BIOAUGMENTATION AND PHYTOREMEDIATION OF HEAVY METAL FROM LEACHATE CONTAMINATED SOIL

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BIOAUGMENTATION AND PHYTOREMEDIATION OF HEAVY METAL FROM LEACHATE CONTAMINATED SOIL

ABSTRACT

Environment contaminated with heavy metals pose a significant problem, mainly due to the toxic effects of these metals throughout the food chain. Landfilling contributes to high volume of leachate generation. In Malaysia, the daily generation of leachate from landfills is about 6 million litres. Landfill leachate is highly heterogeneous and contains heavy metals. Improper waste management allows lateral flow of leachate into soil and cause serious contamination and it later reaches surface and groundwater sources. This posed risks to human health and the environment. Therefore, it is necessary to find solution for the removal of heavy metal from metal contaminated soil. This study aimed to characterize soil and leachate of two selected landfills in Malaysia and further isolate, identify and screen potential microbes for the removal of heavy metals from leachate contaminated soil. The microbes were then formulated into seven different treatments for bioaugmentation of leachate contaminated soil. The effect of inoculum concentration was also evaluated while the field trials were conducted using the best consortia. Phytoremediation to remove heavy metals from the leachate contaminated soil was also carried out using four different plant species. Characterization of both landfill soil and leachate indicated that Pb, Cu, Al, As, Mn, Cr, Zn, Fe and Ni contents were higher than the prescribed limits. Eighteen bacterial species were isolated from the leachate contaminated soil and were further screened for heavy metal sensitivity using the clear zone method. Among the isolates, *Burkholderia vietnamiensis* demonstrated the highest tolerance for metals (>20ppm). The best remediation results on soil collected from Taman Beringin landfill showed the reduction of 61%, 87%, 47%, 75%, 59% and 61% for As, Al, Mn, Fe, Ni and Cr, respectively by Proteo-bacteria group. Similarly, Proteo-bacteria also removed Al (87%), Mn (49%), Cu (65%), Fe (86%), Ni (78.7%) and Cr (67%) from soil from Bukit Beruntung Landfill. Increasing the inoculum concentration from 10% to 30% v/w showed increased capacity of metal removal from the contaminated soil. Results from the 100 days field trials at Taman Beringin landfill revealed that significant reduction of Pb, Mn, Fe, Al, Cu, Cr and Zn occurred when proteo-bacteria consortia was administered to the contaminated plots. The metal concentration was much lower in the microbe amended plots as compared to control plots. Phytoremediation studies revealed that *Cordyline* sp. is the most promising plant and the highest percentage of removal was was for Cu (94.35%), Pb (63%), Ni (88.9%), As (85%), Zn (77.55%), Cr (75%) and Al (67.5%) from the soil collected from Taman Beringin Landfill. For phytoremediation of contaminated soil from Bukit Beruntung Landfill, *Tradescantia spatachea* was the most prominent species with the highest percentage of metal removal for As (87.7%), Cu (81.5%), Fe (48.5%) and *Chlorophylum comosum* for Al (60%), Zn (73%), Cr (54%) and Pb (78.6%). Hence, bacterial isolates, especially those that belong to Proteo bacteria showed higher metal removal from contaminated soil, while *Cordyline* sp., *T. spatachea* and *C. comosum* have high potential to be used for phytoremediation of heavy metal.

Keywords: heavy metal, leachate contaminated soil, bioaugmentation, phytoremediation, microbes

BIOAUGMENTASI DAN FITOPEMULIHAN LOGAM BERAT DARI TANAH YANG TERCEMAR DENGAN LARUTAN RESAPAN

ABSTRAK

Persekitaran yang tercemar dengan logam berat menimbulkan masalah yang besar, terutamanya disebabkan oleh kesan toksik logam berat ini sepanjang rantai makanan. Pelupusan sampah di tapak pelupusan sampah menyumbang kepada penjanaan jumlah larutan resapan pada kadar yang tinggi. Penjanaan harian larutan resapan di Malaysia dari tapak pelupusan adalah kira-kira 6 juta liter. Larutan resapan adalah sangat heterogen dan mengandungi jumlah logam berat yang tinggi. Pengurusan sisa yang tidak betul membolehkan aliran larutan resapan ke dalam tanah dan menyebabkan pencemaran yang serius dan seterusnya ia mengalir ke sumber permukaan dan air. Ini menimbulkan risiko kepada kesihatan manusia dan alam sekitar. Oleh itu, pencarian penyelesaian untuk menyingkirkan logam berat dari tanah yang tercemar logam adalah penting. Kajian ini bertujuan untuk mengenal pasti tanah dan larutan resapan dari dua tapak pelupusan terpilih di Malaysia dan seterusnya mengasingkan, mengenal pasti dan menyaring mikroorganisma yang berpotensi untuk menghilangkan logam berat daripada tanah yang tercemar dengan larutan resapan. Mikroorganisma tersebut, kemudiannya dirumus menjadi tujuh rawatan yang berbeza untuk tujuan kajian pemulihan tanah yang tercemar dengan larutan resapan. Kesan kepekatan inokulum juga dinilai semasa ujian lapangan dijalankan menggunakan konsortium terbaik. Fitopemulihan untuk menyingkirkan logam berat dari tanah yang tercemar dengan larutan resapan juga dijalankan menggunakan empat spesies tumbuhan yang berlainan. Pencirian tanah dan larutan resapan dari dua tapak pelupusan sampah yang berlainan menunjukkan kandungan Pb, Cu, Al, As, Mn, Cr, Zn, Fe dan Ni lebih tinggi daripada had yang ditetapkan. Lapan belas spesies bakteria telah diasingkan dari tanah yang tercemar dengan larutan resapan dan diperiksa lagi untuk kepekaan logam berat menggunakan kaedah “clear zone”. Di antara isolat, *Burkholderia* *vietnamiensis* menunjukkan toleransi tertinggi untuk logam (> 20ppm). Hasil pemulihan tertinggi pada tanah yang dikumpulkan dari Tapak Pelupusan Taman Beringin adalah untuk pengurangan sebanyak 61%, 87%, 47%, 75%, 59% dan 61% untuk As, Al, Mn, Fe, Ni dan Cr oleh bakteria “Proteo”. Begitu juga, bakteria “Proteo” juga menyingkirkan Al (87%), Mn (49%), Cu (65%), Fe (86%), Ni (78.7%) dan Cr (67%) dari tanah tapak pelupusan Bukit Beruntung. Peningkatan kepekatan inokulum dari 10% ke 30% v / w menunjukkan peningkatan kapasiti penyingkiran logam dari tanah yang tercemar. Hasil daripada ujian lapangan selama 100 hari di tapak pelupusan Taman Beringin menunjukkan pengurangan yang signifikan untuk Pb, Mn, Fe, Al, Cu, Cr dan Zn berlaku apabila konsortia bakteria diberikan kepada plot yang terkontaminasi. Kepekatan logam jauh lebih rendah di plot yang dirawat berbanding plot kawalan. Kajian Fitopemulihan menunjukkan bahawa *Cordyline* sp. adalah tumbuhan yang paling terbukti untuk penyingkiran Cu (94.35%), Pb (63%), Ni (88.9%), As (85%), Zn (77.55%), Cr (75%) dan Al (67.5%) daripada tanah yang dikumpulkan dari tapak pelupusan Taman Beringin. Untuk Fitopemulihan tanah yang tercemar dari tapak pelupusan Bukit Beruntung, *Tradescantia spatachea* adalah spesies yang paling menonjol dengan penyingkiran logam tertinggi untuk As (87.7%), Cu (81.5%), Fe (48.5%) dan *Chlorophylum comosum* untuk Al (60%), Zn (73%), Cr (54%) dan Pb (78.6%). Oleh itu, isolat bakteria, terutama yang dimiliki oleh bakteria “Proteo” boleh menyingkirkan logam berat daripada tanah yang tercemar, manakala *Cordyline* sp., *T. spatachea* dan *C. comosum* mempunyai potensi yang tinggi untuk digunakan dalam fitopemulihan logam berat.

Katakunci: logam berat, tanah tercemar dengan larutan lesapan, kajian pemulihan, fitopemulihaan, bakteria

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LIST OF SYMBOLS AND ABBREVIATIONS

>	:	Greater than
<	:	Less than
%	:	Percentage
±		Plus- minus
ABS	:	Absorbance
Al	:	Aluminium
APHA	:	American Public Health Association
As	:	Arsenic
AWMO	:	Asian Waste Management Outlook
BBL	:	Bukit Beruntung Landfill
BCF	:	Bioconcentration factor
BOD	:	Biochemical/Biological Oxygen Demand
Ca	:	Calcium
Cd	:	Cadmium
Cr	:	Chromium
CH_3COOH		Acetic acid
Co	:	Cobalt
CO_2	:	Carbon Dioxide
COD		Chemical Oxygen Demand
Cu	:	Copper
DOE	:	Department of Environment
EPU	:	Environmental Protection Unit
EQA	:	Environmental Quality Act
EU	:	European Union

Fe	:	Iron
H_2	:	Hydrogen
H_20	:	Water
H_2CO_3	:	Carbonic acid
Hg	:	Mercury
ICP-OES	:	Inductively coupled plasma- Optical Emmission Spectrometer
IF	:	Inoculation Fluid
ISWA	:	International Solid Waste Association
IZD	:	Inhibitory Zone Diameter
K	:	Sodium
LFG	:	Landfill Gas
Mg	:	Magnesium
mg/kg	:	miligram/kilogram
MIC	:	Minimal Inhibitory Concentration
Mn	:	Manganese
MSW	:	Municipal Solid Waste
N	:	Nitrogen
Ni	:	Nickel
NSWD	:	National Solid Waste Department
P	:	Phosphorus
Pb	:	Lead
POP	:	Persistent Organic Pollutant
ppm	:	part per million
TBL	:	Taman Beringin Landfill
TF	:	Translocation factor
TOC	:	Total Organic Content

USEPA	:	United States Environmental Protection Agency
VFA	:	Volatile fatty acids
v/v	:	Volume/volume
v/w	:	Volume/weight
WHO	:	World Health Organization
Zn	:	Zinc

LIST OF PLATES

CHAPTER 1: INTRODUCTION

1.1 Background of study

Totally intact and undisturbed environments are gradually disappearing because of human interference. The three basic domains of the earth is air, water and soil. Anthropogenic activities that are guided by the insatiable societal desires are now a global concern, as most green environments have been negatively affected by man-made pollutants. The list of pollutants is long and has been updated every now and then by professional bodies and organizations based on research findings and this substances is released on a daily basis which contributes to global pollution burden. However, the distribution of numerous pollutants in the environment does not imply equal prevalence and influence. Several pollutants are very toxic and dangerous to every form of life whereas other pollutants are considered negligible and example of those are heavy metals.

Environments contaminated with heavy metals pose a significant problem, mainly due to the toxic effects of these metals throughout the food chain. Heavy metals are pollutants that are classified as toxic to the environment, even at minute concentrations. It is high density metallic chemicals that are potentially toxic at low concentrations and poses threat to human health and environment. Source of heavy metals can be resulted from various anthropogenic activities however waste generation and its disposal is one of the major problem that cause serious contamination problem to the environment.

Malaysia has over the years experienced rapid growth in population, urbanization and industrialization (Johari *et al*., 2014; Agamuthu, 2001; Chua *et al*., 2011; Zamali *et al*., 2009) and this has led to increase in the generation of municipal solid waste (MSW) (Budhiarta *et al*., 2012; Fauziah & Agamuthu, 2012). Current global MSW generation from 161 countries are approximately 1.3 billion tonnes per year, and are expected to increase to approximately 2.2 billion tonnes per year by 2025 (World Bank, 2012) while in Malaysia the generation of MSW increased at 3% annually (Agamuthu, 2001). As Malaysia is moving towards to becoming a developed country by the year of 2020, advancement in the country's development also caused an increase in waste generation. Four main types of waste categories are Municipal Solid Waste (MSW), Hazardous Waste, Agricultural Waste and Industrial Waste, however MSW is the most dominant waste generated in Malaysia. The composition of MSW also varied depending on the geographic location, socio-economic conditions, season, waste collection and disposal method (Kanmani & Gandhimathi, 2013).

World Bank has also identified improper management of waste to be one of the three main contributors of environmental degradation in Asian countries (Agamuthu & Fauziah 2011; World Bank, 2012). This has caused an alarming situation for the need of proper waste management to minimize the impacts to the environment and all the inhabitants on the earth. Lack of awareness, facilities and technologies are some of the major factors for improper waste management (Chowdhury, 2009).

Final disposal of MSW is an important component of waste management hierarchy because there are no technologies available to avoid the entire unwanted residue from the waste sector and no endowment is available for zero waste (Fauziah & Agamuthu, 2012). The developed countries adopted more advanced, cleaner and sustainable principles towards waste minimization and handling unlike developing countries which opted for landfilling. Landfilling are the most preferred and dominant method of waste disposal in developing countries and most of the landfill were not properly equipped for leachate collection system and landfill gas collection system. Malaysia currently has 170 waste disposal with only 14 with the status of sanitary landfills. The remaining were listed as non-sanitary landfill or open dumps. Malaysia generated about 31,000 tonnes of MSW daily and 95% of the waste are disposed into landfills (Pariatamby *et al*., 2009).

There are five phases involved in biological degradation of the waste that has been deposited into landfill. During the waste degradation process in landfills, two major output of the process is landfill gas and leachate. The presence of this liquid substance is often subject of concern to both landfill managers and environmental protectionists due to the impact of the leachate on the environment (Emenike *et al*., 2016). Leachate is the liquid/fluid that flows out from waste because of increased moisture levels from water penetration or degradation. The global characterization of leachate, especially from municipal solid waste (MSW) landfills, has shown that it is highly heterogeneous and often contains massive amounts of dissolved organic matter, pesticides, xenobiotics, and heavy metals (Fauziah *et al*., 2013; Kjeldsen *et al*., 2002; Emenike *et al*., 2016; Emenike *et al*., 2012). MSW leachate has been identified to contain more than 200 type of organic compounds and out of that 35 of the compounds has the potential to bring harms to the human health and environment (Paxeous, 2000). The characteristics of leachate depends on the soil type, waste composition, rainfall, degree of compaction, evapotranspiration, landfill type and age (Agamuthu, 2001).

Among the various components of the leachate, heavy metals are the most significantly important because it can induce associated toxic impacts on the ecosystem (Emenike *et al*., 2017). Common heavy metal found in leachate were Pb, Cu, Cr, Ni, Mn, Hg, Fe, Al, Zn and Cd (Emenike *et al*., 2016; Jayanthi *et al*., 2016). The metals concentration may also between landfills and most of landfills in Asian generally show high concentration in the leachate (Agamuthu *et al*., 2011). Furthermore, heavy metal persist in the contaminated site for very long time and it cannot be chemically or biologically degraded unlike other organic contaminants (Wuana & Okieimen, 2011).

In Malaysia, an estimated of 3 million litres of leachate is generated per day from the landfills (Agamuthu *et al*., 2009). It is generated as a results of the waste degradation of the organic materials and the quantity of leachate generated depends on many factors such as precipitation, surface runoff, evapotranspiration, final cover and the moisture content (Mohamad & Agamuthu, 2008; Selic *et al*., 2007).

Malaysia especially, has higher number of landfills without bottom liners and leachate collection system and therefore any sort of leachate treatment is not possible. Due to improper solid waste management, soil has become the main sink for leachate contamination and cause soil pollution. Soil pollution can be defined as the negative change in condition due to natural or anthropogenic activities that can significantly alter the soil composition, texture and structure (Emenike *et al*., 2017).

When solid waste is disposed-off on land in open dumps or in improperly designed landfills (e.g. in low lying areas), it causes contamination to the soil. Soil serves as the backbone of the most terrestrial interactions (Emenike, 2014). The contaminated soil will further cause ground water contamination by the leachate generated by the waste dump surface water contamination by the run-off from the waste dump, bad odour, pests, rodents and wind-blown litter in and around the waste dump, generation of inflammable gas within the waste dump, fires within the waste dump, erosion and stability problems relating to slopes of the waste dump, epidemics through stray animals, acidity to surrounding soil and release of green-house gas on the environment (Adewuyi, 2004). Poor waste management poses a great challenge to the well-being of city residents, particularly those living adjacent the dumpsites due to the potential of the waste to pollute water, food sources, land, air and vegetation. High stability of these metals in water, soil, and even in animals poses a major threat to human health and ecological environment (Hashemi, *et al*., 2012). The leachate contamination to the soil and eventually to water source can occurs by migration of the leachate away from the landfill and the uncontrolled release results in critical environmental pollution.

Groundwater and others form of water bodies are very important part of the ecosystem therefore it is important to minimize any type of contamination to take place. The contaminants can infiltrate deep into underground, pollute groundwater and surface water and there is probability of entering the human food web through plants and aquatic animals that bio accumulate metals, and further transfer to the food chain (Adams *et al*., 2014).

Heavy metal chemically refers to a class of a distinct subdivision of elements characterized with metallic properties. Transition metals, certain lanthanides, metalloids, and actinides, comprise heavy metals. The various properties of heavy metal include a density range of 3.5–7 g/cm^3, atomic weight ranging of 22.98 to < 40, and atomic number of <2 (Afal & Wiener 2014). Similarly, substances at a pure state possess high and useful electrical and thermal conductivities. Five different fractions of metals are present in soil due to differences in their properties, namely soil solution dissolution, binding potential properties for the location exchange of inorganic soil constituent, adsorption to inorganic soil constituents, attachment to insoluble organic matter, and precipitation potential from pure or mixed solids (Ann, 2005). The toxicity of heavy metal depends on several factors including the level of pollutants, route of exposure, and chemical species. Due to their high degree of toxicity, As, Cd, Cr, Pb, and Hg rank among the most toxic metals that are of public health significance. Heavy metals are systemic toxicants that are known to cause multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer. The reduction or removal of heavy metal from the contaminated soil is there for essential before it enters the water bodies and reach the ecosystem and affect the food chain.

Microorganisms have continued to be integral component of the ecosystem. The presence of microbes in atmospheric, terrestrial and aquatic environments enhance different dimensions of metabolism and transformations. This explains the reason for the involvement of microbes in synthesis and degradation activities. Basically, microbes thrive optimally when the site of action or immediate environment is intact, than in the presence of contaminated/polluted environments. The interactions and responses of microbes in presence of leachate as a soil contaminant is important. Microbes' responses to pollution may vary from one environment to another, or among species due to the nature of pollutants and varying concentrations. For instance, microbial growth can be enhanced at low concentrations of Cu, but will be repressed at high concentrations, meanwhile low concentrations of Cd can cause severe toxicity (Lucious *et al*., 2013; Wei *et al*., 2009; Karnachuk *et al*., 2003). Since microbes can significantly assist in the bio-geochemical cycling of toxic heavy metals or remediating metal-contaminated environments, it is imperative to understand the diversity of microbes during heavy metals pollution caused by leachate seepage into soil. Similarly, there is increasing evidence on the metal resistance among naturally resident microbes found in the contaminated sites (Lucious *et al*., 2013). However, studies towards understanding similar resistance within landfill sites still remain limited. Biological methods are environmentally friendly and particularly attractive because of their low cost and relatively simple maintenance (Mirsal, 2008) compared to traditional method such as excavation and off-site disposal method (Agnello *et al*., 2016). Biological remediation strategies and can be used for the remediation of soils affected by different types of pollutants which includes heavy metal as well.

Bioaugmentation is a biological process that biotransforms an environment that is already altered by contaminants to its original or desired status. Microbes can detoxify metal in the environment through various methods; valence transformation, extracellular precipitation or volatilization. It will transform the metal from toxic to less toxic state.

Phytoremediation is an aspects that uses plant for treatment of heavy metal contaminated soil. An effective phytoremediation occurs when the pollutants is within the root zone of the plant (Garbisu & Alkorta, 2003; Chibuike & Obior, 2014). About 420 species from 45 plants have been were identified as hyper accumulators of heavy metals (Alaribe & Agamuthu, 2015). Various plants have been used to remedy polluted soil, yet metal interaction with plant differ with respect to medium or source of metal pollution. Phytoremediation technology is an alternative and cheaper approach for remediation of metal contaminated soil. Plant-based remediation is one of the most significant sustainable techniques to cope with overwhelming consequences of pollutants. The green technology approach is necessary for removal of metal from contaminated soil.

Therefore considering the fact that soil is the main barrier between the leachate from landfills and water surface it is necessary for remediation of soil to take place before it enters the water source. Sustainable bioremediation is important to reduce/remove the metals from the soil.

1.2 Problem Statement

Generation of waste is unavoidable due to the increase in population growth, higher living standards, accelerated urbanization and industrial processes. Municipal solid waste generated in Malaysia was reported to be 1.3 kg per capita per day and 95% of these wastes are sent to landfills (Agamuthu *et al*., 2009). Most of the landfills in Malaysia are with status of non-sanitary and this pose serious threats to the environment. When the waste are not properly dumped it is does not only pollute the environment but also lead to waste borne diseases. The major outputs of landfilling are leachate and landfill gases. Leachate is a liquid product produced by action of leaching when the rain water percolates through any permeable material. If leachate is not properly collected it will flow or migrate to other water bodies. Leachate is produced over time, and with the percolation of rain water, the degradable fractions of the waste decompose and the resulting products are diluted and dispersed into the underlying soil if a site is not contained. Leachate production begins shortly after the process of landfilling and continues possibly for thousand of years. On a small scale, these processes (dilution and dispersion) is effective, as soils have a natural capacity to further decompose organic material and to adsorb many inorganic residues. If the permeability of the soil is low, leachate may collect at the bottom of the refuse layer and may eventually discharge laterally to the surface and contaminate the soil. However if the soil is not permeable enough the leachate will then flow into other water bodies. Leachate contains more than 200 types of elements or inorganic compounds, and about 35 inorganic compounds mostly heavy metals are listed (As, Cd, Co, Cr, Cu, Hg, Mn, Ni and Pb) as having the potential to harm the environment and human health. The heavy metal in soil persist for long time in the after contamination occurred (Subhasini & Swamy, 2013) compared to other organic compounds. Heavy metal contamination of soil may pose risks and hazards to human and the ecosystem through direct ingestion or contacts with the contaminated soil, through food chain (soil – plant – human/ soil – plant –animal – human), consumption of contaminated ground water and reduction in the food quality due to plants grown in contaminated site.

Therefore this study aimed to develop bioremediation and phytoremediation method using potential microbes to remove heavy metal pollutants from soil contaminated with leachate before it enters the water bodies since the intrusion of leachate into the groundwater or any other water bodies negatively affect the whole ecosystem.

Some of the major research gaps in this research is that not many studies has been carried out on metal removal by microbes in landfill soil through bioaugmentation method therefore it is necessary to carried out this research. There were a lot of leachate contamination happened in Malaysia and developing countries which urgently need remediation process and this technique can be highly adopted and practically applied. Secondly, formulation of microbial treatment for bioaugmentation is also important in order to achieve successful removal of metal from the environment. The formulation of treatment in this study is completely new and no studies has been done with this combination especially those belongs to Proteo group, non –proteo group and group based on heavy metal sensitivity test (high sensitivity group and medium/low sensitivity group). No previous studies has been carried out with the four plants selected in this study except for *Tradescantia spatachea* and *Chlorophylum comosum* which is only specific to Pb. The selected plant is an ornamental plant and easily available. Lastly, most studies on bioaugmentation is carried out only in laboratory condition however this study also carried out at the actual landfill condition so that its can be practically applied in future and the results obtained can be a guideline for future researchers.

1.3 Research Hypothesis

1. Bioaugmentation of heavy metal using microbes is expected to be sustainable approach for reduction/ removal of heavy metal of leachate polluted soil.
2. Phytoremediation is green technology approach which is expected for reduction/ removal of heavy metal of leachate polluted soil.
3. Field bio-augmentation study is expected to be key approach/guideline for further *in situ* study.

1.4 Research Objectives

This study is aimed to develop bioaugmentation and phytoremediation method using potential microbes to remove heavy metal pollutants from leachate contaminated soil and the research objectives are:

1. To characterize the physico-chemical properties of soil and leachate from active and non-active non-sanitary landfills
2. To identify microbes from soil contaminated with leachate
3. To identify and formulate potential microbial cocktails to remove heavy metals from soil contaminated with leachate
4. To simulate a system for the application of appropriate bioaugmentation and phytoremediation technique for the heavy metal removal from leachate contaminated soil in laboratory
5. To investigate and apply formulated technique from lab simulation to actual landfill soil (leachate contaminated land)
6. To determine the kinetic model for heavy metal removal during bioaugmentation and phytoremediation experiment as applicable to leachate contamination.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

There are many sources of heavy metal contamination to soil. Different types of anthropogenic activities have led to their wide distribution in the environment, and can negatively impact human health in particular, and the ecosystem in general. Leachate pollution from landfilling is one of such activity which is gradual but persistent, and harbours many environmental pollutants including heavy metals (Agamuthu *et al*., 2014). The increased waste generation pattern especially in the developing countries, often leads to the generation of high volume of leachate (Jayanthi *et al*., 2016). Without proper collection system, raw leachate from landfills will laterally seep into soil compartments to cause soil contamination (Emenike *et al*., 2016). Areas near landfills have a greater possibility of groundwater contamination because of the leachate (Figure 2.1). Such contamination of groundwater resource poses a substantial risk to local resource users and to the natural environment. Therefore, the inevitable task faced by the society is identifying ways to prevent metal pollution in order to conserve the environment. Similarly, a more significant interest is to recover already polluted sites for the associated socio-economic benefits. Usually, the dynamic shift is towards global sustainability in steering remedial or recovery activities using microbes. Hence, the use of microorganisms for cost-effective restoration of environment cannot be over emphasized. Similarly, the use of microbes for metal removal from contaminated media like soil can be achieved with the aid of plants through the concept of phytoremediation. Phytoremediation is another aspects that can be emphasized for removal of heavy metal in metal contaminated soil. Phytoremediation is the direct use of living green plants for in situ, or in place, removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater. An effective phytoremediation occurs when the pollutants is within the root zone of the plant. It is also low cost and solar energy driven clean-up technique.

Figure 2.1: Leachate from landfill flowing to Sg. Kembong system (Meera, 2013)

Bioaugmentation and phytoremediation techniques were adopted for the removal of heavy metal in leachate contaminated soil because the heavy metal problem has now become a very serious global problem mainly from the generation of leachate from the disposed MSW in landfill.

2.2 Municipal Solid waste (MSW)

According to the Malaysia Solid Waste and Public Cleansing Management Act 2007, MSW is defined as "any scrap material or other unwanted surplus substance or rejected products arising from the application of any process; any substance required to be disposed of as being broken, worn out, contaminated or otherwise spoiled; or any other material that according to this regulation or any other written law is required by the authority to be disposed of" (NSWD, 2012). MSW is unavoidable in our daily life. MSW is also known as trash or garbage and mainly consist of items that we use daily such as product packaging waste, food waste, bottles, clothing, batteries, paper and many more. According to Agamuthu (2011), solid waste is defined as inevitable by-products which is solid, or has no use to anyone and unneeded generated by human activity. MSW basically consist of all waste produced, collected, transported and discarded of within the right of municipal authority.

2.2.1 Types and sources of MSW

Types and sources of MSW are important aspect in MSW management. MSW is heterogeneous and the source of MSW is categorized based on the land use and zone. It is categorized into residential, commercial, institutional, industrialization and street sweeping (Emenike, 2013). Residential waste is one of the major source of MSW in developing countries and it consist mostly of food waste while commercial and institutional waste mainly generates paper, plastic or packaging material waste. Table 2.1 shows the detailed type and source of MSW.

Table 2.1: Source, Typical waste generator and Types of Solid Waste (Fauziah, 2009; Agamuthu *et al*., 2004; World bank, 1999)

Source	Typical Waste Generators	Types of Solid Waste
Residential	Single and multifamily houses	Food waste, paper, cardboard, plastics, textiles leather, yard wastes, wood, glass, metals, ashes, special waste (electronic, batteries, oil, tires), and household hazardous waste.
Industrial	Light and heavy manufacturing, fabrication, construction sites, power and chemical plants.	Housekeeping wastes, packaging, food wastes, construction and demolition materials, hazardous wastes, ashes, special wastes.
Commercial	Stores, hotels, restaurants, markets, office buildings, etc	Paper, cardboard, plastics, wood, food wastes, glass, metals, special wastes, hazardous wastes.
Institutional	School, hospitals, prisons, government centers	Same as commercial.
Construction and demolition	New construction sites, road repair, renovation sites, demolition of buildings.	Wood, steel, concrete, dirt, packaging waste etc.
Municipal services	Street cleaning, landscaping, parks, beaches, other recreational areas, water and wastewater treatment plants	Street sweepings, landscape and tree trimming, waste from parks, beaches, recreational areas, sludge.
Processes	Heavy and light manufacturing, refineries, chemical plants, power plants, mineral extraction and processing.	Industrial process wastes, scrap materials, off specification products, slag, tailings.

The sources of MSW in Malaysia vary with the size of locality and economic standards. The individual type of waste and its source is tabulated in Table 2.2.

Table 2.2: Differents type of and sources of MSW (World Bank, 2012)

Type	Sources
Organic	Food scraps, yard (leaves, grass, brush) waste, wood, process residues
Paper	Paper scraps, cardboard, newspapers, magazines, bags, boxes, wrapping paper, telephone books, shredded paper, paper beverage cups
Plastics	Bottles, packaging, containers, bags, lids, cups Glass Bottles, broken glassware, light bulbs, colored glass
Metal	Cans, foil, tins, non-hazardous aerosol cans, appliances (white goods), railings, bicycles
Others	Textiles, leather, rubber, multi-laminates, e-waste, appliances, ash, other inert materials

2.2.2 Solid Waste generation in World/Asia /Malaysia

The generation of waste is continuously increasing due to population growth, high living standard, urbanization, urban migration and industrialization. According to the Waste Atlas report, current global waste generation from 164 countries is 1.9 billion tonnes annually (Waste Atlas, 2016; AWMO, 2017). It is expected to increase by 2.2 billion tonnes in year 2025 (World Bank, 2012; AWMO, 2017).

