Cu	71.33 \pm 3.68 ^a	76.34 \pm 0.71 ^a	62.94 \pm 4.67 ^a	46.81 \pm 2.55 ^b	29.25 \pm 3.21 ^c	23.24 \pm 0.40 ^c
Zn	25463 \pm 732 ^a	24936 \pm 732 ^a	19910 \pm 361 ^b	13379 \pm 343 ^c	6721 \pm 364 ^d	20.50 \pm 3.21 ^c
Cd	32.42 \pm 0.59 ^a	39.69 \pm 1.66 ^b	32.41 \pm 0.72 ^a	21.89 \pm 0.23 ^c	10.53 \pm 0.15 ^d	0.80 \pm 0.24 ^c
Pb	90187 \pm 2870 ^a	76140 \pm 94.52 ^b	61813 \pm 2237 ^c	33440 \pm 1820 ^d	10800 \pm 2623 ^e	6.14 \pm 4.29 ^f
Total metals ($\mu\text{g g}^{-1}$)						
Cu	192.27 \pm 3.70 ^a	119.73 \pm 25.84 ^b	40.00 \pm 6.04 ^c	BD	BD	BD
Zn	30145 \pm 1170 ^a	29968 \pm 1197 ^a	22960 \pm 820 ^b	17502 \pm 387 ^c	9533 \pm 718 ^d	60.75 \pm 15.62 ^e
Cd	91.47 \pm 7.13 ^a	83.20 \pm 8.20 ^a	54.40 \pm 2.63 ^b	36.40 \pm 5.25 ^c	7.60 \pm 1.74 ^d	BD
Pb	101600 \pm 2518 ^a	85867 \pm 1087 ^b	60853 \pm 751 ^c	45707 \pm 3912 ^d	25227 \pm 2036 ^e	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 14 Several parameters of Ryegrass shoots grown in treatments of 2% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	75% PaT + 25% US + 2% C	50% PaT + 50% US + 2% C	25%PaT +75% US + 2% C	100% US + 2% C
Shoots length (cm)	8.20 \pm 0.15 ^a	10.73 \pm 0.37 ^b	11.48 \pm 0.30 ^{b,c}	13.17 \pm 0.88 ^{c,d}	14.23 \pm 0.15 ^d	19.50 \pm 0.29 ^c
Shoots dry weight (g)	0.75 \pm 0.05 ^a	1.49 \pm 0.13 ^b	1.74 \pm 0.14 ^b	1.72 \pm 0.10 ^b	2.57 \pm 0.13 ^c	4.07 \pm 0.17 ^d
Cu	26.04 \pm 2.71 ^a	69.87 \pm 1.70 ^{b,d,e}	69.40 \pm 1.83 ^{b,d,e}	61.49 \pm 2.03 ^{c,d,e}	55.83 \pm 0.15 ^d	65.75 \pm 0.86 ^c
Zn	1767 \pm 83.99 ^a	808.00 \pm 40.57 ^b	637.27 \pm 8.05 ^{b,c}	636 \pm 15.75 ^{b,c}	492 \pm 21.86 ^c	23.94 \pm 0.48 ^d
Cd	5.96 \pm 0.62 ^a	5.46 \pm 0.18 ^{a,b}	4.67 \pm 0.13 ^{a,b,c}	4.29 \pm 0.20 ^{b,c}	3.60 \pm 0.001 ^c	1.34 \pm 0.40 ^d
Pb	122.67 \pm 12.22 ^a	85.50 \pm 4.75 ^b	104.00 \pm 4.16 ^{a,b}	111.43 \pm 4.55 ^{a,b}	103.43 \pm 5.45 ^{a,b}	6.87 \pm 7.73 ^c

