ASSESSMENT OF HEAVY METAL MOBILIZATION AND BIOREMEDIATION OF SOILS FROM SELECTED LANDFILL SITES

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ABSTRACT

This study focused on the speciation and bioremediation of heavy metals from two non sanitary landfills of different status (operating and closed) in Malaysia. Leachate contaminated soils from Bukit Beruntung (operating) and Taman Beringin (closed) landfills were analyzed using sequential extraction procedure. Six heavy metals namely Mn, Zn, Pb, Cr, Ni and Cu were considered. Analytical determinations was performed using inductively coupled plasma mass spectrometry (ICP-MS). The results indicate that in Bukit Beruntung (operating), Zn, Mn and Pb were the highest concentration of metals observed in the mobile phase while only Cu and Cr concentration was predominant in the immobile phase. Similarly, in Taman Beringin (closed), Mn, Zn and Pb were the highest concentration of metals observed in the mobile phase while Cr, Ni and Cu were mostly observed in the immobile phase. The mobility of most metals were higher in Taman Beringin (closed) compared to Bukit Beruntung (operating) which shows that metals found in Taman Beringin (closed) could pose a more serious threat to the environment. The activity or inactivity may have affected metal species in both landfills studied. Bioremediation via bioaugmentation of soils from both landfills was also carried out over a course of 100 days, soil microcosms were taken for heavy metal analysis using sequential extraction procedure after the bioaugmentation (at Day 100). Reduction in Cr, Mn, Cu, Zn in BB and Cr, Mn, Co, Ni, Cu and Zn in TB concentration was observed for metals in both mobile and immobile phase by Day 100. Bioaugmentation of leachate contaminated soils thus showed indigeneous micro organisms reduced metal concentrations in both landfills although mobility of Mn and Zn in BB and Mn in TB was observed and thus could still pose ecological risk.

ABSTRAK

Kajian ini bertumpu terhadap penspesiesan dan biopemulihan logam berat daripada dua tapak pelupusan bukan sanitari yang berbeza status (masih beroperasi dan telah ditutup) di Malaysia. Tanah tercemar dengan resapan terlarut dari tapak pelupusan Bukit Beruntung (masih beroperasi) dan Taman Beringin (telah ditutup) telah dianalisis dengan menggunakan prosedur pengekstrakan berjujukan. Enam logam berat iaitu Mn, Zn, Pb, Cr, Ni dan Cu telah dipertimbangkan untuk dianalisis. Analisis dilakukan dengan menggunakan Terganding Beraruhan Spektrometer Jisim Plasma (ICP-MS). Keputusan menunjukkan bahawa di Bukit Beruntung (masih beroperasi), kepekatan logam Zn, Mn dan Pb didapati paling tinggi dalam fasa bergerak manakala hanya Cu dan Cr mendominasi dalam fasa tidak bergerak. Begitu juga, di Taman Beringin (telah ditutup), logam Mn, Zn dan Pb didapati tinggi dalam fasa bergerak manakala kebanyakan Cr, Ni dan Cu didapati dalam fasa tidak bergerak. Mobiliti kebanyakan logam yang lebih tinggi di Taman Beringin (telah ditutup) berbanding Bukit Beruntung (masih beroperasi) menunjukkan bahawa logam dijumpai di Taman Beringin (telah ditutup) boleh menimbulkan ancaman yang lebih serius kepada alam sekitar. Aktiviti atau tidak aktif mungkin mempunyai spesies logam terjejas dijumpai di dalam kedua-dua tapak pelupusan. Biopemulihan melalui bioaugumentasi tanah dari kedua-dua tapak pelupusan juga telah dilaksanakan selama 100 hari. Mikrokosma tanah telah diambil selepas bioaugumentasi pada hari ke-100 untuk tujuan menganalisa kandungan logam berat menggunakan prosedur pengekstrakan berjujukan. Pengurangan kandungan logam diperhatikan bagi kebanyakan logam bagi kedua-dua fasa bergerak dan tidak bergerak pada hari ke-100. Bioaugumentasi tanah tercemar resapan terlarut itu menunjukkan mikroorganisma asal mengurangkan kandungan logam dalam kedua- dua tapak pelupusan walaupun pergerakan beberapa logam masih diperhatikan.

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BB	:	Bukit Beruntung
TB	:	Taman Beringin
Cu	:	Copper
Pb	:	Lead
Sn	:	Stannum
Ar	:	Arsenic
Hg	:	Mercury
Rb	:	Rubidium
Sr	:	Strontium
Nb	:	Niobium
Zr	:	Zirconium
V	:	Vanadium
Se	:	Selenium
Mn	:	Manganese
Mg	:	Magnesium
Al	:	Aluminium
Co	:	Cobalt
Ti	:	Titanium
Fe	:	Iron
Cd	:	Cadmium
Ni	:	Nickel
CH_4	:	Methane
Ppm	:	Parts per million
Rpm	:	Revolutions per minute

ICPMS	:	Inductively Coupled Plasma-Mass Spectrometry
ug/g	:	Micro gram per gram
F1	:	Fraction 1(Exchangeable Fraction)
F2	:	Fraction 2(Weakly complexed and bound to carbonate)
F3	:	Fraction 3(Bound to iron and Manganese oxides)
F4	:	Fraction 4(Bound to organic matter and sulphide fraction)
F5	:	Fraction 5(Residual fraction)
$MgCl_2$	:	Magnesium chloride
NH_2OHHCl	:	Hydroxylammonium hydrochloride
NaOAc	:	Sodium acetate
HOAc	:	Acetic acid
HCl	:	Hydrochloric acid
$PbCO_2$	:	Lead (II) carbonate
$PbCl_2$	:	lead chloride or laurionite
NH_4OAc	:	Ammonium acetate
WHO	:	World Health Organization
SON	:	Standard Organization of Nigeria
USA	:	United States of America
USEPA	:	United States Environmental Protection Agency
M	:	Molarity
v/v	:	Volume per volume
H	:	Hour
>	:	Greater than
◦C	:	Degrees celcius
Ml	:	Mililitre
M	:	Meter

$Pb_5(PO_4)3X$	:	Pyromorphite minerals
%	:	Percentage
Cl	:	Chlorine
G	:	Gram
Fl	:	Flourine
B	:	Boron
OH	:	Hydroxide
0	:	Zero
VII	:	Seven
PNS	:	Peripheral nervous system
CNS	:	Central nervous system
Eh	:	Redox potential
Min	:	Minutes
Cm	:	Centimeter
BCR	:	Community Bureau of Reference
SEP	:	Sequential extracton procedure
RAC	:	Risk assessment code
$mg\ g^{-1}$	:	Miligrm per gram
Cl^-	:	Chloride ion
II	:	Two
III	:	Three
IV	:	Four
Ba	:	Barium
Ag	:	Silver
Au	:	Gold
Mo	:	Molybdenum

U : Uranium

DO : Diesel oil

EDTA : Ethylenediamine tetraacetic acid

NTA : Nitrilo triacetic acid

HNO3 : Nitric acid

< : Less than

SM&T : European Standard, Measurements and Testing

GCS : Geological Society of Canada

Th : Thorium

Sc : Scandium

Hf : Hafnium

La : Lanthanum

Sb : Antimony

+ : Increase in metal

BA : Leachate contaminated soil amended with BB bacterial strains

TA : Leachate contaminated soil amended with TB bacterial strains

CHAPTER 1: INTRODUCTION

1.1 Heavy metal pollution

The world has been experiencing serious environmental changes, especially since the dawn of industrialization. Global industrial revolution has led to the release of unprecedented toxic pollutants into the environment. This has posed serious threats to human health, flora, fauna, water, soil, air etc. Major contributing factors leading to environmental pollution include burning of fossil fuels, increase in the amount of waste generation, invention of new products leading to new streams of waste for example electrical and electronic equipment waste, nano waste, end of life vehicles and electric hybrid cars etc. In addition, increase in population growth, rapid urbanization, methods of waste disposal,vehicular emissions, industrial activities and processes such as tanning and dyeing, chemical manufacturing, metaliferous mining and smelting, metal plating etc and agricultural activities such as factory farming, application of fertilizers, pesticides etc, have also become significant sources of pollutants causing environmental pollution and degradation (Duruibe *et al.,* 2007; Fong *et al.,* 2008; Ruijuan *et al.,* 2008; Agamuthu and Al-Abdali, 2009; Manaf *et al.,* 2009; Agamuthu and Fauziah, 2010; Mohammad *et al.,* 2011; Wuana and Okieimen, 2011; Ripin *et al.,* 2014; Al Raisi *et al.,* 2014; Islam *et al.,* 2015). Environmental pollution has thus become an issue of global concern. This is due to the fact that natural degradation of pollutants and self-cleansing capacity of most ecosystems is becoming increasingly difficult due to the quantity, complexity and heterogeneity of pollutants released into the environment (Yongkun *et al*., 2013). One of such toxic and recalcitrant pollutant of concern is heavy metals (Sharma *et al.,* 2015).

In the earliest part of the twentieth century, the world was rocked by several heavy metal pollution incidents that led to fatalities, injuries and irreversible damage to pristine ecosystems; from Methylmercury poisoning in Minimata and Niigata, Japan and Northern Iraq to Sandoz chemical spill in Switzerland; Esperance Harbour lead dust incidents to Zamfara lead poisoning epidemic in Nigeria; and Toyoma cadmium poisoning to Rio Guardima nature reserve poisoning in Spain (Tsubaki and Irukayama,1977; Horiguchi *et al.,* 1994; Boos-Hersberger,1997; Burki, 2012; McCafferty *et al.,* 2013; James, 2015). These incidences further stressed the concern regarding heavy metal pollution in the environment.

1.2 Definition of heavy metals

Heavy metals are group of metals and metalloids with atomic density greater than 4000 kg/m³ or 5 times more than water and typically with atomic number higher than 20 (Kulshreshtha *et al.,* 2014; Duffus, 2002). Typical properties of heavy metals include malleability, ligand specificity, conductivity, stability as cations, density and ductility. Examples of heavy metals includes cadmium (Cd), iron (Fe), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), plumbum (lead, Pb), zinc (Zn), arsenic (As), manganese (Mn), nickel (Ni), selenium (Se) and the platinum group metals, which comprises of platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), osmium (Os), and iridium (Ir). Although heavy metals such as Pb, Cd, Hg, and As are metalloids, they are also generally referred to as heavy metals (Chibuike and Obiora, 2014). According to Hashim *et al.* (2011) and Mohammad *et al.* (2011), the most toxic forms of these metals in their ionic species are the most stable oxidation states e.g Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+}, As^{3+} which forms biotoxic compounds when they react with biomolecules of animals and plants.

1.3 Sources of heavy metals in the environment

There are two major sources through which heavy metals enter into the environment; natural sources or geogenic and anthropogenic or man-made sources. In nature, trace quantities of some metals (commonly refered to as biologically essential micronutrients-namely Co, Cr, Cu, Zn, Mn, Fe, Se etc.) is needed by different life forms (Bolan *et al.,* 2011; Mohammad *et al.,* 2011; Park *et al.,* 2011). Although some heavy metals play critical role as micronutrients, metal(loid)s such as Cd, Pb, As, Hg etc. have been shown to be phytotoxic and/or zootoxic (Bolan *et al.,* 2011; Mohammad *et al.,* 2011; Park *et al.,* 2011). Further, heavy metal ions remain the most toxic inorganic pollutants which occur in soils (Bradl, 2004). Additionally, studies have reported that even at low levels, deleterious effects of these metals in humans, animals, plants and microorganisms has been observed, and toxicity of some of these metals increases as it accumulates in soils and water (Bradl, 2004).

1.3.1 Natural sources

Natural sources of heavy metals could be due to geological or pedogenic activities. Through these activities, heavy metals embedded in ores of the earth's crust-in soils, rocks (mostly igneous and sedimentary rocks), coal, sediments, waters, and microorganisms with natural background concentrations are released into the environment (Mohammad *et al.,* 2011; Park *et al.,* 2011; Bolan *et al.,* 2014). However, because of the low solubility of these metals in nature, very minute concentration is released in most cases (Park *et al.,* 2011). Therefore, the metal(loid)s present in the parent materials are often unavailable for plant uptake and have minimum effect on soil organisms (Park *et al.,* 2011). Additionally, often, the concentrations of metal(loid)s released into the soil system by the natural pedogenic (or weathering) processes are largely related to the origin and nature of the parent material. Aside As and Se, heavy metals such as Pb, Ni and Cr derived from (natural) weathering process do not usually have any deleterious impact on the soil environment (Mohammad *et al.,* 2011; Park *et al.,* 2011; Bolan *et al.,* 2014).

1.3.2 Anthropogenic or man-made sources

Anthropogenic sources of heavy metals are a more rampant source of heavy metals in the environment (Bolan *et al.,* 2014). Examples of major sources of these metals in soils and most environmental systems include those primarily associated with industrial processes, manufacturing, acid deposition, tannery, mine waste and organic refuses, such as sewage sludge, agricultural practices that utilizes phosphate, fertilizers, manure and pesticides, landfill leachate, composts, cemeteries, incinerators, electroplating, smelting, laboratories, fossil fuel combustion, traffic-related emissions, agricultural and horticultural materials, warfare and military training, timber preservation, drum reconditioning, waste storage and treatment, metal treatment, sheep and cattle dips, scrap metal yards, urban development such as construction and excavation, chemical manufacturing, production and use in accumulators, mercury lamps, thermometers, batteries etc. (Shazili *et al.,* 2006; Fong *et al.,* 2008; Wuana and Okieimen, 2011; Park *et al.,* 2011; Su *et al.,* 2014).

According to Chik *et al.* (2014), Ripin *et al.* (2014) and Lai *et al.* (2011), sources and fluxes of anthropogenically generated heavy metals have received significant attention worldwide. This concern has also been raised in Malaysia (Khodami *et al.,* 2017; Ong *et al.,* 2016; Kadhum *et al.,* 2015; Agamuthu and Fauziah, 2010). Heavy metal pollution concerns are due to its properties and behavior in the environment. One of the most important properties of these metals which differentiate them from some other toxic pollutants is that they are not biodegradable in the environment. Another problem associated with these metals is their potential for bioaccumulation and biomagnification causing heavier exposure for organisms than is present in the environment alone (Sameera *et al.,* 2011). Additionally, metals exposure to other organisms is as a result of the metals reaching food chains, mainly through plant uptake and animal transfer (Park *et al.,* 2011).

As land application becomes an important part of waste utilization and disposal practices, the soil environment in most landfill sites has increasingly become an important sink for heavy metals.

1.4 Heavy metal pollution from landfills in Malaysia

According to Scott *et al.* (2005) and Ashraf *et al.* (2013), up to 95 % of the total municipal solid waste (MSW) collected worldwide is disposed off in landfills. The story is not different in Malaysia where about 75% of the MSW generated daily is landfilled (Mohamad and Keng, 2013). Despite other waste disposal options such as 3Rs (Reduce, Reuse and Recycle), composting, incineration, Refuse derived fuel (RDF) conversion, gasification etc, to date, the most dominant method of MSW disposal in the country still remains landfilling (Agamuthu and Fauziah, 2011; Fauziah and Agamuthu, 2007; Zainol *et al.,* 2012; Fauziah *et al.,* 2013b). According to Agamuthu and Fauziah (2011), there are about 306 officially recognized landfills/dumping sites in Malaysia and about three times more illegal dumping sites.

Although landfilling as a waste disposal option is able to accommodate all kinds of waste, cost effective and can be seen as a green approach since it involves mainly biological processes (Emenike *et al.,* 2013), the main problem arises from leachate production. Landfill leachate is a complex wastewater with considerable variations in both composition and volumetric flow (Ahmed and Sulaiman, 2001; Trebouet *et al.,* 2001). It is principally produced by percolation of precipitation through waste deposited in a landfill. Landfill leachate consists of large amounts of organic matter including dissolved organic matter, phenols, ammonical-nitrogen, phosphate, heavy metals, sulphide, hardness, acidity, alkalinity, salinity, solids, inorganic salts, and other toxicants (Zainol *et al.,* 2012; Agamuthu, 2014). According to Agamuthu *et al.* (2011b), the volume of leachate generated in Malaysia is about $2.1\text{x}10^7$ m^3 per day and has the potential to leach out about 3,825 g of Fe, 4,095 g of PO_4^{3-} and 23,400 g of Zn into nearby water courses (Agamuthu and Fauziah, 2011). High intensity of rainfall within a short period observed in the country throughout the year is a major contributor to the high volume of leachate generated.

Even though low heavy metal concentration has been recorded in some landfills, landfills in Asia are notorious for having high heavy metal concentrations in leachate regardless of their status (active or in active) (Agamuthu *et al.,* 2011; Fauziah *et al.,* 2013b). The high level of heavy metals found in polluted soils at landfill sites can be traced to the migration of leachate from and within the landfill's waste cell (Agamuthu and Fauziah, 2010; Kanmani and Gandhimathi, 2013). These metals may be retained by soil components in the near surface soil horizons or may become precipitated or co-precipitated as sulfides, carbonates, oxides or hydroxides with Fe, Mn, Ca, etc (Mohammad *et al.,* 2011). Therefore, the level of pollution in soils by these metals is dependent on retention the capacity of the soil, mineralogy, grain size, organic matter and chemical properties of the metal (Moral *et al.,* 2005). In addition, the mobility of these metals is reflected in their capacity to pass from one soil compartment to another where the element is bound less energetically, the ultimate compartment being soil solution, which determines the bioavailability of such metal (Mohammad *et al.,* 2011; Al Maashri, 2012).

Municipal and industrial wastes discarded into landfills leads to the release of leachate (which may contain metal ions amongst other toxic substances) in water and subsequent dispersion into the surrounding areas by surface or subsurface, lateral and vertical movements into the ground. This phenomenon is especially evident around non-sanitary landfills (whether urban, sub-urban or rural) in Malaysia (Fauziah and Agamuthu, 2009; Masirin *et al.,* 2008; Star Online, 2010). Other features of these landfills that lead to environmental pollution are (1) absence of bottom lining systems, (2) nonexistent leachate collection and removal system to prevent leachate seepage into groundwater sources, soils, rivers, streams etc. Additionally, the problem of landfilling in the country is made worse especially around landfill sites that are located near rivers and streams (which are important sources of water for industrial and domestic water supply, agriculture and productivity) (Umar *et al.,* 2010; Fauziah *et al.,* 2013a).

Heavy metal concentration in soils, surface waters and aquatic habitat as a result of leachate flow into these environments from Malaysian landfills has been extensively reported. According to Bahaa-Eldin *et al.* (2008), in soil horizons beneath Ampar Tenang waste disposal site in Dengkil, Selangor, it was observed that heavy metal concentrations (notably Cr, Zn and Pb) were generally high within the near surface soil layer and decreased in concentration with increasing depth. Furthermore, vertical infiltration of leachate from the solid waste and shallow groundwater level strongly impacted the dispersion of the heavy metals in soils below the waste site. In another report, Mohammed *et al.* (2009) highlighted that the Ampar Tenang landfill soil was found to be incapable of preventing the migration of contaminants including heavy metals notably Fe, Mn, Cd, Pb Cu, vertically and horizontally from the source point. The landfill has thus been polluting the groundwater, soil and is also capable of polluting the Labu River closeby.

In another study, Rahman *et al.* (2000), revealed that Ni, Cu, Cr, Cd, Ag and Pb was detected in borehole soils from Taman Rekreasi and Jinjing Utara landfill sites. In the same study also, Cu, Cd, Ag and Pb were also detected in Sungai Besi and Seri Petaling landfills (Rahman *et al.,* 2000). At Kampung Paka 1 and 2 landfill sites, Cu, Cd and Pb were also observed in borehole soils examined (Rahman *et al.,* 2000). Furthermore, they observed that landfill sites which received wastes from commercial and industrial premises had higher heavy metal concentration compared to sites with only MSW (Rahman *et al.,* 2000). They concluded that lack of proper geotextile membrane to prevent leakage of leachate into the soil and surface waters was the main cause of heavy metal pollution in the soil and surface water (Rahman *et al.,* 2000).

Agamuthu and Fauziah (2010) in their studies of Panchang Bedena landfill (an active, non-sanitary landfill) and Kelana Jaya ex-dump site, heavy metal content obtained from different soil depth from the three boreholes at the two landfill sites varied. At Panchang Bedena landfill, Pb concentration was found to have a decreasing trend while Fe, Zn and Ni were found to increase with the depth of the soil samples. In the closed dump site, arsenic was detected at 64.4 mg/kg in surface soil and Hg was detected as high as 11.5 mg/kg. Similar results were obtained from Kubang Badak landfill in Selangor by Kamil and Abdul Talib *et al.* (2010) and Ismail *et al.* (2015).

Similarly, Zaini *et al.* (2013) in their studies of soil samples from Ampar Tenang landfill, revealed that concentration of the heavy metals (Mg, Ca, Mn, Fe, Cu, Zn and Pb) per distance and depth varied. The results indicated that the concentration of Fe was the most dominant per specific distances and depths in North, East and West directions followed by Cu mainly in the West direction.

Studies on the distribution of heavy metals in topsoil of the non-sanitary landfill sites in the Langat river water catchment area, Selangor showed that Cu, Cd, Pb, and Zn was highly concentrated at the boundary of the non-sanitary landfills (Ismail *et al.,* 2015). Surprisingly, none of the elements concentrated in the immediate dumping area (the center point of the site) (Ismail *et al.,* 2015). In contrast to Ismail *et al.* (2015), studies by Ahmed and Suleiman (2001) revealed that lower concentration of Cu, Zn, Cr, Pb, and Ni (except for cadmium) were found in Seri Petaling landfill soils.

Similarly, Rahim *et al.* (2010) reported that solid waste disposed off directly onto an unprotected soil, not only polluted the soil but elevated the concentration of Fe and Pb in groundwater due to high contamination from an open dump site in West, Malaysia.

1.5 Problem statement

Because landfilling is the most common method of waste disposal in Malaysia and open dumping is being practiced (at about 50% of the total landfills), landfills have become major contributors to heavy metal pollution in the country. Coupled with increased waste generation rates of about 2-3% annually and little to no source separation of household wastes before disposal (mixed waste), various types of wastes in MSW stream in Malaysia have become sources of heavy metals within most MSW landfills. Examples of waste fractions that leach out metals includes food cans, discarded utensils and old kitchen appliances, plastics and scrap metals, household hazardous wastes such as fluorescent bulbs, aerosol cans etc, electronic waste such as batteries and old phones and computer etc (Masirin *et al.,* 2008; Agamuthu and Fauziah, 2010; Zainol *et al.,* 2012; Agamuthu, 2014). Additionally, heavy metals in leachate from landfills due to uncontrolled release have been found to contaminate ground and surface water, aquatic organisms, vegetation and soils in the country (Emenike, 2013; Shazili *et al.,* 2006; Fong *et al.,* 2008; Agamuthu and Fauziah, 2010; Ripin *et al.,* 2014).

Even though the soil environment has become a major sink for heavy metals in most landfills in Malaysia, most concerns about leachate contamination majorly points towards groundwater and surface water pollution and little consideration is accorded to soils contaminated with leachate (Emenike, 2013). In addition, extensive studies on the prevalence of heavy metals in Malaysian landfills (Bahaa-Eldin *et al.,* 2008; Agamuthu and Fauziah, 2010; Emenike, 2013; Zaini *et al.,* 2013; Rahman *et al.,* 2000), have tended to focus on the distribution, total concentration and impact of heavy metals on surface and ground water, soils, plants, animals such as fishes and humans. The total metal concentration may only indicate the overall level of metals in the soil and not mobility, toxicity and/or bioavailability of a particular metal (Ackah, 2012). Hence, this calls for the need of chemical speciation to determine the specific physicochemical forms of heavy metals (Tack and Verloo, 1995; Nemati *et al.,* 2011).

Due to a dearth of information on the speciation of heavy metals from Malaysian landfills, this study sought to find out the specific forms, distribution and the mobility of the chemical forms of heavy metals in leachate contaminated soil from Bukit Beruntung and Taman Beringin landfills. Furthermore, bioaugmentation of the leachate contaminated soil was carried out using microorganisms found in both landfills. This study will assist in proper planning, management and remediation of leachate contaminated soils. This will go a long way in preventing the negative consequences of heavy metal contamination on human health and the environment.

1.6 Objectives of the study

The objectives of this study were:

1.To determine and compare the total concentration of heavy metals in leachate contaminated soils from Bukit Beruntung and Taman Beringin landfills.

2.To compare the speciation of heavy metals between an operating non-sanitary (Bukit Beruntung) landfill and a closed non-sanitary landfill (Taman Beringin)

3.To assess the mobility and bioavailability of heavy metals in leachate contaminated soils from Bukit Beruntung and Taman Beringin landfills.

4.To compare the speciation of heavy metals in bioremediated and non-bioremediated soils from Bukit Beruntung and Taman Beringin landfills.

CHAPTER 2: LITERATURE REVIEW

2.1 An overview of heavy metal pollution scholarship

Extensive documentation exists on heavy metal pollution from different sources in different environments from different parts of the world. In most cases, elevated levels of selected metals were observed in the environmental samples well above safe levels for humans, animals, plants etc. The studies on heavy metal pollution in streams and the studies reviewed under each stream is presented as follows.

2.1.1 Heavy metal pollution in waste

Waste material from various industries, households, commercial premises etc has been documented to contain heavy metals. Deleterious impact of these waste sources has been reported in literatures (Benvenuti *et al.,* 1995; Olaniya *et al.,* 1998; Hargreaves, Adl & Warman, 2008; Gautam *et al.,* 2010). A few scholarship on presence of these metals in waste materials is discussed below.

Heavy metal pollution in waste rock dumps, metallurgical wastes and flotation tailings were examined by Benvenuti *et al.* (1995) in the abandoned mining district of Boccheggiano (Southern Tuscany, Italy). More than 50 decades of mining activity has led to heavy metal pollution in the environment in Boccheggiano district (Benvenuti *et al.,* 1995). The concentration of heavy metals in the three examined mine wastes was found to be varied. The highest concentration of metals found in the flotation tailings were 2450 ppm Pb and 6080 ppm Zn (Benvenuti *et al.,* 1995). In metallurgical wastes, 709 ppm Cu and 446 ppm Bismuth (Bi) were the predominant metals while in waste rock dumps, 219 ppm Pb and 705 ppm As were the most dominant (Benvenuti *et al.,* 1995).

Unlike Benvenuti *et al.* (1995), Lokhande *et al.* (2011) evaluated heavy metals in waste water from Taloja industrial belt of Mumbai, India. Effluent samples from textile industries (mostly from dye manufacturing units) accounted for 12.8 mg/L Fe and 33.3 mg/L Cu and was (Lokhande *et al.,* 2011). Further, effluents from paint manufacturing industries had 35.2 mg/L Cr, 33.1 mg/L Zn and 31.4 mg/L Pb and pharmaceutical industries effluent had 35.8 mg/L Cd and 33.6 mg/L Ni (Lokhande *et al.,* 2011). They concluded that high concentration of heavy metals in the effluent samples could pollute nearby water bodies and affect the growth of vegetation and aquatic life (Lokhande *et al.,* 2011). They therefore emphasized the importance of improvement in the industrial waste water treatment methods before discharge into any receiving environment (Lokhande *et al.,* 2011).

Municipal solid waste in landfills also causes heavy metal pollution in the environment (Yarlagadda *et al.,* 1995; Scott *et al.,* 2005; Aucott, 2006; Oluyemi *et al.,* 2009; Waheed *et al.,* 2010; Iwegbue *et al.,* 2010; Bretzel *et al.,* 2011; Rizo *et al., 2*012; Pastor and Hernández, 2012; Alam *et al.,* 2012; Barbieri *et al.,* 2014; Nannoni *et al.,* 2015; Sayadi *et al.,* 2015; Yobouet *et al.,* 2016; De *et al.,* 2016; Aiman *et al.,* 2016; Emenike *et al.,* 2016). An estimate of the concentration of heavy metals in municipal solid waste (MSW) in United States of America by Aucott (2006) is shown in Table 2.1.

Furthermore, direct application of MSW as soil conditioners and fertilizer onto agricultural soils has also resulted in contamination of soils and food crops grown on such soils (Olaniya *et al.,* 1998; Hargreaves, Adl & Warman, 2008; Gautam *et al.,* 2010). Olaniya *et al.* (1998) reported that organic MSW mixed with soil (applied as organic manure) in Calcutta, India revealed that vegetables from such agricultural land contained heavy metals.