In 2016, MSW generation was 1.99 kg per capita per day in United States, 1.45 kg per capita per day in France, 1.34 kg per capita per day in United kingdom, 0.9 kg per capita per day in Japan, 0.37 kg per capita per day in India, 0.58 kg per capita per day in Nigeria and 1.3 kg per capita per day in Malaysia (Kawai & Tasaki, 2016). Socio-economic inclinations may contribute to the variance in the per capita generation of MSW (Emenike, 2013; Agamuthu *et al*., 2011).

Table 2.3 shows the MSW generation trend in Malaysia from 2005 to 2016 and Figure 2.2 shows the MSW generation by selected countries in a year. The variance in the amount of MSW generated between the countries is based on the urbanization, level of income, food habit, social and cultural habits and lifestyle.

Table 2.3: MSW Generation in Malaysia (Pauze, 2016)

Year	2005	2012	2016
Total waste generated (tonne/day)	19,000	33,000	38,200
Waste disposed in Landfill(tonne/day)	18,050	30,129	35,335
Disposal percentage	95%	91.3%	82.5%

In Malaysia, the waste management system practised by local government and the municipalities are inefficient and not sustainable since Malaysian waste generation per capita increased from 0.5 kg day $^{-1}$ in late 1980s to more than 1.3 kg day^{-1} of waste in 2009 (Pariatamby *et al*., 2009). In Kuala Lumpur and Petaling Jaya, the generation increased to 1.5–2.5 kg capita $^{-1}$ day $^{-1}$ (EPU, 2006; Agamuthu *et al*., 2009). The annual waste generation in Malaysia with total population of 28.96 million in 2010 has reached 11 million tonnes (Fauziah *et al*., 2009). It increased at 3% annually. The increase trend in MSW generation is mainly due to rapid development in the urban areas, increase in the income, rural urban migration and changes in the consumption patterns (Agamuthu & Fauziah, 2006; Agamuthu & Khan, 1997).

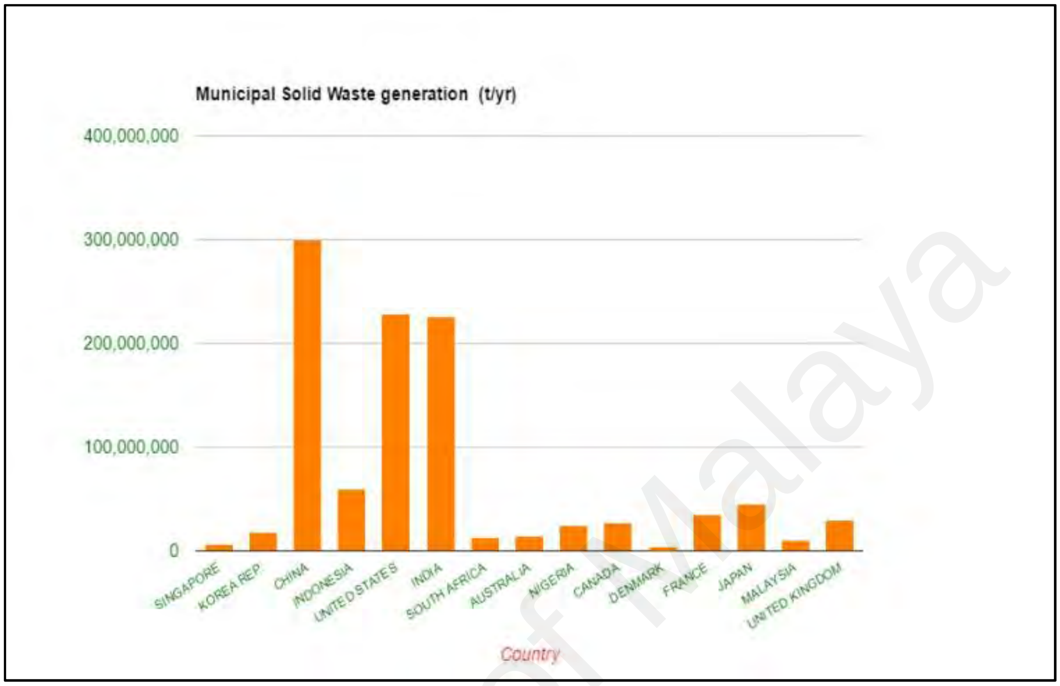

Figure 2.2: Global MSW generation (tonne/year) (Waste Atlas, 2016)

2.2.3 MSW Composition

The composition of MSW is considered as the basic parameter in designing a waste treatment disposal system. MSW consists of heterogeneous mixture of waste that can be categorized as organic or inorganic waste, biodegradable waste, and hazardous or non-hazardous waste. The development status of country affects the waste composition. Globally organic waste is the most dominant component in the MSW composition followed by paper (Figure 2.3). In Malaysia, organic waste constitute 45% of the MSW stream (Table 2.4). Manaf *et al*. (2009) reported that Malaysia has high percentage of organic waste that results in high moisture content. These characteristics reflect the nature

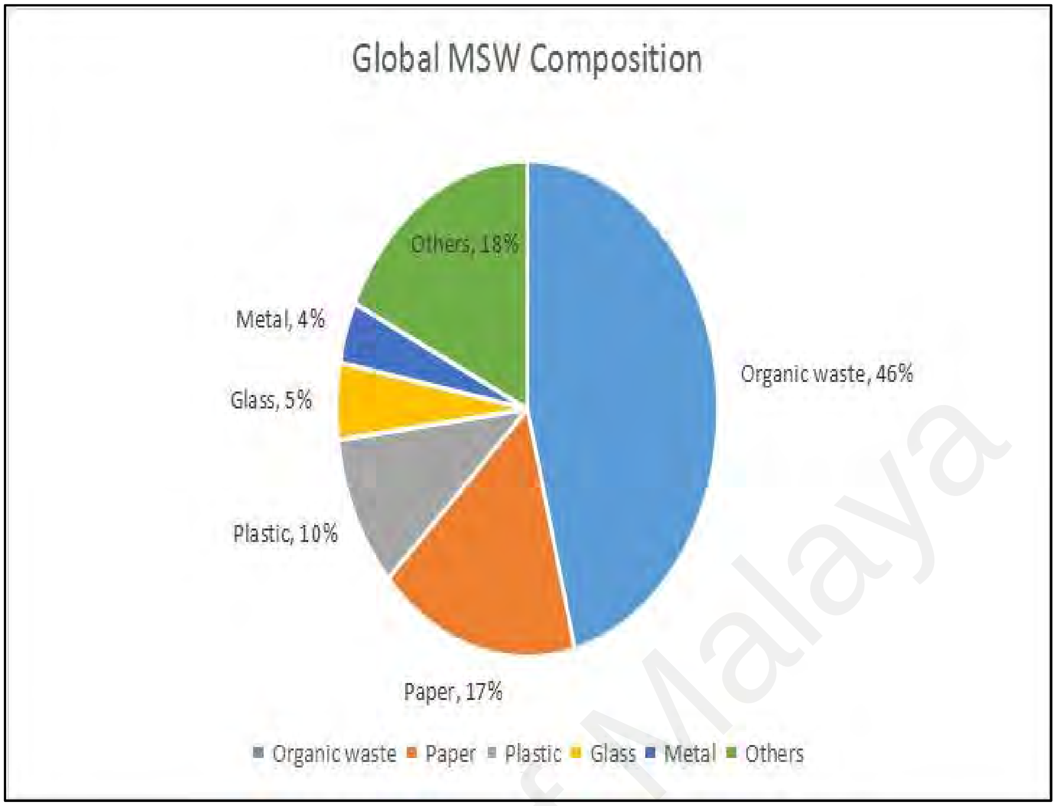

Figure 2.3: Global MSW Composition (Adapted from World Bank, 2012)

Table 2.4: Composition of MSW in Malaysia (1995-2012) (Agamuthu, 2009; Pauze, 2016)

Waste composition	1995 (%)	2000 (%)	2005 (%)	2010 (%)	2012 (%)
Organic	45.7	43.2	44.8	55.0	44.5
Paper	9.0	23.7	16.0	13.0	8.5
Plastic	3.9	11.2	15.0	19.0	13.2
Glass	3.9	3.2	3.0	2.0	3.3
Metal	5.1	4.2	3.3	3.0	2.7
Others	6.4	14.5	17.9	8.0	27.8

and lifestyle of Malaysian's. Besides that, status of income, residential type, affleuncy season and location also plays an important in the MSW composition.

As growth economy and urbanization of a country take place, waste composition changes. Table 2.5 shows the MSW composition in selected countries around the world. It is also evident that other countries such as Denmark, Indonesia, Ghana and Sri Lanka have high organic waste composition as compared to other types of waste. In general, food, paper, and plastics are the main components of MSW which vary with degree of affluence and urbanization of the area.

Table 2.5: MSW Composition of selected countries (World Bank, 2012)

Country	Organic waste (%)	Paper (%)	Plastic (%)	Glass (%)	Metal (%)	Others (%)
Denmark	29	27	1	5	6	32
Indonesia	62	6	10	9	8	4
Thailand	48	15	14	5	4	14
Sri Lanka	76	11	6	1	1	5
Singapore	44	28	12	4	5	7
Nepal	80	7	3	3	1	7
India	35	3	2	1	-	59
China	38	26	19	3	2	12
Ghana	64	3	4	-	1	28

2.3 Current Solid waste management practice

MSW management has become a great concern due to the ever increasing rate in the MSW generation (Agamuthu *et al*., 2009). MSW management is now in an alarming situation due to rapid development mostly in developing countries such as Malaysia, Vietnam, India, Thailand, and Indonesia. An inappropriate MSW management can result in unwanted dumping of waste in public areas such as along the road, drainage or into the water bodies and cause problem to human health.

An effective solid waste management will consider the economics, public health, engineering of disposal site, and other environmental considerations. Figure 2.4 shows the typical waste management.

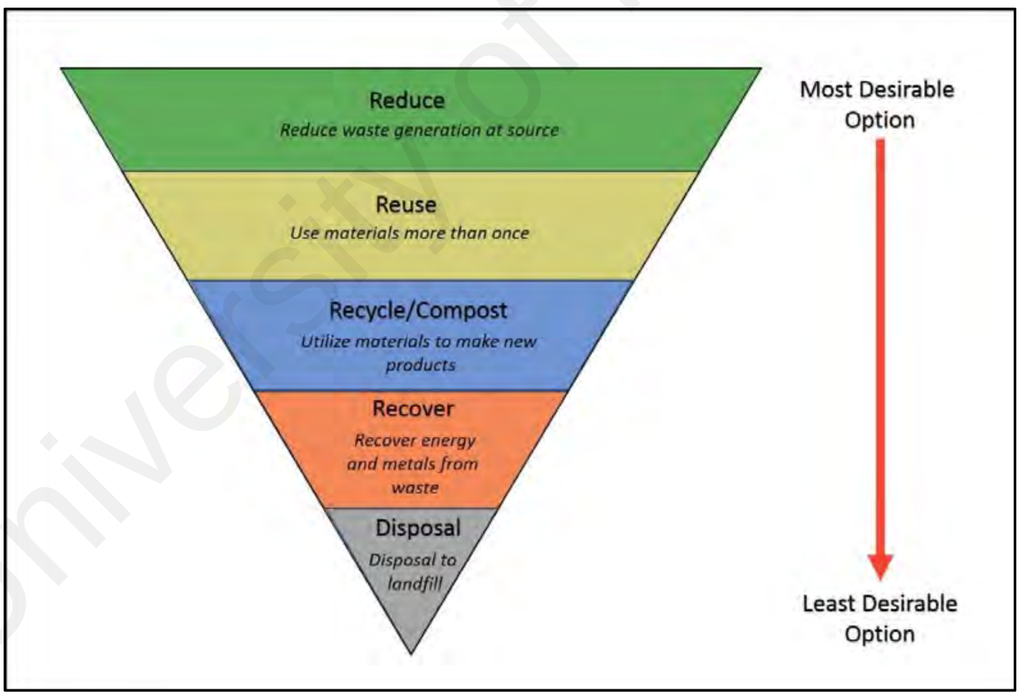

Figure 2.4: Waste Management hierarchy

The most desirable option is to reduce the waste while the least desirable is disposal. Table 2.6 depicted details on each waste management options, which depends on the amount and types of MSW generated, the physical and chemical characteristics of the MSW, geographical location which includes the climatic condition, frequency of MSW collection by local authorities, the number of population with high or medium/low income, land availability, economic aspects, labour, public awareness, recycling strategy, energy availability and demand and environmental impacts (Agamuthu, 2001)

Table 2.6: Different methods of MSW management

Treatment	Definition
Waste prevention	Is the process and the policy of reducing the amount of waste produced by a person or a society.
Recycling	Recycling is a process of using materials (waste) into new products to prevent waste of potentially useful materials, reduce the consumption of fresh raw materials, reduce energy usage, reduce air pollution (from incineration) and water pollution (from landfilling) by reducing the need for "conventional" waste disposal, and lower greenhouse gas emissions as compared to virgin production.
Composting	Process of mixing of decaying organic matter, as from leaves and manure to produce which helps to improve soil structure and provide nutrients.
Incineration	Method of controlled burning of waste material and help in reducing the waste volume.
Landfill	Method where waste is buried between layers of dirt so as to fill in or reclaim low-lying ground.

2.4 Landfilling

Landfill is a place to dispose of refuse and other waste material by burying it and covering it with soil, especially as a method of filling in or extending usable land. It also can be defined as a biochemical reactor with solid waste and water as the major input of the system and landfill gas and leachate as the principal output (Hassan *et al.*, 1999). ISWA (1992) defined landfill as "the engineered deposit of waste onto and into land in such a way that pollution or harm to the environment is prevented and through restoration, land provided maybe used for another purpose".

In Malaysia landfill is the ultimate method of MSW disposal. About 85% of MSW generated in Malaysia is directly disposed into landfills (Agamuthu & Fauziah, 2011). Landfilling is divided into four categories namely sanitary landfill, secure landfill, controlled dump and open dumps. The increase in the number of landfills in Malaysia is due to the increase in the amount of waste generated simultaneously with population growth (Agamuthu & Fauziah, 2011). Each category of landfill serves different types of waste. Malaysia began its operations of disposing waste into landfills or open dumps in the late 1970's (Fauziah & Agamuthu, 2012) and before that, the waste was burned or buried. About 92% of waste generated in Malaysia is disposed into 161 non sanitary landfill or open dumps. Landfill offers lower operation cost as compared to incineration and composting. Landfilling cost about RM50 per tonne while incineration and composting is RM100 and RM216 per tonne of waste, respectively (Agamuthu, 2001).

The number of landfills in Malaysia is shown in Table 2.7. Most of the landfills were built near urban sites for easy accessibilty by local authorities to transport waste. In the early 1980's, new disposal sites were built to accommodate MSW disposed. The use of non-sanitary landfills in the country has caused to several issues such as water and air pollution. Due to this, in 1995, the concept of sustainable development was adopted by Malaysian government to improve the waste disposal facilities. One of the outcomes from this development was the construction of sanitary landfill for MSW disposal (Fauziah & Agamuthu, 2012).

Table 2.7: Number of landfills in Malaysia according to states (Pauze, 2016)

State	Operating Landfill/dumps	Closed landfill/dumps	Total
Kedah	7	8	15
Perak	17	13	30
Perlis	1	1	2
Pulau Pinang	2	1	3
Johor	14	23	37
Melaka	1	7	8
Negeri Sembilan	6	13	19
Pahang	16	16	32
Kelantan	11	8	19
Terengganu	9	11	20
Selangor	8	14	22
Kuala Lumpur	1	10	11
Sabah	19	2	21
Sarawak	49	14	63
Total	**161**	**141**	**302**

2.4.1 Sanitary Landfill

Sanitary landfill is a MSW treatment facility that uses an engineered method for the disposal of waste. It is designed, constructed and operated in a manner to protect human health and the surrounding environment. The main components of a sanitary landfill are bottom liner, waste cell (new or old), leachate collection system, storm water drainage, gas collection system, landfill cover or cap, and ground water monitoring stations (EPA, 2014). The sectional view of a typical sanitary landfill is shown in Figure 2.5 and Figure 2.6.

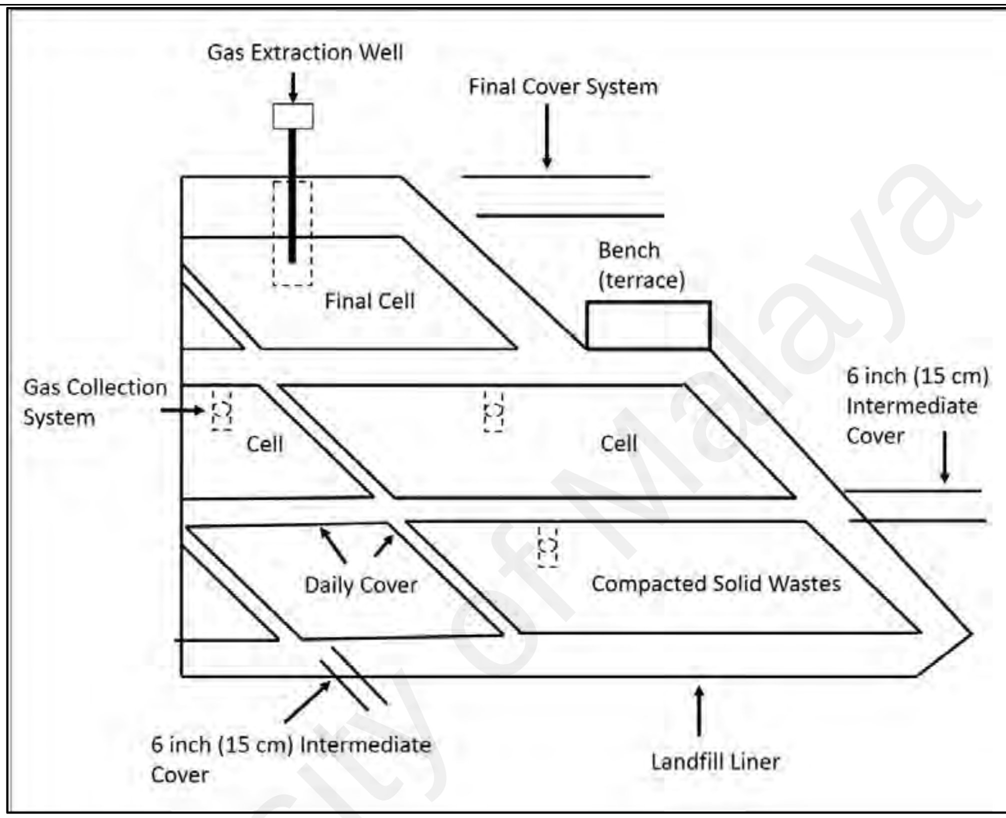

Figure 2.5: The section view of a typical solid waste sanitary landfill (Adapted from Tchobanoglous *et al*., 1993)

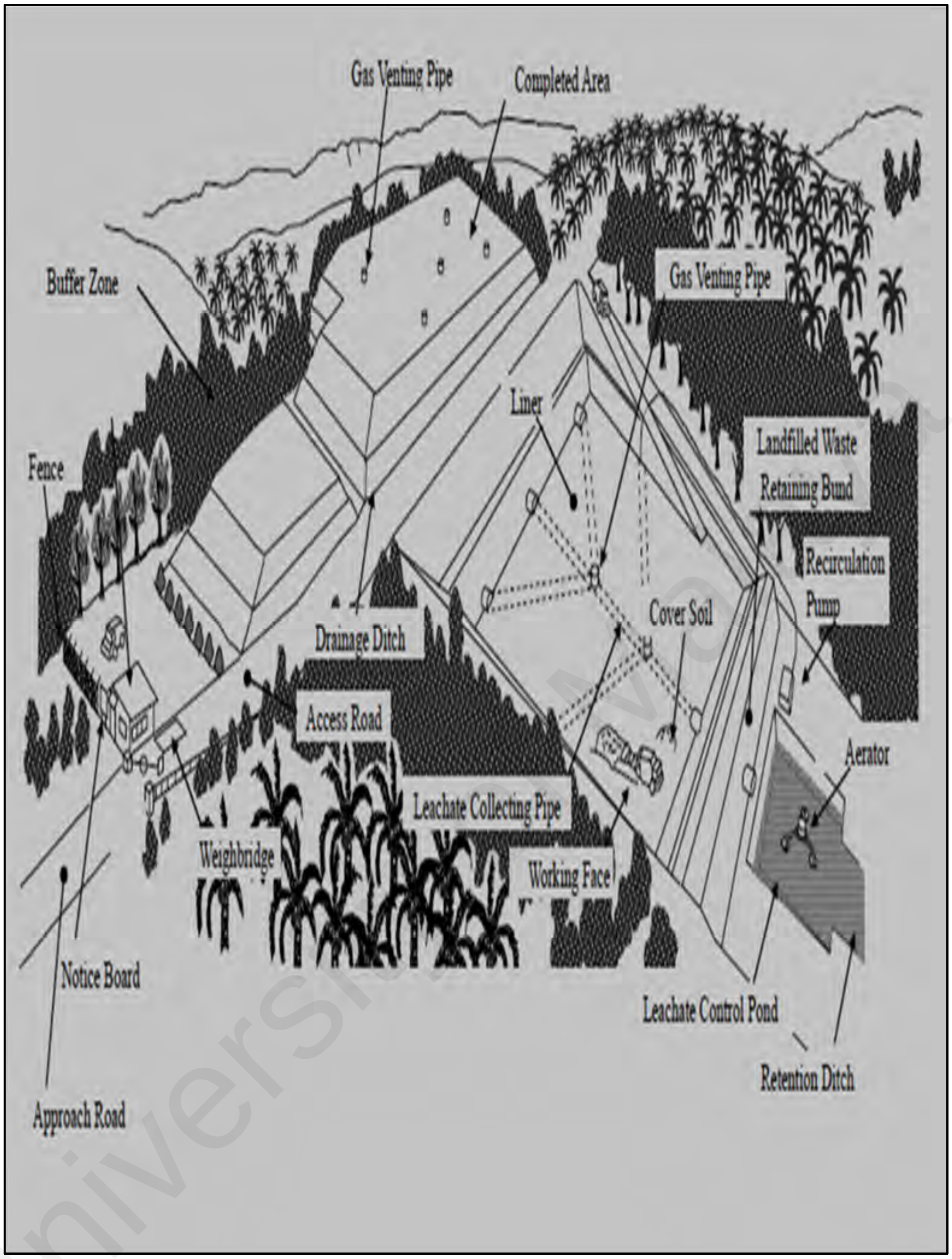

Figure 2.6: The sectional view of municipal solid waste sanitary landfill (NSWMD, 2012)

Sanitary landfill evolved in 1970's whereby it started with the application of daily cover, the compaction of waste is done efficiently and engineered approach is applied for containment of leachate (Emenike, 2013). Sanitary landfill can be divided into three categories which consist of

i) Class I: Hazardous waste

ii) Class II: Designated waste

iii) Class III: Municipal solid waste (MSW)

(Tchobanoglous & Kreith, 2002)

The primary functions of waste containment systems are (Reddi & Inyang, 2000; Emenike, 2013):

a) Minimization of the intrusion of moisture, which can generate and mobilize leachate;
b) Minimization of the transport of waste constituents into the surrounding environment; and
c) Isolation of wastes such that the potential for contact by humans and other animals is minimized.

According to Abdul Rahman Dahlan, Minister of Urban Wellbeing, Housing and Local Government, Malaysia currently has 161 waste disposal sites, with only 14 with the status of sanitary landfill (The Sunday Mail, 2016).

There are two major aspects associated with the building of a sanitary landfill. First is the management aspect which consist of key elements of landfill, planning, design, operation, environmental monitoring, closure and post closure control. The second aspect is technical, which consists of site selection, decomposition, liners, covers, leachate collection and treatment, gas collection and resource recovery or control, closure and post closure (Tchobanoglous & Kreith, 2002; Emenike, 2013).

In a sanitary landfill, waste received will be deposited into 1-2 m layers in low cells, compacted to the smallest volume and covered with 10-15cm thick of soil every day whereas the lining material at the bottom of the landfill is expected to prevent toxic elements and heavy metal from the leachate, from entering the groundwater system.

2.4.2 Non-sanitary landfill or open dumps

In Malaysia nearly 156 landfills were identified as non-sanitary landfills or illegal open dumps (The Sunday Mail, 2016). Open dumping is the improper disposal of any waste including household trash, garbage, tires, barrels, demolition/construction waste, appliances, shingles, pipes or metal. Open dump (Figure 2.7) do not have any control and monitoring system for its leachate and gas generation. Developed countries such as US & EU have totally prohibited the use of open dump for waste disposal (Fauziah, 2009).

About 95% of landfills in Malaysia are non-sanitary landfills or open dumps which can pose critical environmental and social threats (Agamuthu & Fauziah, 2011). Many of these landfill sites are operated beyond their capacity due to inadequate suitable and economical solution to waste disposal (Manaf *et al*., 2009). Non-sanitary landfills are mainly operating in low and middle income countries as a result of limited technical and financial resources (USEPA, 1998).

The advantages of open dump are its easy accessibilities, extended life span, low initial cost, easy access for scavengers for recycling item collection and recovery of materials are high. The disadvantages of open dumps are that, it can cause environmental contamination and overuse of landfill for disposal of waste without control and further more open dumping also gives a lot of severe impacts on environment such as:

(i) Surface and groundwater contamination through leachate,

(ii) Soil contamination through direct contact with waste or leachate,

(iii) Air pollution through the burning of wastes,

(iv) Spreading of diseases by different vectors like birds, insects and rodents,

(v) Stinking odour, and

(vi) Uncontrolled release of methane by anaerobic decomposition of waste.

(vii) Global warming

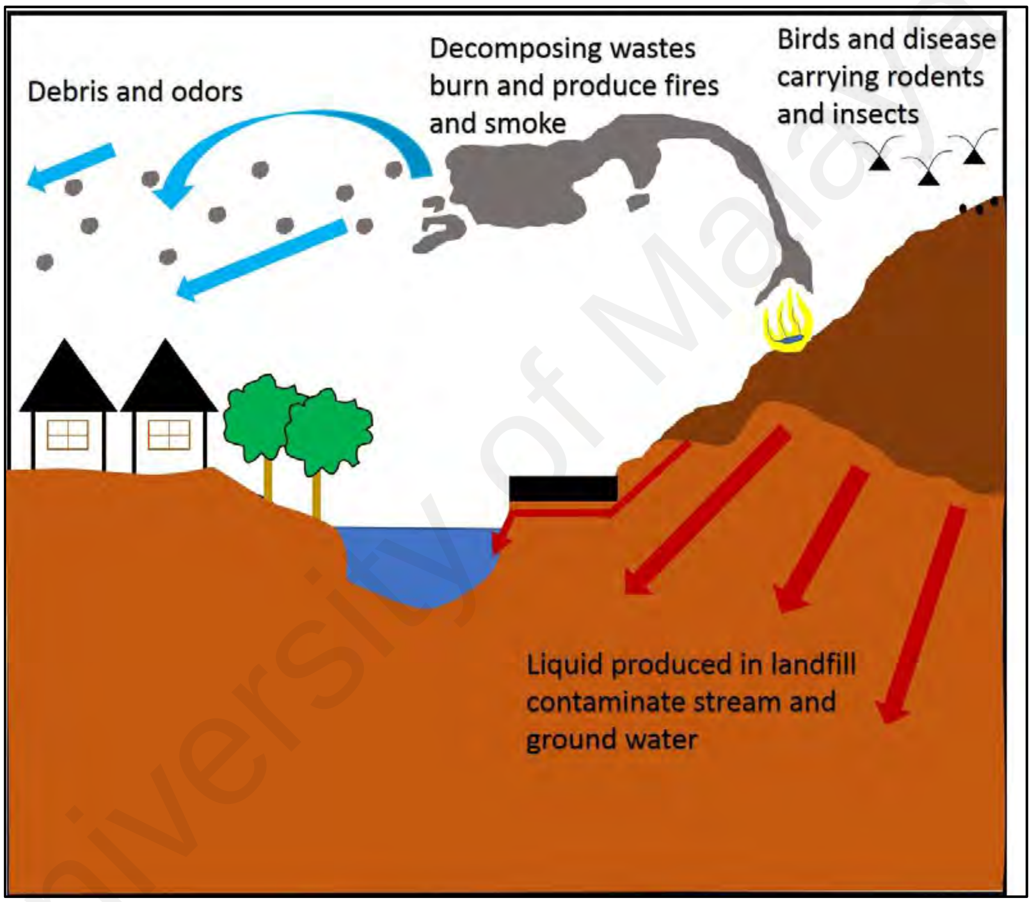

Figure 2.7: Structure of open dump

Non-sanitary landfills are always overloaded with MSW and its operations has always been extended to inadequacy of suitable and economic solutions (Manaf *et al*., 2009). Waste disposal into landfill greatly exposed river water to the risk of contamination from leachate unless proper leachate management is carried out. Groundwater is the major source of water supply for industrial purposes. The presence of high number of non-sanitary landfills and open dumps create an alarming situation due to groundwater contamination, especially heavy metals (Suratman & Sefie*,* 2011). Level of pollution may vary between landfills (Fauziah & Agamuthu, 2005).

The current issue with non-sanitary landfill or illegal dumps needs to be rectified to avoid further contamination to the environment.

2.5 Waste degradation in landfill

Figure 2.8 shows the process of waste degradation in landfill the moment MSW is deposited to the landfill which involve five main stages.

i. First stage of degradation

   Degradation of organic matters by aerobic bacteria takes place where long molecular chains of complex carbohydrates, proteins and lipids that are present in the organic waste, are broken down and oxygen is consumed and CO_2, water (H_2O) and heat are released (Steinlechner *et al*., 1994).