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 15 Several parameters of Ryegrass roots grown in treatments of 2% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 2% C	75% PaT + 25% US + 2% C	50% PaT + 50% US + 2% C	25%PaT +75% US + 2% C	100% US + 2% C
Roots dry weight (g)	0.35 \pm 0.05 ^a	0.46 \pm 0.02 ^a	0.60 \pm 0.03 ^{a,b}	0.64 \pm 0.01 ^{a,b}	0.86 \pm 0.01 ^b	1.31 \pm 0.17 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	378.00 \pm 8.26 ^a	371.59 \pm 11.75 ^a	338.40 \pm 16.38 ^{a,b}	318.85 \pm 11.75 ^b	300.89 \pm 3.56 ^b	47.63 \pm 0.61 ^c
Zn	1657 \pm 31.87 ^a	2667 \pm 56.71 ^b	2770 \pm 72.93 ^b	2246 \pm 195 ^b	1703 \pm 168 ^a	58.23 \pm 7.05 ^c
Cd	70.31 \pm 0.88 ^a	179.41 \pm 11.54 ^b	175.68 \pm 5.10 ^b	142.84 \pm 7.71 ^c	96.53 \pm 3.89 ^a	7.56 \pm 0.62 ^d
Pb	11787 \pm 895 ^a	8896 \pm 161 ^b	7424 \pm 185 ^{b,c}	6542 \pm 256 ^c	7591 \pm 376 ^{b,c}	77.33 \pm 18.67 ^d

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 16 Physical and chemical properties of treatments of 5% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% C	75% PaT + 25% US + 5% C	50% PaT + 50% US + 5% C	25%PaT +75% US + 5% C	100% US + 5% C
pH _{H2O}	6.82 \pm 0.01 ^a	6.99 \pm 0.03 ^b	7.06 \pm 0.02 ^{b,c}	7.10 \pm 0.01 ^c	7.13 \pm 0.02 ^c	6.86 \pm 0.02 ^a
EC <i>mS/cm</i>	0.45 \pm 0.02 ^a	0.95 \pm 0.04 ^b	0.89 \pm 0.02 ^b	0.97 \pm 0.05 ^b	0.93 \pm 0.05 ^b	0.80 \pm 0.09 ^b
O. M %	7.26 \pm 0.04 ^a	9.05 \pm 0.17 ^b	7.97 \pm 0.22 ^a	7.07 \pm 0.25 ^{a,c}	6.16 \pm 0.12 ^{c,d}	5.75 \pm 0.37 ^d
Extractable metals ($\mu\text{g g}^{-1}$)						
Cu	71.33 \pm 3.68 ^a	65.15 \pm 1.83 ^{a,b}	54.33 \pm 2.27 ^b	44.91 \pm 1.41 ^{b,c}	33.05 \pm 3.88 ^{c,d}	24.06 \pm 1.51 ^d
Zn	25463 \pm 732 ^a	23146 \pm 587 ^a	18161 \pm 643 ^b	13351 \pm 452 ^c	7338 \pm 569 ^d	20.63 \pm 4.67 ^e
Cd	32.42 \pm 0.59 ^a	38.31 \pm 1.67 ^b	28.77 \pm 0.87 ^a	21.52 \pm 0.37 ^c	11.53 \pm 0.73 ^d	0.56 \pm 0.15 ^e
Pb	90187 \pm 2870 ^a	69547 \pm 233 ^b	50507 \pm 2679 ^c	32000 \pm 2726 ^d	15840 \pm 2591 ^e	2.55 \pm 2.53 ^f
Total metals ($\mu\text{g g}^{-1}$)						
Cu	192.27 \pm 3.70 ^a	107.20 \pm 5.06 ^b	57.07 \pm 2.67 ^c	BD	BD	BD
Zn	30145 \pm 1170 ^a	38075 \pm 1415 ^b	27051 \pm 441 ^a	18950 \pm 787 ^c	13080 \pm 1845 ^d	114.09 \pm 44.54 ^e
Cd	91.47 \pm 7.13 ^a	92.80 \pm 9.61 ^a	56.40 \pm 5.50 ^{b,c}	30.00 \pm 0.001 ^{c,d}	20.00 \pm 9.81 ^d	BD
Pb	101600 \pm 2518 ^a	91680 \pm 333 ^a	68533 \pm 1916 ^b	50133 \pm 670 ^c	26533 \pm 4489 ^d	153.33 \pm 104 ^e

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$. BD= below detection limit.