Table 2.1: Heavy metals in Municipal Solid Waste (MSW) in United States of America (Aucott, 2006)

Heavy metals	Estimated concentration of heavy metal in MSW(ppm)	Metric tons of waste/year disposed off in U.S. landfills, either as reported directly or assuming 250 million tons MSW disposed/year and concentration as in column to the left
Cadmium(Cd)	4 -20	1,000-2,700
Copper (Cu)	77	19,250
Chromium(Cr)	350	87,500
Lead (Pb)	230-400	57,500-127,000
Mercury(Hg)	1.5	375-400
Nickel (Ni)	57	14,250
Zinc (Zn)	380	95,000

2.1.2 Heavy metal pollution in biota

Aside heavy metal pollution in waste, biotas have also been found to contain a cocktail of heavy metals in plant and animals and their habitat or ecosystem. Hsu *et al.* (2006) revealed that due to the widespread of heavy metal pollution in the environment, heavy metal toxicity among natural populations of organisms in protected Kenting National Park in Taiwan was observed. Terrestrial biota and the associated plant species and soil in the park recorded high bio concentrations of heavy metals (Hsu *et al.,* 2006). This included 260.04 µg/g Cd, 403.87 µg/g Hg and 19.44 µg/g Sn in snails, 158.35µg/g Cd, 23.33 µg/g Hg and 10.88 µg/g Sn in earthworms, 29.87µg/g Cd, 1.06 µg/g Hg and 1.92 µg/g Sn in crabs, 6.75 µg/g Cd, 317.86 µg/g Hg and 8.19 µg/g Sn in lizards, 26.34µg/g Cd, 8.50 µg/g Hg and 9.21 µg/g Sn in bats and 1.73 µg/g Cd, 46.38 µg/g Hg and 11.17 µg/g Sn in snakes- indicating a contaminated terrestrial ecosystem (Hsu *et al.,* 2006). Further, plant species showed high concentrations of 12.62 µg/g Cd, 6.14 µg/g Hg, and 13.80 µg/g Sn (Hsu *et al.,* 2006). Hsu *et al.* (2006) concluded that bioaccumulation of very high levels of heavy metals in these organisms and plant species revealed a strong influence of industrial pollution on the biotic community (Hsu *et al.,* 2006).

Additionally,well-documented historical landuse changes in Tamaki Estuary in Auckland, New Zealand since c1800, showed that the Estuary-from being a relatively pristine natural bush conditions to grain farming and then dairy farming and finally to urban housing and industrial activities, has suffered serious “assault” (Abrahim and Parker, 2008). Cu, Pb, Zn and Cd in fine fraction sediments extracted in eight cores from the Estuary revealed that anthropogenic influence of landuse changes was responsible for polluting the area (Abrahim and Parker, 2008).

In contrast to Abrahim and Parker (2008), Liang *et al.* (2011) evaluated the heavy metal contamination of plants in Dunhua sewage irrigation area (DIA) in China where wastewater has been used to irrigate crops for almost 20 years. Cd, Cr and Pb concentration in the three different crops (sunflower, soybean and corn) varied depending on the organ of the crop- root, stem, leaf, grain. The highest concentration of Cd was found in roots of soybean, Cr and Pb was found mostly in the roots of sunflower. The general trend of Cd, Cr and Pb in the crop was root>stem>leaf>grain.

Unlike Liang *et al.* (2011), Cai *et al.* (2009) took a different approach to investigate the deleterious impact of metals on a biota. Cai *et al.* (2009) examined the impact of lead–zinc smelter on the surrounding environment by analyzing samples from grass and leaves used as cattle feed and tissue samples from cattles that graze nearby the smelter. The bovine tissues showed metal concentrations of 54 mg/kg Zn and 1.31–2.47 mg/kg Cd in liver while 36 mg/kg Zn and 6.64–38.3 mg/kg Cd was observed in kidney (Cai *et al.,* 2009). The bovine tissues showed 1.0 mg/kg and 0.8 mg/kg of Pb in livers and kidneys respectively. Their findings showed that concentration of Cd and Zn exceeded the Chinese standards of these metals in feed, kidney and liver (Cai *et al.,* 2009). Further, the highest concentration of Pb was observed in feed with 132 mg/kg (which was 40 times higher than the Chinese standards) (Cai *et al.,* 2009). They highlighted the danger of transfer of heavy metals across food chain due to bioaccumulation in plants and animals (Cai *et al.,* 2009).

Zukal *et al.* (2015) in their review of worldwide exposure of bats to toxic elements due to contamination of their foraging habitats stressed that heavy metal pollution have been strongly found to negatively influencing ecological, genetic, physiological and behavioral parameters of bats diversity, flight activity, population structure and relative abundance. This is due to toxicological effects on the liver, kidney, immune, nervous and reproductive systems of bats.

2.1.3 Heavy metal pollution in water

Apart from biota pollution, water sources which play critical roles in all living systems have been found to be contaminated by heavy metals. Surface water, ground water, rivers, streams, run-offs and even oceans have not been spared from pollution with heavy metals especially in and around industrial, agricultural areas etc. This section focuses on heavy metal pollution in water samples from different sources.

Jung (2001) in his study highlighted the impact of heavy metal pollution by evaluating water in and around Imcheon Silver and Gold mine in Korea. He observed that metals were continuously dispersed downstream and downslope from the mine tailings by clastic movement through water and wind (Jung, 2001). Elevated concentrations of Cd, Pb, Cu and Zn (9.4 mg/kg, 229 mg/kg, 1640 mg/kg and 6160 mg/kg respectively) were found in water from the tailings (Jung, 2001). In addition, impact of mine waters was observed in soils sampled from paddy fields, uncultivated land and household gardens because they contained higher metal concentrations than those of from nearby control sites (Jung, 2001).

In a different setting, Oluyemi *et al.* (2009) used a slightly different approach in determining the level of heavy metal contamination from open dumping sites. Using proximity of pollution source to drinking water wells, water samples were collected from 44 wells within 25 m radius of six refuse dump sites within the Lagos metropolis, Nigeria (Oluyemi *et al.,* 2009). High concentration of Pb (0.387 mg/l in Oshodi area, 0.234 mg/l in Mushin and 0.424 mg/l in Ikotun) and Cd (0.015 mg/l in Mosafejo, 0.045 mg/l in Mafoluku, 0.113 mg/l in Mushin and 0.120mg/l in Tejuosho) was observed (Oluyemi *et al.,* 2009). Further, in all the sampled area, concentration of Fe in the upper well ranged from 0.201 mg/l to 0.387 mg/l while in the lower well it ranged from 0.145 mg/l to 2.240 mg/l (Oluyemi *et al.,* 2009). Mn concentration in all sampled sites ranged from 0.381 mg/l to 0.773 mg/l in lower wells and 0.166 mg/l to 0.688 mg/l in upper wells (Oluyemi *et al.,* 2009). With the exception of Co (which was not detected in all the studied area) and negligible amount of Ni in the upper wells (concentrations of 0.009 mg/l to 0.229 mg/l), all metals detected in the upper and lower wells within 25 meters radius of six refuse dump sites were above the World Health Organization (WHO) and Standard Organization of Nigeria (SON) recommended limit for potable water (Oluyemi *et al.,* 2009).

Begum *et al.* (2009) in their investigation of one of the major river in India (Cauvery River) observed that anthropogenic activities such as throwing ashes into the river, people taking “holy bath”, industrial and agricultural activities caused heavy metal pollution of the river. Water samples from upstream of the river where industrial, agricultural and “sacred” activities were observed had Pb (2.35mg/kg to 3.56 mg/kg), Cu (0.03 mg/kg to 1.05 mg/kg), Co (1.20 mg/kg to 1.75 mg/kg), Zn (1.20 mg/kg to 4.45 mg/kg) and Ni (0.08 mg/kg to 2.45 mg/kg) (Begum *et al.,* 2009). However Cr and Mn was observed to be below detection limit in some sampled stations upstream of the river (Begum *et al.,* 2009). Compared to the upstream, concentration of Ni, Zn and Pb was found to be higher at the downstream of the river where industrial activities was predominant (Begum *et al.,* 2009). Ni, Zn and Pb concentration was 5.25 mg/kg, 10.70 mg/kg and 9.45 mg/kg, respectively (Begum *et al.,* 2009).

Huber *et al.* (2015) reviewed over 300 articles from six continents (Africa, Asia, Australia, North and South America and Europe) on the total concentration of heavy metals in traffic area run offs. It was revealed that Pb and Cr were mostly particle-bound, while Zn, Cu, Ni, and Cd were found at a higher fraction in the dissolved phase (Huber *et al.,* 2015). In addition, a decreasing trend of Pb over the years was observed which was consistent with the phasing-out of leaded gasoline (Huber *et al.,* 2015). There was no historical trends detected for Zn. The concentrations of heavy metal in runoffs from parking lots were found to differ widely depending on their use (for instance for employees, supermarkets, as rest areas for trucks) (Huber *et al.,* 2015). Run-off from bridge decks had high Zn concentrations and sources were traced to safety fences and galvanizing elements (Huber *et al.,* 2015). Additionally, roads with more than 5,000 vehicles per day were often more polluted than highways because of site-specific factors such as braking and acceleration at traffic signals (Huber *et al.,* 2015). According to Huber *et al.* (2015), worldwide highway runoff concentrations were not significantly influenced by average annual daily traffic and urban/non-urban land uses. Huber *et al.* (2015) also highlighted the significance of storm water treatment before its release into other water bodies and the role of traffic related run-offs as non-point source of heavy metals.

Additionally, one of the recent case of heavy metal pollution was in Flint, Michigan, USA (The Washington Post, 2016). Water samples collected from Flint homes were analysed after outbreak of health issues when the city changed its drinking water source (The Washington Post, 2016). Due to heavy metal pollution of the new water source that served the city, Pb level in water samples taken directly from taps in homes revealed as high as 13,000ppb (The Washington Post, 2016). In addition, Hanna-Attisha *et al.* (2016) reported that due to monumental increase in lead in drinking water source of Flint city, an elevated level of lead in the blood of children in the city was observed (Hanna-Attisha *et al.,* 2016).

2.1.4 Heavy metal pollution in edible food

Edible food has also become contaminated with heavy metals mainly due to bioaccumulation and biomagnification of these metals in agricultural soils, crops etc. Deleterious impact of these metals has been observed in humans due to consumption of contaminated food in some cases. A review of scholarship on heavy metal contamination in food is discussed below.

In different districts of Zhuzhou City, China, Haiyan and Stuanes (2003) sampled lettuce, beet, spinach and celery from agricultural fields and three major vegetable markets (namely Qingshuitang, South and Hexi) in the city. The highest concentration of Cu (71.1 mg kg^{-1} dry weight) in lettuce was from South market (Haiyan & Stuanes, 2003). Cr, Cd, Pb and Zn (2.3 mg kg^{-1} dry weight, 7.3 mg kg^{-1}dry weight, 33 mg kg^{-1} dry weight and 196.3 mg kg^{-1} dry weight, respectively) was highest in Qingshuitang market (Haiyan & Stuanes, 2003). For celery, South market had the highest concentration of Cu (25.6 mg kg^{-1} dry weight) (Haiyan & Stuanes, 2003). Cd, Zn, Cr and Pb (8.3 mg kg^{-1} dry weight, 230.3 mg kg^{-1} dry weight, 4.2 mg kg^{-1} dry weight and 17.9 mg kg^{-1} dry weight, respectively) were highest in Qingshuitang market (Haiyan & Stuanes, 2003). Accumulation of Cd, Pb, Zn and Cr in lettuce and celery from the markets was in the order of Qingshuitang > Hexi > South, only Cu accumulation was in contrast: South > Hexi > Qingshuitang (Haiyan & Stuanes, 2003).

Similarly, vegetables from the agricultural fields also showed contamination with metals. The highest concentration of Cu and Cr (44.1 mg kg^{-1} dry weight and 3 mg kg^{-1} dry weight, respectively) was observed in beet, Cd (7.2 mg kg^{-1} dry weight) in spinach while Pb and Zn (16.7 mg kg^{-1} dry weight and 137 mg kg^{-1} dry weight, respectively) in lettuce (Haiyan & Stuanes, 2003). Finally, vegetable contamination in both studied areas was traced to industrial area surrounding the agricultural fields (Haiyan & Stuanes, 2003). They concluded that growing vegetables or any other agricultural activities around industrial area should be avoided (Haiyan & Stuanes, 2003).

Similarly, heavy metal contamination of vegetables due to irrigation with waste water from a sewage treatment plant in Varanasi, India by Singh *et al.* (2010) showed that concentrations of Cd, Pb and Ni reached unsafe levels for human consumption in all the vegetables sampled. In fruit vegetables, Zn concentration was highest in lady's finger (122.3 µg g^{-1} to 132.7µg g^{-1}) while concentration of Cu concentration was highest in tomato (17.94 µg g^{-1}) (Singh *et al.,* 2010). In leafy vegetables, Ni concentration was highest in spinach (10.45 µg g^{-1} to 39.25 µg g^{-1}). Cd (2.19 µg g^{-1}), Cr (3.69 µg g^{-1}), Cu (13.75 µg g^{-1}) and Pb (12.20 µg g^{-1}) were the highest metal concentration recorded in radish (Singh *et al.,* 2010).

Apart from vegetables, heavy metal pollution of grains such as rice has also been reported. High levels of heavy metals in rice (*Oryza sativa L.*) from a E-waste recycling area in southeast China was reported by Fu *et al.* (2008). Heavy metal contents in rice samples (13 polished rice and relevant hull samples) were analyzed (Fu *et al.,* 2008). All metal concentrations studied, except for Co, in rice hull were higher than those in polished rice (Fu *et al.,* 2008). Cd (0.66 µg g^{-1}), Pb (2.6 µg g^{-1}), As (0.22 µg g^{-1}) and Hg (0.07µg g^{-1}) were recorded in contaminated polished rice samples which were higher than the maximum allowable concentration for milled rice in China (Fu *et al.,* 2008). Serious rice contamination noticed from the study was attributed to erosion of these metals from nearby E-waste recycling activities into rice paddies (Fu *et al.,* 2008).

2.1.5 Heavy metal pollution in air

Heavy metals such as Fe, Cr, Co, Au, Ni, Mn,,Cu, caesium (Cs), barium (Ba), europium (Eu), gallium (Ga), vanadium (V),tungsten (W) and selenium (Se) have been found to exist in fine and coarse fractions in ambient air (Hassanien, 2011). While metals such as Zn, W, Pb, As, Cd, Ga, Mo, Se and antimony (Sb), exist in fine fraction of particulate matter, hafnium (Hf), lanthanum (La), calcium (Ca), aluminium (Al), magnesium (Mg), thorium (Th), scandium (Sc) and titanium (Ti), exist predominantly in the coarse fraction.

Like other parts of the biosphere discussed above, air quality in the atmospheric environment has been affected due to released metals into the environment. Studies to prove this phenomenon is discussed in the consecutive sections.

Jaradat and Momani (1998) took air samples from a major highway in Jordan and observed 0.40 μg/m^3 Cu, 0.94 μg/m^3 Pb and 0.26 μg/m^3 Zn. They also observed that increase in the number of vehicles on the road led to an increase in the level of Cu, Pb and Zn (Jaradat and Momani, 1998). They concluded that automobile emissions are a major source of these metals in the urban atmosphere (Jaradat and Momani, 1998).

In a very comprehensive review of atmospheric heavy metal pollution, an assessment of emission into the atmosphere due to anthropogenic activities by Pacyna and Pacyna (2001) revealed that atmospheric deposition of these metals not only affect air quality but also terrestrial and aquatic environments. Pacyna and Pacyna (2001) review of global and regional emissions inventories further showed that stationary fossil fuel combustion were a major source of Cr, Hg, Mn, Sb, Se and Sn, especially in Asia (accounts for about 40 to 60% of total emission of various metals except for Cd). Similarly, 43 to 44% of the worldwide vehicular emissions of Pb was observed to be from the combustion of gasoline in Asia (Pacyna and Pacyna, 2001). The largest contributions from individual countries came from Mexico, China and Russia each emitting over 8500 tonnes of Pb in 1995 (Pacyna and Pacyna, 2001). While atmospheric Pb emissions were chiefly due to combustion of gasoline (leaded, low-leaded, and even unleaded), atmospheric Indium (In), As, Zn Cd, and Cu were chiefly due to non-ferrous metal production involving roasting and smelting of ores in smelters, melting operations in ferrous foundries, pyrometallurgical processes etc (Pacyna and Pacyna, 2001).

Similarly, as of 1994, emission estimates from iron and steel production revealed that Asia and Europe had the largest emission (Pacyna and Pacyna, 2001). In Europe, the highest metals emitted from iron and steel production were Pb (2255 tonnes), Cr (1037 tonnes) and Zn (778 tonnes) while in Asia, Pb (866 tonnes) Cr (1111 tonnes) and Zn (866 tonnes) were emitted (Pacyna and Pacyna, 2001). Cement production emissions were not as high as in iron and steel production although Asia still topped the list of emitters for Zn (1635 tonnes), Cr (818 tonnes), As and Pb (164 tonnes) and Hg (82 tonnes) (Pacyna and Pacyna, 2001).

Furthermore, according to Pacyna and Pacyna (2001), MSW and sewage sludge incineration contribute to metals in the atmosphere, however quantification on global and regional scale are mostly based on estimates due to difficulty in obtaining data on the amount and composition of waste materials incinerated. Richardson *et al.* (2001) also did a critical review on natural global and regional emissions of trace metals to the atmosphere, they reported that anthropogenic sources accounted for between 50% and 75% of total annual atmospheric Hg loadings worldwide.

Unlike in Asia and Europe, Hassanien (2011) reported that combustion of wood or agricultural waste contributed significantly to atmospheric heavy metal pollution in some Mediterranean countries. His studies conducted in Darya and Tartous, Syria also revealed that 60% of lead in air particulates in both sampled cities was found to be mainly associated with organic materials produced by incomplete burning of vehicles fuels and residential heating while in Shoubra el-Kheima, Egypt, secondary smelters and small foundries were point sources of Mn, Cr, As and Cd (0.2846 $\mu g/m^3$, 0.0022 $\mu g/m^3$, 0.0336 $\mu g/m^3$ and 0.0155 $\mu g/m^3$, respectively) air pollution (Hassanien, 2011).

2.1.6 Heavy metal pollution in soil/sediment

One of the widely reported environment where heavy metal pollution has been observed is- soil and sediment. Although background levels of metals have been recorded in undisturbed, pristine soils and sediments, metal concentration in such cases is often low and does not present any negative consequences (Mohammed *et al.,* 2011). However, soil and sediment samples from industrial and agricultural fields, rivers, seas etc have been reported to contain these metals due to anthropogenic influences (Hanif *et al.,* 2016; Khodami *et al.,* 2017).

Pekey (2007) found that road traffic run-offs, paint industries and coal combustion are among the most significant sources of metal pollution in coastline of Izmit Bay, north-eastern Marmara Sea, Turkey. His study revealed that based on the Sediment Quality Guideline of United States Environmental Protection Agency, sediment from Izmit Bay were heavily contaminated with As (21.5 $\mu g/g$), Cd (6.4 $\mu g/g$), Cu (80.6 $\mu g/g$), Pb (89 $\mu g/g$) and Zn (690 $\mu g/g$) (Pekey, 2007). Similarly, the contamination level of Cr (75 μg/g) and Ni (49.3 μg/g) were between moderately polluted and heavily polluted (Pekey, 2007).

Similar to Pekey (2007), an investigation of the magnitude and ecological relevance of Cd, Cu, Ni, Pb and Zn pollution of River Po sediments in Italy was investigated by Farkas, Erratico and Vigano`(2007). Total metal concentrations in the surficial sediments revealed significant pollution inputs on the whole river stretch investigated especially at the inlet of the River Lambro (Farkas *et al.,* 2007). Cu, Ni and Pb were found to be predominantly associated with the Fe/Mn oxides fraction (whereas Cd and Zn were found to be predominantly associated with the exchangeable fraction). Geoaccumulation index of heavy metals revealed that at the Lambro inlet, the surficial sediments were observed to be moderately polluted with Cu, Pb and Zn ($1 <$ mean Igeo < 2 for each metal) and moderately to strongly polluted with Cd ($2 <$ mean Igeo < 3). The middle reach of River Po was also moderately polluted with Cd ($1 <$ mean Igeo < 2) (Farkas *et al.,* 2007).

Mehmood *et al.* (2009) took a different approach in assessing inputs of heavy metals in Pakistan. They collected phosphate rocks (local and imported) used for the production of phosphorous based fertilizers in Pakistan (Mehmood *et al*., 2009). Pb concentration was particularly high and trace amount of Cu, Co and Ni was also recorded (Mehmood *et al*., 2009). They highlighted the importance of occupational safety especially for workers around the mines where local rock samples are sourced (Mehmood *et al*., 2009). Further, because of the widespread industrial application of phosphate rocks in the manufacturing of many consumer goods such as toothpaste, detergent, cosmetics, shaving creams, light bulbs, optical glass etc, the potential of phosphate rock as a source of heavy metal pollution in the environment by accumulation in air, soil, water bodies, food chain, etc was highlighted (Mehmood *et* *al*., 2009). Hence, there is the need for careful and complete separation of metals from these rocks prior to disposal of phosphogypsum waste (Mehmood *et al*., 2009).

Bhuiyan *et al.* (2010) in their study of agricultural soils in Bangladesh, found elevated concentrations of heavy metals in the soils emanating from a mine drainage surrounding the agricultural fields. The average concentrations of Mn, Zn, Pb, As, Fe, Titanium (Ti), Rubidium (Rb), Strontium (Sr) and Zirconium (Zr) was 427 mg/kg, 59 mg/kg, 433 mg/kg, 853 mg/kg, 1886 mg/kg, 14 mg/kg, 296 mg/kg, 290 mg/kg, 17.55 mg/kg and 877 mg/kg respectively (Bhuiyan *et al.,* 2010). These concentrations exceeded the world normal averages and in the cases of Mn, Zn, As and Pb, they exceeded the toxic limit of the respective metals (Bhuiyan *et al.,* 2010). They highlighted that coal mining activities are major contributors of heavy metals in the environment (Bhuiyan *et al.,* 2010).

Surface soils of a major global manufacturing base in the Chinese province of Guangdong were analyzed to assess the impact of urbanization and industrialization on the soil (Hu *et al.,* 2013). Mean concentrations of heavy metals - Mn, Pb, Ni, Cr, Hg, Fe, and Co in soil samples were 371 mg/kg, 51.4 mg/kg , 26 mg/kg, 67.2 mg/kg, 0.07 mg/kg, 5.092 mg/kg and 8.6 mg/kg, respectively (Hu *et al.,* 2013). Mean concentrations of Cd, Cu, Zn and As were observed to be more than two times higher than the background concentration of these heavy metals in the soils of Guangdong Province (Hu *et al.,* 2013). They concluded that surface soils in the Pearl River Delta in Guangdong, China had been polluted mainly because of anthropogenic influence of utilizing the area for agricultural, industrial and waste disposal/treatment activities (Hu *et al.,* 2013).

2.2 Health and environmental implications of heavy metal pollution

Although some metals such as Cu, Zn, Fe, Mn, Se, Mo, Cr etc are essential to life forms in trace amounts, some are non-essential such as Cd, Hg, Pb, As etc (Ali, Khan and Sajad, 2013). Toxicity of essential and non-essential metals at elevated concentration has been widely reported. According to Ali *et al.* (2013), heavy metals have negative effects on humans and the environment. In humans, certain heavy metals can cause undesirable effects such as oxidative stress (which can lead to cell damage or death) and severe problems even at very low concentrations (Ali *et al.,* 2013). A list of some of the most toxic heavy metals are Hg, Cd, Pb, As, Cu, Zn, Sn, and Cr (Ali *et al.,* 2013). A detailed discussion of some of the health and environmental implications of heavy metal pollution is as follows:

2.2.1 Humans

In humans, exposure to heavy metals is generally through ingestion or inhalation (Ahuja and Jain, 2015). Toxic (acute, chronic, or sub-chronic), neurotoxic, carcinogenic, mutagenic, or teratogenic effects due to heavy metals can occur (Duruibe *et al.,* 2007; Okoro *et al.,* 2012; Tchounwou *et al.,* 2012; Gautam *et al.,* 2014). Cd for example is toxic even at extremely low levels. Cd toxicity is generally indicated when urine levels exceed 10 μg/dl and blood levels exceed 50 μg/dl (Okoro *et al.,* 2012). Toxicity results in increased blood pressure, renal dysfunction, bone defects, namely, osteomalacia, osteoporosis and spontaneous fractures and myocardic dysfunctions (Khan *et al.,* 2012; Okoro *et al.,* 2012). Lead can cause obstructive lung disease, pneumonitis, inhibition of the synthesis of hemoglobin; dysfunctions in the kidneys, joints and reproductive systems, cardiovascular system, and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) (Khan *et al.,* 2012).

Over exposure to Zinc may lead to vomiting, dehydration, abdominal pains, nausea, lethargy and dehydration while high levels of copper exposure may result in problems in the synthesis of haemoglobin, and also affect the kidneys (Mohammed *et al.,* 2011). Unlike other heavy metals e.g Se, Cu, Zn and Fe which are essential micro nutrients at minute concentration, Hg is a toxic metal which has no known function in human biochemistry and physiology. Hg toxicity can result in mental disturbance and impairment of speech, hearing, vision and movement (Okoro *et al.,* 2012). Table 2.2 gives a list of some toxic effects of selected heavy metals on human health.

Table 2.2: Toxic effects of heavy metals on human health (Ali *et al.,* 2013; Ahuja and Jain, 2015)

Heavy	Effects
Arsenic	As (as arsenate),an analogue of phosphate interferes with essential cellular processes such as adenosine triphosphate synthesis and oxidative phosphorylation, linked to hypertension, diabetis mellitus, anemia and leukopenia
Zinc	Lethargy, dizziness, metal fume fever, epilepsy and transient global ischaemia
Copper	Alziemer and Wilson’s disease, elevated levels have also been found to cause brain and kidney damage, liver cirrhosis and chronic anemia, stomach and intestinal irritation
Mercury	Exchange of high sister chromosome and other abnormal mitosis, anxiety, autoimmune diseases, depression, difficulty with balance, drowsiness, fatigue, hair loss, insomnia, irritability, memory loss, recurrent infections, restlessness, vision disturbances, tremors, temper outbursts, ulcers and damage to brain, kidney and lungs

2.2.2 Plants

According to Mohammed *et al.* (2011), presence of heavy metal in soils can lead to accumulation in plant tissues as a result of plant uptake. This may eventually lead to toxicity such as biomembrane deterioration, leakage of ions, change in plant community or even death (Ahuja and Jain, 2015; Mohammed *et al.,* 2011). The toxic levels of these metals in soils have also been observed to inhibit nutrient uptake, inhibit homeostatic processes involved in water uptake and transport, transpiration and root and shoot growth damage or impairment (Mohammed *et al.,* 2011; Ahuja and Jain, 2015). Evidence of heavy metal toxicity has been observed in important crops and the potential to impair animal and/or human health has lead to devastating consequences (Mohammed *et al.,* 2011). Additionally, chlorophyll synthesis and photosynthesis inhibition has been observed to reduce biomass of plants growing on metal-contaminated soil (Mohammed *et al.,* 2011). Similarly, carotenoids inhibition, inhibition of various enzyme activities and induction of oxidative stress including alterations of enzymes in the antioxidant defense system have been observed to lead to reduction in the biomass of plants growing in metal-contaminated soils (Mohammed *et al.,* 2011).

2.2.3 Soils

Because the soil is a dynamic system, resulting from the weathering of the parent rocks, it is subjected to a number of pollutants from natural and anthropogenic sources (Antibachi *et al.,* 2012). However, generally, soils are exposed to metal pollution mostly from anthropogenic sources (Mandal and Segupta, 2006). Further, soils are the largest receiving body (receptor or the sink) of pollutants amongst other components of the ecosystem (Antibachi *et al.,* 2012). Negative impact of heavy metal pollution in soils causes changes in soil pH, humus content and structure. This may ultimately lead to the partial and/or complete loss of soil fertility (Mohammad *et al.,* 2011).