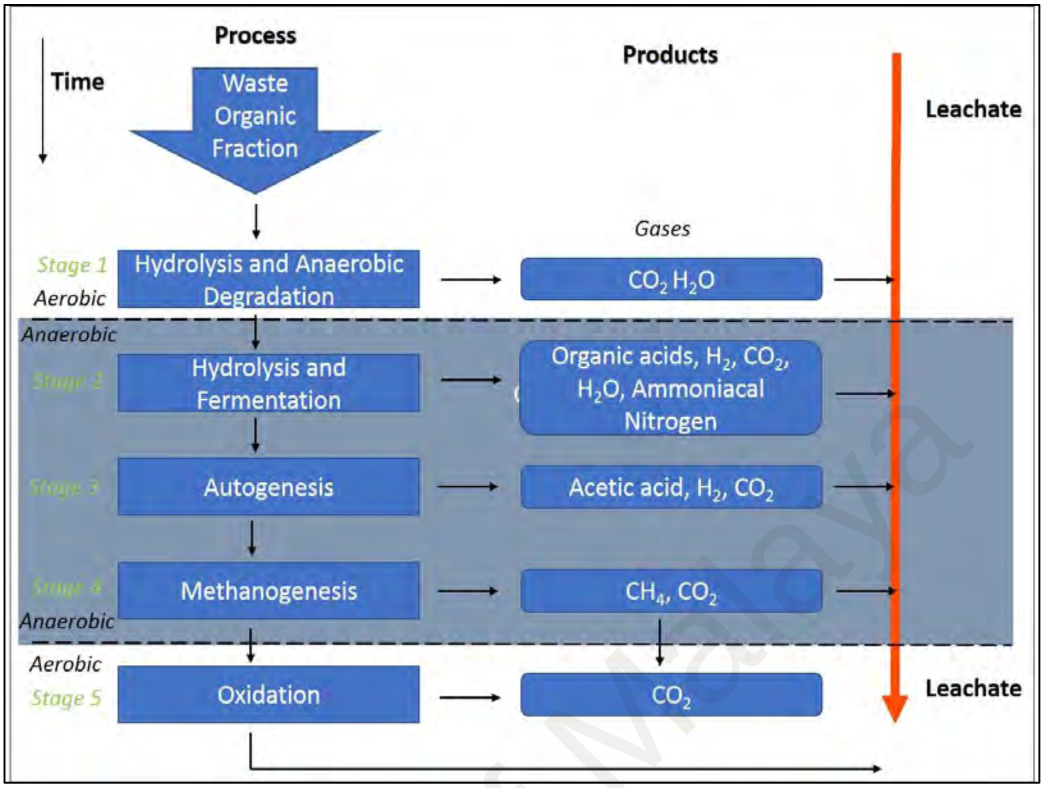

Figure 2.8: Major stages of waste degradation in landfills (HMSO, 1995)

CO_2 is released as gas or is adsorbed into H_2O to form carbonic acid (H_2CO_3), which makes the pH of leachate generated slightly acidic. The process can lasts for days or months, depending on the amount of oxygen present in the waste.

ii. Second Stage of degradation

During the second stage, facultative bacterium hydrolyzed (can survive in aerobic and anaerobic conditions) carbohydrates, proteins and lipids to glucose, amino acid, and fatty acids, respectively by extracellular enzymes produced by facultative and obligatory anaerobic bacterium. Hydrolysis is a necessary process because solid organic compounds must be solubilized before bacteria can do the conversion process. The dissolved organic fragments are fermented to CO_2, hydrogen (H_2), ammonia, and other organic acid (butyric acid, propionic acid, formic acid, valeric acid, etc).The leachate produced is high in ammonia due to deaminisation of proteins.

iii. Third stage of degradation

Third stage of the degradation process is the acidifying stage. During this stage, the organic acids produced from second stage is transformed to acetic acid (CH_3COOH), CO_2, and H_2 by the acetogen microorganisms. During this anaerobic stage, hydrogen sulfides are also produced by the reduction of sulphate compounds by sulphate reducing microorganisms. The landfill gas (LFG) produced will have the "rotten egg smell" (Williams, 2005).

iv. Fourth stage of degradation

The fourth stage is the main stage of LFG production which takes the longest time. Methanogenic microorganisms consume CO_2, H_2 and acetic acid produced from the third stage. The methanogenic take following pathway of:

$4H_2 + CO_2 \longrightarrow CH_4 + 2H_20$ (Reductive methane formation) and

$CH_3COO^- + H^+ \longrightarrow CH_4 + CO_2$ (Decarboxylation)

About 70% of CH_4 is produced from the process. During this process, LFG is generated over a temperature range of 30-65˚C by both mesophilic and thermophilic microorganisms (William, 2005).

v. Final stage of degradation

In the final stage, an aerobic condition occurred with aerobic microorganisms converting CH_4 generated in the previous stage to CO_2 and H_2O; while waste with high concentration of SO_4^{-2} will produce H_2S (William, 2005; Duffy, 2012).

2.6 Leachate generation and its toxicity components

MSW and precipitation are the major input in the landfill whereas landfill leachate and landfill gas are the primary output of landfill. Leachate is the liquid/fluid that is generated from from waste degradation, soil cover, rain and moisture in waste. Leachate is a high strength wastewater that has a major impact and influence on landfill design and its operation. Leachate varies from one landfill to another, and over space and time in a particular landfill with fluctuations that depend on short and long-term periods. Dominant biochemical process occurring in a landfill affects the leachate composition, where some parameters such as pH, bicarbonate, sulfate, iron, manganese, bulk organics (TOC, BOD and COD) and volatile fatty acids (VFAs), being mostly affected. Characterization of leachate, especially from municipal solid waste (MSW) landfills, has shown that it contains different groups of pollutants such as organics: alkenes, aromatic hydrocarbons, acids, esters, alcohols, hydroxybenzene, amides, and others, as well as ammonia nitrogen and high load of heavy metals (Emenike *et al*., 2013; Fauziah *et al*., 2013; Emenike *et al*., 2012; Kjeldsen *et al*., 2002).

Total leachate generated by Malaysian landfills is estimated to be about 6.0 million litres per day. Daily release of leachate generated contains 3835 g/L Fe and 23,400 g/L Zn (Agamuthu *et al*., 2011). The leachate composition is basically influenced by several factors which includes the composition of MSW, temperature in the landfill, degree of waste compaction, the absorptive capacity of the waste, age of waste in the landfill, size of the landfill, seasonal weather variations, level of precipitation, hydro geological conditions, engineering and operational factors of the landfill, pH of the soil in landfill, and landfill chemical and biological activities (Bhalla *et al*., 2012; Iaconi *et al*., 2006; Park *et al*., 2001). The complexity of the leachate characteristics make it difficult to manage (Zainol *et al*., 2012). The manner and rate of contamination depend on many factors, including:

a) Whether the soil is saturated or unsaturated,

b) The type of soil,

c) The type of material flowing through the soil, especially its solubility in water and its specific gravity,

d) The velocity and direction of natural groundwater flow,

e) The rate of infiltration from the source.

(Emenike, 2013).

Figure 2.9 shows the movement of water in a landfill system. It is important to understand the movement of water in the landfill. In a sanitary landfills leachate collection pond contain and accumulate leachate so that it does not flow out of the landfill area before physical, chemical or biological method treatment.

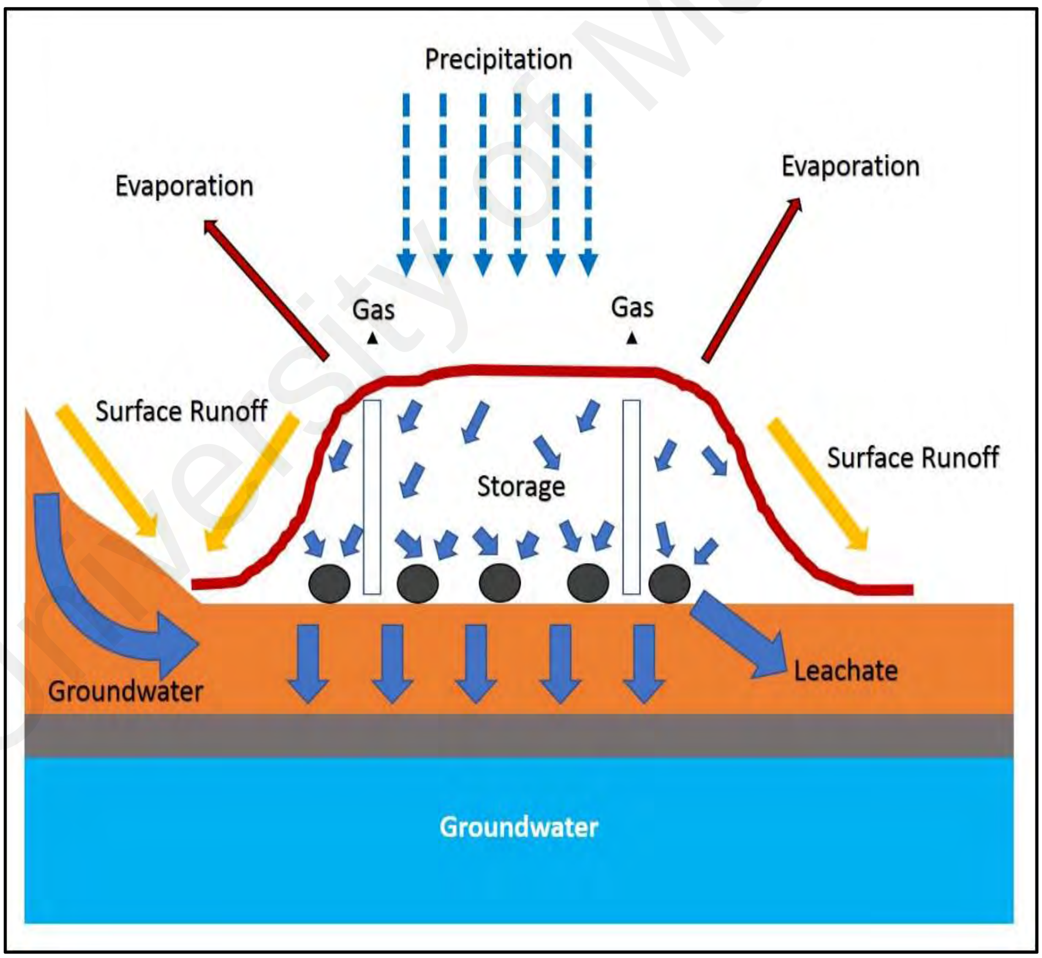

Figure 2.9: Movement of water in the landfill

2.6.1 Leachate composition

An understanding of leachate composition is critical for making projections on the long-term impacts of landfills as well as the treatment options. The potential and degree of risk posed to groundwater, soil and even aquatic life by landfill leachate is extremely difficult to assess without understanding the composition of leachate (Emenike, 2013).The raw leachate components released daily from landfill in Malaysia is shown in Figure 2.10. Leachate generation can go on to 30 to 50 years after the closure of a landfill. While leachate production decreases significantly with placement of the final cover, there is little data on leachate production over long periods of time. Furthermore, in assessing the long-term stability of a landfill, the possibility that the integrity of the landfill cover will decrease must be considered. Should the cover integrity deteriorate, the quantity of leachate could actually increase long after landfill closure (Kjeldsen *et al.*, 2002). Composition of landfill leachate from sites in Malaysia, Denmark, USA and Spain are given in Table 2.8 and Table 2.9 for better understanding and comparison of the leachate composition. Composition of landfill leachate can be divided into four groups:

a) Dissolved organic matter, quantified as Chemical Oxygen Demand (COD) or Total Organic Carbon (TOC), volatile fatty acids (that accumulate during the acid phase of the waste stabilization, (Christensen & Kjeldsen, 1989) and more refractory compounds such as fulvic-like and humic-like compounds.
b) Inorganic macrocomponents: calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ammonium ($NH4^{+}$), iron ($Fe2^{+}$), manganese (Mn^{2+}), chloride (Cl^{-}), sulfate (SO_4^{2-}) and hydrogen carbonate (HCO_3^{-}).
c) Heavy metals: cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+}).

d) Xenobiotic organic compounds (XOCs) originating from household or industrial chemicals and present in relatively low concentrations (usually less than 1 mg/l of individual compounds). These compounds include among others a variety of aromatic hydrocarbons, phenols, chlorinated aliphatics, pesticides, and plastizers.

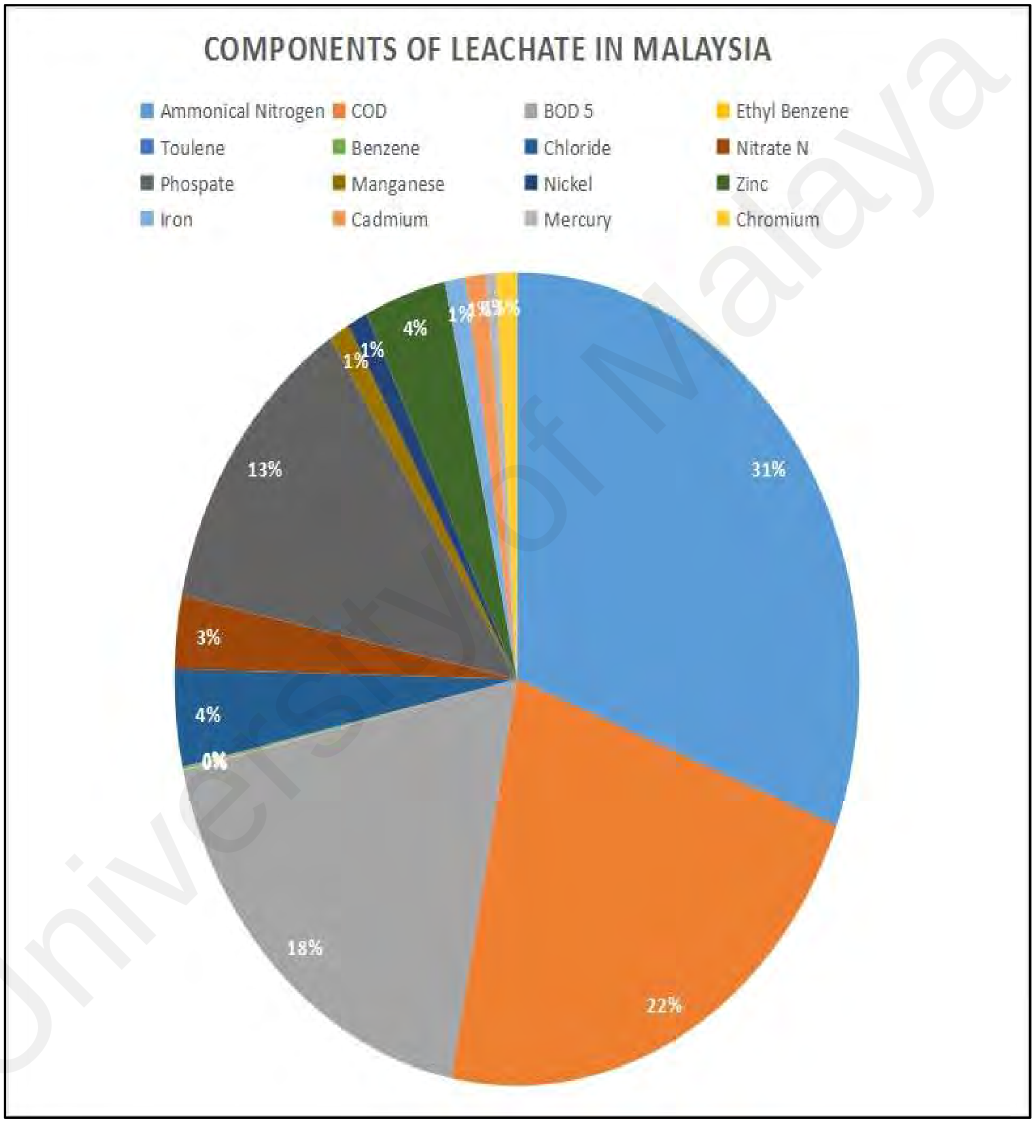

Figure 2.10: Raw leachate components released daily from a landfill in Malaysia. Adapted from Emenike (2013**)**

Table 2.8: Composition of landfill leachate in Malaysia (Emenike, 2013; Emenike *et al*., 2012)

Parameters	**Unit**	**Jeram Sanitary Landfill**	**Air Hitam Landfill**
Apparent colour	-	Black	Bright Brown
Odour	-	Slight ammonic	Stench ammonic
pH	-	7.35	8.2
Temperature	° C	27.5	29.5
Salinity	$^{0}/_{100}$	5.7	8.3
Conductivity	mS/cm	10.04	20
Turbidity	FAU	4150	108
Dissolved oxygen	mg/L	5.8	5.8
BOD	mg/L	27000	3500
COD	mg/L	51200	10234
BOD: COD ratio	mg/L	0.53	0.34
TDS	mg/L	1730	830
Suspended soil	mg/L	688	97
Total organic carbon	mg/L	380	110
Oil & Grease	mg/L	48	7
Chloride	mg/L	4150	4150
Sulphate	mg/L	54.89	37.1
Phosphate	mg/L	113	70.2
Nitrate nitrogen	mg/L	38.6	29.1
Nitrite nitrogen	mg/L	4.8	2.7
Alkalinity	mg/L	1980	9000
Ammonical Nitrogen	mg/L	600	880
Mercury	mg/L	0.05	0.12
Chromium	mg/L	25.24	0.11
Copper	mg/L	3.59	<0.01
Nickel	mg/L	19.51	0.29
Zinc	mg/L	827.7	0.1
Manganese	mg/L	540.6	0.12
Iron	mg/L	97.76	3.10
Calcium	mg/L	20.17	25.6
Potassium	mg/L	530	440
Magnesium	mg/L	11.4	20.3
Sodium	mg/L	58.7	48.6

Table 2.9: Landfill leachate composition of selected countries (Yu *et al*., 2016; Agamuthu, 2001; Corona *et al*., 1998; Johnsen & Carlsen, 1976)

Parameters	**Denmark**	**USA**	**Spain**
pH	4.5- 9	5.4-6.4	8.4
Conductivity (ms/sm)	2500- 35000	-	18.52
Total C (mg/L)	30 -2900	-	-
N(mg/L)	14-2500	56-630	-
Total P(mg/L)	0.1-2.3	5.9-11.3	-
Chlorides (mg/L)	150-4500	-	-
Sulfates(mg/L)	8-7750	-	-
Na(mg/L)	70 -7700	-	938
K(mg/L)	50-3700	-	730
Ammonium (mg/L)	50-2200	-	-
Fe(mg/L)	3- 5500	-	148.5
Mn (mg/L)	0.03-1400	-	10.52
Cu(mg/L)	0.05-10	0.18-1.3	0.34
Pb(mg/L)	0.001-5	0.1-1.4	0.52
Ni (mg/L)	0.015-13	-	-
Zn(mg/L)	0.03-1000	5.3-155	2.16
Cd(mg/L)	-	0.01-0.03	0.23

Characterization of leachate is important because it is the reflection of type of waste dumped into the landfill and leachate is produced through the biological and physico-chemical interaction that occurs during waste degradation process in the landfill (Emenike, 2013). Some of the leachate components are contaminants which have toxic in nature especially persistent organic pollutants (POP), monocyclic aromatic hydrocarbons, heavy metals and others. The concentration of substances in leachate can also vary based on where the leachate sample were collected.

2.6.2 Acceptable conditions for discharge of leachate according to EQA Regulations 2009

According to Environment Quality (Control of Pollutions from Solid Waste Transfer Station and Landfill) Regulations 2009 of EQA 1974, acceptable conditions for discharge of leachate are as in Regulation in Table 2.10 (DOE, 2010). This regulation applies to the solid waste transfer stations and landfills which discharged or released leachate.

Table 2.10: Acceptable Conditions for Discharge of Leachate (Malaysia)

	(1) Parameters	(2) Unit	(3) Standard
i.	Temperature	°C	40
ii.	pH value	-	6.0-9.0
iii.	BOD_5 at 20°C	mg/L	20
iv.	COD	mg/L	400
v.	Suspended Solids	mg/L	50
vi.	Ammoniacal Nitrogen	mg/L	5
vii.	Mercury	mg/L	0.005
viii.	Cadmium	mg/L	0.01
ix.	Chromium hexavalent	mg/L	0.05
x.	Chromium trivalent	mg/L	0.20
xi.	Arsenic	mg/L	0.05
xii.	Cyanide	mg/L	0.05
xiii.	Lead	mg/L	0.10
xiv.	Copper	mg/L	0.20
xv.	Manganese	mg/L	0.20
xvi.	Nickel	mg/L	0.20
xvii.	Tin	mg/L	0.20
xviii.	Zinc	mg/L	2.0
xix.	Boron	mg/L	1.0
xx.	Iron	mg/L	5.0
xxi.	Silver	mg/L	0.10
xxii.	Selenium	mg/L	0.02
xxiii.	Barium	mg/L	1.0
xxiv.	Fluoride	mg/L	2.0
xxv.	Formaldehyde	mg/L	1.0
xxvi.	Phenol	mg/L	0.001
xxvii.	Sulphide	mg/L	0.50
xxviii.	Oil and Grease	mg/L	5.0
xxix.	Colour	*ADMI	100

*ADMI- American Dye Manufacturers Institute

This regulations also indicates that no person shall discharge leachate which contains substances in concentrations greater than the limit allowed onto or into any soil, or into any inland waters in Malaysia.

The non- compliance will be investigated under the Act and if find guilty can be fined not exceeding RM500, 000/USD122, 770 or a jail term of not more than 5 years or both, and additional fine of RM1000/USD 245 for each day of offense continued.

2.7 Heavy metal

Heavy metals in leachate are inorganic elements which has its own importance. Metal is considered as heavy metal when the density is above 5 g/ cm^3. Besides that it is also based on atomic weight, atomic number or other chemical properties. Heavy metals are categorized into three categories namely essential metal, non-essential metal and toxic metal (Banik *et al*., 2014). Essential heavy metal such as Cu, Zn, Mn, Cr, Ni, Fe is required in trace quantities for biological function. Non-essential metal with no known biological effect are Rubidium, Strontium and titanium. Toxic metals such as Hg, Pb, Cd and As are highly toxic and are not required by living organisms (Norouznia & Hamidian, 2014). Heavy metal when present in high concentration can block the essential functional groups, displace other metal ions or even modify the active conformation of biological molecules (Garbisu & Alkorta, 2003). The mobility of heavy metal is strongly affected by the pH, redox potential and the presence of complex agents (Bozkurt *et al*., 2000).

Global annual heavy metal estimation from all sources for Pb is 783,000 metric tonne, for Cd is 22,000 metric tonne, for Cu is 939,000 metric tonne and for Zn is 1, 350,000 metric tonne (Oves *et al*., 2016).

2.7.1 Different source of heavy metals contamination to the environment

Identifying the source of heavy metal is important since heavy metal has toxicity effects to human health, animals, plants and the ecosystem. The schematic flow diagram of heavy metals into waste is illustrated in Figure 2.10.

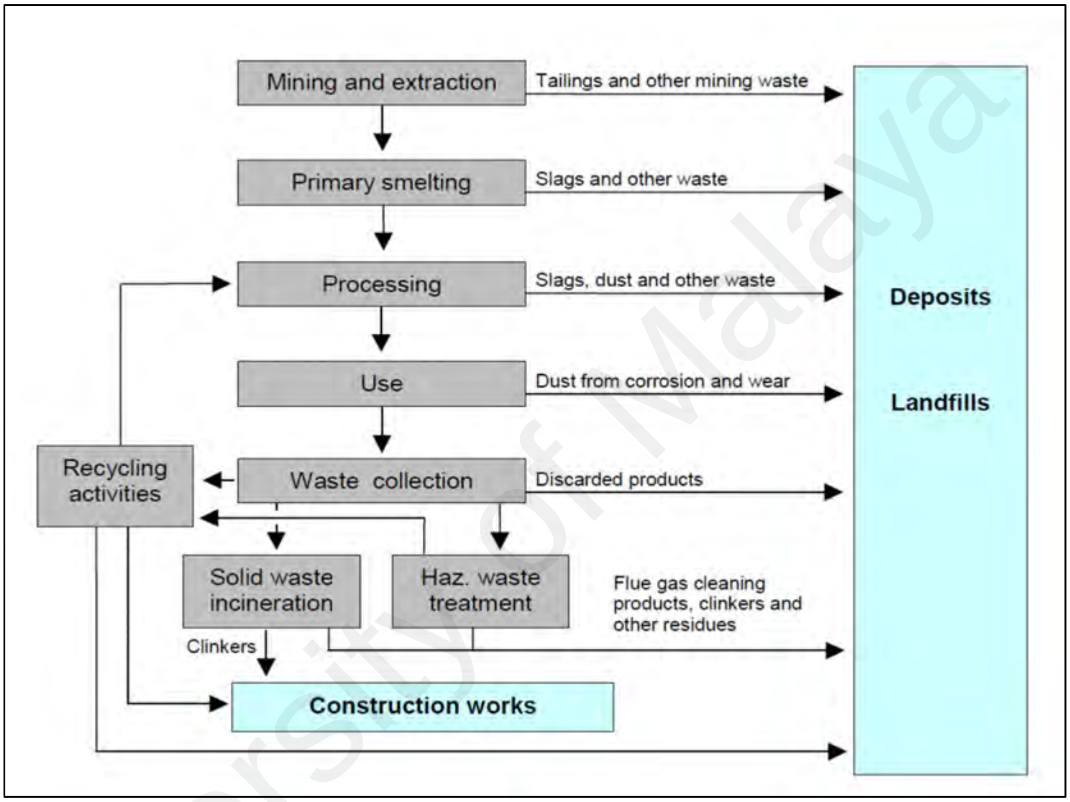

Figure 2.11: Schematic diagram of heavy metals flow into waste. Adapted from European Commission (2002)

Anthropogenic activities contributed one to three fold higher magnitude of heavy metal emission as compared to natural sources (Sposito & Page, 1984). Heavy metal contamination in soil needed more attention because soil exhibit more rapid rates of generation via man made cycles, can be easily transferred from one source to another and direct exposure are higher, metal concentration in the discarded product are high and its bioavailability are higher compared to other environmental media. A simple mass balance of the heavy metals in the soil can be expressed as follows

$$Mtotal = (Mp + Ma + Mf + Mag + Mow + Mip) - (Mcr + Ml)$$

Where “M” is the heavy metal, “p” is the parent material, “a” is the atmospheric deposition, “f” is the fertilizer sources, “ag” are the agrochemical sources, “ow” are the organic waste sources, “ip” are other inorganic pollutants, “cr” is crop removal, and “l” is the losses by leaching, volatilization, and so forth.

Heavy metal in the environment can be sourced from natural or anthropogenic activities. For natural source of heavy metal is mainly from the weathering of underlying bedrock while for anthropogenic sources can be from several sources as following: metalliferous mining and smelting (As, Cd, Pb, Hg), industry (As, Cd, Cr, Co, Cu, Hg, Ni, Zn, atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg, U), agriculture (As, Cd, Cu, Pb, Si, U, Zn) and waste disposal (As, Cd, Cr, Cu, Pb, Hg, Zn). The global heavy metal pollution sites are listed Table 2.11.

Table 2.11: Global heavy metal pollution sites (EEC, 2007; ADEC, 2010; EDMC, 2014; USEPA, 2014; He *et al*., 2015)

Country	**Number of Polluted sites**	**% of heavy metal**
Global	> 10000000	>50
USA	>100000	>70
European Union	>80000	37
Australia	>50000	>60
China	1.0 million km^2	> 80

The source of heavy metal due to anthropogenic activities can be sourced from usage of fertilizers, landfilling, pesticides, biosolids and manures, wastewater, metal mining and air-borne sources. The detailed of the source of heavy metals is further described as below.

Fertilizers - During the process of growing crops, large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K. However the compounds used to supply these elements contain trace amounts of heavy metals (e.g., Cd and Pb) that may significantly rise their content in the soil. Cd and Pb do not have any physiological activity in plants that the application of certain phosphatic fertilizers unintentionally adds more Cd and other potentially toxic elements into the soil, including Fe, Hg, and Pb (Wuana & Okieimen, 2011).

Landfilling - Landfill leachate poses high risk of contamination to the nearby water bodies that could be source for drinking water or habitat for the aquatic organisms because of its toxic and hazardous compounds which includes heavy metals. Landfill leachate contains various type types of heavy metal such as Pb, Zn, As, Mn, Cr, Fe, Ni, Cu, Hg, and Cd. This is because landfills received various type of to supply varying degree of heavy metal into the leachate (Emenike, 2013).

Pesticides - Pesticides contain substantial concentrations of metals. Insecticides and fungicides in UK were based on compounds which contained Cu, Hg, Mn, Pb, or Zn. Pb arsenate was used in fruit orchards for many years to control some parasitic insects. In New Zealand and Australia, As containing compounds were also used extensively to control cattle ticks. To control pests in banana timbers have been preserved with formulations of Cu, Cr, and As (CCA), and there are now many sites with the soil metals concentrations that exceeded the required concentrations. Such contamination has the potential to cause health and environmental problem (Wuana & Okieimen, 2011; Emenike, 2013).

Biosolids and Manures- Biosolid and manures that contain heavy metals such As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn are applied to agricultural lands as fertilizers. Since Cu and Zn as growth promoters and As are added into poultry health products, it may cause metal contamination to the soil from the animal manure.

Land application of biosolid materials is a common practice in many countries. In United States, half of 5.6 million dry tonnes of biosolids are applied to land while in Europe, over 30% of the biosolids are used as fertilizer in agriculture land (Wuana & Okieimen, 2011). In Australia over 175, 000 tonnes of dry biosolids are produced each year by the major metropolitan authorities, and currently most biosolids are used in arable cropping situations where they can be incorporated into the soil (McLaughline *et al*., 2000). If this application continues, there will be high accumulaation of heavy metal in the soils. Common heavy metals found in biosolids are Pb, Ni, Cd, Cr, Cu, and Zn, and the metal concentrations are determined by the nature and the intensity of the industrial activity, as well as the type of process employed during the biosolids treatment. Under certain circumstances, biosolid applied to the land can be leached through the soil and can have the potential to contaminate groundwater (Wuana & Okieimen, 2011).

Wastewater - The application of municipal and industrial wastewater and related effluents to land for agricultural purpose is a common practice in many parts of the world (Emenike, 2013). In several African Asian countries, agriculture based on wastewater irrigation accounts for 50 % of the vegetable supply to urban areas (Bjuhr, 2007). The metal concentrations in wastewater effluents are usually considerably low, but in long term application in land which can eventually result in heavy metal accumulation in the soil.