Table 17 Several parameters of Ryegrass shoots grown in treatments of 5% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% C	75% PaT + 25% US + 5% C	50% PaT + 50% US + 5% C	25%PaT +75% US + 5% C	100% US + 5% C
Shoots length (cm)	8.20 \pm 0.15 ^a	12.50 \pm 0.29 ^b	12.17 \pm 0.73 ^b	14.67 \pm 0.44 ^c	14.73 \pm 0.37 ^c	20.50 \pm 0.29 ^d
Shoots dry weight (g)	0.75 \pm 0.05 ^a	2.34 \pm 0.18 ^b	2.03 \pm 0.12 ^b	2.36 \pm 0.11 ^b	2.18 \pm 0.16 ^b	3.30 \pm 0.06 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	26.04 \pm 2.71 ^a	97.33 \pm 3.71 ^b	96.67 \pm 4.06 ^b	123.33 \pm 14.53 ^b	96.00 \pm 4.53 ^b	11.43 \pm 5.80 ^a
Zn	1767 \pm 83.99 ^a	572.80 \pm 17.12 ^b	546.63 \pm 42.36 ^b	517.91 \pm 10.22 ^b	453.86 \pm 16.83 ^b	31.43 \pm 1.53 ^c
Cd	5.96 \pm 0.62 ^a	4.33 \pm 0.18 ^b	4.36 \pm 0.12 ^b	3.94 \pm 0.43 ^b	3.14 \pm 0.15 ^b	1.43 \pm 0.14 ^c
Pb	122.67 \pm 12.22 ^a	97.33 \pm 3.71 ^a	96.67 \pm 4.06 ^a	123.33 \pm 14.52 ^a	96.00 \pm 4.54 ^a	11.43 \pm 5.80 ^b

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$.

Table 18 Several parameters of Ryegrass roots grown in treatments of 5% compost was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% C	75% PaT + 25% US + 5% C	50% PaT + 50% US + 5% C	25%PaT +75% US + 5% C	100% US + 5% C
Roots dry weight (g)	0.35 \pm 0.05 ^a	0.80 \pm 0.01 ^b	0.82 \pm 0.06 ^b	0.97 \pm 0.04 ^b	0.87 \pm 0.07 ^b	1.24 \pm 0.08 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	378.00 \pm 8.26 ^a	309.13 \pm 2.82 ^b	314.11 \pm 8.34 ^b	304.91 \pm 1.69 ^b	307.54 \pm 2.52 ^b	48.92 \pm 0.82 ^c
Zn	1657 \pm 31.87 ^{a,b,c}	2451.84 \pm 250 ^b	1960 \pm 118 ^{a,b}	1367 \pm 94.50 ^{a,c}	858.39 \pm 407 ^{c,d}	57.08 \pm 4.45 ^d
Cd	70.31 \pm 0.88 ^a	150.61 \pm 9.06 ^b	149.12 \pm 1.58 ^b	103.73 \pm 6.96 ^c	72.36 \pm 1.70 ^a	8.09 \pm 0.79 ^d
Pb	11787 \pm 895 ^a	7424 \pm 231 ^b	6869 \pm 296 ^{b,c}	4607 \pm 635 ^{c,d}	4219 \pm 456 ^d	66.67 \pm 9.24 ^c

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at $P < 0.05$.

Table 19 Physical and chemical properties of treatments of 2% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean ± S.E.M; n = 3).