2.2.4 Food chain contamination

According to Silva *et al.* (2005), food sources have become an essential route for accumulation of heavy metals. They further added that negative impact of heavy metals in the food chain occurs when certain elements present in an organism of lower trophic level are efficiently transferred to organisms in the higher trophic level thereby becoming more concentrated at the top of the food chain (Silva *et al.,* 2005). Furthermore, Mohammed *et al.* (2011) reported that due to non biodegradable nature of heavy metals, biomagnification of these metals can occur when high levels are retained in soils where they become easily bioavailable to plants, animals or the soil mesofauna such as earthworms. For example, shrews at a metal contaminated site that feed on earthworms become exposed to high concentrations of these metals in the soils. Therefore, consumption of soil through earthworm ingestion results in high body burdens for shrews. Further up the food chain, birds that feed on shrews become exposed to these metals (Mohammed *et al.,* 2011). Additionally, Järup (2003) reported that methyl mercury pollution of freshwaters leads to accumulation in fishes and when humans feed on such fish they become exposed to mercury. Food chain contamination has thus presented serious threat to human health (Silva *et al.,* 2005).

2.2.5 Microbial communities

High concentrations of heavy metals in soil have an adverse effect on microorganisms and microbial processes (Leyval, Turnau & Haselwandter, 1997). Toxicity in microbial communities have been observed to be manifested in different ways (Leyval *et al.,* 1997; Shukla *et al.,* 2010; Mohammed *et al.,* 2011). Reduction in the total microbial biomass because of the alteration of the soil and changes in the microflora has been observed in polluted soils (Shukla *et al.,* 2010; Mohammed *et al.,* 2011). In most cases, the number of microorganisms and soil biological activity decreases (Shukla *et al.,* 2010; Mohammed *et al.,* 2011). In contrast to bacteria, sometimes, the proportion of microscopic fungi and actinomyces have been observed to increase at high concentrations of heavy metal although species diversity become significantly lower, especially in microscopic fungi (Shukla *et al.,* 2010; Mohammed *et* *al.,* 2011). Tolerant or resistant species of microbes are also seen to evolve in some instances leading to changes in the microbial community structure. Further, metal ions have been shown to either completely inhibit the microbial population by inhibiting the organism's various metabolic activities such as cell membrane disruption, protein denaturation and inhibition of cell division (Shukla *et al.,* 2010; Mohammed *et al.,* 2011).

2.2.6 Aquatic ecosystems

According to Valavanidis and Vlachogianni (2010), marine ecosystems such as oceans provide a vital sink for many heavy metals and their compounds. However, the natural cycling rates of many metals are being disturbed by anthropogenic release of Pb, Zn, Cd, Hg and Cu from industrial, domestic and urban effluents (Rao, 2008; Valavanidis and Vlachogianni, 2010). In aquatic ecosystems, heavy metals can be absorbed by suspended solids then strongly accumulated in sediments and aquatic organisms (Tang *et al.,* 2014). Due to slow breakdown of heavy metals in the environment, food chain energetics, and low degradation or excretion by organisms, biomagnification along aquatic food chains maybe experienced (Hsu *et al.,* 2011; Tang *et al.,* 2014). Concentrations of heavy metals (Al, As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se and Zn) in the major groups of marine organisms (marine algae, porifera, coelenterates, annelids, mollusks-soft parts, crustaceans, echinoderms, fish and seabirds) has caused devastating effects (Valavanidis and Vlachogianni, 2010; Hopkins *et al.,* 2013; Tang *et al.,* 2014; Smith *et al.,* 2016). For example Hopkins *et al.* (2013) assessed the effects of Hg exposure on reproduction of snapping turtles (*Chelydra serpentina*) inhabiting a river historically contaminated with Hg. Their investigation revealed that total Hg concentrations in tissues of gravid female snapping turtles ranged from 0.01 ppm to 4.99 ppm (wet weight) in blood, 0.05 ppm to 32.29 ppm (dry weight) in muscle, 0.15 ppm to 161.11 ppm (fresh weight) in nail, and 0.01 ppm to 6.61 ppm (dry weight) in eggs. They observed that Hg had a negative influence on turtle reproduction (Hopkins *et al.,* 2013).

Furthermore, Hsu *et al.* (2011) analysed water samples from a stream receiving wastewater from a semi-conductor treatment plant in Taiwan. Results of their studies revealed that particulate concentrations of some metals showed an increase at the intersection between the stream and the municipal wastewater discharge ditch with peak concentrations of 413 µg/g Zn, 84 µg/g Ni and 15 µg/g Mo at intertidal zone and 15 µg/g As for coastal zone (Hsu *et al.,* 2011). In addition, they also observed that dissolved concentrations for some heavy metals (e.g., Cu, Zn, and Ni) were two to ten times higher than those previously observed in 1992 during the early stages of the semi-conductor plant operations and thus concluded that heavy metal pollution from the plant is deteriorating the stream (Hsu *et al.,* 2011).

In order to avoid the deleterious effect of heavy metals and plan remediation, understanding the behavior of these metals is paramount. Understanding the behavior of these metals will further elucidate (1) the rate of reaction and movement of these metals; (2) the reaction of these metals (and compounds formed) in the environment; (3) the rate of accumulation of these metals in soils and most importantly; (4) the way these metals affect man (Förstner, 1991).

2.3 Behavior of heavy metals in soil

According McLean and Bledsoe (1996) and Aydinalp and Marinova (2003), heavy metals in soil are usually found in different forms namely:

(a) in the structure of primary mineral

(b) in the structure of secondary minerals dissolved in the soil solution

(c) occupying exchange sites on inorganic soil constituents

(d) specifically adsorbed on inorganic soil constituents

(e) associated with insoluble soil organic matter

(f) precipitated as pure or mixed solids.

Because the soil environment plays the critical functions of providing nutrient-bearing environment necessary for plant survival, serves as a medium for degradation and transfer of biomass etc, the ability of soils to adsorb metal ions from aqueous solution is of special interest and importance, hence the need to understand the behavior of heavy metals in soils. Additionally, understanding of the behavior of heavy metals in soils is of paramount importance especially due to the fact that it has impact on agricultural issues such as irrigation, soil fertility etc and also raises environmental questions such as waste deposition and remediation of polluted soil (Bradl, 2004).

Heavy metal(loid) ions can undergo various reactions and has different dynamics in the soil (Hashim *et al.,* 2011; Park *et al.,* 2011; Bolan *et al.,* 2014; Seshadri *et al.,* 2015). Reactions involving metal(loid) ions is dependent on some environmental factors and soil properties such as complexation with other dissolved constituents, organic matter, sorption ability, soil pH, cation exchange capacity of geological materials and even root exudates (Aydinalp and Marinova 2003;Wuana and Okieimen, 2011; Seshadri *et al.,* 2015). In addition, the presence of more than one heavy metal in soils could result in competition among various metal ions present in the soil thereby impacting the behavior of such metals (Srivastava *et al.,* 2005; Silveira *et al.,* 2003).

Leaching into groundwater sources, volatilization and plant uptake have been observed to be some of the ways heavy metals are removed from the soil environment

(McLean and Bledsoe, 1996; Nagajyoti *et al.,* 2010; Bolan *et al.,* 2011; Wuana and Okieimen, 2011; Tangahu *et al.,* 2011; Seshadri *et al.,* 2015). The reaction and dynamics of heavy metal in soils is discussed in the subsequent paragraphs.

a. Adsorption and complexation

Adsorption is a major process through which heavy metals accumulate in some environment (Bradl, 2004). According to Sposito (2008), adsorption is the accumulation of a solute at the interface between the solid phase and aqueous solution phase. Therefore, adsorption (physical attachment or bonding) of metal ions and molecules onto the surface of another component results in the formation of surface complexes (Sposito, 2008). Bolan *et al.* (2011) reported that adsorption of charged solutes by a charged adsorbent can be classified into specific and non-specific retention. In non-specific adsorption process, the charge on the solutes balances the charge on the sorbent through electrostatic attraction and displacement of other like-charged ions from the surface (Seshadri *et al.,* 2015). Further, Park *et al.* (2011) reported that chemical bond formation in any specific adsorption process is between the solute and the functional groups on the adsorbent. Soil pH, redox potential, cation/anion exchange capacity and soil components such as silicate clays, organic matter, iron, aluminium and manganese oxides strongly control heavy metal(loid)s sorption process (Dube *et al.,* 2001; Park *et al.,* 2011; Seshadri *et al.,* 2015). Additionally, Seshadri *et al.* (2015) highlighted that organic ligand ions such as citrate, oxalate, fulvic, dissolved organic carbon and inorganic ligand ions such as HPO_4^{2-}, NO_3^-, Cl^- and SO_4^{2-} present in the soil can form complexes with metalloids thereby decreasing or increasing sorption process. Inorganic anions can either form ion pair complexes with metal(loid)s thereby reducing their sorption or increase the negative charge on soil particles, thereby increasing the sorption of cationic metal(loid)s (Seshadri *et al.,* 2015).

Similarly, Schuster (1991) reported that the behavior of Hg in the soil is mainly controlled by adsorption and desorption processes depending on complexation with important ligands in solution such as OH^-, Cl^- and organic anions. Schuster (1991) further highlighted that the physical fractioning of soil organic matter- either adsorbed or dissolved, greatly determines the behavior and distribution of Hg in soils.

In addition to adsorption, complexation of heavy metals with dissolved organic matter in the environment have been shown to affect the solubility and mobility of these metals (Weng *et al.,* 2002). Complexation reaction between metal(loid)s and organic and inorganic ligand ions leads to the retention of metals in soils leading to the formation of inorganic and organic complexes (Bolan *et al.,* 2011). The general order of affinity of heavy metal(loid)s on organic matter is as follows: $Cu^{2+} > Hg^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+} >$ As(V) > As(III) (Park *et al.,* 2011).

Furthermore, surface complexation indicates semi-covalent bond formation between dissolved ions and surface functional groups,with different hydroxyl groups having different levels of reactivity concerning the dissociation of H^+(Park *et al.,*2011). Metal(loid) ions that directly bind to surface functional groups of soil particles form complexes also known as inner-sphere complexes (Park *et al.,* 2011). However, when water molecules interpose between the surface functional group and metal(loid) ions, the complexes formed thereby are called outer-sphere complexes (Park *et al.,* 2011). Generally, inner-sphere complexes are more stable than outer-sphere complexes due to covalent bonding in inner-sphere complexes (Park *et al.,* 2011). Heavy metal(loid)-organic complexes are affected by temperature, solution, soil pH, ionic strength, dominant cations and soil types,which in turn affect metal-soil chemistry (Park *et al.,* 2011). Also, soil types such as fine grained soils have been observed to have more heavy metal(loid) retention potential than coarse grained soils because the fine grained soils contain larger amounts of active surface sites such as clay minerals, iron and manganese oxyhydroxides, and humic acids than coarse grained ones (Park *et al.,* 2011).

b. Precipitation

Precipitation is an important attribute of metal(loid)s explored during liming. Precipitation helps immobilization of heavy metal(loid)s in the presence of anions such as sulfate,carbonate,hydroxide and phosphate when the metal(loid) concentration and soil pH are high (Park *et al.,* 2011; Seshadri *et al.,* 2015). Coprecipitation and precipitation as salts also contributes to the immobilization of heavy metal(loid)s. Coprecipitation of metal(loid)s takes place especially in the presence of Fe and aluminum oxyhydroxides (Park *et al.,* 2011). For example, co-precipitation of Cd, Cu, Ni, Pb and Zn onto precipitated Fe (hydr)oxides (Park *et al.,* 2011). Furthermore, Pb forms precipitates such as carbonate ($PbCO_3$), chloride ($PbCl_2$) and hydroxide chloride (Pb(OH)Cl) when Pb reacts with Mg/Al layered double hydroxides in aqueous solution (Park *et al.,* 2011). Additionally, Seshadri *et al.* (2015) reported that in some instances, co-precipitation of Pb^{2+} with ferric oxyhydroxides was observed to be more efficient at pH4 than adsorption in removing Pb^{2+} from aqueous solutions at similar sorbate/sorbent ratios and pH. Similarly, sorption of Ni^{2+} and Cr^{3+} onto hydrous iron oxides and arsenate (As(V)) onto ferrihydrite;showed that co-precipitation was more efficient than sorption for metal(loid)s removal from aqueous solutions (Seshadri *et al.,* 2015).

c. Oxidation/reduction

Redox transformation involving oxidation and reduction processes are carried out by microorganisms. These processes have been observed to be critical in determining the mobility and speciation of metal(loid)s in soils (Seshadri *et al.,* 2015). While some metal(loid)s such as As, Cr, Hg and Se readily undergo microbial oxidation and reduction reactions (Seshadri *et al.,* 2015), divalent metals such as Zn, Cd, Pb and Ni do not (Park *et al.,* 2011). Assimilatory and dissimilatory oxidation reactions are observed by metal(loid)s. In assimiliatory oxidation reaction, the metal(loid)s substrate functions as a terminal electron acceptor, thereby enhancing the growth of microorganisms such as bacteria (Park *et al.,* 2011). In contrast to assimiliatory oxidation reaction, in dissimilatory reactions, the metal(loid) substrate has no specific function in the physiology of the microorganisms, and occurs by fortuitous reductions coupled to microbial oxidations of simple organic acids and alcohols, H_2 or aromatic compounds (Park *et al.,* 2011). For instance, some anaerobic bacteria use Se(VI) as a terminal electron acceptor for their growth. This reduces Se(VI) to Se(0) thus serving as an important process of precipitating Se from contaminated water (Park *et al.,* 2011). Similarly, in remediation of contaminated environments, bacteria such as *Bacillus* sp has been shown to enzymatically reduce Cr(VI) to the less mobile and less toxic (less bioavailable) Cr(III) which is strongly retained onto soil particles (Park *et al.,* 2011). Archaebacterium- *Sulfolobus acidocaldarius* has the ability to oxidize As(III) to As(V) (Park *et al.,* 2011). Since As(V) is more strongly retained by inorganic soil components and less toxic than As(III), microbial oxidation by bacteria for instance results in the immobilization and bioremediation of As in the environment (Park *et al.,* 2011). According to Gadd (2004), dissimilatory metal reducing bacteria have also been observed to use a variety of metal(loid)s with an appropriate redox couple, including Fe(III),U(VI) and Mn(IV). Shesadri *et al.* (2015) reported that Hg(II) was reduced to Hg(0) by mercuric reductase, and the dissimilatory metal(loid) reducing bacterium-*Shewanella oneidensis* reduced Hg(II) to Hg(0) in the presence of electron donors. Further, according to Shesadri *et al.*

(2015), organic amendment such as biosolids, poultry manure, soyabean meal and rice bran etc have also been observed to affect the oxidation-reduction reactions of metal(loid)s when added into soils.

d. Methylation/demethylation

Toxic metal(loid)s are removed from soil by converting them to methyl derivatives that are subsequently removed by volatilization in a process called methylation (Shesadri *et al.,* 2015). Methylation of heavy metal(loid)s occurs in two major ways; namely: chemical and biological processes (Park *et al.,* 2011). In chemical methylation, volatilization of As, Hg and Se in soils and sediments results in the release of methane (Park *et al.,* 2011). According to Shesadri *et al.* (2015) and Park *et al.* (2011), methylation of heavy metal(loid)s occurs sometimes with the help of living systems. In biological methylation however, there is a transfer of an intact methyl group from methyl donor to another compound (transmethylation) or a transfer of a molecule such as formaldehyde or formic acid from a methyl source to another compound (fission) (Park *et al.,* 2011). Gadd (2004) reported that biological methylation has been observed to be the most dominant process in most soil and aquatic environments by a range of bacteria and fungi under aerobic and anaerobic conditions. Seshadri *et al.* (2015) added that major microbial methylating agents involved are methylcobalamin (CH_3CoB_{12}) and S-adenosylmethionine (SAM). While CH_3CoB_{12} is involved in methylation of Hg, SAM is involved in the methylation of As and Se. Inorganic As for example is methylated in some organisms by alternating reduction of As(V) to As(III) and addition of a methyl group from SAM, to form methylarsonic acid and dimethylarsinic acid which are less reactive with tissue constituents and are more readily excreted in urine (Park *et al.,* 2011). This metabolic methylation of inorganic As is important in detoxifying As poisoned organisms in aquatic environments (Park *et al.,* 2011). Similarly, methylation and demethylation processes involving sulfate reducing bacteria under anoxic conditions have been observed in methylate Hg in water and soils (Park *et al.,* 2011). This processes thus helps regulate Hg cycle in the environment (Park *et al.,* 2011). Further, according to Park *et al.* (2011), bacterial cultures have also been observed to release trimethyl Pb, monomethyl Cd and dimethyl Hg. Abiotic methylation of Hg can therefore also occur by introduction of non microbial methylating agents such as humic matter and methyltin compounds into Hg contaminated environments (Park *et al.,* 2011).

Additionally, other than microbial agents, cellular detoxification or metabolic processes demethylates methyl Hg into either CH_4, Hg(II) or Hg(0) in microorganisms such as *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., and *Clostridium* sp. (Mohammed *et al.,* 2011; Park *et al.,* 2011). Similar to demethylation of Hg, methylation of Se occurs through biomethylation in which plants or microorganisms convert inorganic and/or organic Se into dimethyl selenide or dimethyl diselenide (Park *et al.,* 2011). This is considered an important detoxification processes of Se (Park *et al.,* 2011). In situ bioremediation of selenium-containing land and water at Kesterson Reservoir, California, was found to achieve through microbial methylation of Se, resulting in Se volatilization and its removal (Gadd, 2004).

f. Biological modification of local soil environments

Aside microbial methylation discussed above, certain microbial processes can lead to modification of soil environments. This could either enhance metal(loid)s solubility, thereby increasing their bioavailability and potential toxicity or in contrast result in the immobilization of metal(loid)s, thereby decreasing their bioavailability and toxicity (Park *et al.,* 2011). According to Park *et al.* (2011), solubilization of metal(loid)s by chemolithotrophic (autotrophic) and chemoorganotrophic (heterotrophic) mobilization mostly by the release of inorganic and organic acids, siderophores and other complexing agents accelerates biodegradation, methylation, demethylation etc. On the other hand, microbially induced metal(loid) immobilization can occur by biosorption, precipitation, reduction, accumulation, intracellular deposition, localization and sequestration (*Park et al.,* 2011).

Microbial products derived, induced or excreted by the presence of certain metal(loid)s can result in metal removal through adsorption (Park *et al.,* 2011). Heavy metal(loid)s binding and detoxification occurs when metallothioneins bind metal(loid)s, phytochelatins, cysteine-containing–glutamyl peptides, metal-thiolate clusters and microbial exopolymers (Park *et al.,* 2011). An example of microbial detoxification of heavy metals was observed in *Azotobacter* sp. and *Micrococcus luteus* where about 490 mg g^{-1} and 310 mg g^{-1} of Pb was immobilized (Park *et al.,* 2011). Similarly, efficient removal of Zn was observed when sulfate-reducing bacteria in a medium containing Zn was precipitated with sulphide in the medium (Park *et al.,* 2011). Additionally, bacterial strain, related to the species- *Stenotrophomonas maltophilia* and resistant to Se(III), from the rhizosphere of the Se hyperaccumulator legume- *Astragalus bisulcatus* have been able to reduce soluble and harmful Se(III) to insoluble and non-bioavailable elemental Se (Park *et al.,* 2011).

Phytoremediation technology is another strategy employed to remove metal contamination in an environment (Chirakkara, Cameselle and Reddy, 2016; Mahar *et al.,* 2016). Phytoremediation (especially in soils) relies on the ability of plant (roots or above the roots) to accumulate heavy metal(loid)s from contaminated soils (Ali *et al.,* 2013; Pinto *et al.,* 2015). In soils, changes in bioavailability and immobilization of heavy metal(loid)s is achieved as a result of root-induced changes in soil properties (Park *et al.,* 2011).

Additionally, more than 400 species of plants with excellent heavy metal(loid)s accumulation (hyper accumulators) and tolerance have been found, with most of them belonging to the Cruciferae, including the genus *Brassica*, *Alyssums* and *Thlaspi* (Su *et al.,* 2014). Examples of some hyperaccumulators include: *Alyssum markgrafii* and *Alyssum murale* which are able to take up 19100 mg kg^{-1} and 4730 to 20100 mg kg^{-1} of Ni respectively. *Thlaspi caerulescens* is able to take up 263 mg kg^{-1} Cd, *Azolla pinnata* is able to take up 740 mg kg^{-1} Cd, *Corrigiola telephiifolia* is able to take up 2110 mg kg^{-1} As and *Eleocharis acicularis* is able to take up 20200 mg kg^{-1} Cu (Ali *et al.,* 2013).

2.4 Studies on speciation of heavy metals in soil and sediments

The soil medium is very complex and heterogeneous. It comprises of solid phases and fluid phases. The solid phases or soil matrix contains the minerals and organic matter while the fluid phases consists of soil water and the soil air. Both phases interact with each other and/with ions entering the soil system (Bradl, 2004). Consequently, heavy metals in soils exist either as separate entities or in combination with other soil components (Chibuike and Obiora, 2014).These components may include exchangeable ions sorbed onto the surfaces of inorganic solids, nonexchangeable ions and insoluble inorganic metal compounds such as carbonates and phosphates, soluble metal compound or free metal ions in the soil solution, metal complex of organic materials, and metals attached to silicate minerals (Chibuike and Obiora, 2014). Therefore, heavy metals in soil have been categorized into five (5) namely; adsorptive and exchangeable bound, bound to carbonate phases, bound to reducible phases (Fe and Mn oxides), bound to organic matter and sulphides, and detrital or lattice or residual bound metals (Ranjendran *et al.,* 2003; Dube *et al.,* 2011; Soliman, 2012).

An important way of identifying the various categories of heavy metals in soil is speciation. Speciation analysis is an analytical procedure used to identify the chemical and physical forms of an element existing in the various component of a sample (Kot and Namiesńik, 2000). According to Ashraf *et al.* (2011), speciation is defined as the identification and quantification of the different, defined species, forms, or phases in which an element occurs and is essentially a function of the mineralogy and chemistry of the sample examined. Quantification is typically done using chemical solutions of varying but specific strengths and reactivity to release metals from the different fractions of the examined sample in a sequential order (Rodriguez *et al.,* 2009). The principle of sequential chemical extraction methods is that various chemical extractants are applied successively to a sample, dissolving the components of the sample matrix in sequential order (Rodriguez *et al.,* 2009). Preferably, a reagent should liberate all the metals from a particular matrix's component (i.e. exchangeable, carbonate, etc.), without affecting other components and loosely held metals are extracted first, followed by those more tightly bonded (Aydinalp and Marinova, 2003; Singh and Kalamdhad, 2013). Also, speciation helps in the determination of the mobility, environmental diffusion, bioavailability and toxicity of a metal (Prudent *et al.,* 1996). The chemistry and speciation of some heavy metals is as shown in Table 2.3.

Table 2.3: Speciation and chemistry of some heavy metals (Hashim *et al.,* 2011)

Heavy metals	Speciation and chemistry
Lead	Pb occurs in 0 and +2 oxidation states. Pb (II) is the more common and reactive form of Pb. Low solubility compounds are formed by complexation with inorganic (such as Cl^-) and organic ligands (humic and fulvic acids, EDTA, amino acids). The primary processes influencing the fate of Pb in soil include adsorption, ion exchange, precipitation and complexation with sorbed organic matter
Chromium	Cr occurs in 0, +6 and +3 oxidation states. Cr (VI) is the dominant and toxic form of Cr at shallow aquifers. Major Cr(VI) species include chromate CrO_4^{2-} and dichromate $Cr_2O_7^{2-}$ (especially $Ba^{2+,}$ Pb^{2+} and Ag^+). Cr (III) is the dominant form of Cr at acidic pH (<4). Cr (VI) can be reduced to Cr (III) by soil organic matter, S^{2-} and Fe^{2+} ions under anaerobic conditions. The leachability of Cr(VI) increases as soil pH increases
Arsenic	As occurs in -3, 0, +3, +5 oxidation states. In aerobic environments, As (V) is dominant, usually in the form of arsenate $(AsO_4)^{3-}$. It behaves as chelate and can coprecipitate with or adsorb into Fe oxyhydroxides under acidic conditions. Under reducing conditions, As(III) dominates, existing as arsenite $(AsO_3)^{3-}$ which is water soluble and can be adsorbed/coprecipitated with metal sulphides
Copper	Cu occurs in 0, +1 and +2 oxidation states. The cupric ion (Cu^{2+}) is the most toxic species of Cu e.g. Cu $(OH)^+$ and $Cu_2(OH)_2^{2+}$. In aerobic alkaline systems, $CuCO_3$ is the dominant soluble species. In anaerobic environments CuS(s) will form in presence of sulphur. Cu forms strong solution complexes with humic acids
Cadmium	Cd occurs in 0 and +2 oxidation states. Hydroxide (Cd $(OH)_2$) and carbonate ($CdCO_3$) dominate at high pH whereas Cd^{2+} and aqueous sulphate species dominate at lower pH (<8). It precipitates in the presence of phosphate, arsenate, chromate, sulphide, etc. Shows mobility at pH range 4.5-5.5
Zinc	Zn occurs in 0 and +2 oxidation states. It forms complexes with anions, amino acids and organic acids. At high pH, Zn is bioavailable. Zn hydrolyzes at pH 7.0-7.5, forming Zn $(OH)_2$. It readily precipitates under reducing conditions and may coprecipitate with hydrous oxides of Fe or manganese

Table 2.3: Speciation and chemistry of some heavy metals (continued) (Hashim *et al.,* 2011)

Heavy	Speciation and chemistry
Mercury	Hg occurs in 0, +1 and +2 oxidation states. It may occur in alkylated form (methyl/ethyl mercury) depending upon the Eh and pH of the system. Hg^{2+} and Hg_2^{2+} are more stable under oxidizing conditions. Sorption to soils, sediments and humic materials is pH-dependent and increases with pH
Iron	Fe occurs in 0, +2, +3 and +6 oxidation states. Organometallic compounds contain oxidation states of +1, 0, -1 and -2. Fe (IV) is a common intermediate in many biochemical oxidation reactions. Many mixed valence compounds contain both Fe(II) and Fe(III) centers, e.g. magnetite and prussian blue

Several speciation studies have been conducted to determine different forms of heavy metals in soils and sediments (Rao *et al.,* 2008; Soliman, 2012; Islam *et al.,* 2015; Taberna *et al.,* 2015; Marković *et al.,* 2016; Chavez *et al.,* 2016; Rosado *et al.,* 2016; Xu *et al.,* 2016). Sequential extraction technique uses various extraction reagents and experimental conditions to investigate the distribution of heavy metals in sediments and soils (Abollino *et al.,* 2002). The most common techniques involve five steps (Tessier *et al.,* 1979; Yuan *et al.,* 2004). According to the Tessier *et al.* (1979), heavy metals are associated with five fractions:

(a) Exchangeable (F1) or Salt-displaceable or Dissolved fraction

Heavy metals in this fraction are held by electrostatic adsorption and thus easily released through sorption and desorption processes (Kumar *et al.,* 2011). Neutral salts e.g magnesium chloride and potassium nitrate at neutral soil pH serve as ion displacing extractants to aid the release of metal ions attached by electrostotatic attraction to negatively charged sites of soil particles (Yong *et al.,* 2012). Furthermore, because metals in this fraction are non-specifically adsorbed and ion exchangeable, they can be replaced by competing cations (Yong *et al.,* 2012). Metals in the exchangeable metal in soils and sediments are labile, highly toxic and the most bioavailable fraction (Wang *et al.,* 2010).

(b) Carbonate or acid extractable (F2) fraction

Metals bound to carbonate minerals are also bioavailable for gut environment of benthic organisms (Wang *et al.,* 2010). Acidified acetate is used as an extractant to release metals precipitated or co-precipitated as natural carbonates (Yong *et al.,* 2012). 1M solution of HOAc-NaOAc adjusted to pH 5 usually dissolves carbonate minerals such as dolomite and calcite releasing the metals bound to them without dissolving organic matter, oxides and clay minerals (Kumar *et al.,* 2011; Yong *et al.,* 2012). Further, Tokalioglu *et al.* (2000) stated that carbonates of sediments containing significant concentration of heavy metals and concentrations have been observed to be pH sensitive.