Metal Mining/Milling Processes and Industrial Wastes - Metal mining and milling of metal ores in industries have contributed to wide distribution of heavy metal contaminants in soil. During metal mining tailings (heavier and larger particles settled at the bottom of the flotation cell during mining) are directly discharged into wetlands resulting in elevated concentrations of heavy metal in the environment (DeVolder *et al*., 2003). Extensive mining of Pb and Zn smelting have resulted in contamination of soil that posed risk to human and ecological health. Other materials generated by a variety of industries such as textile, tanning, petrochemicals from accidental oil spills or utilization of petroleum-based products, pesticides, and pharmaceutical facilities are highly variable in composition. Many of the disposed items to the land are potentially hazardous because of their heavy metals content (Cr, Pb and Zn) or toxic organic compounds (Wuana & Okieimen, 2011)

Air-Borne Sources - Heavy metal can also identified from air emmission namely from stack or duct emissions of air, gas, or vapor streams, and fugitive emissions such as dust from storage areas or waste piles (Wuana & Okieimen, 2011). The metals are released to the air as particulates. Metals such as Pb, Cd or Pb can be volatilized at high temperature to be converted to oxides and present as particulates (Smith *et al*., 1995). Stack emissions can be easily dispersed to a large area by wind and react with precipitation. Fugitive emissions are often distributed over to smaller area because emissions are made near the ground level. The type and concentration of metals emitted from both types of sources will depend on site-specific conditions. The solid particles coming out as smoke from fires and factory chimneys are eventually deposited on land or sea. High amount of Cd, Pb, and Zn has been found in plants and soils adjacent to smelting works. Contamination through anthropogenic activities affects the natural resources and can affect the agricultural field or food production especially in developed countries (Wuana & Okieimen, 2011).

Based on the metal chemistry of the different heavy metals and its effects to the human health and the environment, the contamination of heavy metal is obviously serious issue of concern. The following sections discuss the existing heavy metal contamination reported all over the globe.

2.8 Heavy metal contamination

Heavy metal contamination to the soil from various sources is a global problem. Table 2.12 shows the level of different types of heavy metal pollutant to the soil in different countries which exceed the maximum allowable limit. Soil pollution with heavy metals is multidimensional. Upon entering the soil in large amounts, heavy metals primarily affect biological characteristics. The total content of microorganism changes, their species diversity reduced, and the intensity of basic microbiological processes and the activity of soil enzymes decreases. More than 10 million contaminated sites exist worldwide, with more than 50% of the sites contaminated with heavy metal (loid)s (He *et al*., 2015). Majority of these heavy metal(loid) contaminated sites exist in developed countries such the United States of America (USA), Australia, Germany, Sweden and China owing to their increased use in industrial processes (Foucault *et al*., 2013; Goix *et al*., 2014). Excess heavy metal accumulation in soils is toxic to humans and other animals. Exposure to heavy metals is normally chronic (exposure over a longer period of time), due to food chain transfer. Research conducted by Joint Research Centre (JRC) in 27 countries revealed that highest contamination in the soil and groundwater sourced from heavy metal. Soils have the ability to tolerate these contaminants, through filtering or transformation and once this ability is exceeded, issues such as water pollution, human contact with polluted soil, plants taking up contaminants become more significant (EEA, 2007).

Table 2.12: Worldwide heavy metal polluted soils that exceeded permissible limit

Heavy metal	Concentration in soil (mg/kg)	Maximum allowable limit	Study area	References
Cd	42	3	Southern Italy	Baldantoni *et al*., 2016
	19		India	Tiwari *et al*., 2011
	16		Switzerland	Quezada-Hinojosa *et al*., 2015
	14		Mexico	Torres *et al*., 2012
	14		China	Shi *et al*., 2015
Pb	4500	100	China	Luo *et al*., 2011
	1988		China	Niu *et al*., 2015
	711		UK	Nabulo *et al*., 2011
	452		Uganda	Nabulo *et al*., 2012
	302		Brazil	Carvalho *et al*., 2014
As	7490	20	Spain	Beesley *et al*., 2014
	4357		Italy	Marabottini *et al*., 2013
	354		China	Wei *et al*., 2015
	131		Korea	Myoung Soo Ko *et al*., 2015
	64		Bolivia	Acosta *et al*., 2015
Zn	3833	300	China	Niu *et al*., 2015
	370		Nigeria	Obiora *et al*., 2016
	1168		Germany	Shaheen *et al*., 2014
	905		Portugal	Anjos *et al*., 2012
	393		-	Kwon *et al.,* 2015
Ni	2603	52	Mexico	Torres *et al*., 2012
	373		Spain	Lago *et al*., 2016
	201		Zibmbabwe	Mapanda *et al*., 2007
	200		Turkey	Avci & Deveci, 2013
	153		China	Wang *et al*., 2015
Cu	35,582	100	Mexico	Torres *et al*., 2012
	19581		Australia	Sacristán *et al*., 2016
	448		China	Wang *et al*., 2015
	235		Portugal	Anjos *et al*., 2011
Cr	4309	100	Spain	Arenas-Lago *et al*., 2016
	590		China	Xu *et al*., 2014
	418		Greece	Panagopoulos *et al*., 2015
	224		Germany	Shaheen *et al*., 2014

2.9 Effects of heavy metal contamination

Heavy metal contamination that occurs to the environment mainly causes significant effect to human health, plants and soil. The effects of metals is further described below.

2.9.1 Effects to human health

According to Mclaughin *et al*. (2000) and Ling *et al*. (2007), heavy metal contamination in soil poses threat to human and ecosystem through direct ingestion or contact with contaminated soil. In the food chain, toxicity occur from soil > plant > human or soil > plant > animal > human. It also affects the human health through drinking of contaminated groundwater and the consumption from contaminated plant, and even induces reduced agricultural production (Wuana & Okieimen 2011). Besides that he heavy metal contamination from the environment can directly have impact on human through dust inhalation or skin absorption. Table 2.13 shows the effects of heavy metal on human health.

Table 2.13: Effects of heavy metal on human health (Dixit *et al*., 2015)

Heavy Metal	EPA Regulatory Limit (ppm)	Toxic Effects	References
Ag	0.10	Exposure may cause skin and other body tissues to turn gray or blue-gray, breathing problems, lung and throat irritation and stomach pain.	ATSDR 1990
As	0.01	Affects essential cellular processes such as oxidative phosphorylation and ATP synthesis	Tripathi *et al* 2007
Ba	2.0	Cause cardiac arrhythmias,respiratory failure, gastrointestinal dysfunction, muscle twitching and elevated blood pressure	Acobs *et al* 2002
Cd	5.0	Carcinogenic,mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation in biological systems	Salem *et al* 2000; Degraeve 1981
Cr	0.1	Hair Loss	Salem *et al* 2000
Cu	1.3	Brain and kidney, damage, elevated levels result in liver cirrhosis and chronic anemia, stomach and intestine irritation	Salem *et al* 2000; Wvana & Okiemen 2011
Hg	2.0	Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure	Neustadt & Pieczenik 2007; Ainza *et al* 2010; Gulati *et al* 2010
Ni	0.2	Allergic skin diseases such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxic, neurotoxic genotoxic, affects fertility, hair loss	Salem *et al* 2000; Khan *et al* 2007; Das *et al* 2008; Duda & Baszezyk 2008
Pb	15	Excess exposure in children causes impaired development, reduced intelligence, short-term coordination problems, risk of cardiovascular disease	Salem *et al* 2000; Wyana & Okieimen 2011
Se	50	Dietary exposure of around 300 µg/day affects endocrine function, impairment of natural killer cells activity, hepatotoxicity and gastrointestinal disturbaces	Vinceti *et al* 2001
Zn	0.5	Dizziness, fatigue etc.	Hess & Schmid 2002

2.9.2 Effects to plants

Plants growing in the metal contaminated soils are at high degree of risk because of the bioavailability of these metals (Clemens & Ma, 2016). This results in accumulation of heavy metal in plant parts consumed by humans or through meat, milk or other products obtained from animal feeds on these plants. Table 2.14 shows the specific metal effect to the plant. Mercury, arsenic, lead, cadmium and chromium are ranked among the most toxic metals that are of great public health significance (Tchounwou *et al*., 2012).

Table 2.14: Effect of heavy metal to plant (Mathew, 2005)

Type of heavy metals	**Effects to the plant**
Chromium	Reduces the rate of photosynthesis and enzyme activity in the plant and also cause damage to the plant membrane, chlorosis and root damage.
Cadmium	Decreases seed germination, lipid content and growth of the plant
Copper	Affects the plant growth and reproductive processes, affect the photosynthesis and decrease the thylakoid surface area
Mercury	Decrease the photosynthesis activity, uptake of water into the plant, and the antioxidants enzymes
Nickel	Reduces the seed germination of plant, protein production, and chlorophyll and enzyme production
Lead	Reduces the production of chlorophyll in plant and affects the plant growth.
Zinc	Reduces the seed germination of plant

2.9.3 Effects to soils

Soil plays a major role in the environment. It provide a vital role for the growth of soil microbes that is important for nutrient cycling and plant growth. The soil organic matter helps in maintaining soil quality (Siva & Prasada, 2016).

In contaminated soil, soil microorganisms is the first biota that faces direct impacts from heavy metal. Metals such as Fe, Zn, Cu and Ni are important for soil microbial activities such as metabolism and redox processes. However when the soil are polluted with high concentration of this metals, it undergo inhibitory effect or toxic effects. Exposure of soil microbes to high concentration of heavy metal can reduced the soil respiration activity, decreased in the decomposition of organic matter, reduced in the microbial diversity and decrease in the activity of soil enzymes. Besides that heavy metal also affect the growth, morphology and metabolisms of soil microorganisms through functional disturbance, protein denaturation or destruction of the integrity of cell membranes (Leita *et al*., 1995). Effect of heavy metal to soil microorganisms is further explained in Table 2.15.

Table 2.15: Effect of heavy metal to soil microorganisms (Ayangbenro &Bababola, 2017)

Heavy metal	Effect to microorganisms
Arsenic	Deactivation of enzymes
Cadmium	Damage nucleic acid, denature protein, inhibit cell division and transcription, inhibits carbon and nitrogen mineralization.
Chromium	Elongation of lag phase, growth inhibition, inhibition of oxygen uptake
Copper	Disrupt cellular function, inhibit enzyme activities
Mercury	Decrease population size, denature protein, disrupt cell membrane, inhibits enzyme function
Lead	Denatures nucleic acid and protein, inhibits enzymes activities and transcription
Nickel	Disrupt cell membrane, inhibit enzyme activities, oxidative stress
Zinc	Death, decrease in biomass, inhibits growth

The toxicity of heavy metals on soil microorganisms depends on a number of factors such as soil temperature, pH, clay minerals, organic matter, inorganic anions and cations, and chemical forms of the metal (Friedlova, 2010; Nannipieri *et al*., 1997; Baath, 1989).

2.10 Soil heavy metal contamination from leachate

The substantial release of leachate from landfills and open dump cause significant contamination to the soil. This may lead to changes in the soil behaviour due to toxicity nature of leachate that contains heterogeneous compounds. According to previous studies soil contaminated with leachate have change in the soil characteristics due to chemical reactions between the soil mineral particles and the contaminant in the leachate (Sunil *et al*., 2009). In addition, heavy metals also changes humus content, structure, and pH of soils (Levin *et al*., 1989). Heavy metal in soil can be presented in five different fractions, based on the properties of the individual metals. The various fractions are dissolved in soil solution, attached to exchange sites on inorganic soil constituents, adsorbed to inorganic soil constituent, attached to insoluble organic matter, and precipitates of pure or mixed solids (Ann, 2001).

Besides that soil contamination with leachate can also occur due to improper selection and engineering of landfill. Contamination of leachate serve as an external force affecting the physico-chemical characteristics of soil ultimately contributing towards the poor production of vegetation (Papageorgiou *et al*., 2006). The disturbances of higher intensity sometimes endanger the survival of some species and yield low richness (Hussain & Palmer, 2006).

2.11 Leachate contamination to the nearby water source

Landfills have been identified as one of the major threats to groundwater resources (Fatta *et al*., 1999; USEPA, 1984). Leachate from MSW landfills is highly concentrated whereby even small amounts of leachate can pollute large amounts of groundwater, leaving it unsuitable for domestic water use. Due to migration of leachate, soils have been contaminated with heavy metals such as Pb, Cu, Zn, Fe, Mn, Cr, and Cd and these heavy metals in solid wastes lead to serious problems because it cannot be degraded.

The solid waste dumped in landfills are subjected to either groundwater underflow or infiltration from precipitation. Leachate accumulates at the bottom of the landfill and percolates through the soil and finally reaches the groundwater. Areas nearby landfills have higher possibility of groundwater contamination because of the potential pollution source of leachate. Such contamination of groundwater resource poses a substantial risk to local resource user and to the natural environment. Water sources are important in daily life for mankind, because it has many benefits such as agricultural purposes, for domestic and industrial usage, livestock farming and for mining activities.

Area that has shallow water table and high precipitation are more prone to groundwater contamination. Transport of contaminated leachate through the landfill to the groundwater and surface water happens through two main methods: advection and hydrodynamic dispersion (Frost & Griffin, 1977). The risk of groundwater contamination by leachate that is is determined by the following factors; concentration and toxicity of contaminants, permeability and type of the geologic strata and the direction of groundwater flow.

Rahim *et al*. (2010) investigated the effect of MSW landfill leachate on groundwater quality in Malaysia. Their results showed that the elevated concentration of chloride (355.48 mg/l), nitrate (10.40 mg/l), nitrite (14.59 mg/l), ammonia (11.61 mg/l), Fe (0.97 mg/l), and Pb (0.32 mg/l) indicates that the groundwater quality was extremely affected by the migrated leachate from landfill sites.

In recents years, the impact of leachate on groundwater and other water resource, has attracted a lot of attention. Migration of leachate from landfill to water bodies poses high risk to human health due their toxicity effect. Groundwater wells nearby El Jadida Landfill in Morace was reported to contain high amount of Cr (15-25 ug/L) and Cd (60-100ug/L) (Eaton & Franson, 2005; Magda & Gaber, 2015). Water wells at Sri Lanka also have been reported to be contaminated by leachate whereby the water was found to be highly acidic with Cd level in the range of 25 - 38 ug/L (Bandara & Hettiaratchi, 2010).

Agamuthu (2010) studied the impact of leachate contamination from Kundang landfill to a nearby river and reported that the river were detected to be polluted with high amount of Cr, Pb, and Zn.

It was recently reported that few solid waste landfills were found to have serious and recurring leachate contamination issues. List of the landfills are Taman Beringin Landfill, Sungai Udang Landfill, Pulau Burung Landfill, Tanah Merah estate Landfill and CEP Simpang Renggam Estate (Bernama, 2017). Between August to September 2014, the residents nearby Taman Beringin Landfill complained about leachate flowing from landfill to the nearby river called Sungai Batu. In 2016, an environment group called Sahabat Alam Malaysia reported that Pulau Burong landfill leachate flowed into the nearby mangrove forest. The same landfill is also found to release leachate into the Penang sea. In Johor, Sungai Renggam dumpsite was also reported to contaminate the water supplies and the water of Sungai Renggam was found to be highly polluted with leachate (The Star, 2017).

Therefore from the reported studies, the contamination of leachate to soil and water sources is very prominent. The effects of heavy metals to human health, plants and soil is further discussed in next topic.

2.12 Remediation of heavy metal contaminated soil

The importance of remediation of contaminated soil is to ensure that the environment is safe and healthy for human. Heavy metal contamination poses serious risk to human being and the environment. Remediation of contaminated soil is necessary and traditional technologies such as chemical or physical treatment are not 100% effective and it is very expensive. Table 2.16 shows the different approach of reducing heavy metal in contaminated soil. Bioaugmentation or plant remediation is an emerging technology based on the greener technology.

Table 2.16: Different methods adopted to reduce metal contamination of soils

Method	Description
Phytoremediation	The use of plants to remove metals from soil
Bioaugmentation	The use of microorganisms to remove the heavy metal in the soil
Crop selection	An adequate choice of crop, according to individual species accumulation abilities and contamination of soil to provide the consumer with safe food or food products
Good agricultural practices	Maintains a proper pH and a satisfactory level of organic matter and fertility of soil
Deep plowing	Plowing at the level of 40–50 cm to cover the contaminated soil underneath and to expose the clean layer of soil
Top soil replacement	Removal of ca. 20 cm of top soil and its replacement with clean material from some other place
Total soil replacement	Complete removal of soil and replacement with uncontaminated material. The contaminated material is transported to permitted off-site treatment and disposal facilities
Use of binding materials in soil	Introducing various binding materials to the topsoil to bind metals and make them less available to plants
Chemical and electrolytic method, soil washing	Various hard technical soil cleaning methods using electrolysis, chemicals, thermal applications, washing, etc., usually leading to destruction of basic soil properties including soil microflora (side effect)
Placement of clean soil on surface	Uncontaminated soils are applied onto the soil surface. The thickness of the layer applied depends on intended land use
Dilution of contaminated soil by mixing with clean soil	Mixing the contaminated material with clean soil or subsoil in order to reduce the maximum concentrations of contaminants to below the threshold values
Use of site for urban purposes	If any other use of contaminated agricultural land is not feasible, an alternative use of the land should be considered like for urban purposes such as parking, roads, warehouses, etc
Cultivation of non-edible plant	In order to preserve agricultural practices on contaminated land, nonedible plants might be cultivated, i.e., those for industrial purposes, woods, or biofuels

2.13 Bioremediation

Bioremediation is a method of metabolizing contaminants through oxidative or reductive processes by using microorganisms. The microbes attack the pollutants through enzymatic process and convert them to a less toxic form (Dhankar & Guriyan, 2011). Bioremediation is a green technology solution to overcome heavy metal contamination. Bioremediation is also known as sustainable remediation technologies that rectify and re-establish the natural condition of soil. It is also listed to be among new technologies approach that derives its scientific justification from the emerging concept of Green Chemistry and Green Engineering. It is a fast growing and promising remediation options increasingly being studied and applied in practical use for pollutant clean-up (Dadrasnia *et al*., 2015). The key factor that may influence the selection of bioremediation process can be based on cost of the treatment, long term effectiveness, commercial availability, general acceptance, applicability to high metal concentrations, the applicability to the mixed type of metals, toxicity reduction, mobility reduction and volume reduction (Emenike, 2013).

The microorganisms involved in bioremediation cannot destroy the metal. However, it will transform the metal from one oxidation state or organic complex to another state. Microorganisms possess astonishing metabolic pathways which utilize various toxic compounds as a source of energy for growth and development, through respiration, fermentation, and cometabolism (Ayansina & Olubukola, 2017). During effective bioremediation process, the metal will be transformed to either water-soluble state and less toxic, less water soluble state so that it precipitates and then becomes less bioavailable or removed from the contaminated site, or volatilized and removed from the polluted area (Garbisu & Alkorta, 1997). Several environmental factors that affects the effective bio remediation includes:

a) pH- The optimum pH for bioremediation is between 5.5 - 8.0. At this range, the optimum growth of microbes is achieved to allow metal removal (Vidali, 2001).

b) Temperature - At suitable temperature the rate of bioremediation increases. It can range from temperature of 5°C to 45°C and the rates double for each rise of 10°C.

c) Microbial diversity- Diversity of microorganism and the consortia such as *Pseudomonas*, *Aeromonas*, *Flavobacteria*, *Chlorobacteria*, *Corynebacteria*, *Acinetobacter*, *Mycobacteria*, *Streptomyces*, *Bacilli*, *Arthrobacter*, and *Cyanobacteria*.

d) Moisture content - Suitable moisture content is necessary so that the microbial activity is not disrupted due to water log in the soil.

e) Soil structure- Different textures of soil such as silt, sand or clay affects the microbial processes in bioremediation.Well-structured soil enhances the supply of water, air and nutrient to the soil microbes (Mani & Kumar, 2014).

f) Nutrients- The availability of nutrient is important in a efficient bioremediation process. Supplementing the proper nutrient to enhance the remediation is necessary where addition of nitrogen, phosphate and potassium can stimulate the cellular metabolism and growth of the microbes (Atagana *et al*., 2003; Mani & Kumar, 2014).

g) Bioavailability of pollutants- It is important to know the form of heavy metal in a contaminated site whether it is in solid, semisolid, liquid, or volatile form. Besides that the types of heavy metal and toxicity level also influenced the remediation process

The first patent of biological remediation. was registered in year 1974, being strain of *Pseudomonas putida* (Girma, 2015). Table 2.17 shows the list of selected microbes that have been found to utilize heavy metals at higher rate.

Table 2.17: Microbes with heavy metal utilization potential

Microbes	Heavy metal	References
Bacillus sp	Cu, Zn	Gunasekaran *et al*., 2003
Aspergillus niger	Cd, Zn	Gunasekaran *et al*., 2003
Zooglea sp	Ni, Cu	Sar & D'Souza, 2001
Pseudomonas aeuroginosa	Cu, Ni	Gunasekaran *et al*., 2003
Citrobacter sp	Cd, Pb	Gunasekaran *et al*., 2003
Pleurotusostreatus	Cd, Cu,Zn	Gunasekaran *et al*., 2003
Chlorella vulgaris	Cu, Ni, Pb,Hg, Zn	Gunasekaran *et al*., 2003
Ganodermaapplantus	Cu,Hg, Pb	Gabriel *et al*., 1996
Staphylococcus sp	Pb, Cr, Cu	Kumar *et al*., 2011
Streptomyces sp	Cr	Kumar *et al*., 2011
Pseudomonas fluoroscens	Ni	Lopez *et al*., 2002
Ralstonia eutropha	Cd, Hg	Lopez *et al*., 2002
Methylococcus capsulatus	Cr	Hasin *et al*., 2010
Escherichia coli	As	Kostal *et al*., 2004

2.13.1 Microbes in heavy metal polluted soil

The function of soil as a vital system and the support for biological activity productivity is depends on the extent of microflora activity. Despite normal soil is the serene habitat for microbes, fungi, or algae some microbes relatively survive in landfill environment which highly polluted with heavy metals and such may suggest that favourable condition for metabolism exists (Emenike *et al*., 2016). Microorganisms have a great deal of undiscovered and unexplored potential for remediation of soil pollutants and increasing the production of low input agricultural crops. Selection of microbes for remediation of metal polluted should be done based on an understanding of mechanisms involved in the adsorption and mobilization of heavy metals and trace elements in the soil to restore soil health. Researcher has isolated different types of microbes that are resistant to heavy metal from metal contaminated soil (Carlot *et al*., 2002). Examples of microbes isolated from an heavy metal contaminated sites were *Vibrio sp*, *Pseudomonas aeuroginosa* and *Bacillus sp* (Emenike, 2013). Sulaiman *et al*. (2015) isolated microbes that belongs to group of *Escherichia coli*, *Pseudomonas sp*, *Proteus sp*, *Bacillus spp*, *Arthrobacter sp*, *Klebsiella pneumonia*, *Enterobacter cloacae* and *Staphylococcus sp* from leachate from Awotan dumpsite at Ibadan, Nigeria.

The presence of resistant microbes in metal contaminated environment were evident based on the isolation of certain group of microbes by researchers. This microbes may have potential for heavy metal removal.

2.13.2 Mechanisms of Microbes in heavy metal removal

Even though normal soil environment is the habitat for soil microorganisms, some of the microbes were able to adapt and grow in environment polluted with heavy metal. The microbes may be present in the soil even before the soil were contaminated.

Microorganisms is able to uptake heavy metal from the environment. Microbes developed ways of taking up the essential metals and deal with the toxic metals by pumping out the metal ion out of their cells (Timberly *et al*., 2014). Interaction between bacteria and heavy metal is based on the difference of charges. The net negative charge in bacteria and the cationic charge of metals allows the interaction to happen. This is based on that nucleation sites on bacteria cell wall has the ability to bind metal of opposite charges. When this occur, the bacteria will be able to bind large concentration of heavy metal and precipitate on the cell wall (Beveridge, 1989; Monarchese *et al*., 2012).

The resistance of microbes towards heavy metal is encoded on the chromosome while some are encoded on mobile genetic elements such as plasmid and transposons. The resistance of microbes towards heavy metal are divided into three main categories:

a) General resistance mechanisms that do not require metal stress
b) General resistance mechanisms that are activated by metal stress
c) Resistance mechanisms that are dependent on a specific metal for activation

(Nies, 1999)

In order to survive in heavy-metal polluted environments, many microorganisms have developed means of resistance to toxic metal ions (Nies & Silver, 1995; Nies, 1999). These mechanisms include metal exclusion by permeability barriers, active transport of the metal away from the cell organism, intracellular sequestration of the metal by protein binding, extracellular sequestration, enzymatic detoxification of the metal to a less toxic form and reduction in metal sensitivity of cellular targets (Bruins *et al*., 2000; Nies & Silver, 1995; Silver, 1996).

Microbial cell wall consist of polysaccharides, lipid, protein and many functional group that can bind metal ions which includes carboxylate, hydroxyl, amino and phosphate group (Dixit *et al*., 2015). The analysis of the cell wall components in microorganisms, which vary among the different microorganisms, helps in assessing metal uptake by different microorganisms. The peptidoglycan layer in Gram-positive bacteria, which contains alanine, glutamic acid, meso-di-aminopimelic acid, polymer of glycerol and teichoic acid, and that of the Gram-negative bacteria, which contains enzymes, glycoproteins, lipopolysaccharides, lipoproteins, and phospholipids, are the active sites involved in metal binding processes (Fomina & Gadd, 2014; Lesmana *et al*., 2009; Gupta *et al*., 2015). Metals and metalloids are attached to these ligands on cell surfaces, which displace essential metals from their normal binding sites. Once the metal and metalloid are bound, microbial cells will transform them from one oxidation state to another, thus reduce their toxicity (Chaturvedi *et al*., 2015).

The detoxification mechanisms may be directed against one metal or a group of related metals. The mechanisms may vary depending on the type of microorganism (Nies & Silver, 1995). Most microorganisms are known to have specific genes for resistance to toxic ions of heavy metal toxicity. Mostly, the resistance genes are found on plasmids or on chromosomes (Nies, 1999). Plasmid-encoded metal resistance determinants have been reported to be inducible (Silver *et al*., 1981; Rosen, 2002).

The responses of microbial communities to heavy metal depends on the concentration and availability of heavy metals. It is a complex process which is controlled by multiple factors such as type of metal presence in the environment, the nature of medium such as soil, air, or water, and the species of microbes belongs to (Girma, 2015). Potential metal biosorbents under the class of bacteria include *Bacillus* sp, *Pseudomonas* sp, *Streptomyces* sp and P. *aeroginosa* (Dankar & Rachna, 2015).

2.13.3 Specific resistance mechanisms of microbes towards heavy metal

Ni is accumulated by the fast and unspecific CorA (metal transport system, MIT) Mg2+ transport system in bacteria (Hmiel *et al.*, 1989). The specific Ni transporters are either HoxN chemiosmotic transporters or ATP-binding cassette (ABC) uptake transporters, which use periplasmic Ni-binding protein (Nies, 1999). Ni is detoxified by sequestration and/or transport (Nies, 1999). One of the Ni resistance bacteria, *Ralstonia* sp. CH34 and related bacteria is based on Ni efflux driven by RND transporter.

Cu are required as co-factors by many enzymes, such as oxidases and hydroxylases however it become highly toxic when present in excess (Lu *et al*., 1999). Cu is possibly accumulated by the CorA-Mg2+ transporter and additionally by P-type ATPases under Cu starvation. The mechanisms for metabolism of Cu occur naturally in all living organisms, and chromosomally encoded (Liu *et al*., 2002). Specific resistance to Cu in bacteria are often plasmid encoded (Cooksey, 1993). The plasmid and chromosomal systems usually interact with each other to maintain Cu homeostasis in bacteria (Rogers *et al*., 1991; Brown *et al*., 1995; Fong *et al*., 1995; Gupta *et al*., 1995). Inside the cells of organisms Cu may be bound by various compounds to form Cu complexes. P-type ATPases seem to detoxify Cu via efflux in some species (Nies, 1999).

Zn is a component in a variety of enzymes and deoxyribose nucleic acid- (DNA binding proteins like Zn-finger proteins (Chou *et al.*, 1998; Nies, 1999). Unspecific uptake of Zn^{2+} is mediated by CorA (MIT) Mg^{2+} transport systems in some bacterial species and by the fast and unspecific MgtE system in other species. There are two systems involved for Zn detoxification in bacteria. The P-type ATPases (which transport only Zn across the cytoplasmic membrane) and RND-driven transporters (transporting Zn across the complete cell wall of Gram-negative bacteria. For example, *E. coli*, the ZntA P-type ATPases may be responsible for the efflux of Zn (Beard *et al*., 1997; Rensing *et al*., 1997; Nies, 1999) and the related ZiaA transporter in the cyanobacterium *Synechosystis* sp (Thelwell *et al*., 1998; Nies, 1999). Moreover, P-type ATPases mediating Cd resistance, efflux Zn as well in most cases (Nies, 1999). Slow efflux of Zn in *Staphylococcus aureus* is catalyzed by cation-diffusion facilitator (CDF) transporters, which also mediate the resistance to Co (Xiong & Jayaswal, 1998).

The mechanisms by which these microorganisms resist and reduce Cr (VI) are variable and are species dependent. There are several Cr-resistance mechanisms that are displayed by microorganisms. These include active efflux of Cr compounds, metabolic reduction of Cr (VI) to Cr (III), and either intercellular or extracellular precipitation (Joutey *et al*., 2015). Microbial Cr (VI) removal typically involves three stages: binding of Cr to the cell surface, translocation of Cr into the cell, and reduction of Cr (VI) to Cr (III). Cr (VI) reduction by microorganisms may proceed on the cell surface, outside the cell, or intracellularly, either directly via chromate reductase enzymes, or indirectly via metabolite reduction of Cr (VI). The uptake of Cr ions is a biphasic process. The primary step is known as biosorption, a metabolic energyindependent process (Joutey *et al*., 2015).

As enter bacterial cells via transporters of other compounds. In E. *coli,* arsenate uptake is always mediated by phosphate transporters, Pit and the Pst pumps (Bennet & Malamy, 1970; Rosenberg *et al*., 1997; Nies, 1999; Rosen, 2002) Under ample phosphate conditions, the less specific Pit system fulfills the phosphate needs of the cell and leads to arsenate accumulation (Elvin *et al.*, 1987). Under conditions of phosphate starvation, a highly specific Pst-system (for phosphate uptake) is induced (Surin *et al.*, 1987) and it uses the PstS phosphate-binding protein and the PstABC ATPase complex for inner membrane uptake. But the Pit system appears to be the predominant system for arsenate uptake (Willsky, 1980; Rosen, 2002).