Parameters	100%.UPaT	100% PaT + 2% Fe ⁰	75% PaT + 25% US + 2% Fe ⁰	50% PaT + 50% US + 2% Fe ⁰	25%PaT +75% US + 2% Fe ⁰	100% US + 2% Fe ⁰
pH	6.82 ± 0.01 ^a	6.67 ± 0.04 ^b	6.77 ± 0.01 ^{a,b}	6.81 ± 0.02 ^a	6.83 ± 0.02 ^a	6.79 ± 0.04 ^a
EC <i>mS/cm</i>	0.45 ± 0.02 ^{a,b}	0.44 ± 0.03 ^{a,b}	0.53 ± 0.06 ^a	0.45 ± 0.04 ^{a,b}	0.40 ± 0.01 ^b	0.13 ± 0.01 ^c
O. M %	7.26 ± 0.04 ^a	6.86 ± 0.08 ^b	6.07 ± 0.04 ^c	5.20 ± 0.07 ^d	4.28 ± 0.06 ^c	3.41 ± 0.06 ^f
Extractable metals (µg g ⁻¹)						
Cu	71.33 ± 3.68 ^a	97.60 ± 4.39 ^b	82.87 ± 4.07 ^{a,b}	67.13 ± 4.43 ^a	39.60 ± 1.45 ^c	25.29 ± 4.89 ^d
Zn	25463 ± 732 ^a	23407 ± 434 ^a	18579 ± 268 ^b	14674 ± 574 ^c	4361 ± 202 ^d	28.81 ± 6.68 ^c
Cd	32.42 ± 0.59 ^a	36.86 ± 0.55 ^b	30.04 ± 0.36 ^c	22.08 ± 0.30 ^d	10.92 ± 0.77 ^e	0.57 ± 0.09 ^f
Pb	90187 ± 2870 ^a	75200 ± 1711 ^b	55573 ± 3437 ^c	35947 ± 1768 ^d	10400 ± 1342 ^e	3.34 ± 3.33 ^f
Total metals (µg g ⁻¹)						
Cu	192.27 ± 3.70 ^a	153.73 ± 2.22 ^b	89.07 ± 3.93 ^c	44.20 ± 2.91 ^d	4.17 ± 3.91 ^e	BD
Zn	30145 ± 1170 ^a	39536 ± 809 ^b	28192 ± 1705 ^a	18021 ± 930 ^c	11711 ± 2019 ^d	67.76 ± 13.51 ^e
Cd	91.47 ± 7.13 ^a	94.40 ± 12.32 ^a	66.00 ± 6.16 ^{a,b}	36.80 ± 3.56 ^{b,c}	17.20 ± 6.44 ^c	BD
Pb	101600 ± 2518 ^a	96933 ± 1709 ^a	63840 ± 4382 ^b	51173 ± 1208 ^c	25173 ± 1160 ^d	4.00 ± 3.02 ^e

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 20 Several parameters of Ryegrass shoots grown in treatments of 2% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100%.UPaT	100% PaT + 2% Fe ⁰	75% PaT + 25% US + 2% Fe ⁰	50% PaT + 50% US + 2% Fe ⁰	25%PaT +75% US + 2% Fe ⁰	100% US +2% Fe ⁰
Shoots length (cm)	8.20 \pm 0.15 ^a	8.50 \pm 0.15 ^{a,b}	10.00 \pm 0.58 ^b	11.92 \pm 0.46 ^c	12.50 \pm 0.29 ^c	14.42 \pm 0.30 ^d
Shoots dry weight (g)	0.75 \pm 0.05 ^a	1.71 \pm 0.09 ^b	1.75 \pm 0.12 ^b	2.14 \pm 0.09 ^{b,c}	2.39 \pm 0.13 ^c	2.39 \pm 0.08 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	26.04 \pm 2.71 ^a	78.67 \pm 2.49 ^b	73.90 \pm 2.75 ^{b,c}	65.60 \pm 2.02 ^{c,d}	60.62 \pm 1.04 ^d	58.17 \pm 1.09 ^d
Zn	1767 \pm 83.99 ^a	1159 \pm 25.40 ^b	904.0 \pm 15.91 ^c	722 \pm 22.53 ^d	454.0 \pm 14.31 ^e	23.07 \pm 1.31 ^f
Cd	5.96 \pm 0.62 ^{a,b}	7.07 \pm 0.14 ^b	5.20 \pm 0.23 ^a	4.46 \pm 0.30 ^{a,c}	3.09 \pm 0.36 ^c	0.23 \pm 0.15 ^d
Pb	122.67 \pm 12.22 ^a	124.0 \pm 6.11 ^a	138.0 \pm 11.37 ^a	134.67 \pm 9.26 ^a	118.29 \pm 5.24 ^a	5.78 \pm 4.07 ^b