(c) Reducible fraction (F3)

Fe-and Mn-oxides are present as concretions or nodules, as a coating on soil particles or in cracks or veins (Kabata-Pendias, 1984) and have relatively large area and surface site density which play important role in the mobility and behavior of trace metals (Wang *et al.,* 2010; Kumar *et al.,* 2011). Poorly crystallized metal oxides such as Al, Mn and Fe oxides bind to metals due to their strong scavenging efficiency for metals (Wang *et al.,* 2010; Kumar *et al.,* 2011). Despite being thermodynamically unstable under the anoxic circumstances, they are released under acidic pH (Tokalioglu *et al.,* 2000).

(d) Oxidizable fraction or fraction bound to organic matter and sulphides (F4)

Metals may bind to organic materials such as detritus, living organisms or coatings on mineral particles (Tokalioglu *et al.,* 2000). Therefore, organic matter and sulphides are important factors controlling the mobility and bioavailability of heavy metals (Wang *et al.,* 2010). For instance, sulphides are major solid phases controlling the concentration of dissolved heavy metals (Wang *et al.,* 2010). Aside soil organic matter effect on controlling the mobility of heavy metals, soil organic matter greatly affects sorption of heavy metal because they contain functional groups that are capable of complexing metals (Thomas, 2015). Thomas (2015) further reported that although metal bounded to the organic matter are temporarily inaccessible, they can be solubilized by chemical oxidation.

(e) Residual fraction (F5)

Metals in this fraction are mainly from primary and secondary minerals which occlude or "close up" metals within their crystal structures (Tokalioglu *et al.,* 2000). In natural conditions, metals in this fraction are practically inaccessible for living organisms and are said to be immobile (Soliman, 2012). Due to the fact that the concentration of metals in the residual fraction is largely controlled by the mineralogy and extent of their weathering (in the form which are not soluble under experimental conditions), they are considered to be held within the mineral matrix (Soliman, 2012). Therefore, metals in the residual fraction are less toxic because they are not readily bioavailable (Wang *et al.,* 2010; Soliman, 2012). In addition, a high concentration of metals in this fraction is an indication of lower pollution intensity from anthropogenic sources.

In general, the F1 and F2 fractions of metals are most readily available to the living environment. The F3 and F4 fractions of metals are available under extreme condition, while F5 fraction are not easily available for uptake by plants under natural conditions (Singh and Kalamdhad, 2013).

In order to understand the specific forms of heavy metals in soil causing deleterious impact to humans, animal, plants and the environment as a whole, speciation analysis is carried out on samples obtained from contaminated environments. A review of studies on speciation of heavy metals in soil and sediments is discussed below.

There exist numerous other variations of speciation techniques (although these method sometimes involved modifications of the Tessier *et al.* (1979) method). This includes a four steps (BCR, Bureau Commune de Reference of the European Commission), Short Extraction Procedure by Maiz, Galán Procedure, Geological Society of Canada (GCS) Procedure, six steps extraction etc. In these modified methods, the timing, reagents used are changed yet following the same steps of Tessier *et al.* (1979) method (Rao *et al*., 2008; Mizutani *et al*., 2016; Żyrnicki *et al*., 2016). Scholarship on the speciation of heavy metals is divided into two streams, in soils and in sediments. Discussion of the two streams are as follows.

2.4.1 Speciation of heavy metals in soils

The review under this stream represents scholarship on speciation of heavy metals in soils from agricultural fields, dumpsites, industrial zones etc. Navas and Lindhorfer (2003) in their studies on speciation of heavy metals from semi-arid soils of Ebro Valley in Spain found that very little amounts of Fe and Mn were present in the exchangeable phase and Zn was only present in negligible amounts in very few samples. Therefore, low quantities of Fe and Mn can be taken up by plants in these alkaline soils (Navas and

Lindhorfer, 2003). Cr was not detected in the bioavailable forms in all soils. This eliminates the toxic risk both in the trophic chain and from its migration downwards the soil profile (Navas and Lindhorfer, 2003). However, the largest contents of Fe, Zn and Cr were retrieved from the residual phase where metals are strongly bound to minerals and Mn was bound to the carbonate and oxide phases of the soils (Navas and Lindhorfer, 2003).

In a different environment, Asagba *et al.* (2007) in their study on the screening of heavy metal contaminated soils from an automobile spare-parts market in Nigeria revealed the presence of elevated concentrations of Pb, Cd and Cu. On speciating, the highest concentrations of Pb and Cu were found in the residual fraction while the highest value of Cd was found in the carbonate fraction (Asagba *et al.,* 2007). Similarly, Charles *et al.* (2013) found that the carbonate fraction of Pb represented more than 25% of the total Pb found in the soils. A major portion of Cu was associated with organic fractions whereas Cd was associated with exchangeable fraction (Charles *et al.,* 2013). Pb was more mobile and bioavailable as compared to the other metals studied (Charles *et al.,* 2013).

In a separate study, Abdus Salam (2009) in his speciation study of heavy metals in a dumpsite in Nigeria, found out that about 70% of Mn, Fe, Zn, Cd and Pb were found in the exchangeable bound to carbonate and bound to iron/manganese oxide fractions. These fractions represented the mobile portion of the total metals that were lethal to the ecosystem (Abdus Salam, 2009). The metal enrichment factor revealed that Zn, Cd and Pb were of anthropogenic source while Fe was of natural and anthropogenic sources (Abdus Salam, 2009). The dumpsite therefore posed negative consequences to the soil and groundwater environment (Abdus Salam, 2009).

In the Ranipet industrial area of India, more than 45% of the metals (Cd, Co, Pb and Zn) were in the residual form (Saraswathy *et al.,* 2010). The next high percentage of the metals examined were in the oxidisable form and only about 5% of the metals were found in the exchangeable form (Saraswathy *et al.,* 2010).

Using a modified sequential extraction technique, Wang *et al.* (2010) in their studies of speciation of heavy metals from soils along the Xihe River in Shenyang, China revealed that the residual fraction was dominant for As, Cu, Cr, Zn while Pb and Cd mainly existed in the exchangeable fraction and in low concentration in the residual fraction. Hg was found to exist in the residual fraction and the potential migration ability of the heavy metals was in the order of Cd>Hg>Pb>Zn>Cr>Cu>As (Wang *et al.,* 2010).

Doddaiah *et al.* (2012) in their studies at different points of the industrial zone of Mysore city, India revealed that all metals (Fe, Zn, Cu, Cr, Ni) were mainly associated with the oxidizable and residual fraction whereas only a small fraction of Fe, Cu, Cr, Zn and Ni was extracted in soluble, exchangeable and carbonate fractions. They concluded that these metals may pose a threat of bioaccumulation in plants (Doddaiah *et al.,* 2012).

In Pakistan, speciation of heavy metals from agricultural soils showed that Ni and Cr were more prevalent in the Fe–Mn oxide fraction, Zn and Cd as the carbonate bound fraction and Cu in the organic bound form (Rafique *et al.,* 2011). However, concentrations of Pb were similar across the carbonate, Fe–Mn oxide and organic bound fractions (Rafique *et al.,* 2011). The highest average concentration of Zn was found as carbonate bound fraction and Cu as the organic bound (Rafique *et al.,* 2011).

Ashraf *et al.* (2012) in their chemical speciation of Pb, Zn, Cu, Cr, As, and Sn in soils of former tin mining catchment in Malaysia, used the Tessier sequential extraction procedure. The results showed small amounts of Cu, Cr, and As in the exchangeable phase, indicating that they are readily available for biogeochemical cycles in the ecosystem (Ashraf *et al.,* 2012). While low quantities of Cu and As could be taken up by plants, Zn was not detected in the bioavailable forms and Pb was only present in negligible amounts in very few samples (Ashraf *et al.,* 2012). The absence of mobile forms of Pb eliminates the risk of accumulation in the trophic chain and its migration downwards the soil profile (Ashraf *et al.,* 2012). Their results also indicated that most of the metals had high abundance in residual fraction indicating lithogenic origin and low bioavailability (Ashraf *et al.,* 2012). The average potential mobility for the metals were in the following order: Sn > Cu > Zn > Pb > Cr > As (Ashraf *et al.,* 2012).

Osakwe *et al.* (2012) used a six- step sequential extraction procedure to analyse soils from five selected municipal waste dumpsites in Asaba metropolis, Delta State, Nigeria The chemical forms of Cu, Pb, Mn and Zn in soils were investigated in order to obtain information on the bioavailability and mobility of the metals in the soil matrix (Osakwe *et al.,* 2012). Their results revealed that Cu and Zn were associated with organic and Fe –Mn oxide fractions respectively with respective averages of 47.91% and 63.21% while Pb and Mn were speciated into residual fractions with averages of 60.32% and 82.24% respectively (Osakwe *et al.,* 2012). The residual fraction also contained relatively high percentages of Cu 33.18% and Zn 20.81% (Osakwe *et al.,* 2012). The mobility factors of all the metals in all the soil profiles were generally low ranging from 1.97% to 39.99% indicating generally low mobility and therefore bioavailability (Osakwe *et al.,* 2012). They concluded that Mn and Pb do not pose any environmental risk or hazard because the metals were predominately found in the residual fraction (not bioavailable since they were occluded within the crystal lattice layer of silicates and well crystallised oxide minerals) (Osakwe *et al.,* 2012). Zn and Cu however, poses threats of being mobile and thus bioavailable when the soil conditions such as pH and redox potentials change (Osakwe *et al.,* 2012). This is because Zn and Cu were mainly associated with organic and Fe –Mn oxide fractions (Osakwe *et al.,* 2012).

Esshaimi *et al.* (2013) observed that in an abandoned mine in Morocco, after the application of the sequential extraction recommended by the BCR, Pb was mainly associated with the reducible fraction. Zn was mainly associated with the exchangeable, water- and acid-soluble in soil and oxidizable fraction in tailings, thus being more mobile and potentially more dangerous for the environment (Esshaimi *et al.,* 2013).

Wali *et al.* (2015) using the BCR technique in their speciation of heavy metal in soils contaminated by phosphogypsum in Tunisia revealed higher concentrations of trace metals in topsoil samples (0 to 20 cm) than in subsoil samples (20 to 40 cm and 40 to 60 cm) for most metals. While Zn in the topsoil was mostly associated with the non-residual fraction at all sites, about 60% of Pb was bound to the non-residual, exchangeable and reducible fractions at all sites and soil depths (Wali *et al.,* 2015). Cr, Cu, Ni and Fe were mainly in the residual fraction, whereas Mn was largely present in the bioavailable fraction. Therefore, mobility and bioavailability were greatest for Zn and Pb and thus implied that these metals posed serious threat to nearby vegetation (Wali *et al.,* 2015).

Chemical speciation of heavy metals in greenhouse soils from Çanakkale, Turkey by Sungur *et al.* (2016) revealed that Ni, Pb and Cd were found mainly in the mobile fractions. Immobile fractions of Cu, Cr and Zn were more predominant in greenhouse soils. Hence, the order of mobility was: Cd (87.6%) > Pb (76.2%) > Ni (51.2%) > Cr (49.8%) > Zn (49.0%) > Cu (26.9%) (Sungur *et al.,* 2016). Intensive agricultural inputs such Nitrogen and Phosphorus fertilizers in greenhouse production was identified as a source of these metals in greenhouse soils (Sungur *et al.,* 2016). The second stream of speciation analysis of heavy metals reviewed focuses on sediments. Discussion on it is as follows.

2.4.2 Speciation of heavy metals in sediments

The review in this sub-section focused on speciation of heavy metals in sediments from rivers, estuaries etc. Jones and Turki (1997) worked on distribution and speciation of heavy metals in surface sediments from the Tees estuary, North East England. They observed that the sediments were largely organic-rich clayey silts in which metal concentrations exceeded background levels (Jones and Turki, 1997). Cd was found predominately in the exchangeable fractions while Cu was found to be mostly associated with the oxidizable and residual fractions (Jones and Turki, 1997). Cr, Pb and Zn were associated with the reducible, residual, and oxidizable fractions. Negligible amount of Co and Ni was detected (Jones and Turki, 1997).

Similarly, Yuan *et al.* (2004) applied BCR-sequential extraction protocol to obtain metal distribution patterns in marine sediments from the East China Sea. The results showed that both the total contents and non-residual fractions of Cd and Pb were extremely high (Yuan *et al.,* 2004). More than 90% of the total concentration of V, Cr, Mo and Sn existed in the residual fraction and thus these metals do not pose serious environmental hazard to marine ecosystem (Yuan *et al.,* 2004). Also, more than 60% of Fe, Co, Ni, Cu, and Zn were mainly present in the residual fraction. Mn, Pb, and Cd were dominantly present in the non- residual fractions in the top sediments (Yuan *et al.,* 2004).

Morillo *et al.* (2005) in Spain used the BCR-sequential extraction technique to determine the distribution of metals (Cu, Zn, Cd, Pb, Fe, Ni, Cr, and Mn) in samples from the southwest coast of Spain. Based on the chemical distribution of metals, the percentage of Zn was highest in the acid-soluble fraction (the most labile), especially in the central coastal area (Morillo *et al.,* 2005). Further, due to close proximity of the central coastal to the Tinto and Odiel river mouths, a significant increase in the mobility of Cd and Cu was also observed (Morillo *et al.,* 2005). The residual fraction of Cd and Cu had negligible amount. However, the acid-soluble fraction increased for Cd and the oxidisable fraction for Cu (Morillo *et al.,* 2005). In addition, Fe, Cr and Ni were found to have the highest percentages in the residual fraction (84%, 89% and 75%, respectively), which implied that these metals are strongly bound to the sediments and not easily bioavailable (Morillo *et al.,* 2005).

Wang *et al.* (2010) used a modified Tessier sequential extraction method to investigate the distribution and speciation of Cd, Cu, Pb, Fe, and Mn in the shallow sediments of Jinzhou Bay, Northeast China. Results of sequential extraction revealed that 39% to 61% of Cd was found in exchangeable fractions. This shows that Cd in the sediments posed a high risk to the local environment (Wang *et al.,* 2010). Cu and Pb were found to be at moderate risk levels (Wang *et al.,* 2010). According to the relationships between percentage of metal speciation and total metal concentration, it was concluded that the distributions of Cd, Cu and Pb in some geochemical fractions were dynamic. Pollutants migration and stability of metals in marine sediments from Jinzhor Bay decreased in the order: Pb > Cu >Cd (Wang *et al.,* 2010).

Another notable sequential extraction method for heavy metal speciation in soils and sediments is the European Standard, Measurements and Testing (SM&T) program, formerly known as the Community Bureau of Reference (BCR, Bureau Commune de Reference of the European Commission). This procedure is largely similar to that produced by Tessier *et al.* (1979). The difference is in the first fraction of the procedure where instead of evaluating the exchangeable and carbonate bound separately, the BCR procedure combines both in the first fraction (Zimmermen and Weindorff, 2010).

Nemati *et al.* (2011) reported that sequential extraction procedure proposed by the European Standard, Measurements and Testing (SM&T) program was applied for speciation of heavy metals in river sediments collected along the course of Sungai Buloh and the Straits of Malacca in Selangor, Malaysia. Eight elements (V, Pb, Cd, Cr, Co, Ni, Cu and Zn) from seven stations (Station 1 to Station 7) and at different depth of the river were analyzed using the modified BCR Sequential Extraction Procedure (SEP) in combination with ICP-MS to obtain the metal distribution patterns in both region (Nemati *et al.,* 2011). Their findings indicated that heavy metal contamination at Station 2 and Station 3 was more severe than at other sampling sites, especially for Zn, Cu, Ni and Pb (Nemati *et al.,* 2011). In addition, a decrease in metal concentration from top layer to the bottom layer was observed (Nemati *et al.,* 2011). Co, Zn and Pb were highest in samples from the Straits of Malacca (Station 4 to Station 7) while V and Cr were lower, similar to Sungai Buloh sediments (Nemati *et al.,* 2011). Based on Risk Assessment Code, the sediments showed a low risk for V, Cr, Cu and Pb, but medium risk for Co, Zn (except Station 3), Cd at Station 1 and Station 2 and Ni at Station 1, Station 3 and Station 5. Zn at Station 3 and Cd at Station 3 to Station 7 showed high risk to the environment (Nemati *et al.,* 2011).

According to Tatone et al. (2016), speciation of sediments from Uruguay River using a 4-step sequential extraction procedure revealed that Pb was 63%, Cr was 74%, Ni was 75%; Zn was 80%; Cu was 86%; Cu was 86% and Fe was 90% were predominantly associated with the F4 (residual fraction), and thus strongly linked to the mineral matrix, and therefore unavailable to aquatic organisms. However, Mn (82%) was mainly found in the non-residual fractions. The trend of heavy metal mobility and bioavailability observed decreased from Mn (82%) ≫ Pb (37%) > Cr (26%) > Ni (25%) > Zn (20%) > Cu (14%) > Fe (10%). Tatone *et al.* (2016) concluded that sediments of Uruguay River were relatively unpolluted since metals were found in residual fraction indicating lithogenic sources. Mn predominance in the non residual fraction was attributed to the fact that Mn tends to be present in less stable phases such as ion-exchangeable Mn^{2+}, easily reducible Mn oxides and Mn enclosed in carbonate minerals.

In contrast to all the above studies, Rosales *et al.* (2016) carried out a single element (Cr) speciation of sediments from Guadalentin River in Spain. Until 2003, discharge of waste water from tannery industries contaminated the river (Rosales *et al.,* 2016). High concentrations of Cr and Cr(VI) (11099 mg kg^{-1} and 79 mg kg^{-1}, respectively) were found in river sediments (Rosales *et al.,* 2016). Further, all sampled sediments along the river stretch studied had concentrations of Cr well above the background levels and reference values (Rosales *et al.,* 2016). In addition, speciation analysis also indicated that the highest degree of pollution by Cr was located near the discharge point of the tannery industry (prominent source of Cr in the Guadalentin River) (Rosales *et al.,* 2016). Because Cr(III) salt was used in the tanning industry, 95.8% of Cr was found in the form of Cr(III) while hexavalent chromium was only 4.13% of the total Cr (Rosales *et al.,* 2016).

Because anthropogenic sources are the main reason of these metals in most environments (as shown in the review above), removal of these metals have become extremely important to avoid their negative consequences. This leads us to an important strategy used in de contamination of metal contaminated soils-Bioremediation.

2.5 Bioremediation of heavy metals

Bioremediation has been identified as a sustainable means of soil remediation. It uses natural biological activity of living organisms such as animals like earthworms, microorganisms (bacteria and fungi) and/or plants to detoxify and render environmental contaminants harmless (Khan *et al.,* 2011; Sinha *et al.,* 2010). The pivotal role bioremediation strategies play in preventing hazardous consequences of environmental contaminants to human health and/or the environment cannot be over emphasized (Vidali, 2001; Rajendran *et al.,* 2003).

Since heavy metals are not biodegradable, during bioremediation, they are transformed from one organic complex or oxidation state to another. Change in the oxidation state of heavy metals (during transformation) or their re-speciation into other forms renders them less toxic or less bioavailable by making them easily volatilized, more water soluble (and thus easily removed through leaching) or less water soluble (thus allowing them to be easily removed through precipitation) (Valls and De Lorenzo, 2002; Garbisu and Alkorta, 2003; Chibuike and Obiora, 2014).

Although heavy metal removal can be achieved through physicochemical methods such as excavation and landfill thermal treatment, acid leaching, electro reclamation, chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, freeze crystallization, electrodialysis, cementation, starch xanthate adsorption, and solvent extraction (Sinha *et al.,* 2010; Mohammed *et al.,* 2011), bioremediation compared to these other methods is relatively low-cost, requires low-technology, has high public acceptance and can often be carried out on site (Rajendran *et al.,* 2003). There are various kinds of bioremediation technologies namely; biostimulation, bioventing and bioaugmentation. These technologies are increasingly becoming the preferred choice of sustainable soil remediation strategy (Sprocati *et al.,* 2012). Examples of microbes that have demonstrated excellent ability in reducing and/or removing heavy metals from contaminated soils includes *Aspergilus niger, Pseudomonas* spp., *Alcaligenes* spp., *Ganoderma applantus, Pleurotus ostreatus, Rhizopus arrhizus, Stereum hirsutum, Phormidium valderium, Corynebacterium* spp., *Flavobacterium* spp., *Azotobacter* spp., *Rhodococcus* spp., *Mycobacterium* spp., *Nocardia* spp., *Methosinus* sp., *Methanogens, Bacillus* spp., and *Arthrobacter* spp. etc (Girma, 2015). The list of some microorganisms and various metals they utilize is shown in Table 2.4.

Table 2.4: List of micro-organisms and various metals they utilize (Girma, 2015)

Heavy metals	Micro-organisms
Cu, Zn	*Bacillus spp.*
U, Cu, Ni	*Pseudomonas aeruginosa, Zooglea spp.*
Au, Cu, Ni, U, Pb, Hg, Zn	*Chlorella vulgaris*
Cd, Zn, Ag, Th, U, Cu	*Aspergilus niger*
Cd, Cu, Zn	*Pleurotus ostreatus*
Ag, Hg, Cd, Pb	*Rhizopus arrhizus*
Cd, Co, Cu, Ni, Pb	*Stereum hirsutum*
Cu, Hg, Pb	*Ganoderma applantus*
Co, Ni, Cd, U, Pb	*Citrobacter spp*
Cd, Pb	*Phormidium valderium*

2.6 Mechanism of Microbial interaction with heavy metals

Because of the important role microorganism play in the bioremediation of contaminated environment, it is important to understand the mechanism of microbial interaction with these metals. According to Sinha *et al.* (2010) and Mohammed *et al.* (2011), microorganisms have various mechanisms of controlling heavy metal(loid) ion imbalance using two types of uptake systems. The first uptake system which has no substrates specificity is constitutively expressed, fast and driven by the chemiosmotic gradient across the cytoplasmic membrane of bacteria (Mohammed *et al.,* 2011). The second type of uptake system however, is slower, has high substrate specificity and sometimes it uses Adenosine triphosphate (ATP) hydrolysis as the energy source (which is only produced by the cell during starvation, or during a special metabolic situation etc.). Incidences of metal ions toxicity to microorganisms is as a result of high concentrations of nonessential metal transport across the cell by a constitutively expressed unspecific system (also known as the open gate) (Mohammed *et al.,* 2011).

Due to the fact that metal ions are not biodegradable or modified like toxic organic compounds, metal resistance system by micro-organisms have been observed to be in different ways. They are:

(a) exclusion by permeability barrier,

(b) intra and extra-cellular sequestration,

(c) active efflux pumps,

(d) enzymatic reduction, and

(e) reduction in the sensitivity of cellular targets to metal ions (Mohammed *et al.,* 2011).

Remediation (microremediation) of contaminated environments is thus achieved with a combination of one or more of these resistance mechanisms. In addition, these mechanisms allows micro-organisms to thrive in metal-contaminated environments (Mohammed *et al.,* 2011).

2.7 Studies on microbial remediation of heavy metals in soil

Microbial remediation of metal contaminated soils using microorganisms has been investigated in different soils. Du *et al.* (1995) in their investigation of microremediation of a soil contaminated with petroleum hydrocarbons and heavy metals revealed that nutrient supplementation (nitrogen and phosphate) and aeration played crucial role in enhancing biodegradation. While biodegradation of petroleum hydrocarbons was observed to occur under aerobic than under anaerobic conditions (Du *et al.,* 1995), low pH conditions induced by biodegradation also lead to heavy-metal removal from soils (Du *et al.,* 1995).

In a different set up, Wasay *et al.* (1998) developed a bioremediation process using the fungus (*Aspergillus niger*) to produce weak organic acids for the leaching of Hg, Pb, Mn and Cr from contaminated soils. *Aspergillus niger* was cultivated on three types of soils - a sandy clay loam, a loamy and clay loam for 15 days at 30°C at a pH >4 to favor the production of citric acid rather than oxalic acid which hinders Pb leaching (Wasay *et al.,* 1998). The findings of the study revealed that 91% Hg, 85% Pb, 41% Mn and 37% Cr was leached from clay loam soil (Wasay *et al.,* 1998). In loamy soils, the leaching of Cd and Pb was found to reach 99.7% and 83%, respectively (Wasay *et al.,* 1998). For the sandy clay loam, Cd and Zn, Cu and Pb were leached upto levels 99%, 94% and 58% respectively (Wasay *et al.,* 1998).

Alisi *et al.* (2009) investigated the feasibility of bioremediation of a soil contaminated with heavy metals and diesel oil (DO) from a metallurgic area of Bagnoli (Naples, Italy). They used a microbial formula named ENEA-LAM consisting of ten bacterial strains that have been observed to have multiple resistance for heavy metals (Alisi *et al.,* 2009). Their analysis showed that most of the bacterial strains inoculated into the soil at the beginning died while some minor native strains, undetectable in the soil at the beginning of the experiment, developed in due course (Alisi *et al.,* 2009). They highlighted that bioaugmentation approach allows newly established microbial community to strike a balance between the native and introduced organisms found in the soil (Alisi *et al.,* 2009). They concluded that the use of microbial formula specifically tailored to contaminants efficiently facilitates and speeds up the bioremediation of matrices co-contaminated with hydrocarbons and heavy metals (Alisi *et al.,* 2009).

Similar to Alisi *et al.* (2009), Sprocati *et al.* (2012) used a microbial formula (ENEALAMOSS) composed of allochthonous strains isolated from a chronic polluted soil and selected to be functionally linked to the pollutants present in the test-soil (Sprocati *et al.,* 2012). They achieved about 75% biodegradation in soil co contaminated with diesel oil and heavy metals (Sprocati *et al.,* 2012).

Zhan *et al.* (2015) in their experiment used two different microbial agents on soils contaminated with heavy metals. The microbial agents were carbonate and phosphate microbe agent. Carbonate microbe agent (composed of carbonate mineralized bacteria or *Bacillus* and urea in a certain proportion) showed better remediation of Cr^{2+} and Pb^{2+} in the contaminated soil than phosphate microbe agent (Zhan *et al.,* 2015). While Cd^{2+} and Zn^{2+} were better remedied by phosphate microbe agent (composed phosphate mineralized bacteria or *Bacillus* and glycerophosphate in a certain proportion), both microbe agent excellently reduced the concentration of Cu^{2+} (Zhan *et al.,* 2015). Additionally, a decrease in the concentration of heavy metals in the vegetables and seedlings and an improved growth momentum and bud rate was observed in the plant experiment (Zhan *et al.,* 2015). They highlighted the significance of using different microbial agents in remedying heavy metal pollution in soils.

Agnello *et al.* (2015) evaluated four bioremediation strategies: natural attenuation, phytoremediation with alfalfa (*Medicago sativa* L.), bioaugmentation with *Pseudomonas aeruginosa* and bioaugmentation-assisted phytoremediation, for the treatment of a co-contaminated soil with heavy metals (Cu at 87 mg kg^{-1}dry weight, Pb at 100 mg kg^{-1} dry weight and Zn 110 mg kg^{-1} dry weight) and petroleum hydrocarbons (3800 mg kg^{-1} dry weight) (Agnello *et al.,* 2015). Alfalfa plants were able to tolerate and grow in the co-contaminated soil as demonstrated in the increase in shoots and roots (56% and 105% respectively) (Agnello *et al.,* 2015). Plant biomass and selected physiological parameters shows no plant stress (Agnello *et al.,* 2015). The concentration of heavy metals in alfafa plants were in the following order: Zn > Cu > Pb (Agnello *et al.,* 2015). Bioaugmentation (of planted soil) with *Pseudomonas aeruginosa* generally led to a decrease of plant metal concentration and translocation (Agnello *et al.,* 2015). The highest degree of total petroleum hydrocarbon removal was obtained for bioaugmentation-assisted phytoremediation treatment (68%), followed by bioaugmentation with *Pseudomonas aeruginosa* (59%), phytoremediation with alfalfa (47%) and natural attenuation (37%) (Agnello *et al.,* 2015). They highlighted that a combination of micro organisms and plants should be explored for the treatment of co-contaminated soil as compared to bioaugmentation or phytoremediation or natural attenuation applied alone (Agnello *et al.,* 2015).