2.13.4 Bioremediation of heavy metal polluted soil

The conventional method of remediating heavy metal is by excavation followed by solidification or stabilization. This is a temporary method to control the heavy metal pollutant in the environment (Bahi *et al.*, 2012) which is expensive, inefficient and will generate toxic waste (Iram *et al*., 2013).

Bioremediation is a general mechanism that involves processes and actions that take place in order to bio transform an environment that has been contaminated with pollutants to its original status (Girma, 2015). The microbes involved in bioremediation develop and adopt different mechanisms in order to remove the pollutants from the environment. The microbes use the mechanism of biosorption, bioaccumulation, biotransformation, and biomineralization. Microbes involved in the removal of heavy metal uptakes the heavy metals actively through bioaccumulation or passively through adsorption (Hussein *et al*., 2001).

Figure 2.12 summarises the various means by which bacteria react to the presence of metals (M2^{+}) in the medium, with reference to the cellular compartment that harbours the response. These mechanisms include the intra- or extracellular binding (and thus immobilisation) of the metal with a cognate protein (frequently a metallothionein) or a matching anion, the biotransformation of the toxic ion into a less noxious or more volatile form, and the dissimilatory reduction of the metal (Valls & Lorenzo, 2002).

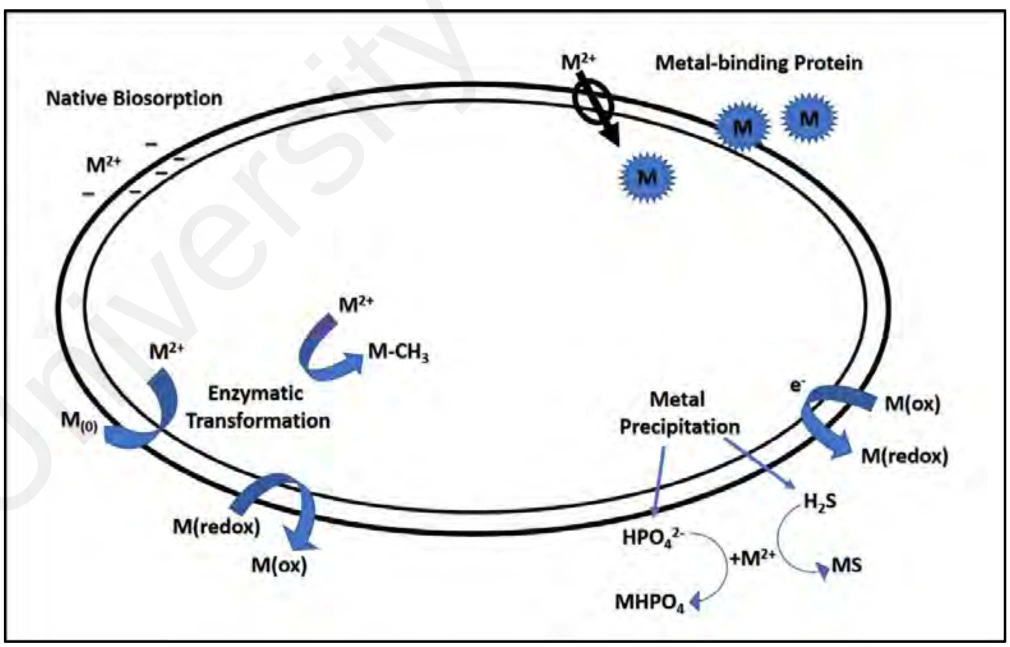

Figure 2.12: The mechanism of microbes in metal removal. Adapted from Valls & Lorenzo (2002)

2.13.5 Methods involved in the bioremediation of heavy metal contaminated soil

High amount of heavy metal in soils and their toxicity effect cause long term environmental and health issues. Metal tends to persist and accumulate and cannot be easily removed or destroy unless biological approach is carried out. Removal of heavy metal from contaminated land is necessary to reduce the pollutants from the site. Two methods involved in the removal of the metals are in-situ bioremediation and ex-situ bioremediation.

2.13.5.1 *In situ* bioremediation

The removal of pollutant directly at the place where the contamination occurs is called in-situ bioremediation. This can be achieved by supplying and circulating suitable amount of oxygen and nutrients in the contaminated area and enhance the naturally occuring bacteria in the system (Vidali, 2001; Evan & Furlong, 2003; Chauhan & Jain, 2010; Rayu *et al*., 2012). *In situ* bioremediation is further classified into two approach namely "intrinsic bioremediation" and "engineered bioremediation" (Hazen, 2010; Girma, 2015).

Intrinsic bioremediation is carried out by inducing the indigenous microorganisms in the soil or contaminated area by supplying necessary amount of nutrient and oxygen to increase their metabolic activities. In an engineered bioremediation, specific microbial consortia is introduced into the system to accelerate the removal of pollutants. The introduction of genetically modified bacteria is also possible in this bioremediation. Selecting bacteria with rapid growth potential can enhance the bioremediation process (Singh *et al*., 2010; Mani & Kumar, 2014).

In situ bioremediation is considered a cost effective method with minimal site disturbance and there is no need for excavation. However, this method takes longer period to achieve successful bioremediation and weather dependent too.

2.13.5.2 *Ex situ* bioremediation

Ex situ techniques involves the removal or excavation of contaminated soil in the ground for treatment. The ex-situ remediation is classified into two categories which consists of solid phase system and slurry phase system. The treatment involves in the solid phase system is land farming, soil bio piles and composting whereas in the slurry phase system the treatment involved the use bio-reactor.

In soil bio piles treatment, the soil excavated from the contaminated site is amended and formed into compost piles or cell above the ground level and the piles is closed. The closed treatment is equipped with aeration system to enhance the growth of both aerobic and anaerobic bacteria (Li *et al*., 2004; Mani & Kumar, 2014). In land farming treatment processes the excavated soil from the contaminated site is spread onto a prepared bed and tilled periodically until the pollutants are completely removed. By using this techniques the degradation of microbial activity can be stimulated for the removal of the heavy metal pollutants. Besides that, this technique requires minimal monitoring and maintenance cost and clean up liabilities (EPA, 2003; Girma, 2015). However this treatment is limited to superficial treatment of 10 to 35 cm of soil only (Mani & Kumar, 2014). As for composting, basically it involves the mixing of excavated soil from the site with organic waste such manures or agricultural waste. The mixing of the organic waste to the soil is aimed to enhance the microbial population in the soil and further help in the removal of contaminants from the soil (Girma, 2015; Mani & Kumar, 2014; Paliwal *et al*., 2014).

For slurry phase bioremediation, the excavated soil from contaminated site is combined with water and other additives in a bioreactor (large tank) and mixed thoroughly to keep the microorganisms in contact with the contaminants (Paliwal *et al*., 2012). This process is also to be a rapid process as compared to other treatment options (Girma, 2015). Nutrients and oxygen is added and the conditions in the bioreactors is controlled to create an optimum condition for the microbial activity to take place. After the completion of the treatment, the water slurry is separated from the solid for further treatment or disposal (Cunningham & Philip, 2000). The advantage of this method is that it is easily controllable and manageable. However the disadvantages is that it requires pre-treatment by soil washing or physical extractions (Girma, 2015).

2.13.6 Bioremediation approaches

Scientists are trying to research on the approaches of removing heavy metal from the contaminated site cause of its impacts to living organisms, plants and the environment. Integrative approach or system biology bioremediation is introduced recently to remediate the contaminated site. There are several bioremediation approach involved such as bioaugmentation, biostimulation, bioventing, biosparging, and bioattenuation.

Bioaugmentation is an approach that involves the introduction of microorganisms that possessed bioremediation potential to a polluted environment to assist in the remediation process by indigenous microbes. Bio augmentation is also considered as a low-cost bioremediation strategy in which an potent bacterial isolate or microbial consortium capable of removing the pollutant from the contaminated sites (Abioye, 2011). Bioaugmentation in a contaminated site with preferred microbes is expected to tackle the heavy metal contamination. The behaviour of microbes inoculated may vary based on the condition of the pollutant, which might be one type of metal pollutant or mixed. The selection of suitable microbes, the concentration of inoculum augmented and heterogeneity of the inoculum are also very important factors (Emenike *et al*., 2016; Sprocati *et al*., 2012). According to Emenike *et al*. (2016), bioaugmentation should aim to rearrange the group of microbes inoculated from the contaminated area by identfying those dominant ones with the ability to do clean-up of the pollutants. The addition of the microbes is aimed to increase the rate of bioremediation. Previous studies have indicated that the use of microbial consortia of aromatic-degrading bacteria has been more effective in removing pollutants as compared to selected single strains (Ghazali *et al*., 2004; Goux *et al*., 2003).

According to Forsyth *et al*. (1995) bioaugmentation should be applied in soils (1) with low or non-detectable number of contaminant-degrading microbes, (2) containing compounds requiring multi-process remediation, including processes detrimental or toxic to microbes and (3) for small-scale sites on which cost of non-biological methods exceed the cost for bioaugmentation.

Bioaugmentation studies were carried out using gram-negative bacteria belonging to genus *Pseudomonas* (Heinaru *et al*., 2005), *Flavobacterium* (Crawford & Mohn 1985), *Sphingobium* (Dams *et al*., 2007), *Alcaligenes* (Haluska *et al*., 1995) and *Achromobacter* (Ronen *et al*., 2000), gram-positive bacteria belonging to the genera *Rhodococcus* sp. (Briglia *et al*., 1990), *Mycobacterium* (Jacques *et al*., 2008) and *Bacillus* (Silva *et al*., 2009). Potential fungi used in bioaugmentation are represented by species from genus *Absidia* (Garon *et al*., 2004), *Achremonium* (Silva *et al*., 2009), *Aspergillus* (Dosantos *et al*., 2008), *Verticillium* (Silva *et al*., 2009), *Penicillium* (Mancera-Lopez *et al*., 2008) and *Mucor* (Szewczyk & Dlugonski, 2009). Microorganisms are metabolically versatile and are capable of degrading a wide type of metals.

According to Emenike *et al.* (2016), *Lysinibacillus* sp, *Bacillus* sp, and *Rhodococcus* sp isolated (gram positive bacteria) from landfill leachate can influence the metal removal efficiency when blended together. The bioaugmentation process enhanced the reduction in concentration of the heavy metals within the soil spectrum of substrates including heavy metals.

Biostimulation is a process where the environment will be modified to stimulate the existence of bacteria capable for bioremediation. It can be carried out with the addition of phosphorus, nitrogen, oxygen, or carbon supply. The addition of nutrient is to increase the population or activity of naturally occurring microorganisms available for bioremediation. The primary advantage of biostimulation is that the present native microbes induce bioremediation due to adaptation to the subsurface environment, and optimal spatial distribution within the subsurface. Some factors that limits the activity of biostimulation in soil is the nutrients, pH, temperature, moisture, oxygen, properties of soil and types of contaminants (Atagana, 2008; AlSulaimani 2010; Bundy *et al*., 2002). Biostimulation study has been carried out using bacteria isolated from heavy metals contaminated site located at Bhayander India (Fulekar *et al*., 2012). The biostimulated bacteria consortium was effective in the remediation of Cd, Cu, and Fe at higher concentration of 100 mg/L up to 98.5%, 99.6%, and 100%, respectively. Chromium bioremediation and outlined heterogeneous group of bacteria isolated from contaminated sites to remediate chromium was reviewed by Kanmani *et al*. (2012). According to Kanmani *et al*. (2012) the isolated bacteria exhibits plasmid-mediated chromate resistance and the reduction was enzymatically mediated. With genetic engineering technology, there it is possible to obtain high performance bacteria at extreme conditions.

Bioventing is an *in situ* bioremediation where it is carried out by stimulation of airflow by delivering oxygen to unsaturated (vadose) zone in order to increase bioremediation and increasing activities of indigenous microbes. In bioventing, amendments are made by adding nutrients and moisture to enhance bioremediation with the ultimate goal being to achieve microbial transformation of pollutants to a harmless state (Philp & Atlas, 2005).

Bioattenuation is an *in situ* treatment method, which make use of the natural processes to contain the spread of contamination from chemical spills and also to decrease the concentration of pollutants at contaminated sites. This means that environmental contaminants remain undisturbed in place in order to give opportunity for natural degradation/reduction/transformation on the contaminant. Natural attenuation is one part of a site cleanup that also includes the control or removal of the source of the contamination. The effectiveness of bioattenuation depends on sites, but at varying rates and degrees of effectiveness, depending on the types of pollutants, and its physical, chemical and biological characteristics of the soil and ground water. It may reduce mass of contaminant (biodegradation and chemical transformations) by reducing concentration of pollutants (through simple dilution or dispersion) or bind contaminants to soil particles so the contamination will not spread or migrate very far (adsorption). This process is an effective and inexpensive clean up option and the most appropriate way to remediate some contamination problems. Bioattenuation depends on the natural processes to dissipate contaminants through biological transformation.

2.13.7 Advantages and disadvantages of bioremediation

The advantages and disadvantages of bioremediation is summarized as in Table 2.18.

Table 2.18: Advantages and disadvantages of bioremediation (Arpita *et al*., 2014)

Advantages of Bioremediation	Disadvantages of bioremediation
• Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contamination increase in number when contaminant is present; when the contaminant degraded, the biodegradative population declines. • Less energy is required as compared to other technologies. • Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste. • Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. • Instead of transferring contaminants from one environment medium to another, for e.g. from land to water or air, the complete destruction of target pollutants is possible.	• Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. • Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration. • Contaminants may be present as solids, liquids and gases. • Dynamic process, difficult to predict future effectiveness. • Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation

As one of a bioremediation method, phytoremediation techniques also play a very important role in heavy metal removal from soil.

2.14 Phytoremediation of heavy metal contaminated soil

Idea of phytoremediation was first incepted by Chaney in 1983 (Hajar & Amir, 2014). Phytoremediation makes use of natural processes where the plants in combination with their microbial rhizosphere degrade and take up pollutants (organic and inorganic) from soil or water. Phytoremediation is a very innovative and effective alternative method for heavy metal removal from the environment. It is considered as very green, safe and solar orientated technology. Besides that, phyoremediation is also a very cost effective method as compared to other technologies (Hajar & Amir, 2014) and can be defined as the science of plantation forestry with clean up strategies.

The aim of phytoremediation can be categorized into three based on the economic implications: (i) plant-based extraction of metals with financial benefit i.e. Ni ; (ii) risk minimization (phytostabilization); and (iii) sustainable soil management in which phytoremediation steadily increases soil fertility to allow crop growth with added economic value (Vangronsveld *et al*., 2009 ; Garbisu & Alkorta, 2003; Van Aken, 2009). High-biomass and rapid growing plants such as poplar, jatropha and willow can also be exploited for the dual purpose of energy production and phytoremediation (Abhilash *et al*., 2012; Prasad, 2003; Chaudhry *et al*., 1998; Chaney, 1983 ; Pilon-Smits, 2005).

The heavy metal uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contaminant degradation abilities of the entire plant body is important in phytoremediation (Tangahu *et al*., 2014). Besides that, the selection of proper plant species are very important in phytoremediation. The plants must grow easily and widely, and able to accumulate heavy metal extensively (Ali *et al*., 2013). Factors which affects the uptake mechanisms of heavy metal in phytoremediation are plant species of plants, properties of the medium, root zone of the plant, environmental conditions, chemical properties of the contaminants, bio availability of the heavy metal and added agent (Tangahu *et al.*, 2014). Different mechanisms of phytoremediation of heavy metal is shown in Figures 2.13 and 2.14.

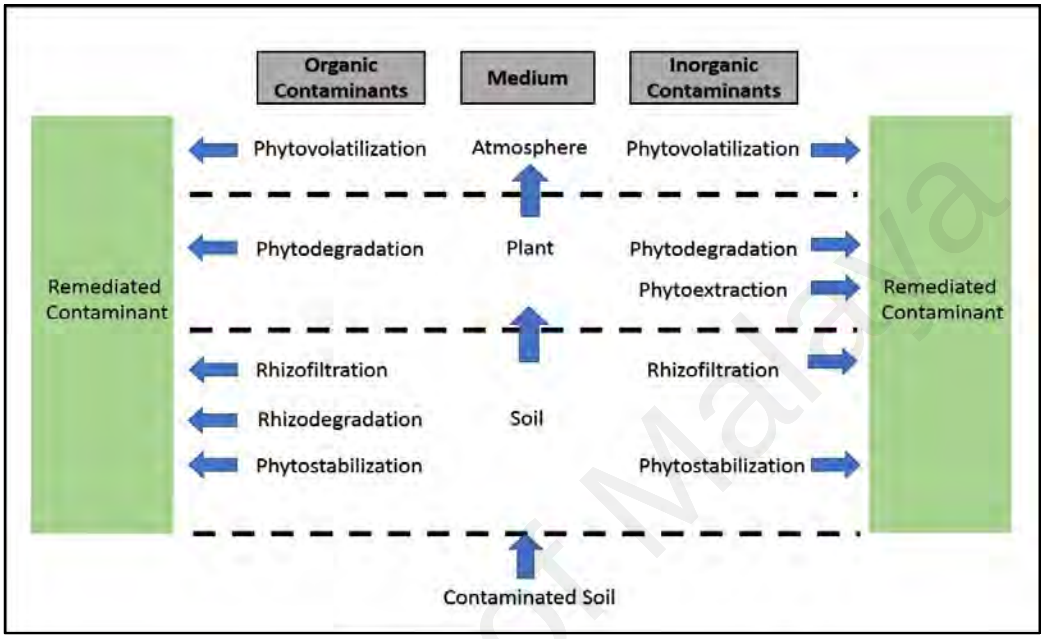

Figure 2.13: Uptake mechanism by phytoremediation technology. Adapted from Interstate Technology and Regulatory Council (2009).

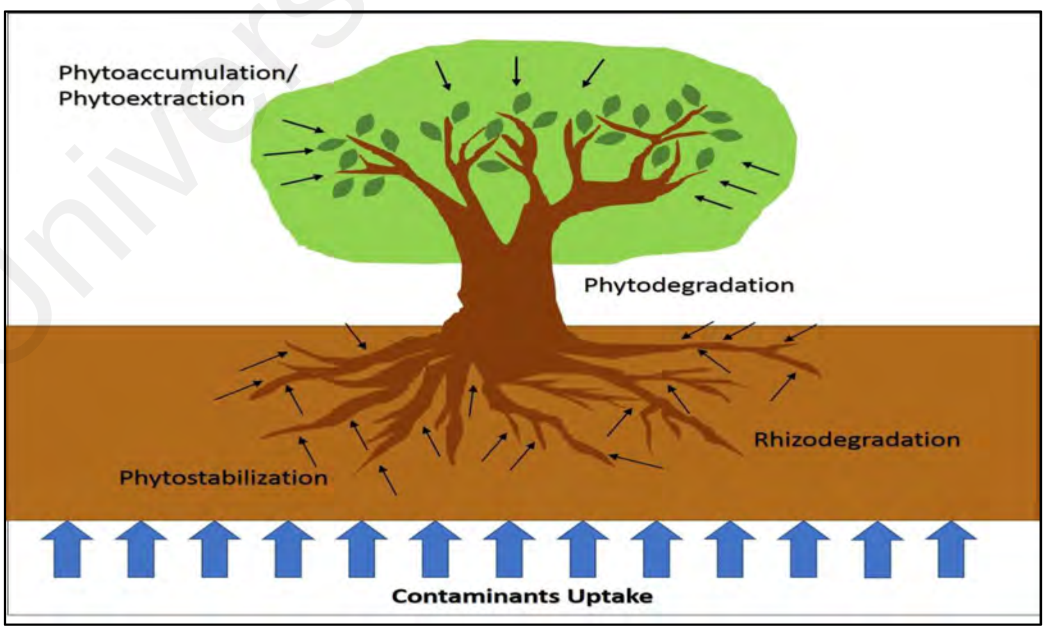

Figure 2.14: The mechanisms of heavy metals uptake by plant through phytoremediation technology

In the case of phytoremediation of leachate, the plant where able to extract and sequester excess nutrients and the pollutants such as heavy metal and control the leaching of those pollutants to other water sources or soil. Many studies has been carried to test the accumulation of heavy metal in different plant species in leachate from landfills (Alaribe & Agamuthu, 2010; Meera, 2013; Zalesny *et al*., 2007) and the results indicate that significant amount of heavy metal were removed from the leachate.

To date there are up to 400 species of hyperaccumulator plants belonging to 45 families (Reeves & Baker, 2000). Table 2.19 shows examples of hyper accumulator plant.

Table 2.19: List of hyper-accumulator plant

List of hyperaccumulator plant	Heavy metal accumulated	References
Aeolanthus biformifolius	Cu	Chaney *et al*., 2010
Achillea millefolium	Hg	Wang *et al*., 2012
Alyxia rubricaulis	Mn	Chaney *et al*., 2010
Alyssum heldreichii	Ni	Bani *et al*., 2010
Azolla pinnata	Cd	Rai, 2008
Betula occidentalis	Pb	Koptsik, 2014
Brassica juncea	Pb	Harris *et al*., 2009
Corrigiola telephiifolia	As	Garcia-Salgado *et al*.,2012
Deschampsia cespitosa	Pb, Cd, Zn	Kucharski *et al*., 2005
Eleocharis acicularis	Cu, Zn, As, Cd	Ha *et al.,* 2011
Helianthus annuus	Pb	Koptsik, 2014
Ipomoea alpina	Cu	Cunningham & Ow, 1996; Lasat, 2002
Macadamia neurophylla	Mn	Sheoran *et al*., 2009
Medicago sativa	Pb	Koptsik, 2014
Phyllanthus serpentinus	Ni	Chaney *et al*., 2010

2.14.1 Different methods of phytoremediation application

There are three main methods involved in the application of phytoremediation to a contaminated soil or site. The methods are *in situ* phytoremediation, *in vivo* phytoremediation and *in vitro* phytoremediation. This methods are further described below.

In situ phytoremediation is the use of plant for remediation of contaminated surface of soil water, contaminated sediment with groundwater. This method basically depends on uptake and accumulation of heavy metal by plants and after certain duration the planted plants will be removed from the contaminated site. The removed plant is then either disposed or undergo metal recovery. The roots of plant important role for in-situ phytoremediation (Sun *et al*., 2011). There are some limitations to this type of remediation. Among others, this technique is only applicable to shallow ground of surface water, soil or sediment (Susarla *et al*., 2002). Nevertheless, this method is considered the cheapest as compared to other methods.

***In vivo* phytoremediation** is approach that can be applied to the contaminated site that are not easily accessible. Mechanical treatment method are used at the contaminated site. The soil from contaminated site is extracted and transferred to a temporary area and the plant is used to remove the contaminants. After the completion of the remediation, the soil will be returned to its original place and the plants used is harvested or sent for treatment. The cost of this method is more expensive than in-situ phytoremediation because the contaminated soil must be excavated and transferred to another temporary area (Susarla *et al*., 2002).

***In vitro* phytoremediation** method involves the application of components of live plants for the removal of contaminants from the site. The application is applied either in-situ or at a temporary treatment area. This method is the most expensive because due to the cost of preparing or extracting the plant enzymes. This method also takes longer time as compared to in-situ and in-vivo method because the enzyme remain active for breakdown of contaminants requires active enzymes (Susarla *et al*., 2002).

2.14.2 Mechanisms involved in phytoremediation

Phytoextraction is one of the mechanism of removing contaminant from the environment by plants (Figure 2.15). Plants have the ability to uptake the heavy metal from the environment and sequester them in their cell until the plant is harvested (Annie & Gilbert, 2013). Ideal plant for phytoextraction has the following features of metal tolerance, fast growing, high accumulating biomass, able to accumulate metal at the above the ground level (shoot, stem, leaves) and lastly easily harvestable (Buscaroli *et al*., 2016). Three main purpose of phytoextraction is firstly, to remove the contaminant from the soil or contain it, secondly phytoextraction of elements that have market value and finally gradually improving soil quality to cultivate crops with higher market value (Vangrosveld *et al*., 2009). The removed metal from the contaminated site can be easily recycled or harvested from the plant (Bieby *et al*., 2011). Soil, metal and plant relationship is important aspects in phytoremediation (Lasat, 2002). Continuous or natural way and by inducing with chelating agent are the two approaches applied to remove heavy metal contaminants from the environment (Utmazian & Wenzel, 2006).

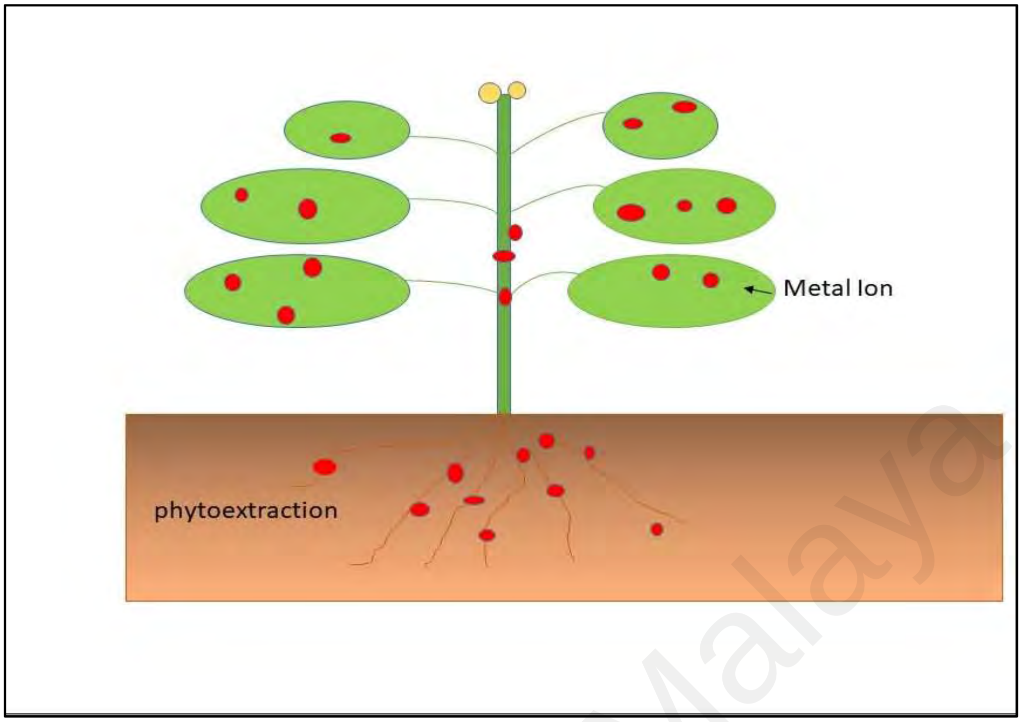

Figure 2.15: Uptake of metal by Phytoextraction

Phytostabilization is the process whereby the plant accumulate the metals into their roots only (as in Figure 2.16) and not to the shoots (Mendez & Maier, 2008). Phytostabilization mechanisms is used to reduce the mobility and bioavailability of pollutants in the environment. Phytostabilization can occur through the absorption, precipitation, complex action, or metal valence reduction (Ghosh & Singh, 2005). The upper layer of the contaminated soil is treated with chemical to adjust the pH of the soil and transform the metal to non- soluble form. The important feature of plant used for phytostabilization is the plants ability to tolerate high concentration of heavy metal, has dense root system, and able to accumulate metal above ground (Van gronsveld *et al*., 2009).

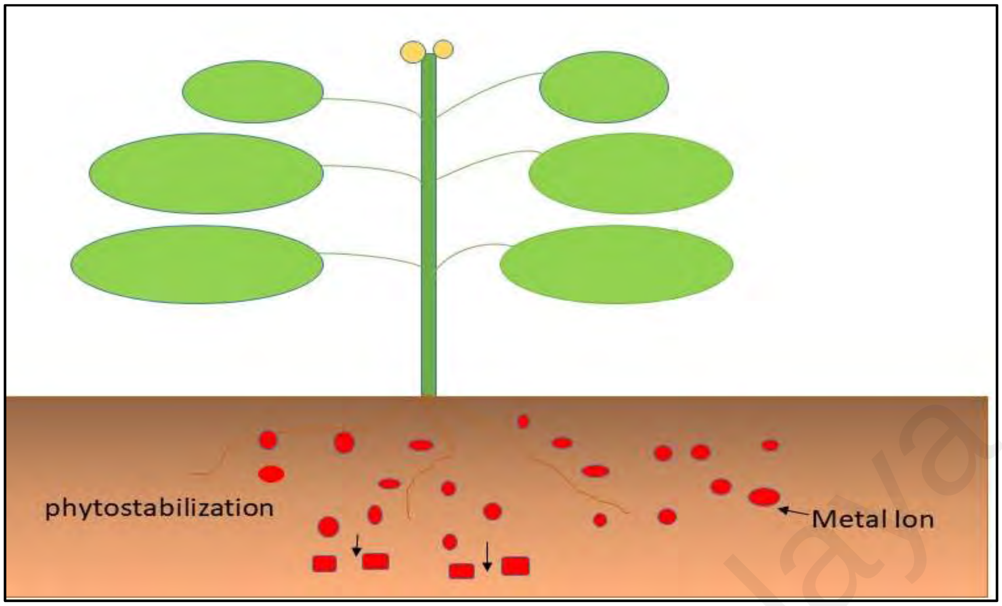

Figure 2.16: Phytostabilization process involves whereby the plant accumulate the metals into their roots

Phytovolatilization is a mechanism that uses the plant to extract the volatile contaminants such as Hg or Se from heavy metal contaminated soil or sediments. Normally in this process the plant ascend them into the air through their folliage (Karami & Shamsuddin, 2010). The pollutants is converted to volatile form and released to the atmosphere through transpiration process (Figure 2.17). It is transferred from soil to another segment which is the atmosphere (Ali *et al.*, 2013).

Mercury is the most studied heavy metals for phytovolatilization. To explore the potential of plants to extract and detoxify mercury, Bizily *et al.* (1999) used an engineered model plant, *Arabidopsis thaliana*, to express a modified bacterial gene, merBpe, encoding organomercurial lyase (MerB) under control of a plant promoter. MerB catalyzed the protonolysis of the carbon-mercury bond, removing the organic ligand and releasing Hg(II), a less mobile mercury species. Transgenic plants expressing merBpe grew vigorously on a wide range of concentrations of monomethylmercuric chloride and phenylmercuric acetate. Those plants that lacks of merBpe gene can be severely inhibited or died at the same organomercurial concentrations. Suggesting that native macrophytes (e.g. trees, shrubs, grasses) engineered to express merBpe may be used to degrade methylmercury at polluted sites and sequester Hg (II) for later removal (Wang & Wen, 2001).