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 21 Several parameters of Ryegrass roots grown in treatments of 2% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 2% Fe ⁰	75% PaT + 25% US + 2% Fe ⁰	50% PaT + 50% US + 2% Fe ⁰	25%PaT +75% US + 2% Fe ⁰	100% US + 2% Fe ⁰
Roots dry weight (g)	0.35 \pm 0.05 ^a	0.35 \pm 0.04 ^a	0.45 \pm 0.05 ^a	0.50 \pm 0.02 ^a	0.81 \pm 0.02 ^b	0.97 \pm 0.05 ^b
Total elements ($\mu\text{g g}^{-1}$)						
Cu	378.00 \pm 8.26 ^a	321.42 \pm 9.35 ^b	320.36 \pm 5.72 ^b	312.61 \pm 5.25 ^b	313.04 \pm 3.98 ^b	62.22 \pm 6.78 ^c
Zn	1657 \pm 31.87 ^{a,b,c}	2535 \pm 181 ^b	2196 \pm 50.0 ^b	1634 \pm 124 ^a	1074 \pm 24.79 ^c	76.78 \pm 23.20 ^d
Cd	70.31 \pm 0.88 ^a	152.36 \pm 10.49 ^b	142.93 \pm 1.36 ^b	107.11 \pm 5.98 ^c	53.42 \pm 3.26 ^a	BD
Pb	11787 \pm 895 ^a	8272 \pm 782 ^b	8085 \pm 492 ^b	7253 \pm 244 ^b	7502 \pm 519 ^b	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 22 Physical and chemical properties of treatments of 5% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean ± S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% Fe ⁰	75% PaT + 25% US + 5% Fe ⁰	50% PaT + 50% US + 5% Fe ⁰	25%PaT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
pH	6.82 ± 0.01 ^a	6.68 ± 0.04 ^b	6.74 ± 0.01 ^{a,b}	6.79 ± 0.01 ^a	6.82 ± 0.02 ^a	6.95 ± 0.03 ^c
EC <i>mS/cm</i>	0.45 ± 0.02 ^a	0.49 ± 0.004 ^a	0.49 ± 0.04 ^a	0.49 ± 0.03 ^a	0.42 ± 0.01 ^a	0.15 ± 0.003 ^b
O. M %	7.26 ± 0.04 ^a	7.10 ± 0.10 ^a	6.21 ± 0.10 ^b	5.38 ± 0.04 ^c	4.40 ± 0.04 ^d	3.75 ± 0.03 ^c
Extractable metals (µg g ⁻¹)						
Cu	71.33 ± 3.68 ^a	105.0 ± 2.52 ^b	88.50 ± 2.69 ^{a,b}	76.43 ± 2.17 ^a	51.68 ± 4.60 ^c	31.91 ± 6.07 ^d
Zn	25463 ± 732 ^a	22505 ± 238 ^b	17365 ± 910 ^c	12051 ± 235 ^d	5795 ± 640 ^e	30.53 ± 4.45 ^f
Cd	32.42 ± 0.59 ^a	35.46 ± 0.49 ^b	26.51 ± 0.36 ^c	17.73 ± 0.08 ^d	8.80 ± 0.80 ^e	0.94 ± 0.024 ^f
Pb	90187 ± 2870 ^a	69067 ± 3469 ^b	49280 ± 3448 ^c	33120 ± 2407 ^d	7680 ± 1538 ^e	5.47 ± 2.07 ^f
Total metals (µg g ⁻¹)						
Cu	192.27 ± 3.70 ^a	172.67 ± 3.62 ^b	124.80 ± 9.12 ^c	87.87 ± 11.92 ^d	0.13 ± 0.9 ^e	BD
Zn	30145 ± 1170 ^a	33845 ± 439 ^b	25189 ± 1032 ^c	17961 ± 773 ^d	6849 ± 372 ^e	47.61 ± 5.70 ^f
Cd	91.47 ± 7.13 ^a	79.00 ± 4.65 ^a	47.20 ± 1.44 ^b	38.80 ± 6.44 ^b	4.70 ± 0.06 ^c	BD
Pb	101600 ± 2518 ^a	79093 ± 1419 ^b	66800 ± 1499 ^c	48667 ± 2359 ^d	18267 ± 879 ^e	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