Emenike *et al.* (2016) used nine bacterial species to evaluate their efficiency in removing certain heavy metals from leachate contaminated soil. Three microcosms in triplicates were set up under different conditions: soil contaminated and amended with nine isolated bacteria strains (*Pseudomonas putida biotype B, Stenotrophomonas maltophilia, Flavimonas oryzihabitans, Lysinibacillus sphaericus, Lysinibacillus sphaericus, Acinetobacter schindleri, Brevundimonas vesicularis, Microbacterium maritypicum and Rhodococcus wratislaviensis)*; soil contaminated and amended with three strains (*Bacillus thuringiensis, Lysinibacillus sphaericus and Rhodococcus wratislaviensis*); and contaminated soil without any amendment (control with no addition of any micro organisms) (Emenike *et al.,* 2016). Their findings revealed that the highest removal efficiency (86%, 73% and 71% for Cu, Zn and Pb, respectively) was observed in soil amended with three strains (*Bacillus thuringiensis, Lysinibacillus sphaericus* and *Rhodococcus wratislaviensis*) (Emenike *et al.,* 2016). Heavy metal removal efficiency in soil amended with nine isolated bacteria strains was 64%, 54% and 59% for Cu, Zn and Pb respectively (Emenike *et al.,* 2016). In the microcosm without the microbial formula, the reduced percentage of heavy metals (ranging from about 42% to 53%) observed was attributed to natural‘‘bioattenuation’’ phenomenon (Emenike *et al.,* 2016). They concluded that the microbial consortia formulated and introduced as bioaugmentation agents were able to enhance heavy metals removal in soil contaminated with leachate from a MSW landfill (Emenike *et al.,* 2016).

CHAPTER 3: MATERIALS AND METHOD

3.1 Reagents/Extractants

All reagents used were of analytical grade unless stated otherwise. Acetic acid (glacial, 100% Fischer Scientific, Loughborough, Leicestershire, UK), Hydrogen peroxide (30% Fischer Scientific, Loughborough, Leicestershire, UK), Magnesium Chloride ($MgCl_2$) and hydroxylammonium chloride (ACROS Organics, NJ, USA). Also, ammonium acetate and Nitric acid (HNO_3) (65% Supra pure Merck, Darmstadt, Germany) were of supra pure quality. A multi-element standard solution IV for Inductively Coupled Plasma-Mass Spectrometry (Fluka, Switzerland) was used to prepare series of working standard solutions. Blank determinations were carried out using the same reagents in equal quantities as described in the analytical procedure throughout the whole experiments.

3.1.1 Preparation of Chemical Solutions

All extractants were prepared using double deionized water (Milli-Q Millipore 18.2 M Ω/cm resistivity). All glassware and containers used in the preparation of chemical solutions were soaked in dilute nitric acid and rinsed with double deionized water. Extractants were prepared according to the following procedures:

1 M magnesium chloride was prepared by dissolving 203.31 g magnesium chloride hexahydrate ($MgCl_2$) crystal in 500 ml of deionized water. The pH of the solution was then adjusted to pH 7 by adding drop by drop of 1 M NaOH solution (until the desired pH is reached) and then made upto 1000 ml with deionized water. 1 M sodium acetate was prepared by dissolving 82.03 g sodium acetate (NaOAc) in 500 ml of deionized water. The pH of the solution was then adjusted to pH 5 by adding drops of 1 M acetic acid (HOAc) solution (until the desired pH is reached) and then made upto 1000 ml with deionized water. 0.04 M hydroxylammonium hydroxide in 25% v/v acetic acid was

prepared by dissolving 2.7801 g of hydroxylammonium hydroxide (NH_2OH-HCl). The solution was then made upto 1000 ml with 25% v/v acetic acid solution. 25% (v/v) glacial acetic acid was prepared by mixing 250 ml acetic acid with 750 ml of deionized water. 3.2 M ammonium acetate in 20% (v/v) nitric acid was prepared by dissolving 246.62 g of ammonium acetate and then diluted to 1000 ml with 20% (v/v) nitric acid. 20% (v/v) nitric acid (HNO_3) was prepared by mixing 200 ml of concentrated nitric acid with 800 ml of deionized water. 0.02 M nitric acid (HNO_3) was prepared by diluting 1.395 ml of concentrated nitric acid (HNO_3) which was then made upto 1000 ml with deionized water. 0.5 M nitric acid (HNO_3) was prepared by diluting 34.868 ml of concentrated nitric acid (HNO_3) which was then made upto 1000 ml with deionized water. Hydrogen peroxide (H_2O_2) with 30% v/v: 30% H_2O_2 was used as purchased from Merck. 12 M hydrochloric acid and 15.8 M HNO_3 was used as purchased from Merck (65% purity).

3.2 Apparatus

A centrifuge (Kubota 2420) was used to completely separate the extracts from the soil residues after treatment with respective extractant(s). The operating parameters for working elements were set as recommended by the manufacturer. Metals determination for eight heavy metals (Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb) was carried out by using an Agilent 7500a Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent Technologies, Japan) which was equipped with a Babington nebulizer. A glass double-path spray chamber and a standard quartz torch operated at conditions listed in Table 3.1. In addition, a microwave digester (MARS-5), an orbital Shaker (OS-340), hot water bath (Wisebath, WSB-18) and a pH meter (Hanna instruments, HI2213) were used during analysis.

Table 3.1: Instrumental parameters for trace element determination

Parameters	Conditions
RF power	1350 W
RF matching	1.6 V
Carrier gas flow rate	1.10 L/min
Peristaltic pump flow rate	0.1 rps
Sample Uptake Time	30 sec
Sample Uptake Rate	0.4 r sec^{-1}

3.3 Sample collection and preparation

In this study, the sampling sites were (a) an operating non-sanitary landfill namely Bukit Beruntung (BB) landfill and (b) a closed non-sanitary landfill namely Taman Beringin (TB) landfill. Soils from both landfills were excavated in accordance to 2014 ASTME – 1197 standard guidelines for conducting terrestrial soil-core microcosm test at 0-30 cm depth from the areas which were contaminated with leachate. For BB landfill, three different sampling stations were selected designated as Station 1, Station 2 and Station 3 while for TB landfill, collection were done at as Station 1, Station 2 and Station 3 as shown in Table 3.2. Choice of sampled stations in both landfills was based on preliminary assessment such as visual observation, topographic outlay etc. The general condition analyzed from both landfills studied is as listed in Table 3.3. Figure 3.1 and Figure 3.2 shows map of the sampled stations at each landfill.

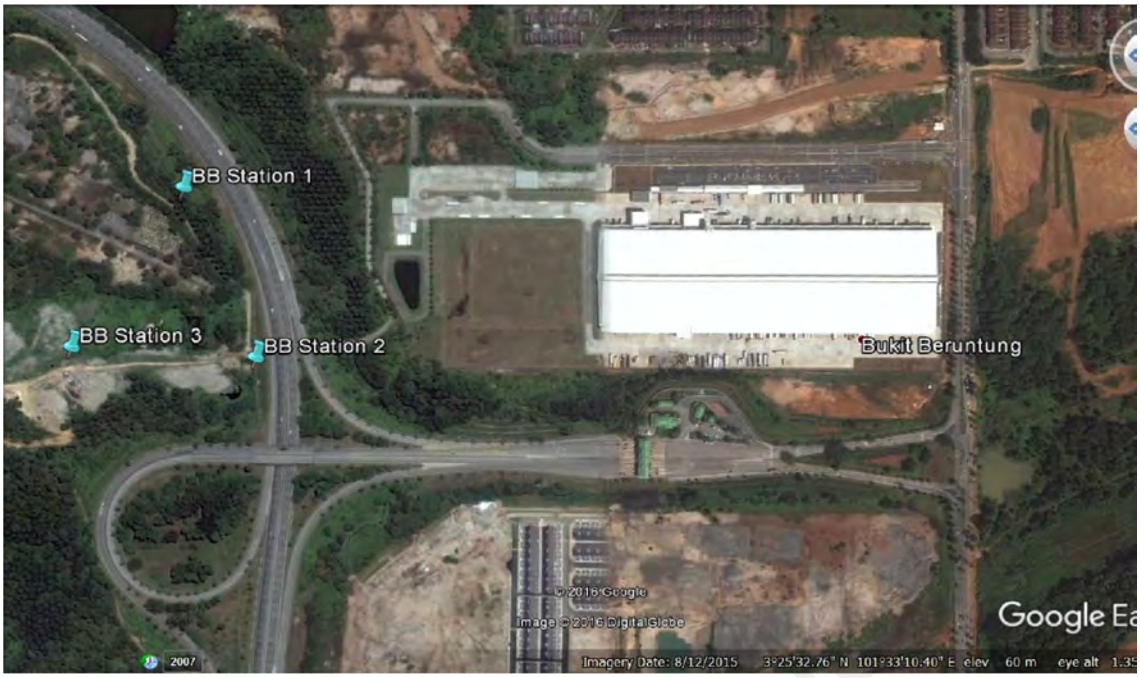

Figure 3.1: Map of Bukit Beruntung landfill showing the three sampled stations

Figure 3.2: Map of Taman Beringin landfill showing the three sampled stations

In BB landfills, samples from Station 1 represents soil samples from inside the dumping area while in TB landfill, Station 1 represent soil samples from inside the waste cells. Station 2 represents soil samples from open area within active dumping cells in BB landfill while in TB landfill, Station 2 represents soil samples from open area within the waste cells. Station 3 represents soil samples from the entrance to the dumping areas in BB landfill while in TB landfill, Station 3 represents soil samples from entrance to the landfill (Figure 3.1 and Figure 3.2). Sampling of soil from both landfill was carried out twice.

Soil samples were placed in clean plastic containers, sealed, labelled and taken to the laboratory for analysis. Large and unwanted objects such as stones, broken glass, plastic, leaves, rags, insects, worms etc. were removed and the remaining material was dried at 40 °C in an oven. Dried samples were grinded with ceramic mortar and pestle to pass through a stainless steel sieve of > 1.18mm to reduce the particle size to that needed for the sequential extraction procedure (Wang *et al.,* 2014). The dried samples were then stored in clean, dry air tight containers for subsequent use. Extra care was taken to avoid contamination during sampling, drying, grinding, sieving and storage.

Table 3.2: Description of sampling sites showing status and grade

Landfill	Status	Grade	Sampled points	Latitude	Longitude
Bukit Beruntung (BB)	Operating	Non sanitary	Station 1	3° 42’49 .21 N	101° 54’ 55.87 E
			Station 2	3° 42’ 49.81 N	101° 54’ 53.35 E
			Station 3	3° 25’ 31.88 N	101° 32’ 48.92 E
Taman Beringin (TB)	Closed	Non sanitary	Station 1	3° 13’ 40.17 N	101° 39’ 43.48 E
			Station 2	3° 13’ 42.86 N	101° 39’ 37.16 E
			Station 3	3° 13’ 37.91 N	101° 39’ 51.74 E

Table 3.3: General condition of the landfills studied (Emenike, 2013)

Landfills	Bukit Beruntung	Taman Beringin
Landfill type	Non sanitary	Non sanitary
Status	Active (operating)	Inactive (closed)
Location	3° 32.14'N, 101° 25.80'E	3° 13.78'N, 101° 39.72'E
Period of landfilling	2001- to date	1995-2005
Classification based on age	Mature	Stabilized
Leachate treatment system	Biological	Physical and Biological
Waste type	Household,commercial, industrial	Household, commercial, industrial and others
Daily average of waste disposed (tonnage)	1500	1800-2000
Distance to nearby stream/river (meters)	5	No nearby stream/river
Fate of landfill gas generated	No facility	No facility
DOE requirement for effluent discharge	Standard A	Standard B

3.4 Determination of total heavy metal content

The most common method for determining the concentration of metal contaminants in soil is via total elemental analysis-USEPA Method 3051 (Evanko & Dzombak, 1997; Wuana and Okieimen, 2011; Al Farraj *et al.,* 2013; Asrari, 2014). In this study, 1 g of soil sample from each station was put into a Teflon vessel. 10 ml of HNO_3 was added and the vessels were tightly capped and placed in a microwave and digested for 10 minutes according to USEPA 3051 (total-recoverable) (Table 3.4). After cooling, the digested solutions were filtered using Whatman 42 filter paper and brought to a total volume of 50 ml with deionised water in a volumetric flask (Wuana and Okieimen, 2011; Al Farraj *et al.,* 2013). The total heavy metal concentration was measured using ICP-MS. Each sample from the various stations of the landfill sites were analysed in triplicates.

Table 3.4: Microwave digestion operating conditions (Al Farraj *et al.,* 2013)

Step	Power (W)	% max	Time to raise temperature (minutes)	Temperature (°C)	Running time (minutes)
1	400	100	10	200	5
2	400	100	5	210	5
3	400	100	1	220	5

3.5 Speciation of heavy metals using Sequential extraction technique

In this study, a sequential extraction process based on Tessier *et al.* (1979), Tsang *et al.* (2007) and standard ISO 11466 (1995) was performed for the determination of eight heavy metals (Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb) in soil samples. An initial weight of

1.0 ± 0.005 g was used in the sequential extraction process and all sample analyses were run in triplicates. The extraction procedures employed are detailed as follows:

Fraction 1 (F1) (Exchangeable metal fraction)

1.0 ± 0.005 g sample was extracted with 10 ml of 1 M magnesium chloride ($MgCl_2$) at pH 7 for 2 hours at room temperature with continuous agitation.

Fraction 2 (F2) (carbonate bound metal fraction)

The residue from F1 was continuously agitated with 10 ml of 1 M sodium acetate (NaOAc) adjusted to pH 5 with acetic acid (HOAc) at room temperature for 5 hours.

Fraction 3 (F3) (Fe-Mn oxides bound metal fraction)

Residue from F2 was mixed with 20 ml of 0.04 M Hydroxylammonium hydrochloride (NH_2OH-HCl) in 25% (v/v) HOAc for 6 hours at 96°C with occasional agitation. After cooling, the sample was diluted to 20 ml with deionised water.

Fraction 4 (F4) (Organic matter and sulphide bound metal fraction)

Residue from F3 was extracted with 3 ml of 0.02 M nitric acid (HNO_3) and 5 ml of 30% hydrogen peroxide (H_2O_2) (adjusted to pH 2 with HNO_3), heated to 85°C for 2 hours, then extracted with another 3 ml aliquot of 30% H_2O_2 (adjusted to pH 2 with HNO_3) at 85°C for 3 hours with intermittent agitation. After cooling 5 ml of 3.2 M NH_4OAc in 20 % (v/v) HNO_3 was added, with continuous agitation for 30 minutes.

Fraction 5 (F5) (Residual metal fraction)

The residue from F4 was digested with 9 ml of 12 M hydrochloric acid (HCl) followed by 3 ml of 15.8 M HNO_3 added dropwise to reduce foam. After that, 5 ml of 0.5 M

HNO_3 was added and kept for 16 hours at room temperature to oxidize the organic matter in the soil. The mixture was then heated and maintained for 2 hours before cooling.

The sequential extraction was carried out in polypropylene centrifuge tubes of 50 ml capacity. After each successive extraction, samples containing the extractants were centrifuged at 3500 rpm for eight minutes at room temperature. The supernatant liquid were then filtered using a Whatman filter paper into 15ml polypropylene bottles for the determination of metals using ICP-MS analysis. Prior to the start of the next extraction step, 10 ml deionised water was used to wash samples and then the washing solution was discarded after centrifugation. All the experiments were carried out in triplicates to reduce systematic error.

3.6 Bioremediation of leachate contaminated soil from BB and TB landfill sites using Bioaugmentation technique

In this study, the bioaugmentation technique was the preferred choice for the remediation of leachate contaminated soil from BB and TB landfill. The rational for selection of the bioaugmentation technique is due to the advantages this technology offers. First, it allows the use of specific microbes in sufficient number to complete the biodegradation of the desired contaminant in any given environment. Second, when a specific microbial population is injected the degradation process can start immediately, unlike in biostimulation, for example where there is a delay after injection of nutrients as the microbial population propagates and also nutrient are not specific. Lastly, using native soils has the advantage of allowing the microbes more chances of survival and propagation when reintroduced into the site (i.e for ex-situ mediation later on) (Alisi *et al.,* 2009).

The bioaugmentation set up was carried out in the laboratory and the microbial formula used was obtained from the Central Analysis Laboratory in University of Malaya. A total of fourteen (14) strains of bacteria from TB landfill and eighteen (18) for BB landfill was used. The number of microbes used in the bioaugmentation was based on the total number of indigenous bacteria isolated from soil samples in each landfill. This is in order gain to insight into whether indigeneous microorganism observed in each landfill is able to reduce metal concentration in leachate contaminated soil. The following steps were taken to inoculate the blend of microorganisms into the leachate contaminated soil in the laboratory:

a. Removal of unwanted debris such as stones, plastic, broken glass, grasses etc from leachate contaminated soil
b. Next, two microcosms in triplicates were set up under different conditions: BA and TA, leachate contaminated soil amended with all the isolated bacteria strains from BB and TB landfill respectively; and BB Control and TB Control, leachate contaminated soil without any amendment with micro organisms
c. Microcosms BA and TA were watered with 200 ml of the inoculums (obtained from equal volumes of pooled discrete species) each containing about 3 x 10^9 CFU g^{-1}
d. After the microbial formula was introduced into the designated microcosm's soils, it marked the start of the bioaugmentation experiment.
e. Thereafter, a portion of the soil microcosms was taken at the end of the experimental time-course (100 days) for heavy metal analysis using sequential extraction procedure.
f. The bioaugmentation set up was carried out in the laboratory. In order to maintain soil moisture during the experimental time-course, regular watering with about 10ml of distilled water was done at intervals. Caution was exercised during watering to avoid leaching of metal contents and contamination of the soil.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Total metal concentrations in soils from Bukit Beruntung and Taman Beringin landfill

To reiterate, the first objective of this study was to determine the total concentration of heavy metals in leachate contaminated soils from Bukit Beruntung (BB) and Taman Beringin (TB) landfills. The results obtained for each landfill is discussed separately in the subsequent sections.

4.1.1 Total metal concentrations in soils from Bukit Beruntung (BB) landfill

Bukit Beruntung Landfill is one of the landfills under the district council of Hulu Selangor located in the state of Selangor (Suratman *et al.,* 2011). The area was a secondary forest area prior to its use as a landfill. It is a class I landfill (non sanitary type) because it has no geotextile lining to prevent leachate flow into groundwater sources, lacks monitoring wells etc (Suratman and Sefie, 2010). It receives approximately 80 tonnes of waste daily (Fauziah and Agamuthu, 2007). The landfill covers an area of about 5 acres and started its operation since 1992 (Emenike, 2013). The site receives mostly domestic and commercial wastes collected from Bukit Beruntung, Serendah and Bukit Beruntung industrial area. BB landfill has been recommended for safe closure since it has surpassed its operational capacity, however it is still receiving waste and currently an active landfill (Suratman *et al.,* 2011). Table 4.1 shows the total heavy metal concentration in soils from Bukit Beruntung (BB) in microgram per gram (µg/g).

Table 4.1: Total concentration of heavy metals at Station 1 to Station 3 in BB landfill (µg/g)

Sampled points	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
Station 1(inside the dumping area)	2.09±0.12	3.25±0.29	ND	0.37±0.03	6.37±1.58	3.39±1.06	ND	3.62±0.13
Station 2 (open area between active dumping cells)	0.66±0.13	0.93±0.11	ND	0.02±0.04	0.38±0.07	1.20±0.79	ND	2.13±0.27
Station 3 (entrance to the landfill)	3.90±0.71	6.32±0.50	ND	1.00±0.53	3.87±0.19	15.1±2.11	ND	4.37±0.23

ND-not detected

Results of total metal analysis using the USEPA 3051 revealed that variation exists between the total heavy metal content present in the soil sample from the different sampled stations at BB landfill (Table 4.1). Station 1 (i.e inside the dumping area) had Cu at 6.37 µg/g as the most dominant while Station 2, which is the open area between dumping cells had Pb at 2.13 µg/g predominate over all other metals. Zn at 15.1 µg/g was the most predominant metal at the entrance to the landfill (Station 3). In general, the trend observed in BB landfill showed that for all metals (except Cu, Co and Cd), the average highest concentration of total heavy metal content was found to be mostly predominant at the entrance to the landfill (Station 3) (Table 4.1).While only Cu with 6.37 µg/g was dominant inside the dumping area (Station 1), Co and Cd were not detected in all stations (Table 4.1). The high concentration of heavy metals at the entrance to the dumping areas (Station 3) in this study could be as a result of the illegal dumping of waste observed at the entrance to the landfill and subsequent migration of leachate from decomposing waste into unprotected soil in the landfill. This finding is similar to Ismail *et al.* (2015) where Cu, Cd, Pb, and Zn was highly concentrated at the boundary of non-sanitary landfills in Langat water catchment area and none of the metals concentrated in the immediate dumping area (the center point of the site). Meanwhile, the open area between the dumping cells (Station 2) had the least concentration of heavy metals in BB landfill (Table 4.1). Sources of dominant metals such as Cu, Zn and Pb in BB landfill could be attributed to types of waste found there such as packaging materials, metal and steel products, cleaning and food products, discarded batteries, plastics and pigments, electronic wastes etc (Korzun and Heck, 1990; Ngole and Ekosse, 2012, Sakawi *et al.,* 2013).

Continuous waste dumping activity could increase the concentration of heavy metals in soil in BB landfill in the future due to uncontrolled release of leachate into the soil environment. This finding is similar to Ahmed and Sulaiman (2001) who found similar distribution of Zn and Pb in soils from Seri Petaling Landfill. Similar to this studies also, Bahaa-Eldin *et al.* (2008) found elevated concentration of heavy metals (especially Cr, Zn and Pb) in soils from Ampar Tenang waste disposal site in Dengkil, Selangor due to the migration of leachate from decomposing garbage.

Overall, the concentration of heavy metal in BB landfill is in the order: Zn > Cu > Mn > Pb > Cr > Ni. The order of heavy metals observed in BB is similar to Kennou *et al.* (2015) where Zn concentration was found to be highest in soil from a controlled dump. Similarly, Prechthai, Parkpian and Visvanathan (2008) also observed that Mn, Cu and Zn were highest in Nonthaburi dumpsite. Adamcova *et al.* (2016) and Thomas (2015) also observed similar metals in a landfill in Czech Republic and Nigeria, respectively.

4.1.2 Total metal concentrations in soils from Taman Beringin landfill (TB) landfill

Taman Beringin landfill is located at North Jinjang which is about 10 km North West of Kuala Lumpur city centre. It received wastes from municipal, commercial, agricultural, recreational, domestic and mixed industrial wastes originating from households and industrial premises (Zakaria *et al.,* 2005). The landfill covers an area of about 16 hectares. The landfill, which is a non sanitary type, started its disposal activities since 1991 and was closed in early 2005 (currently an inactive landfill) (Emenike, 2013).

The results showed that the metal values in TB varied over a wide range: from 1.85 µg/g to 12.9 µg/g for Cr, 44.7 µg/g to 175 µg/g for Mn, 0.43 µg/g to 3.15 µg/g for Co, 0.23 µg/g to 3.92 µg/g for Ni, 0.63 µg/g to 7.84 µg/g for Cu, 0.13 µg/g to 0.79 µg/g for Zn and 2.14 µg/g to 2.98 µg/g for Pb. Variation in metal concentration in landfills could be as a result of the solid organic compounds and their ability to stabilize metal elements (Marzieh *et al.,* 2010). This finding is similar to Kasassi *et al.* (2008) where concentration of metals were observed to vary widely in soils from a closed unlined landfill in Greece.

In contrast to BB, in TB, the concentration of heavy metals (Cr, Co, Ni, Cu and Zn) inside the dumping area (Station 1) was higher compared to other sampling sites (Station 2 and Station 3) except for Pb and Mn which were predominately observed in Station 2 and Station 3 respectively (Table 4.2). Similar to BB, Cd was not detected in all sampling stations in TB landfill (Table 4.2).

Generally, for most metals in TB, the concentration was observed to be highest in Station 1. This could be because a long period of inactivity has allowed direct mingling of leachate with the underlying soil since waste placement in TB landfill. Further, inactivity in the landfill may have contained metal in soils because direction of leachate flow is not as witnessed in BB landfill. This result is similar to Kamil and Abdul-Talib (2010) who observed elevated concentration of Pb, Cr, Mn, Zn and Cu in soil from closed Kudang Badak landfill. Overall, the concentration of heavy metal in TB landfill is in the order: Mn > Cr > Cu > Ni > Co > Pb > Zn. The order of concentration of Cu, Ni, Cr and Co was observed to be similar to that Al Raisi *et al.* (2014) who found these metals in an unlined landfill in the Sultanate of Oman.

A comparison of heavy metal concentration between BB and TB revealed that, in general, heavy metal concentration in TB was much higher than that of BB. This could be because more metals may have leached out from the buried waste in TB compared to that in BB landfill where continuous waste dumping activity is still occurring. Although both landfills are non sanitary and received similar kinds of wastes, the type of heavy metals occurrence at the three sampling sites in each landfill is quite different.

Table 4.2: Total concentration of heavy metals at Station 1 to Station 3 in TB landfill (µg/g)

Sampled points	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
Station 1(inside the waste cells)	12.9±0.01	88.5±0.06	3.15±0.01	3.92±0.01	7.84±0.01	0.79±0.01	ND	2.73±0.01
Station 2(open area between the waste cells)	1.85±0.01	44.7±0.35	0.43±0.04	0.23±0.01	0.63±0.01	0.13±0.01	ND	2.98±0.01
Station 3 (entrance to the landfill)	10.0±0.01	175±0.31	2.11±0.01	1.81±0.01	4.20±0.01	0.26±0.01	ND	2.14±0.01

ND-not detected

This could be due to the differences in weather and environmental condition at each landfill (Kulikowska and Klimiuk, 2007).

4.2 Speciation of heavy metals in a closed non-sanitary landfill and an operating non-sanitary landfill

This study's second objective was to assess the speciation of heavy metals in an operating non-sanitary i.e BB landfill and a closed non-sanitary i.e TB landfill. The F1, F2 and F3 represents the mobile forms of heavy metal in BB and TB landfill. Metals observed in these fractions are bioavailable and potentially toxic. The last two fractions (F4 and F5) are the non mobile forms of metals observed in BB and TB landfill. Hence, metals extracted in the F4 and F5 are generally considered neither mobile nor bioavailable because they are bound to silicates and primary minerals in uncontaminated soils and sediments (Ashraf *et al.,* 2011). Therefore, F4 and F5 metals in BB and TB does not pose immediate threat to the environment. Consequently, a high percentage of metal in the F1, F2, F3 fractions in BB and TB is therefore an indication of pollution in an area mainly due to anthropogenic activities of dumping waste materials in the area while a high concentration of metals in the F4 and F5 fractions indicates that metal content in the landfill is from natural sources (Ashraf *et al.,* 2011).

4.2.1 Concentration of metals in each fraction at different stations in BB landfill

The concentration of metals in each fraction at different stations in BB landfill revealed that in Fraction 1 (F1 or Exchangeable metal fraction), Mn with 1.06 µg/g in Station 1 dominates over all other metals found in this fraction. Zn at 0.13 µg/g was detected only in Station 1. Pb was present in Station 1 and Station 2 at 0.08 µg/g and 0.04 µg/g, respectively. While, Mn was the only metal in this fraction detected in all three stations. Cr, Co, Ni, Cu and Cd were not detected in this fraction in all samples stations (Table 4.3). In fraction 2 (F2 or Carbonate bound metal fraction), no metal was detected in Station 1 and Station 2. In Station 3 however, Zn with 0.75 µg/g was the most dominant metal F2 detected followed by Mn with 0.18 µg/g and trace amount of Pb (0.01 µg/g) (Table 4.3). In Fraction 3 (F3 or Fe-Mn oxides bound metal fraction), the highest concentration of metal detected in BB was observed. Zn at 2.34 µg/g was the most dominant metal in Station 3. A similar study by Essaku *et al.* (2005) on MSW species revealed that Zn was mainly found in the Fe-Mn oxide bound fraction. Further in BB, similar to F1 and F2, Co, Ni, Cu and Cd were not detected in this fraction. In addition, Mn in F3 was not detected in Station 2 (Table 4.3). In fraction 4 (F4 or Organic matter and sulphide metal fraction), Mn was not detected in Station 1 and Station 2. Pb at 0.06 µg/g was detected in Station 2. Similar to other fractions, F4 of Co, Ni and Cd was not detected in all samples (Table 4.3). In Fraction 5 (F5 or Residual Fraction), the highest concentration of Cu at 1.00 µg/g, Cr at 0.41 µg/g and Pb at 0.60 µg/g was observed yet F5 of Co, Ni and Cd was not detected in all samples (Table 4.3).