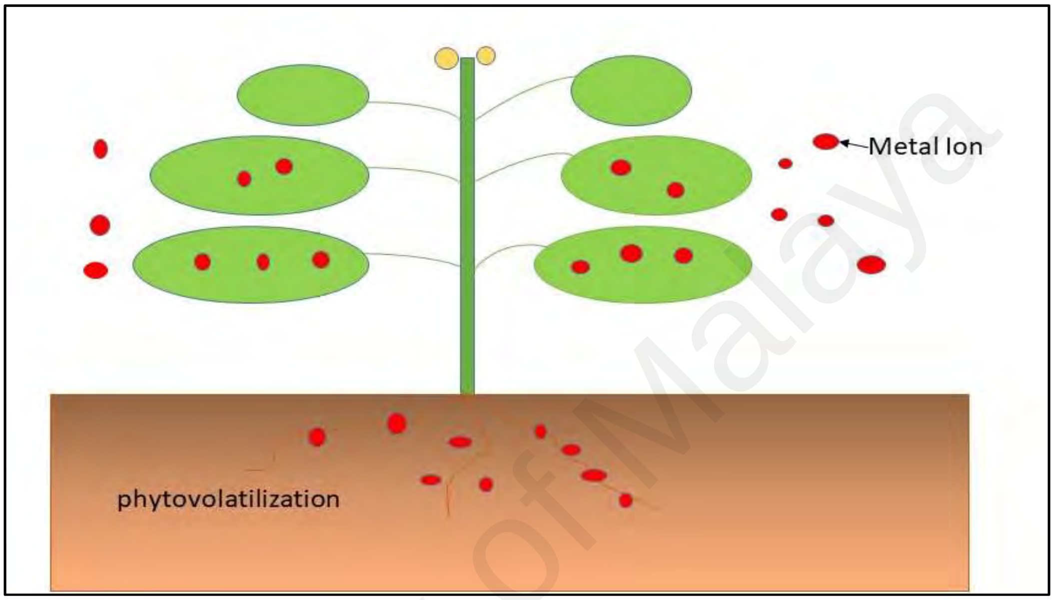

Figure 2.17: Phytovolatilization process take place whereby the pollutant were converted to volatile form and released to the atmosphere

Phytodegradation mechanisms uses both plant and microorganisms for degradation, uptake or metabolization of contaminants. The root of plant and microbes is associated to remove the contaminants from the soil. In phytodegradation mechanisms the contaminants undergo subsequent breakdown, mineralization, or metabolization by the plant itself through various internal enzymatic reactions and metabolic processes (Yousaf *et al.*, 2011). Degradation process can still occur in an environment free of microorganisms (Feroz *et al.*, 2012). Potential phytodegradation plants are able to grow in sterile soil and also in soil that has concentration levels that is toxic to microorganisms (Feroz *et al.*, 2012).

Phytofiltration is the removal of pollutants from contaminated water or wastewater by plants. The removal of heavy metal or contaminants can be through rhizofiltration, blastofiltration or caulifiltration of waste water (Pivetz, 2001; Ahmad Pour *et al*., 2012). Rhizofiltration (Figure 2.18) is a complex mechanisms which involves the interactions of roots, root exudates, rhizosphere soil and microbes result in degradation of organic contaminants to non-toxic or less-toxic compounds.

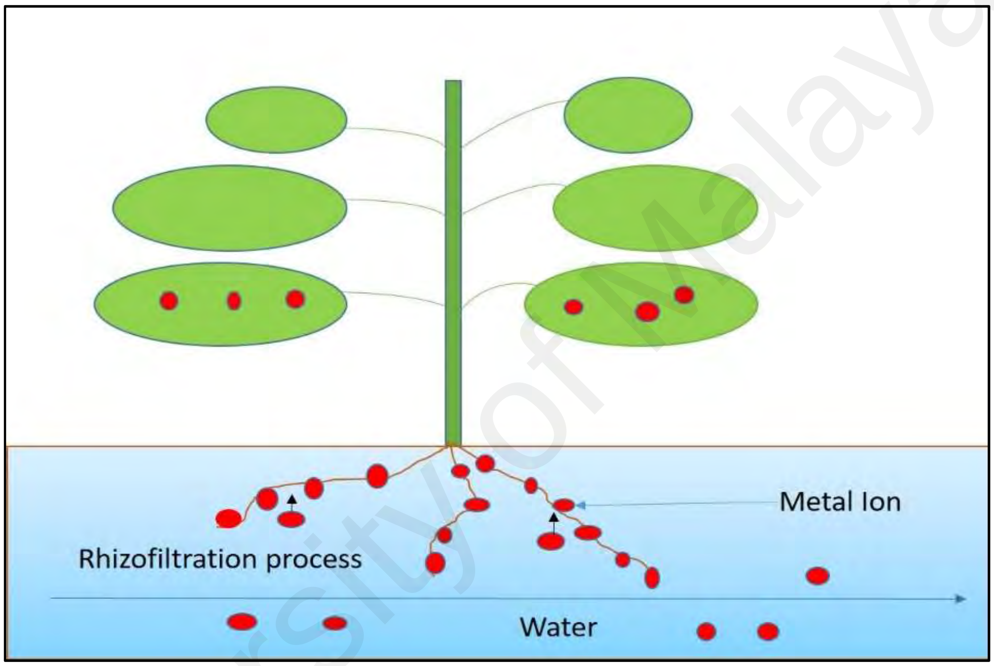

Figure 2.18: Rhizofiltration process

2.14.3 Advantage and disadvantage of phytoremediation

Table 2.20 shows the advantages and disadvantages of phytoremediation. Phytoremediation basically can provide advantages in removal of heavy metal from an contaminated site rather than other physical or chemical method however this technique is commonly known to be slower due to nature of process.

Table 2.20: Advantages and Disadvantages of Phytoremediation (Raskin and Ensley, 2000; Merime *et al.*, 2015)

Advantages of phytoremediation	Disadvantages of phytoremediation
Cost	**Time**
Low capital and operating cost	This technique is slower than other methods of remediation and highly dependent on weather variation
Metal recycling/mining provides further economic advantages	Most of the plants used in phytoremediation are slow growers
Performance	**Performance**
Permanent treatment solution	In term of performance, 100% of reduction or removal cannot be achieved
In situ application	May not suitable if the contaminants are in mixed version
Able to remediate bio availability metal	Very high concentration of contaminants can be toxic to the plant
Capable of mineralizing organic compounds	Soil phytoremediation is only applicable to contamination that occurs in surface soil
Can applied to various type of contaminants	
Eliminate secondary air or water borne wastes	
Other	**Space**
Easily accepted by public	Groundwater and wastewater application requires large surface area
Compatible with risk based remediation	**Others**
Can be applied to field/ in situ remediation	Regulators are unfamiliar with the new technology
	Lack of recognized economic performance data

2.14.4 Challenges of phytoremediation

Phytoremediation is an attractive option for soil heavy metals removal but it also has some challenges (Mahar *et al*., 2016; Clemens, 2001; Tong *et al*., 2004; LeDuc & Terry, 2005; Karami & Shamsuddin, 2010; Sangeeta & Maiti, 2010; Naees *et al*., 2011; Ramamurthy & Memarian, 2012). Soil phytoremediation takes several years for complete removal of heavy metal. The efficiency of phytoextraction is limited or slower because most metal hyperaccumulator plants are low in biomass and has slower growth rate. The accumulation capacity heavy metal plants used in phytoremediation may be compromised and ineffective due to pests and disease attack in climate affected tropical and sub-tropical regions. Besides that, it is difficult to mobilize more tightly bound fraction of metal ions from soil i.e., limited bioavailability of the contaminants in the soil. Agronomic practices and soil amendments may negatively influence the mobility of contaminants. Sustainable phytoremediation depends mainly on the climatic and weather conditions. Phytoremediation is an good approach for contaminated sites with low to moderate levels of metal contamination due to unsustainable plant growth in highly contaminated soils (Ann, 2005).

2.14.5 Selection of plants for phytoremediation

Selection of suitable plant is necessary for an efficient phytoremediation of heavy metal. The ability of plant to remove the pollutants from a contaminated site depends on the amount of metal that the plant can accumulate, growth rate of the plant and the planting density. The plants used must be highly tolerable to heavy metal and able to accumulate different types of metals and not specific to only one or two metal pollutants. According to Pilon-Smith (2005), the selected plant must be able to uptake high level of heavy metal, have the translocation ability and able to accumulate in the harvestable part of the plant. Addition to that, the depth of plant root, the type of soil involved and climate at the contaminated site are important factors in plant selection (Meriem *et al*., 2015). Different type of plants have different uptake ability where grasses the depth of cleaning is < 3 feet, shrubs is < 10 and < 20 feet for deep rooting trees.

2.14.6 Fate of Absorbed Metals in Plant

Plants may take up excess heavy metal which eventually affect different physiological process. Stunted growth, leaf epinasty, necrosis, chlorosis (as a result of inhibition of chlorophyll synthesis) and discoloration of leaves are symptoms in plants affected by severe pollutant toxicity due to altered processes at the cellular level (Diaz *et al*., 2001). Plant acts as accumulator and excluders in the removal of heavy metal contaminant from the environment. When the plant act as "accumulator" it survives by concentrating contaminant in the aerial tissues of the plant and biodegrade or biotransform into inert form in the tissue of the plant. As excluder, the plant restrict the contaminant uptake into their biomass (Sinha *et al*., 2004). Plants bioavailability towards heavy metal is basically based on their uptake mechanism, translocation ability and the ability to store the toxic in the plant tissue. The heavy metal ion is uptake or translocated by the proton pump, co and antitransporters, or through channel (Voijant *et al*., 2011). In an effective phytoremediation, heavy metal accumulated in the plant should be in the harvestable part of the plant. As an example, Brakefern plant absorbs 95% of As into the stem, shoot and leaves of the plant and least uptale at the root part. In another study, Indian mustard showed the ability to accumulate high amount of metals in the root of the plant compared to other part of the plant (Salt, 2002). Different plants have different uptake ability of heavy metal into their various part of the plant cell.

2.14.7 Cost of phytoremediation

Analysts have estimated that the cost of cleaning one hectare of highly metal contaminated land at a depth of one meter would range from $600,000 to $3,000,000 depending on the extent of the pollution and the toxicity of the pollutants (Huang & Cunhingham, 1996). The cost of phytoremediation could be 20 times less expensive, making this practice far less prohibitive than conventional methods (Lasat, 2000). The cost of phytoremediation of a contaminated site or soil is estimated to be about $17 to $100 for each cubic meter. Different treatment method and the cost of treatment for remediation of heavy metal contaminated is depicted in Table 2.21.

Table 2.21: Cost of different treatment method for heavy metal remediation

Treatment	Cost ($ /tonne)
Vitrification	75-425
Landfilling	100-500
Chemical treatment	100-500
Electrokinetics	20-200
Phytoremediation	5-40

2.14.8 Utilization of phytoremediation by-product and phyto-mining

In phytoremediation, plant tends to uptake the metal into different part of the cell. The disposal or management of the contaminated plant material is possible by thermochemical conversion process (Ghosh & Singh, 2005). The combination of phyto-extraction with biomass generation can be commercialized. Phytomining aims to generate revenue by recovering marketable amount of metals from plant biomass through the use of plants to valuable heavy metal from the contaminated site or soil (Rascio & Navaria, 2011).

2.14.9 Plants used in this study

Four plant species were selected to study the removal of heavy metal from leachate contaminated soil. All the four plant tested were not reported to have been used in phytoremediation and the plants are easily available ornamental plants. Since many studies has been carried out using hyperaccumulator this study deemed to identify plants that are easily available and capable of accumulating high amount of metals. The description of the studied plants is listed below:

Cordyline sp (Plate 2.1) plant is also known as "Red sister" plant. The plant has pink, plum, maroon and deep burgundy foliage. The plant has a broadleaf evergreen shrub which resembles a short palm tree which is native plant at tropical Southeast Asia. The unbranched stems support the strap like leaves. During summer or full sun, the color of plant become more intense. The plant grows in clumping form. In most of the temperate regions, this plant is grown as indoor plant. The plant grows well in moist soil with partial sun.

Plate 2.1: *Cordyline* sp.

D. variegated (Plate 2.2) is an ornamental plant from the family of *Verbenaceae* and the common name of the plant is variegated golden dew drop or variegated honey drops. This plant originated from West Indies. *Duranta* is am evergreen plant and it consist of variegated foliage. It can grow up to 15 to 25 feet height. This plant is also known as variegated sky flower. It required medium water supplies for good growth (www. smgrower.com).The foliage of this plant is ovate and has variegated leaves that is about 2.5cm long and its colour is light green with cream to light yellow spot. The stem of the plant is the woody. The genus *Duranta* was named after Dr Castor Durantes, an Italian physician of the 15th century (florafaunaweb.nparks.gov.sg).

Plate 2.2: *Duranta variegated*

Spathacea (Plate 2.3), commonly referred to as Moses in the Cradle, Moses in the Boat, or Oyster Plant is a greenery favorite. This plants belongs to the family of *Commelinaceae*, genus of *Tradesscantia* and species of *Spatachea*. *Spatachea* produced a tri-colored foliage of white, pink and green stripes on the top with purple on the under side. This tender perennial's upright mounding growth habit, heat tolerance and vivid colours makes this a great choice for landscape and mixed containers! Its colours will come to life through early spring, summer and fall, right up until the first frost while producing small white blooms all season. The plant has vivid foliage, rapid vigorous growth, heat tolerance, low maintenance, and upright mounding growth habit for landscape, beds, and containers.

Plate 2.3: *Tradescantia spathacea*

C. comosum (Plate 2.4) also known as spider plant, airplane plant, St. Bernard's lily, spider ivy, ribbon plant (Poole *et al.*, 1991) is a flowering perennial herb. It is native to tropical and southern Africa, but has become naturalized in other parts of the world, including western Australia (World Checklist of Selected Plant Families*,* 2011*).C. comosum* is easy to grow as a houseplant; variegated forms are the most popular. This plant also able to reduce indoor air pollution in the form of formaldehyde, and approximately 70 plants would neutralize formaldehyde production in a energy-efficient house (Wolverton *et al*., 1984).

Plate 2.4: *Chlorophylum comosum*

2.15 Bio-concentration Factor and Translocation factor

2.15.1 Bio-concentration factor (BCF)

Bio-concentration factor (BCF) is the capability of a selected plant to uptake heavy metal from the environment or in particular soil. It indicates the potential of plant for accumulation of heavy metal (Pratas *et al*., 2012; Hajar & Amir, 2014). BCF is defined as the heavy metal concentration in dry mass in relation to its concentration in external substratum (Favas & Pratas, 2012). Amount of metal extracted and bioaccumulation factor (BCF) can be used to evaluate the plant's phytoextraction efficiency and calculated accordingly (Ashraf *et al*., 2012). BCF for hyperaccumulators is more than 1, and in some cases can be increase up to 100.

2.15.2 Translocation Factor

Translocation factor is defined as the ability of the plant in transporting the heavy metal from root to aerial parts of the plant (Ali *et al*., 2013). TF value with value more than 1, implies that the plant have high potential of metal transport within the plant system.When *A. littoralis* plant were used for Cd removal from soil the TF > 1 and therefore could be labeled as Cd-hyperaccumulator (Baker & Brooks *et al*., 1989; Zhou *et al*., 2004). Similarly when *Avicennia* sp plant found at both mangrove also mostly has translocation factor more than 1. It seems that this plant has good translocation potency in order to move metals, especially Cu from one organ to another (Takarina & Tjiong, 2015).

2.16 First order kinetics modelling and half-life calculation

First order kinetic modelling were adopted to use in this study based on previous studies by Emenike *et al*. (2016). Selection of suitable kinetic modelling and rate constant is important for accurate estimation of heavy metal removal at a time for both bioaugmentation and phytoremediation studies. This is important for the determination of concentration of metal removed in a time and how long it will take for complete removal of metal to take place. First order kinetic models were also widely used for other biodegradation experiment such as hydrocarbon (Abioye *et al*. (2013); Arezoo (2013)). Emenike *et al.* (2016) reported removal rate constant of Cu (0.0212 g day^{-1}), Al (0.0127 g day^{-1}), Cd (0.053 g day^{-1}), Mn (0.0105 g day^{-1}), and Pb (0.0124 g day^{-1}) for bioaugmentation set up for 100 days with addition of *Bacillus* sp. , *Lysinibacillus* sp. and *Rhodococcus* sp. Arezoo (2013) reported removal rate constant of Zn 0.099 $month^{-1}$ for phytoremediation of Zn by *Dracaena* plant amended with soy cake. The kinetics of bioaugmentation can be divided into two whereby the first one is the factors influencing the amount of transformed compounds with time and second one is the approach seeks the types of curves describing the transformation and determines which of them fits the degradation of the given compounds by the microbiological culture in the laboratory microcosm and sometimes in the field (Maletic *et al*., 2009). The half-life was the time after which half of the original amount of substance present had been transformed (Fritsche & Hofrichter, 2005), Half-life was then calculated from the model by Yeung *et al*. (1997). Mujidat *et al*. (2013) has also calculated half-life for phytoextraction potential of *Vetiveria zizanioides* on heavy metals and reported value for Pb, Zn and Cd calculated according to the available data at the completion of the third month are 7.2, 6.4 and 29.64 months, respectively.

CHAPTER 3: METHODOLOGY

3.1 Soil and Leachate characterization

Municipal solid waste (MSW) landfill sites were selected for this study based on their non-sanitary landfill status. Bukit Beruntung Landfill (BBL) is landfill with“operational” status and is actively operating and receiving waste since 2001 whereas Taman Beringin Landfill (TBL) is non-operational status and closed its operation in 2005. Based on the age classification BBL are categorized as matured landfill and TBL is stabilized landfill. Soil samples were excavated at (0-30cm) depth from each of Taman Beringin (TBL) (3^0 13.78´N; 101^0 39.72´E; non-operational) and Bukit Beruntung (BBL) (3^0 32.14´N; 101^0 25.80´E; operational) landfills in accordance with the 2004 ASTME–1197 standard guidelines for conducting terrestrial soil-core microcosm tests (Sprocati *et al*., 2012). Soil was collected from 0 – 30 cm depth using soil cores and transferred into plastic containers and stored in room temperature before the soil is analysed.

Figure 3.1 and Figure 3.2 illustrates the sampling point at the landfill marked using google map. The marked sampling was chosen based on the structure of the landfill which represents the characteristics of soil of the entire landfill. The excavated triplicate samples were analyzed for pH using a multiprobe meter (YSI Professional Plus, USA),. Elemental concentrations of metals in the soil were analyzed based on the USEPA 3050B guidelines (method decribed in 3.6.1) except for mercury (Hg), which was analyzed based on the USEPA 3052 method. All assessments were analyzed in triplicates (including different trials). Similarly, the raw leachate samples were collected from the environment and analyzed for parameters similar to the soil samples. Part of the leachate assessment included on-the-spot analysis of raw leachate collection for several parameters, especially pH (HANNA HI 8424). Similarly, several other physico-chemical properties of the leachate samples determined in the laboratory were BOD_5, COD, total N, P, K, and the metal distribution. The assessment was conducted based on APHA (1998) standards. Preliminary investigation and assessment of the landfill site, included soil testing, topographic outlay, and visual observation, determined the degree of heterogeneity and siting of the sampling spots.

Figure 3.1: Map of sampling point of Taman Beringin Landfill (the sampling point is marked with yellow mark)

Figure 3.2: Map of sampling point of Bukit Beruntung Landfill (The sampling point is marked with yellow mark)

3.2 Isolation of microbes from landfill soil

Bacterial species were isolated by mixing 1 g of soil sample from Taman Beringin landfill or Bukit Beruntung andfill soil with 10 ml of normal saline water (0.9% NaCl) as stock. The mixture was shaken vigorously (3 h at 180 rpm) with the aid of a Lab-line 3521 orbit shaker, and the resulting suspension was subjected to 20 times serial dilution. Dilutions (0.1 ml) were dispensed on freshly prepared nutrient agar under aseptic conditions (Kauppi *et al*., 2011). The inoculated media plates and associated replicates were incubated at 37 °C for 24 h. Developed colonies were further sub-cultured to ensure the purity of samples prior to identification as in Plate 3.1.

Plate 3.1: Examples of microbes isolated from landfill soil

3.3 Identification of microbes

Biolog GEN III Microplate protocol was used to test the isolated microbes according to Bochner (1989a), Bochner (1989b) and Emenike *et al*. (2016). The GEN III MicroPlate test panel provides a standardized micro method using 94 biochemical tests to profile and identify a broad range of Gram-negative and Gram-positive bacteria. It consist of 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a "Phenotypic Fingerprint" of the microorganism that can be used to identify it at the species level. Biolog's Microbial Identification Systems software (OmniLog Data Collection) is used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate.

3.3.1 Detailed methodology of Biolog Identification

The isolated bacteria were grown on agar medium and then suspended in a special "gelling" inoculating fluid (IF) at the recommended cell density. Then the cell suspension is inoculated into the GEN III MicroPlate, 100 µl per well, and the MicroPlate is incubated to allow the phenotypic fingerprint to form. All of the wells start out colorless when inoculated. During incubation there is increased respiration in the wells where cells can utilize a carbon source and/or grow. Increased respiration causes reduction of the tetrazolium redox dye, forming a purple color. Negative wells remain colorless, as does the negative control well (A-1) with no carbon source. There is also a positive control well (A-10) used as a reference for the chemical sensitivity assays in columns 10-12 as shown in Table 3.1. After incubation, the phenotypic fingerprint of purple wells is compared to Biolog's extensive species library. If a match is found, a species level identification of the isolate is made.

Table 3.1: Layout of Assays for Microplate (GEN III)

A1 Negative control	A2 Dextrin	A3 D-maltose	A4 D-trehalose	A5 D-cellobios	A6 Gentiobiose	A7 Scurose	A8 D-turanose	A9 Stachyose	A10 Positive control	A11 pH 6	A12 pH 5
B1 D-raffinose	B2 α-D-lactose	B3 α-D-lactose	B4 β-mthayl-D-glucose	B5 D-salicin	B6 N-acetyl-D-glucossamin	B7 N-acetyl-β-D-mamosamin	B8 N-acetyl-D-galactosamin	B9 N-acetyl neuraminic	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-glocose	C2 D-mannose	C3 D-fructose	C4 D-galactose	C5 3-methyl glucose	C6 D-fucose	C7 L-fucose	C8 L-rhamnose	C9 Inosine	C10 1% sodium lactate	C11 Fusidic acid	C12 D-serine
D1 D-sorbitol	D2 D-mannitol	D3 D-arabitol	D4 Myo-inositol	D5 glycerol	D6 D-glusose 6-PO_4	D7 D-fructose-6-PO_4	D8 D-aspartic acid	D9 D-serine	D10 Troleandom ycin	D11 Rifamycin sv	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-prol	E3 L-alanine	E4 L-arginine	E5 L-aspartic acid	E6 L-glutamin acid	E7 L-histidine	E8 L-pyroglutamic acid	E9 L-serine	E10 Linocymcin	E11 guanidine Hcl	E12 Niaproof 4
F1 Pectin	F2 D-galaturonic	F3 D-galatonic acid lactone	F4 D-gluconic acid	F5 D-glucuronic acid	F6 Glucurona mide	F7 Mucic acid	F8 Quinic acid	F9 S-saccharic acid	F10 Vancomycin	F11 Tetrazolium violet	F12 Tetrazolium blue
G1 p-hydroxy-phenylatic acid	G2 Methyl pyruvate	G3 D-lactic acid	G4 L-lactic acid	G5 Citric acid	G6 α-keto-glutaric acid	G7 D-malic acid	G8 L-malic acid	G9 Bromo-succinic	G10 Nalidixic acid	G11 Lithium chloride	G12 Potassium tellurite
H1 Tween 40	H2 γ-amino-butyric acid	H3 α-hydroxy-butyric acid	H4 β-hydroxy-D lybutyric	H5 α-keto-butyric acid	H6 Acetoacetic acid	H7 Propionic acid	H8 Acetic acid	H9 Formic acid	H10 Aztreonam	H11 Sodium butyrate	H12 Sodium bromate

3.4 Heavy metal resistivity test

Isolated bacteria were aseptically re-grown by inoculating each species into individual test tubes containing 5 ml of nutrient broth at 37 °C for 18–24 h. Each inoculum was later introduced into test tubes containing 4.5 ml of normal saline water for standardization (NCCLS, 1993) to obtain 0.1 ABS (absorbance)/0.5 McFarland at 860 nm. However, the final inoculums required for the heavy metal sensitivity assessment were obtained by dispensing 0.1 ml of the resultant standard into corresponding test tubes containing 9.9 ml of normal saline water for each test organism to provide an approximate cell density of 5 x 10^5 CFU/ml. Furthermore, the chemical characteristics of heavy metals used for the resistivity test are in Table 3.2.

Table 3.2: Characteristics of heavy metals used

Metal	Salt	Product Supplier	Mol.wt (g/mol)	Atomic wt (g)
Pb	$PbCl_2$	Merck	278.1	207.2
Mn	$MnSO_4$	Friendemann Schmidt	169.02	54.93
Fe	$FeSO_4 . 7H_2O$	HumbG Chemicals	278.02	55.85
Hg	$HgSO_4$	Bendosen	296.65	200.59
Zn	$ZnSO_4 . 7H_2O$	AnalaR	287.55	65.38
Cu	$CuSO_4$	Bendosen	159.60	159.60
Cd	$CdCl_2$	Friendemann Schmidt	228.85	112.41
Ni	$NiCl_2 . 6H_2O$	Bendosen	237.73	58.69
Cr	$K_2Cr_2O_7$	HumbG Chemicals	294.19	103.8
Al	$Al_2(SO_4). 16H_2O$	Systerm	630.39	53.92

3.4.1 Heavy metal sensitivity test

Therefore, the metal tolerance (single condition) for each bacterial isolate was determined by agar diffusion. The standard suspension of each organism (5×10^5 CFU/ml) was used to seed each sterile plate, which contained 20 ml of nutrient agar. Pre-diffusion was allowed before a core borer was used to make 6 mm diameter wells (4) on the seeded plates. Four concentrations (5, 10, 15, and 20 ppm) of each metal were prepared. Metals (70 µl) were dispensed into corresponding wells. Hence, each plate accommodated four concentrations of a specified heavy metal and was allowed to stand for 1 h for pre-diffusion. Plates were then incubated at 37 °C for 24 h. The minimum inhibitory concentrations (MIC) of the heavy metals on the microbes were determined based on observed growth pattern (Plate 3.2). Diameters of the corresponding clear zones that characterized the concentrations of the heavy metals that showed no visible growth were measured to determine the inhibition zone diameter (IZD) and method was adopted and modified from Sabdono *et al*. (2012) and Rani *et al*. (2010). When the microbes shows no zone of inhibition in the growth in the plated inoculated with microbes the result is interpreted as absolute growth (++), for the plate with the growth that shows less than half growth from the distance from the edge of zone to the edge of where the metal is inoculated it is interpreted as mild growth with some inhibition (+-) and plate that shows no growth of microbes and complete inhibition is interpreted as no growth (--) (Sabdono *et al*. (2012); Rani *et al*. (2010)). Data are expressed as means of the three (3) replicates. Comparison of metal resistance among isolated microbes was analysed using a one-way ANOVA followed by a linearity plot to evaluate the correlation between resistance and inhibition zone diameter. A p value below 0.05 was regarded as statistically significant.

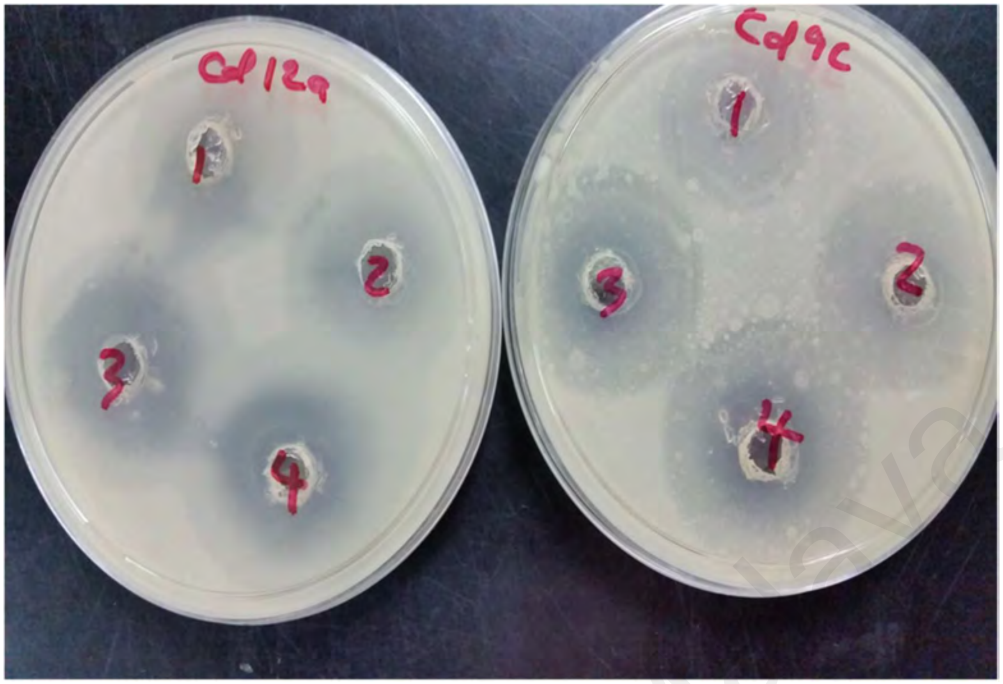

Plate 3.2: Example of heavy metal sensitivity test plate inoculated with microbes for Cadmium

3.5 Formulation of bacterial treatment for bioaugmentation experiment

The formulation of bacterial group is expected to enhance the removal of heavy metals from the contaminated soil. Eight groups of treatment including control were formulated The formulated treatments are groups that contained all isolated bacteria; gram negative bacteria; gram positive bacteria; highly sensitivity bacteria (based on sensitivity test); medium/ low sensitivity bacteria; proteo-bacteria; non-proteo bacteria.