Table 23 Several parameters of Ryegrass shoots grown in treatments of 5% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% Fe ⁰	75% PaT + 25% US + 5% Fe ⁰	50% PaT + 50% US + 5% Fe ⁰	25%PaT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
Shoots length (cm)	8.20 \pm 0.15 ^a	8.83 \pm 0.44 ^a	11.00 \pm 0.29 ^b	11.17 \pm 0.60 ^b	11.42 \pm 0.30 ^b	12.67 \pm 0.44 ^b
Shoots dry weight (g)	0.75 \pm 0.05 ^a	1.76 \pm 0.03 ^b	1.69 \pm 0.08 ^b	1.79 \pm 0.07 ^b	1.86 \pm 0.07 ^b	2.36 \pm 0.13 ^c
Total elements ($\mu\text{g g}^{-1}$)						
Cu	26.04 \pm 2.71 ^a	75.07 \pm 1.78 ^{b,c}	81.67 \pm 5.70 ^b	71.26 \pm 0.41 ^{b,c}	65.54 \pm 1.89 ^c	64.74 \pm 1.67 ^c
Zn	1767 \pm 83.99 ^a	700.40 \pm 21.37 ^b	560 \pm 40.33 ^{b,c}	465 \pm 31.75 ^c	381 \pm 11.48 ^c	21.85 \pm 2.48 ^d
Cd	5.96 \pm 0.62 ^{a,b}	6.33 \pm 0.26 ^a	5.87 \pm 0.29 ^{a,b}	4.37 \pm 0.15 ^{b,c}	4.37 \pm 0.25 ^{b,c}	3.71 \pm 0.22 ^c
Pb	122.67 \pm 12.22 ^a	124.67 \pm 12.22 ^a	127.33 \pm 2.41 ^a	122.86 \pm 15.12 ^a	113.54 \pm 11.81 ^a	6.52 \pm 3.27 ^b

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05.

Table 24 Several parameters of Ryegrass roots grown in treatments of 5% Fe⁰ was added to the mixtures of Pandora tailings and uncontaminated soil (values presented as mean \pm S.E.M; n = 3).

Parameters	100% UPaT	100% PaT + 5% Fe ⁰	75% PaT + 25% US + 5% Fe ⁰	50% PaT + 50% US + 5% Fe ⁰	25%PaT +75% US + 5% Fe ⁰	100% US + 5% Fe ⁰
Roots dry weight (g)	0.35 \pm 0.05 ^a	0.45 \pm 0.01 ^a	0.47 \pm 0.01 ^a	0.67 \pm 0.04 ^b	0.73 \pm 0.03 ^b	0.80 \pm 0.04 ^b
Total elements ($\mu\text{g g}^{-1}$)						
Cu	378.00 \pm 8.26 ^a	401.50 \pm 20.92 ^a	332.40 \pm 23.74 ^a	205.78 \pm 17.45 ^b	133.33 \pm 4.29 ^{b,c}	77.24 \pm 11.19 ^c
Zn	1657 \pm 31.87 ^a	3012 \pm 169 ^b	2707 \pm 13.83 ^b	1825 \pm 78.16 ^a	1529 \pm 126 ^a	55.12 \pm 3.22 ^c
Cd	70.31 \pm 0.88 ^a	114.56 \pm 2.59 ^b	105.17 \pm 3.33 ^b	54.31 \pm 2.05 ^c	20.09 \pm 1.46 ^d	BD
Pb	11787 \pm 895 ^a	10312 \pm 353 ^{a,b}	10003 \pm 144 ^{a,b}	9586 \pm 268 ^b	9610 \pm 500 ^{a,b}	BD

Different lower case letters within the same row indicate a significant difference according to the Tukey's HSD test at P < 0.05. BD= below detection limit.