Table 4.3: Concentration of metals by Tessier procedure (µg/g) in BB landfill

	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
STATION 1								
FRACTION 1	ND	1.06±0.16	ND	ND	ND	0.13±0.02	ND	0.08±0.04
FRACTION 2	ND	ND	ND	ND	ND	ND	ND	ND
FRACTION 3	0.02±0.02	0.01±0.01	ND	ND	ND	0.20±0.62	ND	0.05±0.01
FRACTION 4	0.02±0.02	ND	ND	ND	0.04±0.01	ND	ND	0.08±0.01
FRACTION 5	0.32±0.36	0.15±0.23	ND	ND	1.00±1.13	ND	ND	0.60±0.56
STATION 2								
FRACTION 1	ND	0.60±0.09	ND	ND	ND	ND	ND	0.04±0.01
FRACTION 2	ND	ND	ND	ND	ND	ND	ND	ND
FRACTION 3	ND	ND	ND	ND	ND	0.16±0.54	ND	0.01±0.00
FRACTION 4	ND	ND	ND	ND	ND	ND	ND	0.07±0.01
FRACTION 5	0.03±0.01	ND	ND	ND	ND	ND	ND	0.23±0.02
STATION 3								
FRACTION 1	ND	0.31±0.08	ND	ND	ND	ND	ND	ND
FRACTION 2	ND	0.18±0.01	ND	ND	ND	0.75±0.12	ND	0.01±0.01
FRACTION 3	0.09±0.01	0.21±0.01	ND	ND	ND	2.34±0.05	ND	0.29±0.01
FRACTION 4	0.14±0.01	0.08±0.00	ND	ND	0.15±0.01	0.54±0.13	ND	0.23±0.02
FRACTION 5	0.41±0.06	0.38±0.04	ND	ND	0.37±0.06	ND	ND	0.60±0.04

ND- not detected

4.2.2 Concentration of metals in each fraction at different stations in TB landfill

In fraction 1 (F1 or Exchangeable metal fraction), similar to BB, Mn at 7.72 µg/g in Station 1 dominates over all other metals found in F1. Co at 0.70 µg/g was another metal detected while Cr, Ni, Cu, Cd and Pb were not detected in this fraction in all samples (Table 4.4). The main difference between BB and TB in this fraction is that in BB, Pb was detected in two of the samples while in TB, Pb was not detected at all. Similarly, Co was detected only in TB and not detected at all in BB landfill. In fraction 2 (F2 or Carbonate bound metal fraction), Mn and Co were the only metals detected in two samples (Station 1 and Station 3, respectively). Station 3 had a very high concentration of Mn at 50.9 µg/g and Co at 0.12 µg/g in F2. Cr, Ni, Cu, Zn and Pb were not detected (Table 4.4). Wang *et al.* (2010) reported that metal ions such as Cu^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+} are adsorbed onto surfaces of carbonate minerals and incorporated into the crystal lattice. This could be the reason for the high concentration of Mn and Co in this fraction similar to what is observed in this study. While Zn was the most predominant metal in F2 in BB, it was not detected in TB. In fraction 3 (F3 or Fe-Mn oxides bound metal fraction), the highest concentration of Mn at 133 µg/g was observed. The F3 also represented the highest concentration of metal detected in TB landfill. High concentration of Mn could be attributed to the precipitation of amorphous hydrous oxides of Mn during aging of MSW landfills (Essaku *et al.,* 2005). Also, in this study, the highest concentration of Pb at 0.70 µg/g in Station 3 was recorded in F3. Additionally, in contrast to other fractions, only Cd was not detected in all sampled sites in F3 in TB landfill (Table 4.4). This is probably because under extremely reducing conditions, diffusion mechanisms could lead to the release of metals into the environment (Essaku *et al.,* 2005), thus F3 showed the highest concentration of metal observed in BB and TB landfill sites. This finding is similar to Karim *et al.* (2014) where Cd was not detected in Matuail and Khulna dumping sites in Bandgladesh. In fraction 4 (F4 or Organic matter and sulphide metal fraction), the highest concentration of Ni at 0.72 µg/g and Cu at 3.35 µg/g in F4 was detected in Station 1. This is similar to Essaku *et al.* (2005) whose assessment of metal species in MSW revealed that about 60% Cu was found in the organic matter and sulphide fraction. The possible reason for this is the high stability constants of Cu complexes with organic matter (Essaku *et al.,* 2005). Similar to BB, F4 of Co, Ni, Zn and Cd in Station 2 and Ni, Zn and Cd in Station 3 was not detected in TB (Table 4.4). The high concentration of Cu in Station 1 could also be attributed to metal scraps, cardboards and papers (ink) found in waste. In fraction 5 (F5 or Residual Fraction), the highest concentration of Cr with 2.81 µg/g and Co with 1.17 µg/g was found in F5. Only Cd was not detected in all samples in TB landfill (Table 4.4).

Table 4.4: Concentration of metals by Tessier procedure (µg/g) in TB landfill

	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
STATION 1								
FRACTION 1	ND	7.72±0.00	0.07±0.00	ND	ND	ND	ND	ND
FRACTION 2	ND	4.37±0.00	0.04±0.00	ND	ND	ND	ND	ND
FRACTION 3	1.21±0.01	29.9±0.03	0.89±0.00	0.39±0.00	0.74±0.00	0.44±0.00	ND	0.56±0.00
FRACTION 4	1.71±0.00	2.72±0.01	0.14±0.00	0.11±0.00	3.35±0.00	0.03±0.001	ND	0.27±0.00
FRACTION 5	2.81±0.01	9.20±0.02	0.36±0.00	0.72±0.00	1.54±0.00	ND	ND	0.15±0.00
STATION 2								
FRACTION 1	ND	0.85±0.00	ND	ND	ND	ND	ND	ND
FRACTION 2	ND	3.16±0.00	ND	ND	ND	ND	ND	ND
FRACTION 3	0.33±0.00	19.9±0.04	0.14±0.00	0.12±0.00	0.13±0.00	0.14±0.00	ND	0.59±0.00
FRACTION 4	0.06±0.00	0.82±0.00	ND	ND	0.12±0.00	ND	ND	ND
FRACTION 5	0.53±0.00	2.67±0.00	ND	ND	0.18±0.00	ND	ND	0.31±0.00
STATION 3								
FRACTION 1	ND	2.52±0.00	ND	ND	ND	ND	ND	ND
FRACTION 2	ND	50.9±0.06	0.12±0.00	ND	ND	ND	ND	ND
FRACTION 3	1.21±0.00	133±0.73	1.17±0.00	0.42±0.00	1.09±0.00	0.04±0.00	ND	0.70±0.00
FRACTION 4	0.93±0.00	6.31±0.07	0.06±0.00	ND	0.80±0.00	ND	ND	0.06±0.00
FRACTION 5	1.89±0.09	5.08±0.07	1.48±0.02	0.28±0.01	0.88±0.20	ND	ND	0.01±0.08

ND-not detected

4.3 Comparison of concentration of metals in each fraction at different stations between BB and TB landfill

Comparison of metal species between BB and TB landfill revealed that in general, in BB (which is an operating landfill), Zn was the most dominant metal found at the entrance to the landfill (Station 3) in the mobile fraction. This was followed by Mn which was predominantly found inside the dumping area (Station 1). In the immobile fraction, however, Cu was detected mostly inside the dumping area (Station 1) and was followed by Pb which was also predominant inside the dumping area (Station 1) and at the entrance to the landfill (Station 3) (Table 4.3). In contrast to BB, in TB (which is a closed landfill), Mn was the most dominant metal observed in all fractions. In the mobile fraction, it was mostly concentrated entrance to the landfill (Station 3). However, in the immobile fraction, Mn was predominantly observed inside the dumping area (Station 1) (Table 4.4).

Speciation analysis therefore revealed that differences exist between the mobile and immobile forms of heavy metals found in the operating and closed non sanitary landfill examined in this study. While in BB (which is an operating landfill), different metals were observed to dominate the different fractions in the mobile and immobile forms, in TB landfill (which is a closed landfill), only a particular metal i.e Mn was observed to dominate over all other metals in the mobile and immobile fractions. The specific forms of metals observed in the mobile and immobile fraction in BB and TB could be due to the fact that both landfills are in different stages of waste degradation. Further, aside the fact that the landfills are of different age, inactivity or activity may have affected the concentration of the metals found in each fraction.

4.4 Potential mobility and bioavailability of heavy metals in BB and TB landfill

This study's third objective was to assess the mobility and bioavailability of heavy metals in leachate contaminated soil from BB and TB landfills.

4.4.1 Potential mobility and bioavailability of heavy metals in BB landfill

In this study, the percentage of metals extracted in the most mobile fractions (F1 + F2 + F3) for all stations in BB landfill is shown in Table 4.5 to 4.7. Zn at all three stations showed the greatest amounts in the bioavailable fractions accounting for 100% in Station 1 and Station 2 and 92.2% in Station 3. This was followed closely by Mn with 100% and 87.2% in Station 1 and Station 2, respectively. This investigation also revealed that Cr, Cu, and Pb were chiefly present in the immobile fractions. Table 4.8 shows the order of mobility of heavy metals in BB landfill from the most bioavailable (F1) to the least bioavailable (F5). Zn and Mn were the most mobile while the least mobile metals were Cr and Cu in all stations. A comparison of the distribution of the heavy metals in order to understand the mobility at all stations from BB is presented in Table 4.8.

Table 4.5: Percentage distribution of metals in the sequential extraction at Station 1 in BB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	5.85	5.01	89.1	5.85	94.2
Mn	87.6	ND	0.41	ND	12.0	88.0	12.0
Co	ND	ND	ND	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	3.39	96.6	ND	100
Zn	39.5	ND	60.5	ND	ND	100	ND
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	10.2	ND	6.63	9.57	73.6	16.8	83.2

ND-not detected

Table 4.6: Percentage distribution of metals in the sequential extraction at Station 2 in BB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	ND	ND	100	ND	100
Mn	100	ND	ND	ND	ND	100	ND
Co	ND	ND	ND	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	ND	ND	ND	ND
Zn	ND	ND	100	ND	ND	100	ND
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	12.6	ND	2.01	19	66.4	14.5	79.1

ND-not detected

Table 4.7: Percentage distribution of metals in the sequential extraction at Station 3 in BB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	14.5	21.0	64.5	14.5	85.5
Mn	26.7	15.9	18.1	6.53	32.7	60.8	39.3
Co	ND	ND	ND	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	37.8	37.2	ND	75.04
Zn	ND	20.6	71.6	14.8	0.11	92.2	14.9
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	ND	0.80	25.6	20.1	53.5	26.4	73.6

ND-not detected

Table 4.8: Comparison of distribution heavy metals in terms of mobility at all stations from BB in percentages

Stations	Order of mobility heavy metals (%)	
	Mobile Σ(F1+F2+F3)	Non mobile Σ (F4+ F5)
Station 1	Zn (100) > Mn (87.2)	Cu (100), Cr (94.2), Pb (83.2)
Station 2	Mn (100), Zn (100)	Cr (100), Pb (79.1)
Station 3	Zn (92.2) > Mn (60.8)	Cr (85.5), Cu (75.0), Pb (73.6)

4.4.2 Potential mobility and bioavailability of heavy metals in TB landfill

In this study, the percentage of metals extracted in the most mobile fractions (F1 + F2 + F3) for all stations in TB landfill is shown in Table 4.9 to 4.11. In all stations, Zn and Mn showed the greatest amounts in the bioavailable fractions, ranging from 76% to exactly 100% extracted of the total contents in the F1, F2 and F3 for both metals. The high percentage of mobile fractions of Zn and Mn can be attributed to sources such as scrap metals in the mixed waste (Karim *et al.,* 2014). Consequently, high mobility of Zn and Mn could cause environmental pollution because these metals are easily available for biological functions. Pb and Co in all stations (except Co in Station 2) showed high percentage mobility of over 50% and thus their potential to cause ecological risk (Karim *et al.,* 2014).

Meanwhile, immobile fractions (F4 and F5) of Cr and Cu in all stations were higher than mobile fractions (Table 4.9 to 4.11). Thus an indication that Cr and Cu maybe of lithosphere-origin (Sungur *et al.,* 2015) and the soil in TB is relatively unpolluted by these metals. Although metals extracted in the F4 and F5 are generally considered to be neither mobile nor bioavailable, it is unlikely that any naturally occurring processes could dissolve these metals (Øygard *et al.,* 2008) and make them bioavailable in TB landfill and its nearby environment. Further, Table 4.12 shows the order of mobility of heavy metals in TB landfill from the most bioavailable (F1) to the least bioavailable (F5). Zn was observed to be the most mobile metal in all stations while Cu was the least mobile metal in all stations except in Station 3.

Table 4.9: Percentage distribution of metals in the sequential extraction at Station 1 in TB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	21.6	30.2	48.3	21.6	78.5
Mn	14.3	8.11	55.4	5.06	17.1	77.9	22.1
Co	4.98	2.36	59.6	9.30	23.8	67	33.1
Ni	ND	ND	31.9	9.26	58.9	31.9	68.2
Cu	ND	ND	13.1	59.6	27.4	13.1	86.9
Zn	ND	ND	92.7	6.43	0.85	92.7	7.28
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	55.8	26.9	15.3	55.8	42.2

ND-not detected

Table 4.10: Percentage distribution of metals in the sequential extraction at Station 2 in TB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	36.4	6.02	57.6	36.4	63.7
Mn	3.10	1.11	72.6	2.99	9.74	76.8	12.7
Co	ND	ND	100	ND	ND	ND	ND
Ni	ND	ND	100	ND	ND	ND	ND
Cu	ND	ND	30.3	27.7	42	30.3	69.7
Zn	ND	ND	100	ND	ND	100	ND
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	65.9	ND	34.1	65.9	34.1

ND-not detected

Table 4.11: Percentage distribution of metals in the sequential extraction at Station 3 in TB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr	ND	ND	30	23.1	46.9	30	70
Mn	1.27	25.7	67.3	3.18	2.57	94.2	5.80
Co	ND	7.92	79.5	3.75	8.86	87.4	12.6
Ni	ND	ND	60.4	ND	39.6	60.4	39.6
Cu	ND	ND	39.4	28.9	31.7	39.4	60.6
Zn	ND	ND	100	ND	ND	100	ND
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	90.6	8.10	1.36	90.6	9.45

ND-not detected

Table 4.12: Comparison of distribution heavy metals in terms of mobility at all stations from TB in percentages

Stations	Order of mobility heavy metals (%)	
	Mobile Σ(F1+F2+F3)	Non mobile (F4 + F5)
Station 1	Zn (92) > Mn (77.9) > Co (67) > Pb (55.8) > Cu (31.9) > Cr (21.6)	Cu (86.9), Cr (78.5)
Station 2	Zn (100) > Mn (76.8) > Pb (65.9) > Cr (36.4) > Cu (30.3)	Cu (69.7), Cr (63.7)
Station 3	Zn (100) > Mn (90.6) > Co (87.4) > Ni (60.4) > Cu (39.4) > Cr (30.0)	Cr (70.0), Cu (60.6)

4.4.3 Comparison of potential mobility of the heavy metals in BB and TB landfill

In this study, a comparison of the overall potential mobility of the heavy metals observed in BB and TB landfill is as shown on Figure 4.1.

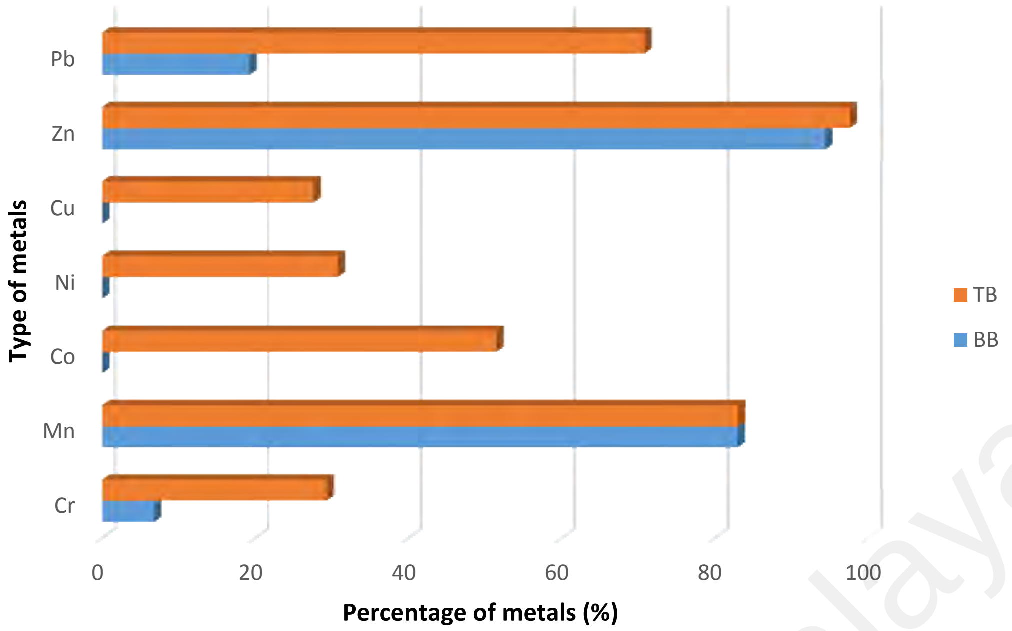

Figure 4.1: Potential mobility of metals in BB and TB landfill

The overall trend of mobility of metals in each landfill can be summarized as follows:

In BB: Zn>Mn>Pb>Cr

In TB: Zn>Mn>Pb>Co>Ni>Cr>Cu

Even though the area in which the most dominant mobile forms of each heavy metal was observed to differ between the two landfill, the trend of mobility of the heavy metals is similar. The trend in the mobility of the heavy metals shows that Zn, Mn and Pb have the same order of mobility in both landfill. This could be due to the similarity in the composition of waste materials received in both landfills and that the non sanitary condition that prevail in both landfill allows the mobility of Zn, Mn, Pb to supersede others metals studied. Furthermore, a comparison of the trend of potential mobility of heavy metals between BB and TB shows that for most metals namely Cr, Ni, Co, Cu and Pb, there is a difference in mobility (Figure 4.1). In addition, metals in TB showed more mobility than in BB and this could be due to the age and status (inactivity) of the landfill.

Further, the location (area) of mobile and immobile forms of the dominant metal varied in each landfill. In TB, Cr was mostly found in the mobile form in the open area within the waste cells while in contrast to BB, Cr was mostly found in the mobile form at the entrance to the dumping area. For Ni, Co, Cu and Pb the most mobile forms were observed at the entrance to the landfill in TB while in BB, Cu and Pb were mostly observed in the immobile forms inside the dumping area while Ni, Co and Cd was not detected in either forms in all samples. This further indicates that the status of the landfill strongly influences the species of metals observed in the mobile and immobile form.

The implications of mobility of heavy metals in an environment is their potential for environmental toxicity due to bioavailability to flora and fauna. In this study, metals in TB showed more mobility and therefore may pose more threat to the environment although in BB, disposal activities is still on-going, the concentration of heavy metals in the mobile phase may not present immediate threat and thus proper post-closure plans of the landfill could help prevent the deleterious impact of leaching of these metals into the environment. Further, health and environmental issues can be magnified considering the National Urban Policy in Malaysia to redevelop a total of 296 ex-landfills (such as TB) into residential housing projects, public parks and commercial areas by 2020 (Simis and Awang, 2015).

4.5 Indicators of pollution

In order to assess the degree of contamination in the sampled sites of BB and TB landfill, two indices of pollution: contamination factor (C_f) and risk assessment code (RAC) were calculated for each of the heavy metals found in this study. The individual contamination factors (C_f) and risk assessment code (RAC) of metals in soil samples from BB (Table 4.13) and TB (Table 4.14) are further discussed.

4.5.1 Contamination factor (Cf) and Risk assessment code (RAC) of metals in BB and TB landfill

4.5.1.1 Contamination factor (C_f) of metals in BB and TB landfill

The contamination factor (C_f) is one of the indices of pollution used to assess the level of heavy metal contamination in soils and sediments (Salah *et al.,* 2012; Ololade, 2014). Hakason (1980) proposed that contamination factor (C_f) <1 indicates low contamination, 1≤ (C_f) <3 indicates moderate contamination, 3≤ (C_f)< 6 indicates considerable contamination and (C_f) > 6 very high contamination. In this study, the individual contamination factor (C_f) of heavy metals was determined by dividing the sum of each heavy metal concentration in the F1, F2, F3, F4 by its concentration in the residual phase (F5) (Nemati *et al.,* 2011) as shown in Table 4.13 and Table 4.14 for BB and TB landfill, respectively.

The individual contamination factors of each metal at all stations in BB landfill is shown in Table 4.13. In BB landfill, the highest individual contamination (C_f) was Zn and Mn at the entrance to the landfill and inside the dumping area, respectively while Cr, Cu and Pb have the lowest C_f indicating low contamination. In general, C_f results shows that the level of contamination at BB landfill is quite low and most metal pose no immediate threat to the environment.

The individual contamination factors of each metal at all stations in TB landfill is as shown in Table 4.14. In TB, similar to BB, the highest C_f was Zn however it was mostly observed inside the dumping area indicating very high contamination. Similarly, Pb and Mn were both found to have very high contamination in the open area between sealed waste cells and the entrance to the landfill. Moderate contamination from Cr and Cu was observed inside the dumping area and at the entrance to the landfill. Ni inside the dumping area had the lowest C_f indicating low contamination. In general, C_f results showed higher level of contamination at TB landfill than BB and most metal may pose a threat to the environment. The C_f values agrees with the trend of mobility observed at each landfill.

Table 4.13: C_f and RAC values for heavy metals present in all three stations at Bukit Beruntung landfill

	Cr	Mn	Cu	Zn	Pb
Station 1					
C_f	0.21	7.32	0.04	-	0.36
RAC	-	87.6	-	39.5	10.2
Station 2					
C_f	-	-	-	-	0.51
RAC	-	100	-	-	12.6
Station 3					
C_f	0.69	2.06	0.39	90.3	0.80
RAC	-	42.7	-	20.6	0.89

- = not applicable

Table 4.14: C_f and RAC values for heavy metals present in all three stations at Taman Beringin landfill

	Cr	Mn	Co	Ni	Cu	Zn	Pb
Station 1							
C_f	1.07	4.86	3.21	0.70	2.65	117	-
RAC	-	14.3	4.98	-	-	-	-
Station 2							
C_f	0.74	9.26	-	-	1.38	-	30.0
RAC	-	3.10	-	-	-	-	-
Station 3							
C_f	1.13	37.9	10.3	1.53	2.16	-	72.8
RAC	-	1.27	-	-	-	-	-

- = not applicable

4.5.1.2 Risk Assessment Code (RAC) of metals in BB and TB landfill

The risk assessment code is another indices of pollution that applies scale of proportions of the metals in the bioavailable fraction (which could be taken by plants, animals causing environmental toxicity) to determine the availability of sedimentary metals (Li *et al.,* 2016). According to RAC classifications described by Perin *et al.* (1985), for any metal, soil/sediment which can release less than 1% of the total metal in the exchangeable and carbonate fractions will be considered safe for the environment and soil/sediment with 11% to 30% carbonate and exchangeable fractions will pose medium risk to the environment. In contrast, soil/sediment releasing 31% to 50% carbonate and exchangeable fractions will pose high risk to the environment and more than 50% of the total metal in the exchangeable and carbonate fractions has been considered highly dangerous, and can easily enter the food chain (Abdullah, 2012; Sarkar *et al.,* 2014; Saleem *et al.,* 2015). In this study, the RAC was calculated by summing the percentage fraction of metal exchangeable and/or associated with carbonates for the eight heavy metals studied in both landfill (Table 4.13 and Table 4.14, respectively). Table 4.15 and 4.16 shows the RAC classification for all stations in BB and TB respectively.

Table 4.15: RAC classification of heavy metals present in all three stations at Bukit Beruntung landfill

	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
Station 1	N	VH	N	N	N	H	N	L
Station 2	N	VH	N	N	N	N	N	M
Station 3	N	H	N	N	N	M	N	N

N-No risk; L – Low risk; M- Medium risk; H- High risk ; VH –Very high risk

The results obtained indicate that in BB, Mn posed a very high risk to the environment in all sampled stations and Zn inside the dumping area also showed high risk to the environment (Table 4.15). Pb was found to pose moderate risk to the environment while Cr, Co, Ni, Cu and Cd poses no risk to the environment.

Table 4.16: RAC classification of heavy metals present in all three stations at Taman Beringin landfill

	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
Station 1	N	M	L	N	N	N	N	N
Station 2	N	L	N	N	N	N	N	N
Station 3	N	M	L	N	N	N	N	N

N-No risk; L – Low risk; M- Medium risk; H- High risk ; VH –Very high risk

In TB, Mn was found to pose moderate risk according to the RAC classification (Table 4.16). Although mobility of some metals in TB were observed as discussed above, the concentration of metals in the exchangeable and carbonate fraction was not detected (except for Mn and Co). Therefore, only the percentage of metal concentration in the Fe and Mn oxide metal bound fraction accounted for the high mobility of metals in TB. Because RAC classification are dependent solely on the concentration of metals in the exchangeable and carbonate fraction, no risks was observed for most metals in TB (Table 4.16).

4.6 Chemical speciation of heavy metals in bioaugmented and non-bioaugmented soils from BB and TB landfills

This study's fourth objective was to compare the speciation of heavy metals in bioremediated and non-bioremediated soils from Bukit Beruntung (BB) and Taman Beringin (TB) landfills. In this study, in order to evaluate the bioaugmentation potential of indigeneous microorganisms in BB and TB, sequential extraction procedures was used to identify changes associated with metal speciation in bioaugmented soil. The results obtained at "Day 0" (prior to the addition of the microorganism at the beginning of the bioaugmentation) were compared to "Day 100" (which marked the end of the bioaugmentation) and with control soils (leachate contaminated soils without the addition micoorganisms). Detailed discussion of each metal and their speciation before and after bioaugmentation in each landfill is given in subsequent sections. In addition, the percentage reduction of the heavy metals at Day 100 compared to Day 0 was calculated using the following formula:

Removal % = $[(C_o - C_f)/ C_o] \times 100$……………equation 4.1

where C_o and C_f are the initial and final concentration (µg/g) of metal in soil, respectively (Uzun and Güzel, 2000; Salehzadeh, 2013).

4.6.1 Chemical speciation of each heavy metal in bioremediated and non-bioremediated soils from BB landfill

(a) Chromium

The chemical fractions or specific forms of Cr before and after bioaugmentation is shown in Table 4.17. In the non bioaugmented soil at Day 0 or microcosm at the beginning of the experiment, Cr in F1 (0.82µg/g) and F3 (0.43 µg/g) recorded the highest concentration of the mobile forms (Table 4.17). In the immobile form, Cr (0.67 µg/g) in F5 was more dominant than in F4 (0.40 µg/g). After bioremediation at Day 100, the immobile fraction was found to be more prevalent. Further, the concentration of Cr in F1 was 0.06 µg/g indicating a 92.7% reduction in mobile Cr. In F3 (0.16 µg/g), over 90% reduction was observed. According to Avudainayagam *et al.* (2003), reduced Cr is usually bound to various ligands in soil solution and hence rendered insoluble, immobile and unreactive.

Soil microcosm with microbial formula recorded reduction of 54% to 93% compared to control soil where reduction was between 37% to 87%. Reduction in concentration of Cr in control soil microcosm at Day 100 was observed. This could be due to natural bioattenuation phenomenon (Emenike *et al.,* 2016).