Each microbes was grown as pure strain in Nutrient Agar at 33°C for 2 days before inoculated into desired amount of Nutrient Broth and grown until reached the stationary phase in a rotating shaker at 150 rpm. The discrete suspension of 1.5 ABS at 600nm were then pooled together to get equal proportions of inoculums before introduced into soil.

3.6 Bioaugmentation experiment set up with 10 % of inoculum

The leachate contaminated soil from Taman Beringin Landfill and Bukit Beruntung Landfill was collected for the bioaugmentation study according to the standard method of 2004 ASTME–1197 standard guideline. The experiment consisted of eight treatments including one control experiment. It is a multicondition experiment. The experiment was carried out with 2 kg of leachate contaminated soil amended with 10% v/w (200ml to 2 kg of soil) of microbial inoculum and method adopted from previous work on bioaugmentation experiment (Emenike, 2013). Each treatment consisted of about 3×10^9 CFU/g of inoculum, and the experiment was conducted in triplicates for all treatments. Soil moisture content was maintained by added distilled water in regular basis. Similarly, the microbial density, heavy metal concentration and other soil parameters for the leachate contaminated soil were carried out at every 20 days interval for 100 days (Emenike *et al*., 2016). The experiment duration is fixed to 100 days based on the previous research work on bioaugmentation carried out by several researchers on removal of heavy metal or other contaminants in soil (Abioye, (2011); Sprocati *et al*. (2012); Emenike, (2013)).

3.6.1 Soil heavy metal analysis

Soil heavy metal concentration was analysed every 20 days for all the treatment using ICP-OES according to USEPA 3050B guidelines (Sprocati *et al*., 2012; Emenike *et al*., 2016).Therefore the USEPA 3050B Method is followed for the analysis of soil. 1.5g of soil/plant sample were placed in a beaker prior to undergo digestion process. Several types of acids were being added into the soil sample. This include 4mL of dilute nitric acid solution (which made up by 2mL of Nitric Acid 65% and 2mL of deionized water), 2mL of Nitric Acid 65% and 2mL of Hydrochloric Acid 37%. The mixture of soil and acids was then heated using hot block at 85°C for 30 minutes. Once the heating process completed, the soil sample was then placed in desiccator for cooling purpose and then the sample volume was markup to 50mL by adding the deionized water. The digested sample was aspirated into Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) for, As, Cd, Pb analysis while for Hg analysis, the sample was analyzed by CVAAS (FIMS-400). Once the instrumental reading was obtained, the concentration of heavy metals in dry weight basis was determined.

3.6.2 Bacterial count

The microbes count was carried out by mixing 1 g of soil sample from Taman Beringin landfill and Bukit Beruntung landfill soil with 10 ml of normal saline water (0.9% NaCl) as stock. The mixture was shaken vigorously (3 h at 180 rpm) with the aid of a Lab-line 3521 orbit shaker, and the resulting suspension was subjected to 20 times serial dilution. Dilutions (0.1 ml) were dispensed on freshly prepared plate count agar under aseptic conditions (Kauppi *et al.*, 2011). The inoculated media plates and associated replicates were incubated at 37 °C for 24 h. Developed colonies were counted after 24 hours.

3.6.3 Soil parameters

Soil pH and soil redox potential was measured using multi probe YSI Professional Plus, USA at ratio of 1:2.5 soil: water ratio (Malik *et al.*, 2010). Calibration of the YSI probe was first carried before any reading was taken using standard buffer solutions. Five (5) grams of each soil type was weighed into a beaker and distilled water was added (12.5 ml) and the solution stirred vigorously for 15 seconds. This was left to stand for 30 minutes. The electrodes of the YSI probe were placed in the slurry, swirled carefully, and the pH and redox potential read and recorded.

3.6.4 Leachate Analysis

3.6.4.1 Biochemical oxygen Demand (BOD)

BOD analysis is carried out for leachate using APHA 1998 method. The analysis is carried is out within 2 hours of sample collection. BOD dilution water was prepared 24 hours prior to the sample collection time. BOD water contains 1ml of phosphate buffer solution, Magnesium sulphate solution, Calcium chloride solution, Ferric chloride solution which is diluted into 1 L of distilled water. This BOD water is kept at room temperature for 24 hours prior to BOD testing. The raw leachate collected from landfill was diluted 200 times with the prepared BOD water and the pH is adjusted with addition of HCI or NaOH solutions. The DO0 was recorded using DO6 Dissolved Oxygen palm top meter. The BOD bottle were filled with the diluted leachate till reach the brim in gradual manner to avoid presences of trapped gas bubbles. The bottle is then incubated at 20°C for 5 days, after which the DO5 was measured. The final BOD_5 is calculated using formula $BOD_5 = DO_5 \times$ Dilution factor.

3.6.4.2 Chemical Oxygen Demand (COD)

The COD analysis of leachate was also calculated using APHA 1998 method. The raw leachate collected is diluted 400 times with distilled water. Therefore, 2ml of the diluted leachate solution is COD vial (COD HACH vial HR Digestion solution for COD of 0-1500 mg/L range) and capped tightly. The vial is vigorously shake and then placed into a COD digester (HACH DRB 200) unit and allowed be digested for 2 hours. After the digestion of sample is complete the vial is placed into HACH COD HR Program in Spectrophotometer (DR 4000 UV-VIS) and the COD reading was taken from the monitor.

3.7 Bioaugmentation set up to study effect of inoculum concentration on metal remediation

The leachate contaminated soil from Taman Beringin Landfill and Bukit Beruntung Landfill was collected for the bioaugmentation study according to the standard method of ASTM Guidelines. Similar to 10% set up, the experiment was carried out with 2 kg of leachate contaminated soil however the microcosm was amended with 20% v/w (400ml to 2kg of soil) and 30% v/w (600 ml to 2kg of soil) of microbial inoculum. The experiment was conducted in triplicates for all treatments. Soil moisture content was maintained by added distilled water in regular basis. The microbial density, heavy metal concentration and other soil parameters for the leachate contaminated soil were carried out at Day 0, 60 and 100 as described in above method (Emenike *et al*., 2016).

3.8 Field Bioaugmentation experiment set up

The field bioaugmentation experiment was carried out at non-operating landfill with leachate contaminated soil (heavy metal pollution). The concentration of heavy metal contaminants was determined before the soil was used for bioaugmentation set up. The best treatment from the lab scale experiment was chosen for the set up (Proteo-bacteria). The experiment set up was carried out using polluted soil and analyzed using USEPA 3050B test method (refer to 3.6.1). The selected point were constructed with temporary barrier to minimize leaching of leachate into the system however no other parameters or natural activities in the landfill was controlled. The constructed barrier is illustrated in Plate 3.3. The bioaugmentation set up was also carried out with control sample (without any treatment). The microbial consortium formulated was introduced to the leachate contaminated soil every 20 days using an inoculation to a desired amount of about 20L with the concentration 1.50 ABS at 600nm, while monitoring the heavy metal concentration (sample will be collected in triplicates at three different point at each point at 10cm, 20cm and 30cm depth) using soil auger. Microbial density and soil parameters was carried out in 20 days intervals for 100 days as described in section 3.6.2.

Plate 3.3: Construction of barrier to minimize leachate flowing into the experimental plot

3.9 Phytoremediation experiment set up

The leachate contaminated soil from Taman Beringin Landfill and Bukit Beruntung Landfill was collected for the phytoremediation study according to ASTM Standard method. Soil was collected from 0 to 30cm depth of soil using soil cores. The phytoremediation potential of four selected plants were tested in this study (Table 3.3) and the reason for chosing this four plants is basically to identify new hyperaccumulators which never has been used to phytoremediation of heavy metal from leachate contaminated soil. Plants were collected from University of Malaya Nursery. The selected plants were duly chosen to be same height. The plant was later transplanted into contaminated soil using by uprooting from the normal soil. Each plant was placed in polybag containing 2.25 kg of leachate contaminated soil with predetermined heavy metal concentration. Experiment was conducted for 120 days. The experiment was carried out without any plant as a control. At the end of experiment, plant wet weight, plant dry weight and plant height were measured and heavy metal concentration of different components of plants and of soil before and after harvest were performed. Similarly, the microbial density, heavy metal concentration and other soil parameters for the leachate contaminated soil was carried out at every 30 days interval using similar method described in section (3.6.1, 3.6.2 and 3.6.3) bioaugmentation experiment set up.

Table 3.3: Phytoremediation experiment set up

Details of treatment	No of Samples
2.25 kg of Soil + *Cordyline* sp.	3
2.25 kg of Soil + *D. variegated*	3
2.25 kg of Soil + *T. spathacea*	3
2.25 kg of Soil +*C. comosum*	3
2.25 kg of Soil (Control)	3

3.9.1 Description of phytoremediation experiment set up area

This study was conducted in open environment at the roof top of Institute of Postgraduate's studies of University of Malaya, Malaysia. The experiment trials were conducted under a netted plant shelter to protect the test plants from direct rain and sunlight as in Plate 3.4.

Plate 3.4: Experiment set up at roof top of Institute of Postgraduate studies of University of Malaya

3.9.2 Bio-concentration Factor and Translocation factor

3.9.2.1 Bio-concentration factor (BCF)

Bio-concentration factor (BCF) is calculated using the following formula:

$$BCF = \frac{Metal\ concentration\ in\ plant}{Metal\ concentration\ in\ the\ soil}$$

BCF= Bio- concentration factor

Heavy metal in plant= Concentration of heavy metal in harvested part of plant (mg/kg)

Heavy metal in soil = Concentration of heavy metal in soil (mg/kg)

3.9.2.2 Translocation Factor

Translocation factor is defined as the ability of the plant in transporting the heavy metal from root to aerial parts of the plant (Ali *et al*., 2013). TF value with value more than 1, implies that the plant have high potential of metal transport within the plant system.

$$TFshoot = \frac{Metal\ in\ shoot}{Metal\ in\ root}$$

TF= Translocation factor

Metal in shoot = Concentration of heavy metal in shoot of plant (mg/kg)

Metal in root = Concentration of heavy metal in root of plant (mg/kg)

3.9.3 Plants height and weight determination

Fresh weights (shoot and roots) were determined by weighing the plant parts using Sartorius ENTRIS 224-1S analytical balance and for dry weight for roots and shoot of the plant sample was weighed after drying in oven at 80°C for 3 days until constant weight (Mangkoedihardjo *et al*., 2008; Parrish *et al*., 2004; Saadati *et al*., 2012). Height was measured from ground level to the base of the apical bud on the terminal shoot as showed in Plate 3.5 (Zalesny *et al*., 2007) using measuring ruler.

Plate 3.5: Plant after uprooted from soil

3.9.4 Soil and plant heavy metal analysis

Soil and plant heavy metal concentration was analysed every 30 days for all the treatment using ICP-OES according to USEPA 3050B guidelines (Emenike *et al*., 2016) and the method was similar to section 3.6.1.

3.10 First order rate constant and half-life calculation

First order kinetic modelling were adopted to use in this study based on previous studies by Emenike *et al*. (2016). The heavy metal removal calculation using the model as follow

$$k = -\frac{1}{t}\left(\ln\frac{C}{C_0}\right)$$

k= first-order rate constant for metal uptake per day
t = time in days
C = concentration of residual metal in the soil (mg kg^{-1})
C_0= initial concentration of metal in the soil (mg kg^{-1})

The half-life was the time after which half of the original amount of substance present had been transformed (Fritsche & Hofrichter, 2005), Half-life was then calculated from the model by Yeung *et al*. (1997).

Half-life of heavy metal removal is calculated as

$$half\ life = \ln 2/k$$

k= first-order rate constant for metal uptake per day

3.11 Statistical Analysis

The statistical analysis of data was conducted using analysis of variance (ANOVA) in the SPSS software 21.0 with the LSD post-hoc test at p-value =0.05.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterization of leachate

Leachate from Taman Beringin Landfill (TBL) and Bukit Beruntung Landfill (BBL) contained significantly high amount of heavy metal and the characteristics of leachate is shown in Table 4.1.

Table 4.1: Characteristics of leachate from Taman Beringin Landfill and Bukit Beruntung Landfill

Test parameter	Test method	Taman Beringin Landfill*	Bukit Beruntung Landfill*	Standard (Environmental Quality Regulations 2009,Malaysia
pH	Probe insertion	7.57 ±0.8	7.09 ±0.63	6.00-9.00
BOD (mg/L)	APHA 5210 B	127 ±45	259 ±37	20
COD (mg/L)	APHA 5220	482 ±103	985 ±185	400
Total N (%)	ASTM E778-87	0.25 ±0.08	0.32 ±0.05	5
Total K (mg/L)	ASTM E926-94	11.6 ±2.1	40.4 ±6.04	N.A
Total P (mg/L)	ASTM D5198-92	18.3±0.7	24.3 ±0.7	N.A
As (mg/L)	USEPA 3050 B	< 0.01	0.21±0.04	0.05
Ca (mg/L)	USEPA 3050 B	242.1 ±42	91.2 ±11.6	N.A
Fe (mg/L)	USEPA 3050 B	134.6 ± 16	60 ±18.2	5.0
Mn (mg/L)	USEPA 3050 B	3.1 ±0.32	5.1 ±0.5	0.2
Mg (mg/L)	USEPA 3050 B	52.2 ±8.7	96.6 ±16	N.A
Na (mg/L)	USEPA 3050 B	29.7 ±5.1	242.1 ±22.8	N.A
Cu (mg/L)	USEPA 3050 B	0.5 ±0.1	2.62 ±0.8	0.2
Zn (mg/L)	USEPA 3050 B	24.3 ±3	236 ±11.8	2.0
Pb (mg/L)	USEPA 3050 B	<0.01	1.12 ±0.04	0.10
Cd (mg/L)	USEPA 3050 B	0.4 ±0.1	0.4±0.1	0.01
Hg (mg/L)	USEPA 3052	0.03	0.04	0.005
Cr (mg/L)	USEPA 3050 B	6.2 ±1.4	17.3 ±1.19	0.20
Ni (mg/L)	USEPA 3050 B	0.85 ±0.1	12 ±4.4	0.20
Al (mg/L)	USEPA 3050 B	5.47 ±1.2	13.1 ±3.2	N.A

*: mean values (n=3)

Both landfills are categorized as non-sanitary landfills and further description is tabulated in Table 4.2. Comparison with Malaysian standards from Department of Environment, Malaysia under Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 and International standards showed that almost all the metal contents in both landfills leachate exceeded the prescribed limit.

Table 4.2: General conditions of the two landfill sites

Condition Class	Taman Beringin landfill	Bukit Beruntung landfill
Landfill type	Non-Sanitary (non-operational)	Non-Sanitary (operational)
Period of landfilling	1991 – 2005	2001 - date
Age classification	Stabilized	Mature
Daily average of waste disposed (tonnage)	1800 - 2000	1500
Waste type	Household, commercial and industrial	Household, commercial and industrial
Form of leachate treatment	Physical and biological	Biological
Distance to river/stream (m)	5	NA
Fate of generated landfill gas	No facility	No facility
Coordinates of sampling spots	A 3^0 13’ 40.17 N 101^0 39’43.48 E B 3^0 13’ 43.86 N 101^0 39’51.74 E C $3^0$13’37.91 N $101^0$39’51.74 E D $3^0$13’36.44 N $101^0$39’46.72 E	A $3^0$42’49.21 N $101^0$54’55.87 E B $3^0$42’49.81 N $101^0$54’53.35 E C $3^0$25’31.88 N $101^0$32’48.92 E

TBL ceased operation in year 2005. However the concentration of heavy metal in the landfill leachate exceeded the limit allowed even after 12 years of closure. Among the metal analyzed, Iron (Fe) showed the highest concentration of 134.6± 16 mg/L, followed by Zinc (Zn) with a concentration of 24.3 ± 3 mg/L. Other metals such as Aluminium (Al), Chromium (Cr) and Manganese (Mn) were 6.2± 1.4 mg/L, 5.47± 1.2 mg/L and 3.1± 0.32 mg/L respectively.

BBL is an active landfill which is constantly receiving waste at the rate of 1500tonne/day, and this may contribute to the leaching of heavy metals. Zn (236 ± 11.8 mg/L) was found to be highest in the landfill leachate as compared to Fe (60 ± 18.2 mg/L) and Cr (17.3 ± 1.9 mg/L). Abdul Aziz *et al*. (2004) also reported higher concentrations of Fe in the study conducted in non-sanitary landfill. Besides, another study by Sumaiya *et al*. (2014) also reported high Fe content in the leachate produced at Sultanate of Oman landfill (39.85 mg/L). The steel material dumped at the site may be the cause for the high concentrations of Fe. The allowable limit for Fe in leachate is 5.0 mg/L, however leachate of BBL landfill showed higher Fe concentration above the limit. The level of soluble metals tends to be higher in active landfills and similar result has been reported by Emenike *et al*. (2013), Yusof *et al*. (2009) and Lagerkvist (2003). Alaribe and Agamuthu (2010) also reported high heavy metal concentration from leachate collected from Ampar Tenang landfill which proves that the landfill in Malaysia received industrial and household hazardous waste that may contribute to the elevated heavy metal concentration in the leachate.

Other parameters such as pH were also analyzed and the average pH value for TBL and BBL were pH 7.57±0.8 and pH 7.09±0.63, respectively. According to Kanmani and Gandhimathi (2013), metal will be less soluble when the pH increases due to precipitation of metal ions as in soluble hydroxide at high pH value.

Total Nitrogen (TN) of leachate was also determined and the percentage of TN for TBL was 0.25% and 0.32% for BBL however this value is acceptable with the standards value. This is possibly due the age of both landfills. BOD_5 and COD are another important aspect in the leachate characterization. BOD_5 of TBL was lower as compared to BBL. BBL shows almost 50% higher value of BOD_5 compared to TBL leachate. The value of BOD_5 for TBL were 127±45 mg/L, while for BBL it was 259±37 mg/L. High value of BOD_5 in BBL may be associated its active status. High BOD_5 value indicates that the organic materials in the leachate is highly biodegradable. This is agreeable with other researchers that analysed leachate sample from landfill in Malaysia and obtained high concentrations of BOD_5 in active landfills (Emenike, 2013; Mohammed & Agamuthu, 2008).

4.2 Characterization of leachate contaminated soil

The heavy metal concentration of soil from TBL and BBL is shown in Table 4.3. The concentrations of As, Fe, Mn, Cr, Al and Ni were higher in TBL whereas for the highest metal concentration was for Cu, Zn, Pb and Cd in BBL. This result can be associated with the different operational status of both landfills. The high metal concentration in TBL and BBL soil may be due to leachate contamination of the soil around the landfill area that tends to persist in the soil.

The metal concentrations in the TBL follow the order of Al (49600mg/kg) > Fe (42900mg/kg)> Mn (281mg/kg)> As (103mg/kg)>Cu (59mg/kg)>Zn (49 mg/kg) > Cr (46 mg/kg)> Ni (21 mg/kg)> Pb (18mg/kg). This could mainly be due to the nature of solid waste dumped to the landfill types of waste including bottle caps, blades, and pharmaceuticals, galvanizing, paints, pigments, insecticides and cosmetics along with garbage were dumped into the landfill (Kanmani & Gandimathi, 2013). The metal concentration found in TBL may imply that extractable/mobile metal ions have been immobilized over time and may exist as complexes.

Table 4.3: Characterization of soil from Taman Beringin Landfill and Bukit Beruntung Landfill

Test parameter	Test method	Unit	Taman Beringin landfill	Bukit Beruntung landfill
			Mean*	Mean*
pH			7.57	7.09
Total N	ASTM E778-87	%	0.62	0.46
Total K	ASTM E926-94	mg/kg	396.9	935.5
Total P	ASTM D5198-92	mg/kg	568	1858
As	USEPA 3050 B	mg/kg	103	7
Ca	USEPA 3050 B	mg/kg	1608	8614
Fe	USEPA 3050 B	mg/kg	42900	20400
Mn	USEPA 3050 B	mg/kg	281	66
Mg	USEPA 3050 B	mg/kg	127.2	618.8
Na	USEPA 3050 B	mg/kg	4.54	269.9
Cu	USEPA 3050 B	mg/kg	59	85
Zn	USEPA 3050 B	mg/kg	49	319
Pb	USEPA 3050 B	mg/kg	18	67
Cd	USEPA 3050 B	mg/kg	<0.01	2.64
Hg	USEPA 3052	mg/kg	<0.02	<0.002
Cr	USEPA 3050 B	mg/kg	46	13
Ni	USEPA 3050 B	mg/kg	21	9
Al	USEPA 3050 B	mg/kg	49600	3040

[a] :mean values (n=3)

The order of heavy metal concentrations in BBL soil followed the order of Fe (8614 mg/kg) > Al (3040 mg/kg) > Zn (319mg/kg) > Cu (85 mg/kg) > Pb (67 mg/kg) > Mn (66 mg/kg) > Cr (13 mg/kg) > Ni (9 mg/kg) > As (7 mg/kg) > Cd (2.64 mg/kg). Soil collected from both landfill exhibit high metal concentration of soil. The levels of soluble metals in active landfills are often higher (Lagerkvist, 2003; Yusof *et al*., 2009; McBean *et al*., 1995; Calli *et al*., 2005). Both landfills are considered to be in acidogenic phase because the pH values were pH 6.8 and pH 7.1 for the TBL and the BBL, respectively. The upper limit of the acidogenic phase is pH 4.5–7.5 (Kjeldsen *et al*., 2002).

4.3 Isolation and identification of microbes isolated from leachate contaminated soil

Microbes isolated from soil collected from TBL and BBL are shown in Table 4.4. The list shows diverse genera of bacteria that grows in leachate contaminated soil. Their presence in highly contaminated soil with heavy metal shows that they may have the potential to remedy heavy metals. *O. intermedium*, *S. acidaminiphilia*, *A. ebreus*, *B. diminuta*, *Cloacibacterium sp* , *A. caviae* DNA 4, *D. tsuruhatensis*, *P. alcaligenes*, *C. gleum*, *P. mendocina* and *S. marcescens marcescens* are gram negative microbes whereas, *B. vietnamiensis, B. aryabhattai, R. ruber, B. pumilus, B. kochii, J. hoylei* and *B. cereus* belong to gram positive microbes. Isolation of bacteria from metal polluted environment would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioaugmentation purpose (Malik, 2004).

O. intermedium strain is a gram negative, short rod shaped, spore forming and strictly aerobic organism isolated from leachate contaminated soil. The presence of this microbe in the leachate contaminated soil is suggestive that it is a common microbe found in metal polluted soil (Xiumei *et al*., 2014). Having been isolated from metal polluted soil, the organism also can be related for its relevance for metal reduction in soil. Similarly, the organism has also been reported involved in bioaugmentation of heavy metal (Cheng *et al*., 2010; Pandey *et al*., 2013; Faisal & Hasnain, 2006; Pandey *et al*., 2012; Ozdemir *et al*., 2003; Waranusantigul *et al*., 2011).

Table 4.4: Microbes isolated from leachate contaminated soil

Isolated microbes	Gram stain	Proteo	Non- proteo
Ochrobacterium intermedium	Negative	Proteo	-
Stenotrophomonas acidaminiphilia	Negative	Proteo	-
Acidovorax ebreus	Negative	Proteo	-
Brevundimonas diminuta	Negative	Proteo	-
Cloacibacterium sp.	Negative	-	Non-proteo
Aeromonas caviae DNA 4	Negative	Proteo	-
Delftia tsuruhatensis	Negative	Proteo	-
Pseudomonas alcaligenes	Negative	Proteo	-
Chryseobacterium gleum	Negative	-	Non-proteo
Pseudomonas mendocina	Negative	Proteo	-
Serratia marcescens marcescens	Negative	Proteo	-
Burkholderia vietnamiensis	Positive	Proteo	-
Bacillus aryabhattai	Positive	-	Non-proteo
Rhodococcus ruber	Positive	-	Non-proteo
Bacillus pumilus	Positive	-	Non-proteo
Bacillus kochii	Positive	-	Non-proteo
Janibacter hoylei	Positive	-	Non-proteo
Bacillus cereus	Positive	-	Non-proteo

S. acidaminiphilia was also found in the leachate contaminated soil. It is a gram-negative, motile, non-sporulating bacteria with straight to curved rods that possess polar flagellum. The resistive nature of the microbes towards heavy metal pollution indicates its bioaugmentation potential for heavy metal and similarly, Chien *et al*. (2007), Pages *et* *al*. (2008), Alonso *et al*. (2000), Ryan *et al*. (2007) and Crossman *et al*. (2008) reported the relevance of the organisms for heavy metal removal from contaminated soil.

B. pumilus is a Gram-positive, aerobic, spore-forming bacillus isolated from leachate contaminated soil in this study. The nature of being gram positive organism indicates its ability for uptake of metals in contaminated soil, possibly because it has outer layer of the peptidoglycan cross-links in *B. pumilus* is covered by teichoic and lipoteichoic polyglycosyl phosphates with mono- and disaccharides as their monomers that can play a role in adhesion to different surfaces like the host cells. Similarly, Chen *et al*. (2011) also reported the importance of this organism for metal reduction.

Another bacteria isolated from the leachate contaminated soil was *B. kochii.* It is Gram positive, strictly aerobic, motile, catalase-positive, endospore-forming rods. Dominance of *Bacillales* in heavy metal polluted soil is also in agreement with findings of Seralathan and Kui (2008) and Singh *et al*. (2010).

Also found in the leachate contaminated soil was *B. cereus*, which belongs to gram positive group, rod shaped and facultative aerobic organism. It is widespread in the environment and similarly, Hookoom and Puchooa (2013) has also isolated the same organism from waste dumping area. The presence of *B. cereus* in the leachate contaminated soil can be attributed to its potential for metal remediation and in agreement with findings of Huiqing *et al.* (2016) and Costa *et al.* (2001).

S. marcescens marcescens is another species of organism found to be in the leachate contaminated soil. It is gram negative, rod shaped and motile organism. Its isolation from contaminated soil may possibly attribute to its potential for bioaugmentation of heavy metal in contaminated soil and it was found to previously used for remediation of metal such as Pb, Cd, Zn, Hg, Fe and Al (Owolabi & Hekeu , 2015; Christani *et al*. 2012; Khan *et al*., 2017, Sahar, 2012).

Another organism isolated in this study from leachate contaminated soil was *A. caviea* DNA group 4. It is is a gram negative, motile and rod shape bacteria. The isolation of this microbe from metal contaminated soil reasoned its potential for metal remediation and it has been previously reported to be tolerant to metals such as Shamim *et al*. (2013), Miranda and Castillo (1998) and Owolabi and Hekeu (2015).

D. tsuruhatensis is gram negative bacteria of bacilli group isolated from the landfill leachate contaminated soil. It has irregular and cream coloured colonies. The ability to grow and survive in metal contaminated soil point out its potential to be tolerant towards heavy metals. This is agreeable to previous findings of Bautista *et al*. (2012) and Ubalde *et al*. (2012), who also isolated the organism from mine tailings and metal contaminated soil.

P. alcaligenes is another microbe isolated from leachate contaminated soil. It is an aerobic gram negative soil bacteria. The existence of this microbe in metal contaminated soil may possibly be due to its ability to resist towards heavy metal and can be a good bioremediation agent and this is supported by findings of Liu *et al*. (2011) and Mahony *et al*. (2006), who has reported the potential of *P. alcaligenes* for removal of heavy metal from polluted soil and water.

Another important microbe isolated in this study was *P. mendocina*. It is an gram negative, aerobic and in rod shape. Though this microbe was isolated from leachate contaminated soil it also has been widely isolated from different locations of farmland soil and it is known to be strain of bacteria that possess resistance towards heavy metals (Chong *et al*., 2012). This further confirmed that the organism may be a potential candidate organism for metal remediation and this is in agreement with findings of Ramos *et al*. (2003) and Chong *et al*. (2012).

B. vietnamiensis is gram positive bacteria, isolated from the leachate contaminated soil. Similarly, it has been also previously isolated from metal contaminated soil (Idris *et al*., 2004; Mengoni *et al*. (2001), Schlegel *et al*. (1991) and Jiang *et al*. (2008) while it also can be related as potential organism for metal reduction in contaminated soil. This is agreeable to previous findings of Cheng *et al*. (2016) who studied the potential of *B. vietnamiensis* for removal of metal by using bio materials.

Cloacibacterium sp. is a gram negative bacteria isolated from leachate polluted soil. The presence of this bacteria in the soil indicates its ability to tolerate heavy metal. It has been previously isolated from contaminated wastewater (Allen *et al*., 2006).

A. ebreus is a motile and gram negative microbe found in leachate contaminated soil. Not much information has been documented on this species over its tolerance towards heavy metal however its relevance in metal removal may further investigated due to its survival in metal polluted soil.

B. diminuta is non-lactose fermenting environmental gram negative bacilli isolated from leachate contaminated soil. This microbe bacterium is motile with single polar flagellum. The organism may have the potential for removal of heavy metal from contaminated soil and it has also been categorized as heavy metal rhizobacteria (Hamzah *et al*., 2015). This suggest its possible use for metal remediation.

J. hoylei is gram positive, non-motile and non-endospore forming cocci bacteria isolated from leachate contaminated soil. The colonies of *J. hoylei* is cream in colour. No literature has reported the presence of *J. hoylei* in heavy metal polluted soil however its ability to grow and survive in leachate contaminated soil may further exhibit its role for metal bioaugmentation, however further investigation is duly necessary.

B. aryabhattai is also gram positive and motile bacteria isolated from the leachate polluted soil. The isolation of the organism from the contaminated shows its relevance for metal remediation and this is in agreement with findings of Tendulkar *et al*. (2016).

R. rubber is a non-motile, gram positive bacteria isolated in this study from the leachate contaminated soil and similarly it can be commonly found in the environment such as soil and water. The ability of the organism for heavy metal removal was not reported so far however its relevance in degrading organic pollutant has been studied (Kuyukina & Iushina, 2010). The presence of this microbe in the highly polluted leachate soil gave an idea that this bacteria may have resistance towards heavy metal in the soil and may further be developed for remediation study.

Lastly *C. gleum* is a gram negative bacteria isolated from leachate contaminated soil and shows its relevance for metal remediation. However not much has been explored as potential bacteria for metal remediation.