Table 4.17: Concentration of Cr in the various chemical fractions of bioaugmented and non bioaugmented soil from BB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.82	0.09	0.43	0.40	0.67	2.41
100	Bioaugmented soil at Day 100	0.06	0.09	0.16	0.18	0.31	0.80
Control	Non bioaugmented soil at Day 100	0.11	0.09	0.16	0.20	0.42	0.98
	Percentage Reduction from Day 0	**92.7**	**-**	**62.8**	**55**	**53.7**	

- = no reduction in percentage of metal

The distribution and percentages of Cr in the various chemical fractions in BB landfill before and after remediation is shown in Figure 4.2. The percentage of the F1 which was 34% in non bioaugmented soil at Day 0 was found to have decreased to 7.5% in bioaugmented soil at Day 100. The F5 however was observed to have increased at Day 100 (38.8%). This could be due to immobilization of Cr after bioaugmentation. Similar to this study, Krishna and Philip (2005) and Jeyasingh and Philip (2005) also observed Cr immobilization by indigenous microbial population in chromium contaminated soils in India. In control soil, reduction in Cr (11.2%) was also observed, however it was at a lower rate compared to 7.5% in bioaugmented soil at Day 100.

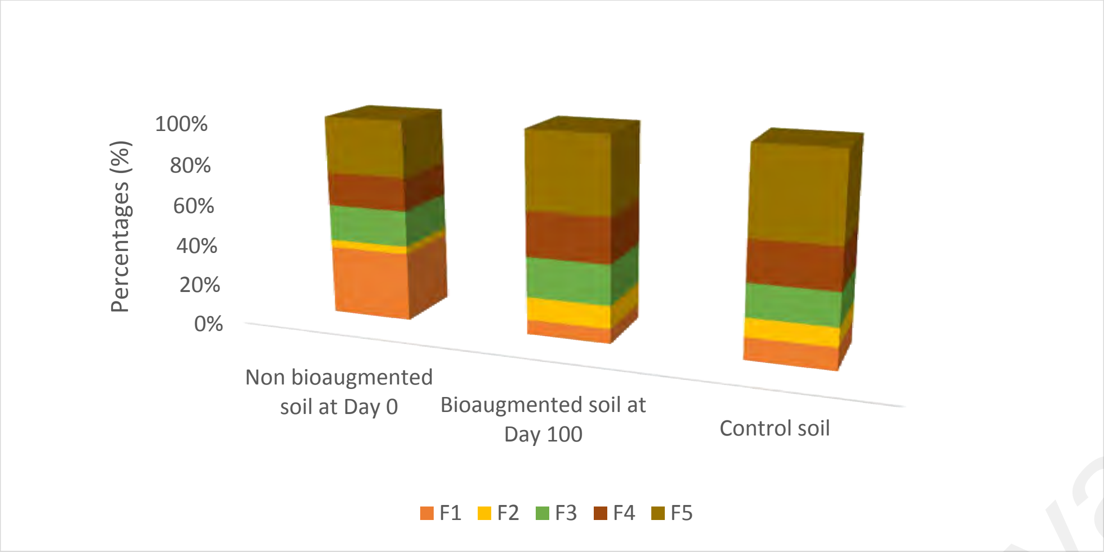

Figure 4.2: Cr distribution and percentage before and after remediation in BB landfill

Therefore, efficiency of the bioaugmentation observed in this study in reducing the mobile and bioavailable form of Cr (in F1 and F3) indicates that indigenous microorganisms in BB landfill can decrease the concentration of Cr in leachate contaminated soil and thus prevent ecotoxicity of Cr. Krishna and Philip (2005) also showed that isolated microbial consortia from Cr contaminated soils showed good Cr reduction capacity.

(b) Manganese

In this study, in contrast to Cr, the mobile form of Mn was observed to be higher than the immobile forms in bioaugmented soil at Day 100 (Table 4.18). F2 had the highest concentration of Mn with 11.6 µg/g in BB landfill followed by F3 with 8.01 µg/g and F1 with 1.21 µg/g (Table 4.18). Even though in bioaugmented soil at Day 100, 56% to 70% reduction in the concentration of Mn was observed in F1 and F2, the F2 at 3.56 µg/g and F3 at 5.08 µg/g still remained the most dominant form of Mn in BB (Table 4.18). Reduction in the percentage of Mn can be attributed to the microorganisms used in the bioaugmentation (Sinha *et al.*, 2010).

Table 4.18: Concentration of Mn in the various chemical fractions of bioaugmented and non bioaugmented soil from BB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	1.21	11.6	8.01	0.65	0.91	22.3
100	Bioaugmented soil at Day 100	0.53	3.56	5.08	0.30	0.43	9.90
Control	Non bioaugmented soil at Day 100	0.70	4.45	4.68	0.26	0.31	10.4
	Percentage reduction from Day 0	**56.2**	**69.2**	**36.6**	**53.8**	**52.7**	

The distribution and percentages in the various chemical fractions of Mn before and after remediation in BB landfill is shown in Figure 4.3. Similar to Cr, the distribution of Mn was found to be 5.42% in F1 and 51.7% in F2 at Day 0 in non bioaugmented soil (Figure 4.3). After bioaugmentation at Day 100, a decrease in percentage of F1 (5.35%) and F2 (36%) was observed while an increase in the F3, F4 and F5 occurred (Figure 4.3). Therefore, addition of the microbial formula altered the distribution of Mn in soil. The percentage of F1 and F3 in control soil did not decrease when compared to bioaugmented soil at Day 100 (Figure 4.3).

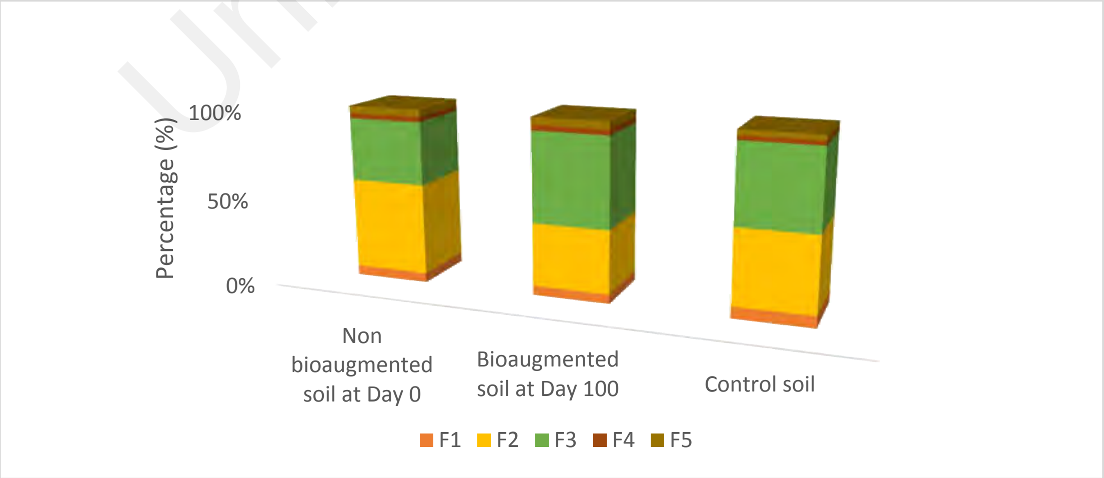

Figure 4.3: Mn distribution and speciation before and after remediation in BB landfill

(c) Copper

In this study, Cu desorption decrease was observed to be in the following order: F4>F5>F2>F1>F3 in non bioaugmented soil at Day 0 (Table 4.19). The highest concentration of Cu was observed in the F4 and F5. According to Kanmani and Gandhimathi (2012), Cu has been well known to be an immobile metal in soil. Although high percentage reduction (over 80%) of the mobile and immobile forms of Cu were observed in bioaugmented soil at Day 100, the most prevalent form of Cu were still associated with F4 and F5 at Day 100 (in contrast to Mn) (Table 4.19 and Figure 4.4). Similar to other metals, natural bioattenuation phenomenon may have played a key role in reducing Cu concentration in control soil (Figure 4.4).

Table 4.19: Concentration of Cu in the various chemical fractions of bioaugmented and non bioaugmented soil from BB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.09	0.42	0.03	2.22	1.71	4.47
100	Bioaugmented soil at Day 100	0.01	0.06	ND	0.80	0.28	1.15
Control	Non bioaugmented soil at Day 100	0.00	0.10	0.02	0.02	0.34	1.15
	Percentage reduction from Day 0	**88.9**	**85.7**	**100**	**64**	**83.6**	

ND- not detected

The distribution and percentages of Cu in the various chemical fractions was observed to be similar before and after remediation in BB landfill (Figure 4.4). It followed the pattern: F4>F5>F2>F1>F3 in both non bioaugmented soil at Day 0 and bioaugmented soil at Day 100 (Figure 4.4). However, a decrease in percentage distribution of Cu was observed in bioaugmented soil at Day 100 in F1 (0.87%), F2 (5.22%), F3 (0%) and F5

(24.35%) compared to in non bioaugmented soil at Day 0 where the percentage distribution was F1 (2.01%), F2 (9.36%), F3 (0.67%) and F5 (38.26%) (Figure 4.4).

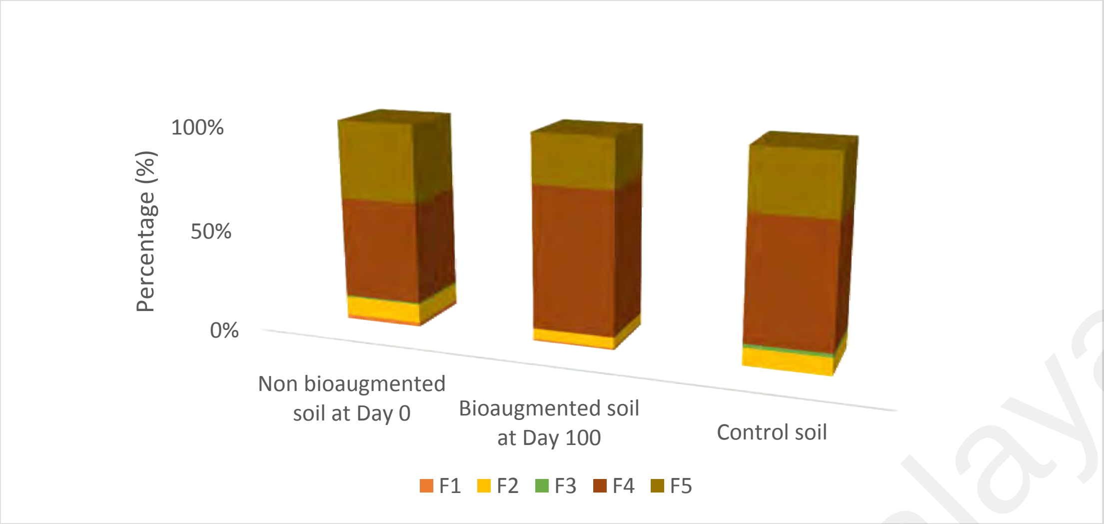

Figure 4.4: Cu distribution and speciation before and after remediation in BB landfill

(d) Zinc

Zn was only observed in four fractions in BB. The most dominant form was the mobile form before and after remediation (Table 4.20). The mobile forms- F2 and F3 recorded 0.35 µg/g and 1.76 µg/g of Cu, respectively while the immobile forms F4 and F5 recorded 0.46 µg/g and 0.49 µg/g in non bioaugmented soil at Day 0 (Table 4.20). Reduction in metal concentration at F2, F4 and F5 were observed in bioaugmented soil at Day 100 (0.16 µg/g, 0.17 µg/g and 0.01 µg/g respectively) (Table 4.20 and Figure 4.5). Suprisingly though, metal concentration at F3 in bioaugmented soil at Day 100 was found to have increased to 1.93 µg/g (Table 4.20). This could be due to remobilization of the Zn by micro organisms (Gadd, 2005). Additionally, Krishna *et al.* (2014) reported that Zn removal efficiency by certain bacteria has been observed to fluctuate due to factors such as pH and thus this could also explain the increase in F3 of Zn and decrease in the immobile fractions at Day 100.

Table 4.20: Concentration of Zn in the various chemical fractions of bioaugmented and non bioaugmented soil from BB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	ND	0.35	1.76	0.46	0.49	3.06
100	Bioaugmented soil at Day 100	ND	0.16	1.93	0.17	0.01	2.27
Control	Non bioaugmented soil at Day 100	ND	0	1.78	0.22	0.04	2.04
	Percentage reduction from Day 0	**ND**	**54.9**	+	**63**	**98**	

ND- not detected; += increase in metal

The results of the distribution and percentages of Zn in the various chemical fractions before and after remediation in BB landfill is shown in Figure 6. The results show that the order of distribution of Zn in non bioaugmented soil at Day 0 is different from that in bioaugmented soil at Day 100. In bioaugmented soil at Day 100, the order was F3>F4>F2>F5>F1 while in non bioaugmented soil at Day 0, it was F3>F5>F4>F2>F1. Therefore, addition of microbial formula decreased the concentration of Zn from 11.4% to 7.05% in F1 and from 16% to 0.44% in F5 (Figure 4.5). In control soil however, natural bioattenuation could have led to complete reduction of the F1 and F2 of Zn (Figure 4.5).

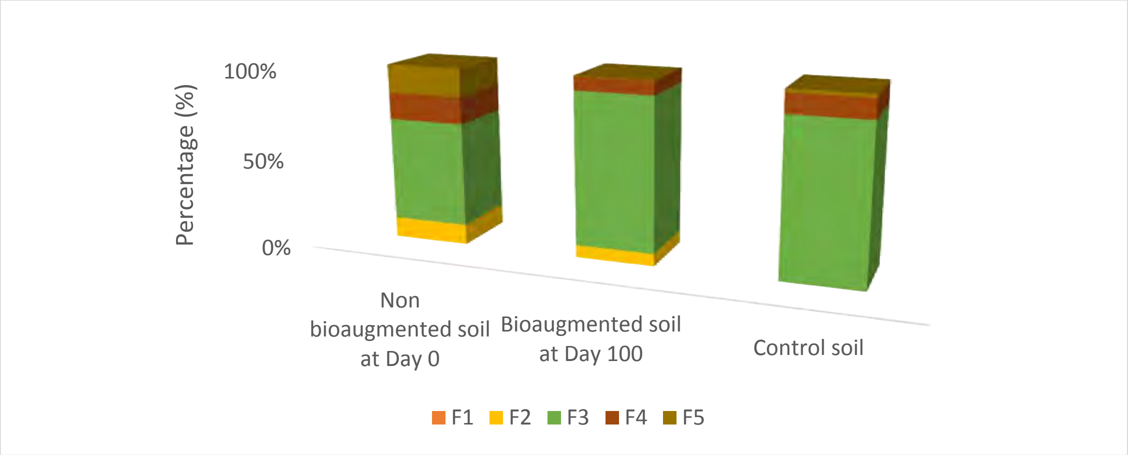

Figure 4.5: Zn distribution and speciation before and after remediation in BB landfill

(e) Lead

The trend of Pb concentration in all fractions in BB landfill was observed to be very different from all other metals found in this study. Concentration of metals in bioaugmented soil at Day 100 and control soil were observed to have increased compared to non bioaugmented soil at Day 0 (Figure 4.6). The immobile form of Pb was the most dominant in non bioaugmented soil at Day 0 (0.36 µg/g in F4 and 0.40 µg/ in F5) and in bioaugmented soil at Day 100 (0.69 µg/g in F4 and 1.00 µg/g in F5) (Table 4.21).

Table 4.21: Concentration of Pb in the various chemical fractions of bioaugmented and non bioaugmented soil from BB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.03	0.06	0.54	0.36	0.40	1.39
100	Bioaugmented soil at Day 100	0.24	0.07	0.26	0.69	1.00	2.26
Control	Non bioaugmented soil at Day 100	0.09	0.08	0.25	0.87	1.23	2.52
	Percentage reduction from Day 0	+	+	**51.9**	+	+	

+= increase in metal

In contrast to other metals, Pb distribution differed in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100 (Figure 4.6). In all the various chemical fractions (except for F3) in bioaugmented soil at Day 100, Pb percentage increased (from 28.8 % to 44.3%) in F5 and from 25.9% to 30.5% in F4 (Figure 4.6). This could be due to decrease in Pb-resistant bacteria and increased solubility of the Pb in the soil solution as a similar trend was observed even in control soil.

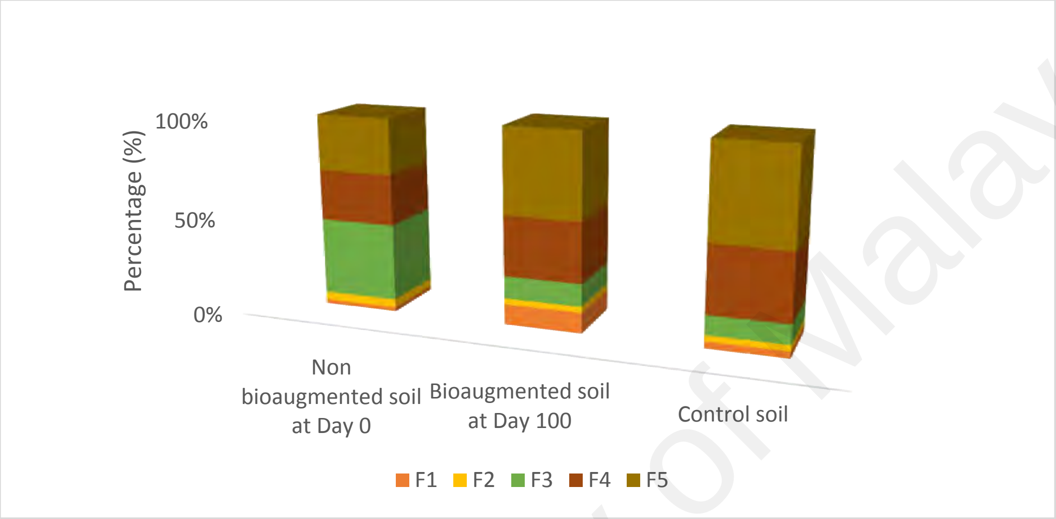

Figure 4.6: Pb distribution and speciation before and after remediation in BB landfill

4.6.2 Chemical speciation of heavy metals in bioaugmented and non-bioaugmented soils from TB landfill

(a) Chromium

The distribution of Cr in TB landfill indicates that its content in various fractions varied in the microcosm at the beginning of the experiment (in non bioaugmented soil at Day 0) (Figure 4.7), F4 and F5 had the highest concentration at 1.61 µg/g and 6.64 µg/g, respectively while F1, F2 and F3 were 0.060 µg/g, 0.07 µg/g and 0.70 µg/g of Cr, respectively (Table 4.20). The F4 and F5 recorded the highest amount of Cr and thus the immobile fraction predominates over the mobile fraction in non bioaugmented soil at Day 0 (0.36 µg/g in F4 and 0.40 µg/ in F5) and in bioaugmented soil at Day100 (0.69

µg/g in F4 and 1 µg/g in F5) (Table 4.22 and Figure 4.7). Furthermore, concentration of Cr was found to have decreased in bioaugmented soil at Day 100 (0.69 µg/g in F4 and 1 µg/g in F5) across all fractions (Table 4.22). The highest percentage removal was observed in F1 in bioaugmented soil at Day 100 (Table 4.22). Similar to this study, Jeyasingh and Philip (2005) reported that indigenous soil microbial community were able to reduce approximately 97% of hexavalent Cr(VI) (mobile and toxic form of Cr) in contaminated soil. Also, Bader *et al.* (1999) reported that reduction in Cr concentration in contaminated sites is due to widespread Cr(VI) reducing bacteria in such soil.This could explain the reduction in Cr observed in this study.

Table 4.22: Concentration of Cr in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.06	0.07	0.70	1.61	6.64	9.08
100	Bioaugmented soil at Day 100	ND	0.05	0.22	0.78	1.86	2.91
Control	Non bioaugmented soil at Day 100	0	0	0	0.13	0.74	0.87
	Percentage reduction from Day 0	**100**	**28.6**	**68.6**	**51.6**	**72**	

ND-not detected

A decrease in percentage distribution of Cr in bioaugmented soil at Day 100 (except in F2 and F4) was observed compared to its distribution in non bioaugmented soil at Day 0 (Figure 4.7). Cr decreased from 73.3% to 63.9% in F5, 7.71% to 7.56% in F3 and 0.66% to 0% in F1 (Figure 4.7). The influence of microorganisms in contaminated soil was also observed to decrease Cr distribution in F1, F2, F3 in control soil (Figure 4.7).

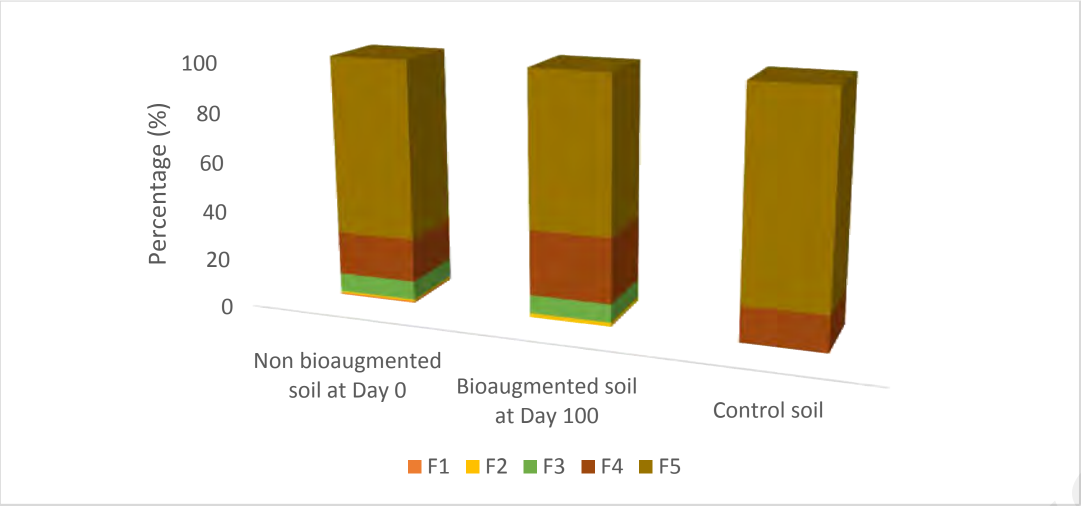

Figure 4.7: Cr distribution and speciation before and after remediation in TB landfill

(b) Manganese

High concentration of Mn was observed in TB landfill. Sources of manganese in MSW can be traced to the disposal of bottle caps, cosmetics, insecticides, paints and pigments along with domestic garbage (Kanmani and Gandhimathi, 2013). The mobile form of Mn was highest with F1, F2 and F3 having 6.56 µg/g, 25.2 µg/g and 22.9 µg/g, respectively in non bioaugmented soil at Day 0 (Table 4.23 and Figure 4.8). Similar to BB, mobile form of Mn still dominated over the immobile form even in bioaugmented soil at Day 100. According to Nadaska (2010) and Habibah *et al.* (2014), Oxide-Mn is readily reduced to available forms and is an important source of Mn for plants and so elevated levels observed in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100 is easily bioavailable in the environment. The removal efficiency in bioaugmented soil at Day 100 was about 49% in F1, 71% in F2 and 64% in F3. Reduction in Mn immobile forms was at lower rate in F4 (19.8%) as compared to other fractions. In F5, a 76.1% reduction was also observed (Table 4.23).

Table 4.23: Concentration of Mn in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	6.56	25.2	22.9	3.03	16.9	74.6
100	Bioaugmented soil at Day 100	3.34	7.27	8.12	2.43	4.04	25.2
Control	Non bioaugmented soil at Day 100	1.05	6.71	6.46	0.35	1.96	16.5
	Percentage reduction from Day 0	**50.5**	**71.1**	**64.6**	**19.8**	**76.1**	

Mn distribution and speciation before and after remediation in TB landfill (Figure 4.8) revealed that Mn reduced from 33.7% to 28.9% in F2 and 22.7% to 6.03% in F5 (Figure 4.8). However, an increase in the percentage of F3 and F4 occurred in bioaugmented soil at Day 100 (Figure 4.8). Similar to BB, addition of the microbial formula altered the distribution of Mn in soil.

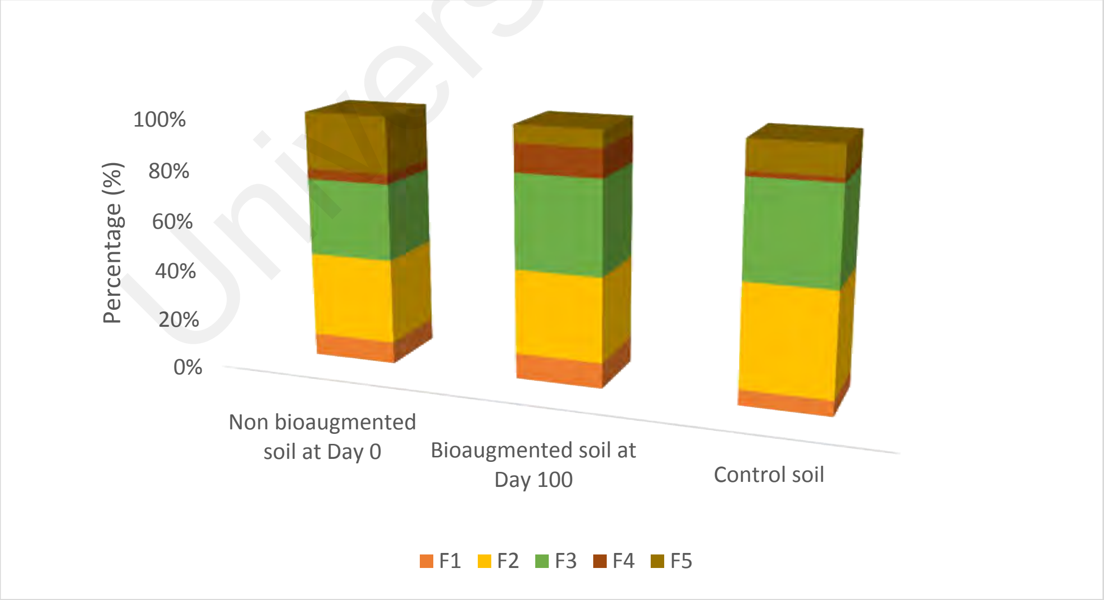

Figure 4.8: Mn distribution and speciation before and after remediation in TB landfill

(c) Cobalt

Speciation of Co revealed that the most dominant fraction was in the immobile form in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100. Additionally, complete reduction (100%) of Co in F1, F2 and F4 was observed in soil with added microorganisms after Day 100 (Table 4.24 and Figure 4.9).

The percentage distribution of Co in the various chemical fractions was different from all other metals inn TB. Co distribution was reduced from 31.3% to 25% in F2 with a corresponding increase in the percentage distribution in F5 from 57.4% to 75%. There bioaugmentation of soil reduced the bioavailable form of Co in TB landfill. Complete reduction of Co in control soil can be attributed natural bioattenuation phenomenon (Emenike *et al.,* 2016).

Table 4.24: Concentration of Co in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	ND	0.06	0.36	0.07	0.66	1.15
100	Bioaugmented soil at Day 100	ND	0	0.02	0	0.06	0.08
Control	Non bioaugmented soil at Day 100	ND	0	0	0	0	0
	Percentage reduction from Day 0	**ND**	**100**	**94.4**	**100**	**90.9**	

ND-not detected

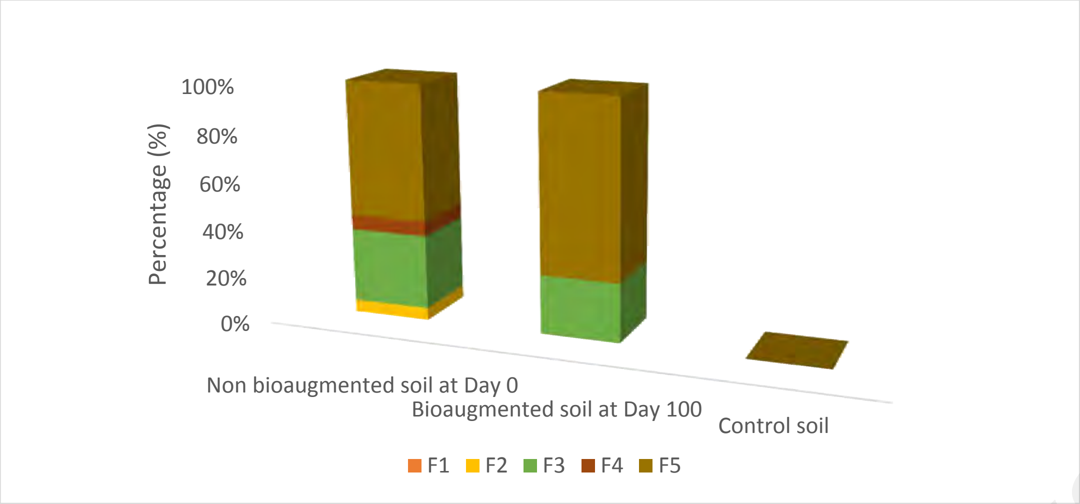

Figure 4.9: Co distribution and speciation before and after remediation in TB landfill

In comparison to other metals in this study, low levels of cobalt recorded maybe due to the fact that typically, MSW landfills such as BB and TB may not receive cobalt containing waste such as fossil fuel and waste combustion, aircraft exhausts, cobalt containing alloys, copper and nickel smelting and refining wastes, cobalt chemicals and fertilizers derived from phosphate rocks which have been observed to be the primary source of anthropogenically induced cobalt in soils (Sternenberger, 2007).

(d) Nickel

Ni was not detected in F1 and F2 in TB landfill and therefore the immobile form was more dominant especially in the residual fraction in non bioaugmented soil at Day 0. The only mobile form of this metal observed was associated with the Fe-Mn oxide bound metal fraction at 0.43 µg/g (Table 4.25). In bioaugmented soil at Day 100, the immobile form of Ni was still observed to be prevalent (Table 4.25 and Figure 4.10). In addition, in bioaugmented soil at Day 100, the percentage reduction of Ni concentration in the F3 was over 97% (Table 4.25), thus eliminating the potential risk of this metal in the environment. Similarly, over 70% reduction in the immobile form of Ni was observed after Day 100 of bioaugmentation (Table 4.25).

Table 4.25: Concentration of Ni in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	ND	ND	0.48	0.22	2.72	3.42
100	Bioaugmented soil at Day 100	ND	ND	0.01	0.05	0.62	0.68
Control	Non bioaugmented soil at Day 100	ND	ND	ND	0.32	0.14	0.46
	Percentage reduction from Day 0	**ND**	**ND**	**97.9**	**77.2**	**77.2**	

ND- not detected

The chemical fraction of Ni changed in bioaugmented soil at Day 100 (Figure 4.10) due to the addition of microbial formula to soils.Further, reduced percentage of Ni was observed in F3 from 14% to 1.47% and an increase in F5 (from 79.5% to 91.2%) was observed in bioaugmented soils at Day 100. Ni immobilization was observed in bioaugmented and control soils due to microbial action in soil.

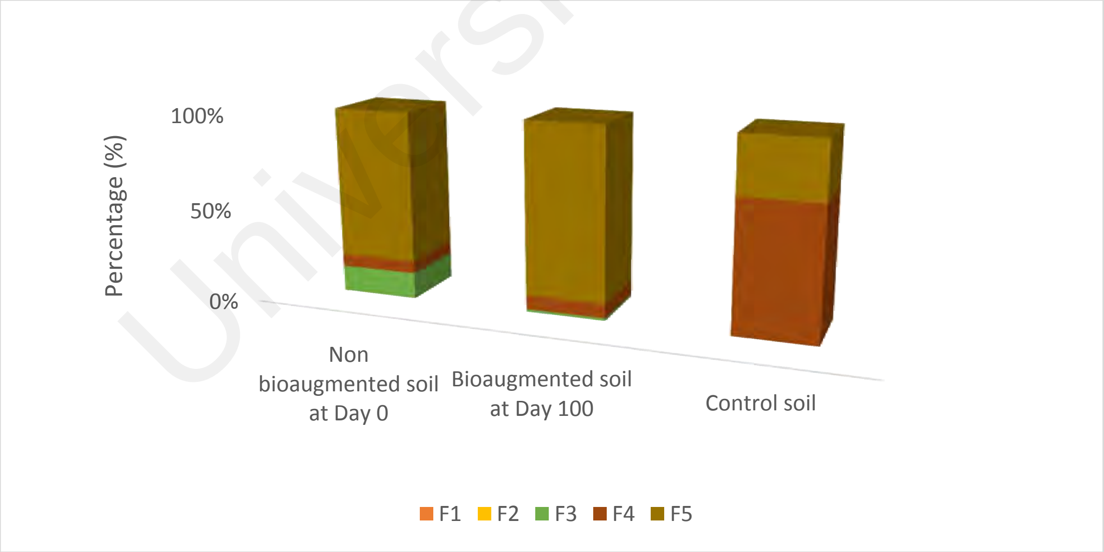

Figure 4.10: Ni distribution and speciation before and after remediation in TB landfill

(e) Copper

Similar to Ni, immobile forms of Cu were most prevalent in non bioaugmented soil at Day 0. In addition, similar to BB, mobile form of Cu was still dominant over the immobile form even in bioaugmented soil at Day 100 with 3.01 µg/g in F4 and 4.78 µg/g in F5 (Table 4.26). In the mobile form, concentration of Cu was 0.04 µg/g, 0.11 µg/g and 0.57 µg/g in F1, F2 and F3 at Day 0, respectively. By Day 100, 100% reduction in F1 and F2 of Cu was observed while over 85% reduction in F3 was recorded thus making the immobile form still prevalent (Table 4.26 and Figure 4.11). Because the concentration of Cu was majorly associated with the F4 and F5, Cu in TB landfill may not pose immediate threat to the environment.

Table 4.26: Concentration of Cu in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.04	0.11	0.57	3.01	4.78	8.51
100	Bioaugmented soil at Day 100	ND	ND	0.08	1.32	1.05	2.45
Control	Non bioaugmented soil at Day 100	ND	0	0	0.32	0.44	0.76
	Percentage reduction from Day 0	**100**	**100**	**86**	**56.1**	**78**	

ND- not detected

The percentage distribution of Cu showed there is a difference in the distribution of Cu in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100 (Figure 4.11). Cu was majorly found in the F5 in non bioaugmented soil at Day 0 (Figure 4.11). After bioaugmentation at Day 100, Cu was majorly found in F4 and this could be because Cu was completely reduced in F1and F2 (Figure 4.11).

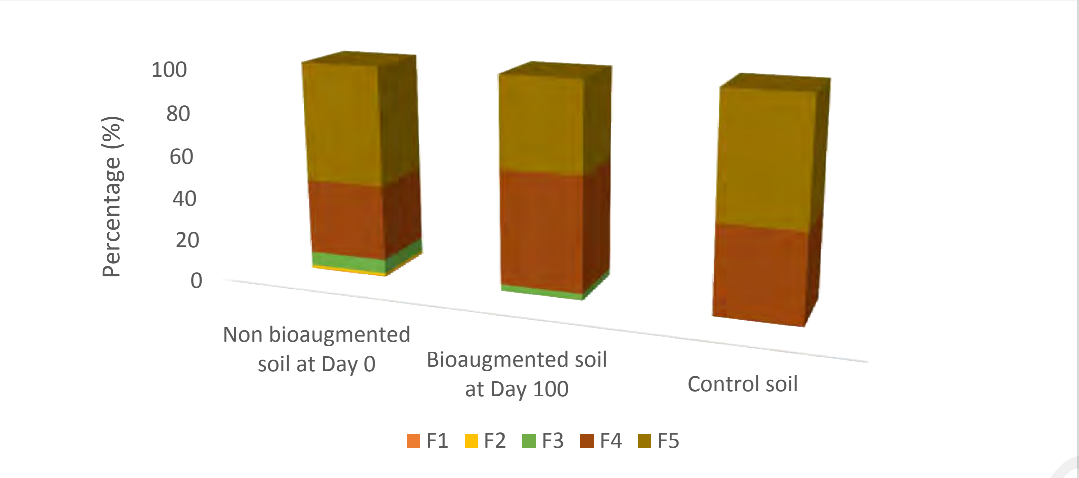

Figure 4.11: Cu distribution and speciation before and after remediation in TB landfill

(f) Zinc

Similar to Ni, the immobile form of Zn predominate over all other fractions in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100. Zn concentration in mobile form was 0.25 µg/g and 2.52 µg/g in F2 and F3, respectively (Table 4.27 and Figure 4.12). A complete reduction of Zn in F2 was observed after Day 100 treatment while almost 70% reduction was observed in F3 (Table 4.27).

Similar to Ni, percentage distribution of Zn showed that in non bioaugmented soil at Day 0, Zn was mostly associated with F5 (65.3%) (Figure 4.12). However, after bioaugmentation at Day 100, the pattern of distribution changed and thus Zn became mostly associated with F3 (43.6%) (Figure 4.12). Complete reduction in percentage distribution of Zn in F2 was recorded bioaugmented soil at Day 100. This can be attributed to the addition of microbial formula in soil.

Table 4.27: Concentration of Zn in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	ND	0.25	2.52	0.76	6.64	10.2
100	Bioaugmented soil at Day 100	ND	ND	0.80	0.39	0.63	1.82
Control	Non bioaugmented soil at Day 100	ND	ND	0.15	0	0	0.15
	Percentage reduction from Day 0	**ND**	**100**	**68.3**	**48.7**	**90.5**	

ND- not detected

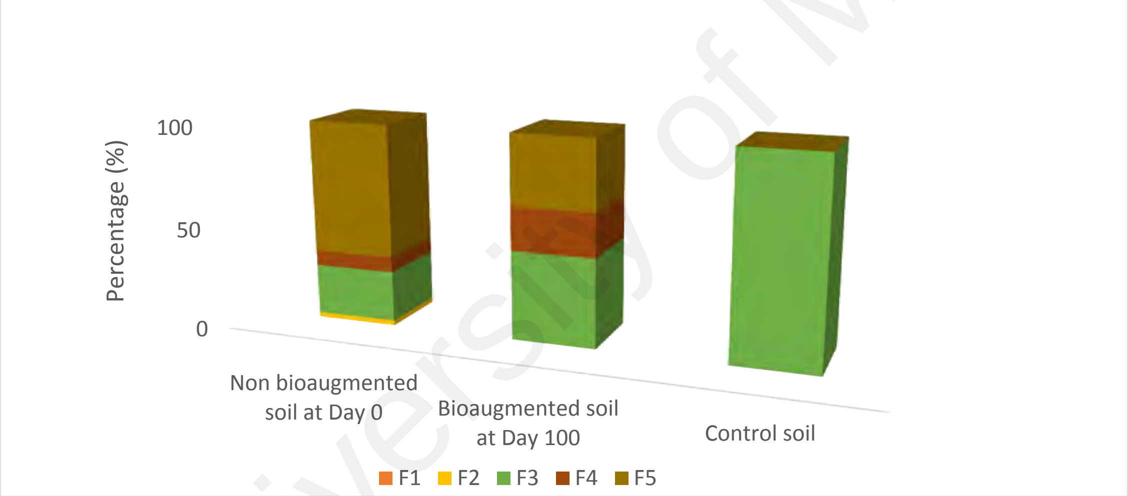

Figure 4.12: Zn distribution and speciation before and after remediation in TB landfill

(g) Lead

Pb concentration in microcosm at the beginning of the experiment at Day 0 was observed to be highest in the immobile form with F4 and F5 having 0.79 µg/g and 1.98 µg/g, respectively (Table 4.28 and Figure 4.13). A contrast in the concentration of Pb in F1, F4 and F5 was observed in microcosm with microbial formula after Day 100 where an increase in Pb concentration occurred (Figure 4.13). Although metals confined in the residual fractions (immobile forms) are not usually expected to be released over short period of time under the conditions usually encountered in nature, Pb observed in TB may still pose immediate threat to the environment. This is because of its increased concentration in F1 after Day 100 (Table 4.28). This result agreed with other studies by Kulikwoska and kluimuik (2007) who reported that there was no decrease in Pb concentration compared to other metals.

Table 4.28: Concentration of Pb in the various chemical fractions of bioaugmented and non bioaugmented soil from TB landfill (µg/g)

Day	Treatment	F1	F2	F3	F4	F5	Total
0	Non bioaugmented soil at Day 0	0.02	0.08	1.03	0.79	1.98	3.90
100	Bioaugmented soil at Day 100	0.08	0.07	0.42	2.39	3.44	2.45
Control	Non bioaugmented soil at Day 100	0	0	0.21	0.09	0.35	0.65
	Percentage reduction from Day 0	+	**12.5**	**59.2**	+	+	

+= increase in metal

The pattern of Pb percentage distribution was observed to be the similar to that in BB landfill in non boaugmented soil at Day 0 and bioaugmented soil at Day 100 (Figure 4.13). Pb distribution was in the order F5>F3>F4>F2>F1 however, a decrease in F2 (from 2.05% to 1.09%) and F3 (26.41% to 6.56%) was observed (Figure 4.13). Increase in Pb could be because the indigeneous micro organisms used in this study did not have any influence on Pb concentration.This study is similar to Braud *et al.* (2006) where soil bioaugmentation not only modified the metal speciation for the most easily extractable fractions (i.e. F1 and F2) but also modified the distribution of metals in the other fractions.

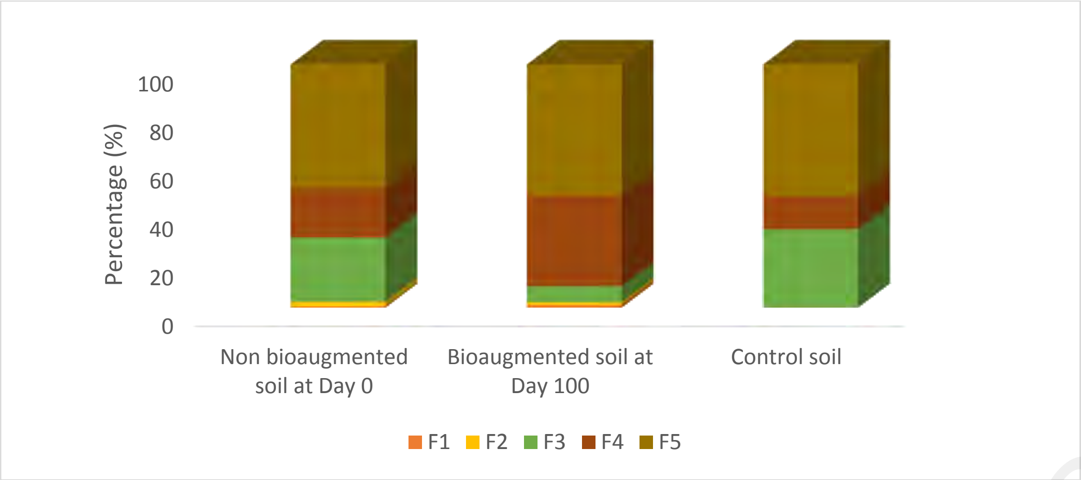

Figure 4.13: Pb distribution and speciation before and after remediation in TB landfill

4.7 Mobility and bioavailability of heavy metals after bioaugmentation of leachate contaminated soils from BB and TB landfill

The mobility of trace metals reflects their capacity to pass from one soil compartment to another where the metal is bound less energetically, the ultimate compartment being soil solution, which determines the bioavailability (Khan *et al.,* 2011). In this study, percentage of metal distribution in the mobile and immobile fractions (Table 4.29 and Table 4.30) and the mobility of metals in BB and TB before and after remediation is shown in (Figure 4.14 and 4.15), respectively.

In BB, the mobility of Cr, Cu and Pb was observed to have decreased after bioaugmentation of leachate contaminated soils from BB (Figure 4.14). Therefore, Cr, Cu, Pb may not pose serious threat to the environment due to their decreased mobility. However, Mn and Zn still remained mobile and thus could pose a threat to the environment (Figure 4.14).

Similar to BB landfill, in TB, after bioaugmentation of leachate contaminated soils from TB, most metal (Cr, Co, Ni, Cu, Zn and Pb) were predominantly found in the immobile form and thus these metals may not pose immediate threat to the environment due to their decreased mobility (Figure 4.15). Only Mn remained mobile and thus could pose a threat to the environment (Figure 4.15).

Table 4.29: Percentage of metal distribution before and after bioaugmentation in BB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr_B	34	3.73	17.8	16.6	27.8	55.6	44.4
Cr_A	7.50	11.3	20	22.5	38.8	38.8	61.3
Mn_B	5.42	51.7	35.9	2.91	4.08	94.2	5.75
Mn_A	5.35	36	51.3	3.03	4.34	92.7	7.37
Co_B	ND	ND	ND	ND	ND	ND	ND
Co_A	ND	ND	ND	ND	ND	ND	ND
Ni_B	ND	ND	ND	ND	ND	ND	ND
Ni_A	ND	ND	ND	ND	ND	ND	ND
Cu_B	2.01	9.36	0.67	49.7	38.3	12	87.9
Cu_A	0.87	5.22	0	69.6	24.3	6.09	93.9
Zn_B	ND	11.4	57.5	15	16	69	31
Zn_A	ND	7.05	85	7.49	0.44	92.1	7.93
Cd_B	ND	ND	ND	ND	ND	ND	ND
Cd_A	ND	ND	ND	ND	ND	ND	ND
Pb_B	2.16	4.32	38.9	25.9	28.8	45.3	54.7
Pb_A	10.6	3.10	11.5	30.5	44.2	25.2	74.8

Subscript A= Percentage of metal distribution after bioaugmentation, Subscript B= Percentage of metal distribution before bioaugmentation, ND= not detected

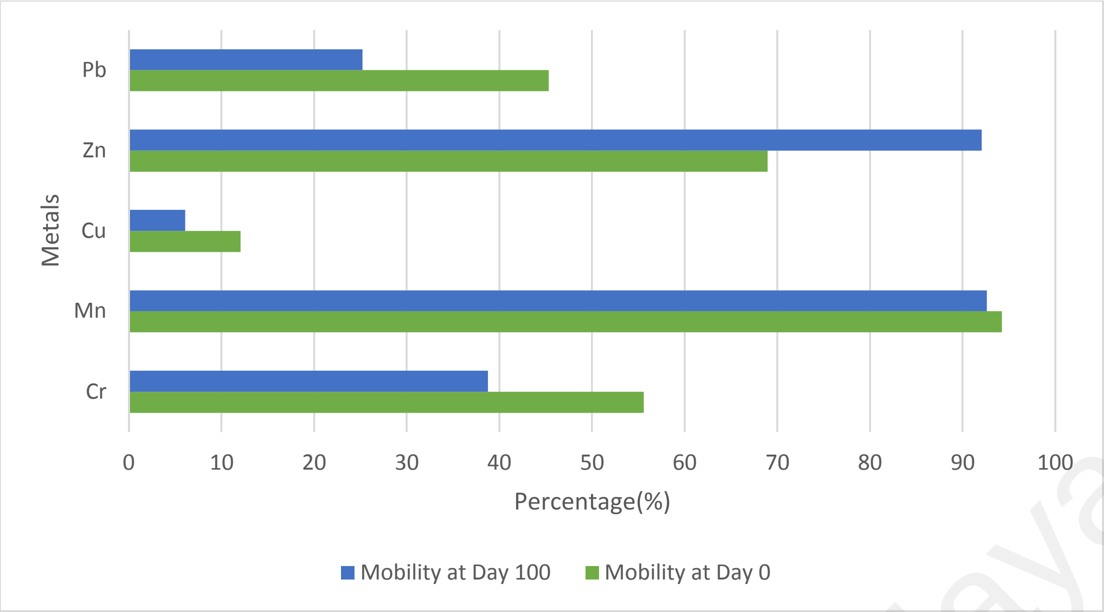

Figure 4.14: Mobility trend of heavy metals before and after bioaugmentation in BB landfill

Table 4.30: Percentage of metal distribution before and after bioaugmentation in TB landfill

Metals	Fractions (%)						
	F1	F2	F3	F4	F5	Σ(F1+F2+F3)	Σ(F4+F5)
Cr_B	0.66	0.77	7.71	17.7	73.1	9.14	90.9
Cr_A	0	1.72	7.56	26.8	63.9	9.28	90.7
Mn_B	8.79	33.7	30.7	4.06	22.7	73.3	26.7
Mn_A	8.71	28.9	32.2	9.64	16	69.8	25.7
Co_B	ND	5.22	31.3	6.09	57.4	36.5	63.5
Co_A	ND	ND	25	ND	75	25	75
Ni_B	ND	ND	14	6.43	79.5	14	86
Ni_A	ND	ND	1.47	7.35	91.2	1.47	98.5
Cu_B	0.47	1.29	6.70	35.4	56.2	8.46	91.5
Cu_A	ND	ND	3.27	53.9	42.9	3.27	96.7
Zn_B	ND	2.46	24.8	7.47	65.3	34.7	65.3
Zn_A	ND	ND	44	21.4	34.6	44	56
Cd_B	ND	ND	ND	ND	ND	ND	ND
Cd_A	ND	ND	ND	ND	ND	ND	ND
Pb_B	0.51	2.05	26.4	20.3	50.8	29	71
Pb_A	1.25	1.09	6.56	37.3	53.8	8.91	91

Subscript A= Percentage of metal distribution after bioaugmentation, Subscript B= Percentage of metal distribution before bioaugmentation, ND= not detected

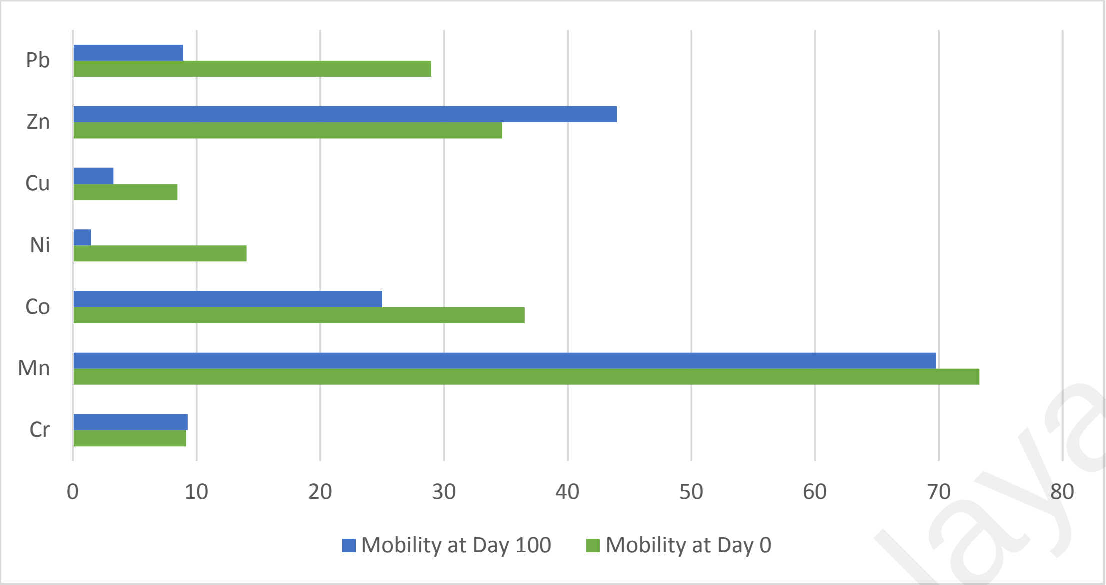

Figure 4.15: Mobility trend of heavy metals before and after bioaugmentation in TB landfill

CHAPTER 5: CONCLUSION

The results of speciation analysis of heavy metals in soils showed that in general, in Bukit Beruntung landfill (which is still operating), the mobile fractions of Zn was the most dominant metal found to be mostly concentrated at the entrance to the landfill. This was followed by Mn which was predominantly found inside the dumping area. However, in the immobile fraction, Cu was detected mostly inside the dumping area and was followed by Pb which was also predominant inside the dumping area and at the entrance to the landfill. Cd and Co were not detected in Bukit Beruntung landfill.

In contrast to Bukit Beruntung landfill, in Taman Beringin landfill (which is closed), Mn was the most dominant metal observed in all fractions although its location varied. In the mobile fraction, it was mostly concentrated at the entrance to the landfill. However, in the immobile fraction, Mn was predominantly observed inside the dumping area. Only Cd was not detected in this landfill.

Speciation analysis of metals in soils from this study revealed that differences exist between the mobile and immobile forms of heavy metals found in the operating and closed landfill even though they are both of non sanitary status. While in Bukit Beruntung landfill, different metals were observed to dominate the different fractions in the mobile and immobile forms, and in Taman Beringin landfill, only a particular metal i.e Mn was observed to dominate over all other metals in the mobile and immobile fractions.

The implications of mobile fractions of Zn and Mn in soils from Bukit Beruntung landfill and Mn in soils from Taman Beringin landfill are that these metals are in the bioavailable form which can easily be taken up by plants. Thus, ecotoxicity as a result of these metals presents a threat to the environment.

Additionally, an assessment of the potential mobility and bioavailability of heavy metals in Bukit Beruntung landfill revealed that the trend of mobility of metals is in the order: Zn>Mn>Pb>Cr. The Risk Assessment Code (RAC) results showed that Mn posed a very high risk to the environment in all sampled stations and Zn inside the dumping area also showed high risk to the environment. Pb was found to pose moderate risk to the environment while Cr, Co, Ni, Cu and Cd pose no risk to the environment. Also, in Taman Beringin landfill, the trend of mobility of metals is in the order: Zn>Mn>Pb>Co>Ni>Cr>Cu. Based on RAC, Zn, Pb, Ni, Cr and Cu poses no risk to the environment while Mn and Co were found to pose moderate risk. A comparison of the two landfills showed that metals in Taman Beringin have more mobility than those in Bukit Beruntung and this could be due to the age and status (inactivity) of Taman Beringin landfill.

This study provides an insight into the specific forms of heavy metal found in an operating and closed non sanitary landfill in Malaysia. This is especially important because there is a dearth of information on the speciation of heavy metals from Malaysian landfills. In addition, this study highlights the importance of speciation studies in order to determine the potential mobility and bioavailability of metals from landfills because the total concentration of metals does not reflect the mobility, bioavailability and toxicity of metals found in an environment.

Furthermore, bioaugmentation of the leachate contaminated soil was carried out using microorganisms found in both landfills for 100 Days. The chemical speciation of heavy metals in non- bioaugmented and bioaugmented soils from Bukit Beruntung landfill revealed that the percentage reduction of the mobile and non-mobile forms (F1, F2, F3, F4 and F5) varied for all metals and the distribution of the specific form of metals changed after bioaugmentation at Day 100. This showed that indigenous microorganisms found in both landfills are able to decrease the mobility of these metals.

In addition, the percentage of metal distribution before (at Day 0) and after bioaugmentation (Day 100) in Bukit Beruntung landfill revealed that mobility of Cr, Cu, Mn and Pb were reduced while Zn remained highly mobile after bioaugmentation at Day 100.

Similarly, in Taman Beringin landfill, the chemical speciation of heavy metals in non-bioaugmented and bioaugmented soils revealed that the percentage reduction of the mobile and non mobile forms (F1, F2, F3, F4 and F5) varied for all metals and the distribution of the specific form of metals changed after bioaugmentation at Day 100. In addition, the percentage of metal distribution before (at Day 0) and after bioaugmentation (Day 100) in Taman Beringin landfill revealed that mobility of Mn, Co, Ni, Cu and Pb were reduced. However, Cr and Zn remained highly mobile after bioaugmentation at Day 100. These results indicate that microorganisms are able to reduce the mobility of most metals, however it will not completely eliminate the risk and the toxicity that maybe caused by these metals.

This study highlights the need for proper assessment of ex disposal sites such Taman Beringin landfill before they are redeveloped into residential buildings, public parks and commercial premises as proposed in the National Urban Policy in Malaysia by the year 2020. Additionally, considering that most landfills are non sanitary, regular monitoring of the pollution impact of heavy metals from non sanitary landfills should include speciation analysis so as to understand the specific forms of metals that may present a serious threat to the environment. This will help to plan the remediation strategies.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. 2016 International conference on Chemical, Metallurgy and Environmental Engineering (CMAEE-16), Sept 21-26, 2016 (Paris) France
2. G. Umm-kulthum, S. H Fauziah and S Mohamad (2016). Assessment of Heavy Metal Mobilization in Soils from Landfill Sites in Malaysia. *International Journal of Advances in Agricultural & Environmental Engineering, 3(2)*, 371-374
3. Poster Presentation at workshop on evaluation and prediction of nutrients availability from biowaste using sensor and cloud technology to meet crop demand in Malaysia 2016, Rimba Ilmu, University of Malaya, Kuala Lumpur
4. Poster Presentation at Sustainability Science Research Cluster (SuSci) Symposium 2016 at Research Management and Innovation Complex (RMIC), University of Malaya