4.4 Screening of microbes using heavy metal sensitivity assessment

Heavy metal resistance has been carried out to obtain the tolerance ability of the isolated microbes from leachate polluted soil towards different types of heavy metals at varying concentrations. This study did not adopt the conventional approach of testing heavy metal on the species until absence of absolute growth but instead it adopted set (5-20 ppm) that accommodates the range typical of most environment. This test are basically preliminary test to screen the microbes and identify their tolerance level towards different heavy metals before the actual bioaugmentation experiment is carried out in laboratory and landfill conditions.

4.4.1 Heavy metal Sensitivity test

Table 4.5 shows the results of the heavy metal sensitivity test. Although the growth across the microbial diversity and metal concentrations were pronounced, the overall growth of the microbes declined as metal concentrations increased. This trend was in contrast to the situation in the control (0.0 ppm of metals), where absolute growth of all the bacteria species was observed.

Strong positive growth was observed for *B.vietnamiensis* up to 20 ppm for all the heavy metal tested except for 20 ppm Cr whereby mild inhibition was observed (Table 4.5). This indicates that this bacteria has strong metal resistance as compared to other microbes studied. Heavy metal resistant microorganisms play an important role in the remediation of heavy metal contaminated soils (Ray & Ray, 2009).

J. hoylei, *D. tsuruhatensis*, *C. gleum*, *B. diminuta* and *Cloacibacterium* sp, *B. kochii*, *B. aryabhattai* and *B. pumilus* showed an inhibition for certain metal even at 5 ppm. Low concentration of metal is enough to induce growth inhibition on bacterial species, though the bacterial species and presence of co-contaminant may also influence the toxic effect and supported by Amor *et al*. (2001).

Comparison between the different types of metals revealed that almost all microbes tested except for *C. gleum* were highly resistant to Hg up to 20ppm. The order of heavy metal resistance by microbes is Hg> Mn > Al > Pb > Cu > Zn > Fe > Ni > Cd> Cr.

This order will reflect the reaction of the microbes when exposed to different types of metals and their resistance mechanism.

Except for *C. gleum* exposure to Mn revealed that all isolates were highly resistant up to 20ppm. *C. gleum* shows inhibition even at 5 ppm concentration. Bacteria exposed to high levels of heavy metal in the environment may have adapted to metal stress and develops various resistance mechanisms (Ahmed *et al*., 2005).

Positive growth was observed for fifteen isolates except for *B. cereus*, *A. caviae* DNA 4 and *C. gleum* with Al exposure. Mild inhibition at 20 ppm were observed for *B. cereus* and *A. caviae* DNA and complete inhibition was observed for *C. gleum* even at low concentration (5 ppm) of Al. The response of microbes to heavy metals depends on the concentration and availability of heavy metal and it is a complex process which is controlled by factors such as type of metal, the nature of medium and species of bacteria (Jayanthi *et al*., 2016). *C. gleum* seems to be highly sensitive to certain metal whereby exposure to higher concentration prohibits its growth.

Table 4.5: Heavy metal sensitivity test for isolated microbes

Bacteria	Concentration(ppm)	Pb	Mn	Fe	Hg	Zn	Cu	Cd	Ni	Cr	Al
A. caviae DNA group 4	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	+-	++	++	++
	15	++	++	++	++	++	++	+-	++	+-	++
	20	+-	++	++	++	+-	+-	+-	+-	+-	+-
P. alcaligenes	5	++	++	++	++	++	++	++	++	+-	++
	10	++	++	++	++	++	++	+-	++	+-	++
	15	+-	++	++	++	++	++	+-	++	+-	++
	20	+-	++	+-	++	+-	++	+-	+-	+-	++
P. mendocina	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	+-	++	+-	++
	15	++	++	++	++	++	++	+-	++	+-	++
	20	++	++	++	++	++	++	+-	++	+-	++
B. pumilus	5	++	++	++	++	++	++	++	--	++	++
	10	++	++	++	++	++	++	++	--	++	++
	15	++	++	++	++	++	++	++	--	++	++
	20	++	++	+-	++	++	++	++	--	+-	++
O. intermedium	5	++	++	++	++	++	++	+-	++	+-	++
	10	++	++	++	++	++	++	+-	++	+-	++
	15	++	+-	++	++	++	++	+-	++	+-	++
	20	+-	+-	++	++	++	++	+-	++	+-	++
S. acidaminiphilia	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	+-	++
	15	++	++	+-	++	++	++	++	+-	+-	++
	20	++	++	+-	++	++	++	++	+-	+-	++

--: no growth; +-: mild growth with some inhibition; ++ absolute growth

Table 4.5, continued.

Bacteria	Concentration(ppm)	Pb	Mn	Fe	Hg	Zn	Cu	Cd	Ni	Cr	Al
B. cereus	5	++	++	++	++	++	++	+-	++	++	++
	10	++	++	++	++	++	++	+-	++	++	++
	15	++	++	++	++	++	++	+-	++	++	++
	20	++	++	++	++	++	++	+-	++	++	+-
D. tsuruhatensis	5	--	++	++	++	--	++	--	--	--	++
	10	--	++	++	++	--	++	--	--	--	++
	15	--	++	++	++	--	++	--	--	--	++
	20	--	++	++	++	--	++	--	--	--	++
C. gleum	5	--	--	--	--	++	++	++	++	--	--
	10	--	--	--	--	++	++	+-	++	--	--
	15	--	--	--	--	++	++	+-	++	--	--
	20	--	--	--	--	++	++	+-	++	--	--
S. marcescens marcescens	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	+-	++	++	++
	15	++	++	++	++	++	++	+-	++	++	++
	20	++	+-	+-	++	++	++	+-	++	++	++
B. vietnamiensis	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	++	++
	15	++	++	++	++	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	++	+-	++
A. ebreus	5	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	+-	++	++	++
	15	++	++	++	++	++	++	+-	++	+-	++
	20	++	++	++	++	++	++	+-	++	+-	++

--: no growth; +-: mild growth with some inhibition; ++ absolute growth

Table 4.5, continued.

Bacteria	Concentration(ppm)	Pb	Mn	Fe	Hg	Zn	Cu	Cd	Ni	Cr	Al
B. diminuta	5	++	++	++	+-	+-	+-	+-	++	++	++
	10	++	++	++	+-	+-	+-	+-	+-	+-	++
	15	++	++	++	+-	+-	+-	+-	+-	+-	++
	20	++	++	+-	+-	+-	+-	+-	+-	+-	++
Cloacibacterium sp	5	++	++	++	++	++	++	+-	+-	++	++
	10	++	++	++	++	++	++	+-	+-	+-	++
	15	++	++	++	++	++	++	+-	+-	+-	++
	20	++	++	++	++	++	+-	+-	+-	+-	++
R. rubber	5	++	++	++	++	++	++	+-	++	++	++
	10	++	++	++	++	++	++	+-	++	+-	++
	15	++	++	++	++	++	++	+-	++	+-	++
	20	++	++	++	++	++	++	+-	++	+-	++
B. aryabhattai	5	++	++	++	++	++	--	+-	++	++	++
	10	++	++	++	++	+-	--	+-	++	+-	++
	15	++	++	++	++	+-	--	+-	++	+-	++
	20	++	++	++	++	+-	--	+-	++	+-	++
B. kochii	5	++	++	++	++	--	++	+-	--	++	++
	10	++	++	++	++	--	++	+-	--	--	++
	15	++	++	++	++	--	++	+-	--	--	++
	20	++	--	+-	++	--	--	+-	--	--	++
J. hoylei	5	++	++	++	++	+-	++	++	++	++	++
	10	++	++	++	++	+-	++	++	++	++	++
	15	++	++	++	++	+-	++	++	++	++	++
	20	++	++	++	++	+-	++	++	++	++	++

--: no growth; +-: mild growth with some inhibition; ++ absolute growth

Table 4.6: One-way ANOVA for heavy metal sensitivity test between different isolates

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
B. vietnamiensis	Between Groups	217.867	9	24.207	4.428	.003
	Within Groups	109.333	20	5.467		
	Total	327.200	29			
R. rubber	Between Groups	2158.667	9	239.852	48.949	.000
	Within Groups	98.000	20	4.900		
	Total	2256.667	29			
B. aryabhattai	Between Groups	3161.867	9	351.319	94.951	.000
	Within Groups	74.000	20	3.700		
	Total	3235.867	29			
Cloacibacterium sp	Between Groups	3210.000	9	356.667	82.308	.000
	Within Groups	86.667	20	4.333		
	Total	3296.667	29			
A. ebreus	Between Groups	1241.333	9	137.926	27.222	.000
	Within Groups	101.333	20	5.067		
	Total	1342.667	29			
B. diminuta	Between Groups	2936.300	9	326.256	123.895	.000
	Within Groups	52.667	20	2.633		
	Total	2988.967	29			
B.cereus	Between Groups	965.867	9	107.319	27.055	.000
	Within Groups	79.333	20	3.967		
	Total	1045.200	29			
D. tsuruhatensis	Between Groups	4016.700	9	446.300	152.148	.000
	Within Groups	58.667	20	2.933		
	Total	4075.367	29			
C. gleum	Between Groups	3140.833	9	348.981	455.193	.000
	Within Groups	15.333	20	.767		
	Total	3156.167	29			
S. marcescens marcescens	Between Groups	1052.167	9	116.907	64.949	.000
	Within Groups	36.000	20	1.800		
	Total	1088.167	29			

Table 4.6, continued.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
A. caviae DNA group 4	Between Groups	836.833	9	92.981	53.643	.000
	Within Groups	34.667	20	1.733		
	Total	871.500	29			
P. alcaligenes	Between Groups	1673.500	9	185.944	143.034	.000
	Within Groups	26.000	20	1.300		
	Total	1699.500	29			
P. mendocina	Between Groups	1516.167	9	168.463	45.531	.000
	Within Groups	74.000	20	3.700		
	Total	1590.167	29			
B. pumilus	Between Groups	1560.000	9	173.333	34.667	.000
	Within Groups	100.000	20	5.000		
	Total	1660.000	29			
O. intermedium	Between Groups	2362.133	9	262.459	123.028	.000
	Within Groups	42.667	20	2.133		
	Total	2404.800	29			
S. acidaminiphilia	Between Groups	1257.867	9	139.763	66.554	.000
	Within Groups	42.000	20	2.100		
	Total	1299.867	29			
B. kochii	Between Groups	2624.805	9	291.645	28.081	.000
	Within Groups	197.333	19	10.386		
	Total	2822.138	28			
J. hoylei	Between Groups	1540.700	9	171.189	67.575	.000
	Within Groups	50.667	20	2.533		
	Total	1591.367	29			

Exposure to Pb at different concentration shows that 78% of the isolates were highly resistant towards Pb above 20 ppm. However *D. tsuruhatensis* and *C. gleum* were showed inhibition at 5 ppm of Pb. Besides that *A. caviae* DNA 4 and *P. alcaligenes* showed mild inhibition at 20 ppm of Pb. The concentration of heavy metal is the key issue when developing its potential for bioremediation (Zhou *et al*., 2014). Resistance of isolates towards Fe indicates that *C. gleum* was the only isolate among the eighteen isolates that showed inhibition at 5 ppm of Fe. All other isolates showed absolute positive growth towards exposure to Fe. Fe is an essential metal for living organisms, however excessive uptake of Fe can be highly toxic to the organisms (Emenike *et al*., 2016). All the isolates except for *C. gleum* was able to tolerate high level of Fe of up to 20 ppm.

The microbial resistance test to different concentrations of Zn revealed that all isolates except *D. tsuruhatensis*, *B. kochii*, *B. diminuta*, *J. hoylei* and *B. aryabhattai* showed positive growth above 20ppm. *B. aryabhattai* showed inhibition in the growth on exposure to more than 10 ppm, whereas *B. diminuta* and *J. hoylei* showed inhibition in their growth in more than 5 ppm of Zn. The growth of *D. tsuruhatensis* and *B. kochii* were inhibited even at low concentration of Zn (< 5 ppm). No growth or complete inhibition was observed for *B. aryabhattai* when exposed to Cu and *B. diminuta* showed inhibition at 5 ppm. All other sixteen isolates showed absolute resistance towards Cu at 20 ppm and above 20 ppm and these agree with Jayanthi *et al*. (2016).

Exposure of the isolates to different concentration of Cd revealed that *A. ebreus*, *B. pumilus*, *S. acidiminiphilia* and *J. hoylei* has highest resistance above 20 ppm. *B. cereus*, *C. gleum*, *S. marcescens marcescens*, *A. caviae* DNA 4 and *P. alcaligenes* showed inhibition at 10 ppm while other isolates showed inhibition towards Cd exposure even at low concentration (5 ppm). *D. tsuruhatensis* was completely inhibited at 5 ppm of Cd. Cd seems to be highly toxic to most of the microbes compared to other type of heavy metals. The changes in responses by the microbes towards metals may be due to some reason particulary on the uptake mechanism to selected metals rather than the characteristics in terms of being gram positive or negative bacteria.

Results of sensitivity test at different concentration of Cr showed that only *B. cereus*, *S. marcescens marcescens* and *J. hoylei* shows high tolerance towards exposure to Cr above 20 ppm. This might be because these three bacteria were highly resistant towards heavy metal. Similarly, *S. marcescens marcescens* and *B.cereus* have been recommended for bioremediation of heavy metal by previous researchers (Sahar, 2012; Costa *et al*., 2001; Abdul Rahim *et al*., 2017). Other isolates showed inhibition towards Cr at different concentration below 20 ppm. Cr seems to be highly toxic to the isolates and extreme exposure of heavy metal can affect the resistance of the isolates towards Cr.

Lastly the isolates were also tested at different concentration of Ni and the results revealed that, almost half (50%) of the isolates were able to tolerate Ni above 20 ppm. They are *B. cereus*, *C. gleum*, *S. marcescens marcescens*, *B. vietnamiensis*, *P. mendocina*, *O. intermedium*, *R. rubber*, *B. aryabhattai* and *J. hoylei*. According to Mergeay *et al*. (2009), *B. vietnamiensis* have plasmid pMOL 30 which contains two large putative genomic islands comprising most of the gene involved in the response or resistance to heavy metals. Furthermore study by Maria *et al*. (2014) using *Rhodococcus* sp. also confirmed that *Rhodococcus* sp. may be useful for the remediation of sites contaminated with high concentrations of the metals (Van & Dijkhuizen, 2004). *Delftia* sp., *B. pumilus* and *B. kochii* showed inhibition in growth even below 5 ppm Ni concentration. The degree of microbial resistivity to metal concentration was characterized by the extent of the bacteria growth similar to study by Mgbemema *et al.* (2012).

Comparison between the different microbes towards metal resistance revealed that *B. vietnamiensis* showed the highest tolerance. The nature of this microbes as gram positive bacteria and aerobic bacteria influenced the interaction that existed when exposed to heavy metals (Jayanthi *et al*., 2016). The overall growth pattern was similar to a study conducted by Mgbemena *et al*. (2012) which indicated that extreme exposure to metal concentrations will negatively affect microbial resistance to pollution. The overall data obtained could serve as comparative data with other tested species.

4.4.2 Minimal Inhibitory Concentrations

Minimal inhibitory concentration (MIC) of the heavy metal ions on the bacteria strains were also evaluated from the inoculated media plates. Table 4.7 represents the MIC of various heavy metals towards the isolated strains. High similarity was observed in the tolerance developed by the strains towards heavy metals. *B. vietnamiensis* demonstrated the highest tolerance towards all the metals (>20ppm) except Cr. This is also agreeable with finding by *Basu et al*. (1997) who reported higher metal resistance by gram positive bacteria. The resistance mechanisms also could be utilized for detoxification and removal of heavy metals in polluted environment. *R. ruber, B. cereus*, *B. vietnamiensis*, *J. hoylei*, *P. mendocina* and *O. intermedium* also demonstrated high tolerance, but did not show much resistance to Cd, Cr and Zn exposure. Previous studies reported that such accumulation leads to the expression of a CadA resistance system, which is located on plasmids p1258 and related plasmids (Novick & Roth, 1968; Nies & Silver, 1995). The resistance is mediated by active ion efflux (Nies & Silver, 1995; Lucious *et al*., 2013). Responses of the microbes to the heavy metals were not too heterogeneous, but the result is consistent with findings of Lucious *et al*. (2013), who reported that both Gram-positive and Gram-negative bacteria can be resistant to heavy metals.

Table 4.7: Minimal Inhibitory Concentrations of heavy metal on the bacterial isolates

Bacteria	Pb	Mn	Fe	Hg	Zn	Cu	Cd	Ni	Cr	Al
A.caviae DNA group 4	20	>20	>20	>20	20	20	10	20	15	20
P.alcaligenes	20	>20	20	>20	20	>20	10	20	5	>20
P.mendocina	>20	>20	>20	>20	>20	>20	10	>20	10	>20
B. pumilus	>20	>20	20	>20	>20	>20	>20	<5	20	>20
O.intermedium	>20	>20	>20	>20	>20	>20	5	>20	5	>20
S. acidaminiphilia	>20	>20	20	>20	>20	>20	>20	15	10	>20
B.cereus	>20	>20	>20	>20	>20	>20	10	>20	>20	20
D.tsuruhatensis	<5	>20	>20	>20	<5	>20	<5	<5	<5	>20
C. gleum	<5	<5	<5	<5	>20	>20	10	>20	<5	<5

Table 4.7: continued

Bacteria	Pb	Mn	Fe	Hg	Zn	Cu	Cd	Ni	Cr	Al
S. marcescens marcescens	>20	20	20	>20	>20	>20	10	>20	>20	>20
B.vietnamiensis	>20	>20	>20	>20	>20	>20	>20	>20	20	>20
A.ebreus	>20	>20	20	>20	>20	>20	>20	15	10	>20
B. diminuta	>20	>20	20	5	5	5	5	15	10	>20
Cloacibacterium sp	>20	>20	20	>20	>20	20	5	5	5	>20
R. ruber	>20	>20	>20	>20	>20	>20	5	>20	10	>20
B.aryabhattai	>20	>20	>20	>20	10	<5	5	>20	15	>20
B. kochii	>20	>20	20	>20	<5	20	5	<5	10	>20
J.hoylei	>20	>20	>20	>20	5	>20	>20	>20	>20	>20

4.4.3 Expression of Inhibition Zone Diameter by the test microbes

Inhibition zone diameter (IZD) of the bacterial species was measured against the heavy metal concentrations during the resistivity test. The IZDs demonstrated the extent of the toxic effect of the heavy metals on the isolated organisms. Furthermore, a correlation exists between metal resistance of the bacterial species and the measured IZD and supported by findings of Sabdono *et al*. (2012) and Rani *et al*. (2010). Observed IZD was found to be inversely proportional to resistivity. For example, *B. aryabhattai* showed pronounced correlation from a plot, $y = -0.2424x + 3.8243$ with an R^2 value of 0.94 (Figure 4.16), despite showing an absolute susceptibility to Cu exposure. The less the inhibition of microbes toward metals the higher the potential of microbes to remediate metal and can be good bioremediation agent. Hence, the distribution nullified the hypothesis that all the bacterial species will exhibit the same response to metal contamination.

Figure 4.1 represents the IZD value of *A.caviae* DNA group 4 exposed to different metals. The figure clearly indicates the maximum IZD value was for Cr. The maximum value of IZD for Cr exposure were 1.1cm at 20ppm. Besides that, *A. caviae* DNA group 4 were also observed to have IZD value of 0.8 cm for Cd at 20ppm concentration. Furthermore *A. caviae* also shows IZD range 0.1 – 0.3 cm towards 20 ppm of Pb, Zn, Cu, Ni and Al. *A. caviae* DNA group 4 was less tolerance towards heavy metal therefore the extent of metal toxicity can be measured by the zone of inhibition that occurred.

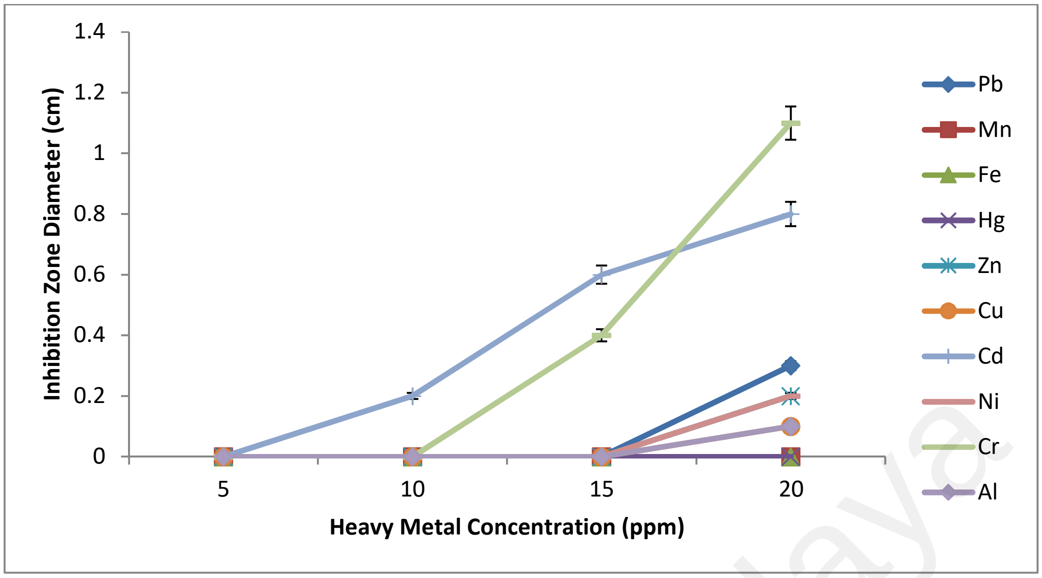

Figure 4.1: Measured inhibition zone diameter (IZD) of *A. DNA group 4* exposed to different metals

The maximum IZD value for *P. alcaligenes* (Figure 4.2) was for the exposure of Cr and value was 1.2 cm. Besides Cr, *P. alcaligenes* was also showing inhibition zone

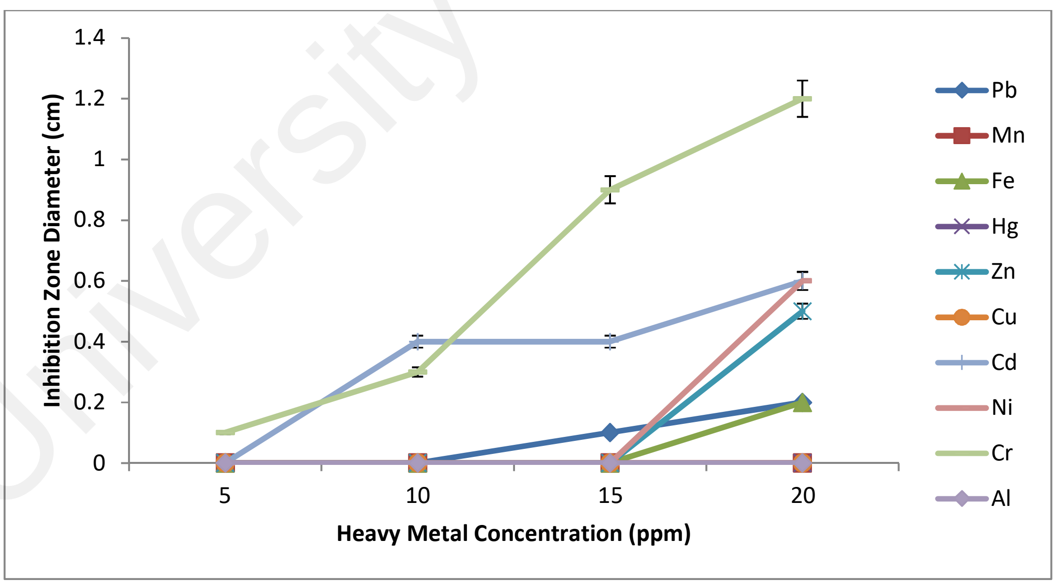

Figure 4.2: Measured inhibition zone diameter (IZD) of *P. alcaligenes* exposed to different metals

towards Pb, Zn, Cd, Ni and Fe. The IZD value was in the range of 0.2 to 0.6cm. The resistance mechanism in the microbes does not clearly provide protection at extremely high concentration of metal ions (Konopka *et al*., 1999; Mgbemena *et al*., 2012). The concentration of Cr seems to affect the growth of microbes whereby for Cr inhibition clear zone was observed at 5 ppm.

In the case of *P. mendocina* (Figure 4.3), inhibition in the growth of the microbes was observed when exposed to Cd and Cr. The maximum IZD value were 1.1 cm with exposure to Cd. The value of IZD was 0.6 cm, 0.8 cm and 1.1 cm, respectively for Cd

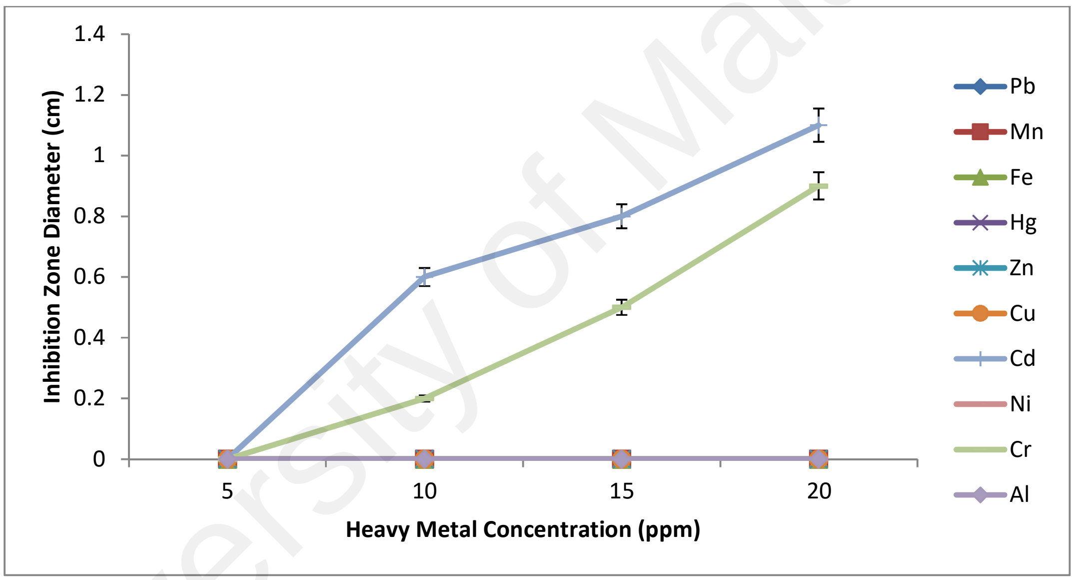

Figure 4.3: Measured inhibition zone diameter (IZD) of *P. mendocina* exposed to different metals

exposure at 10, 15 and 20 ppm. Besides Cd, *P. mendocina* was also showing inhibition towards Cr with lower IZD value than Cd. The zone of inhibition was observed when the microbes were exposed above 5ppm Cr. The range of IZD value were 0.2 cm to 0.9cm. The inhibition of *P. mendocina* towards Cd and Cr can be due to the behaviour of organisms which have inhibitory mechanisms towards some of the metal ions but may be resistant to other metal ions.

B. pumilus was among the isolates that were highly tolerant towards heavy metals. Figure 4.4 shows the measured IZD of *B. pumilus* exposed to different metals. The maximum IZD value was 1.3cm at 20 ppm exposure of Fe. The microbe also showed inhibition at 20 ppm of Cr exposure with minimum IZD value of 0.1 cm. It was highly resistant to all other metal and no zone of inhibition has been observed. *B. pumilus* has been previously studied for metal remediation and being gram positive bacteria that may be the reason for it to be one of the highly resistant group of bacteria.

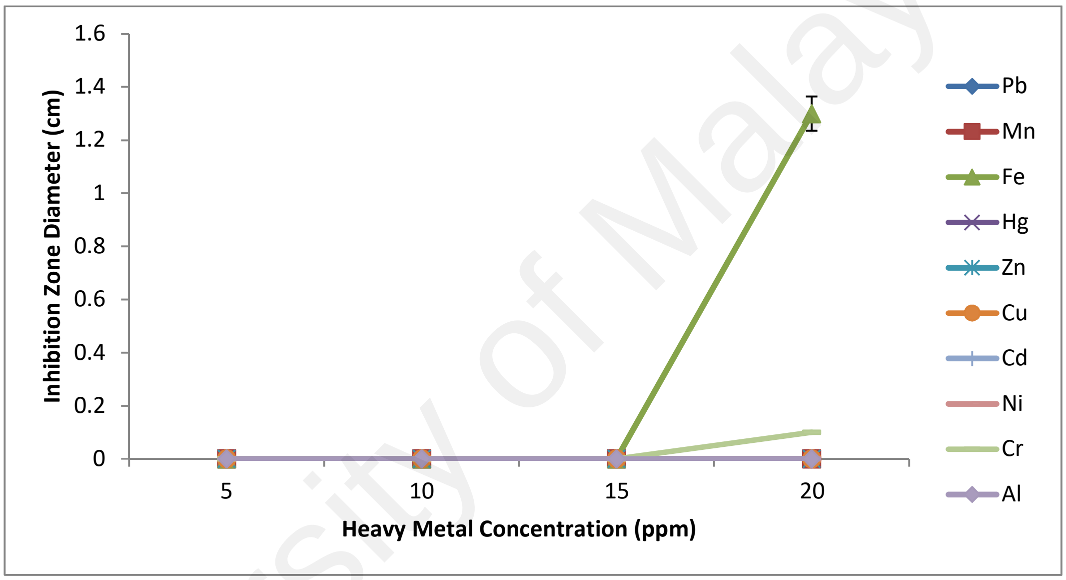

Figure 4.4: Measured inhibition zone diameter (IZD) of *B. pumilus* exposed to different metals

O. intermedium showed high zone of inhibition when exposed to Pb, Mn, Cd and Cr (Figure 4.5). The maximum IZD value was 1.9cm when the isolates were exposure to 20 ppm of Cr. Besides that, *O. intermedium* was also inhibited at low concentration (5 ppm) of Cr that IZD was 1.2 cm. It seems *O. intermedium* was not able to grow even at lower concentration of Cr. It may be because this particular microbe does not have resistance towards Cr. A good correlation was observed between metal resistance of the bacteria and the measured IZD, whereby for Pb the R^2 value was 0.966 from the equation $y = -0.32x + 0.65$.

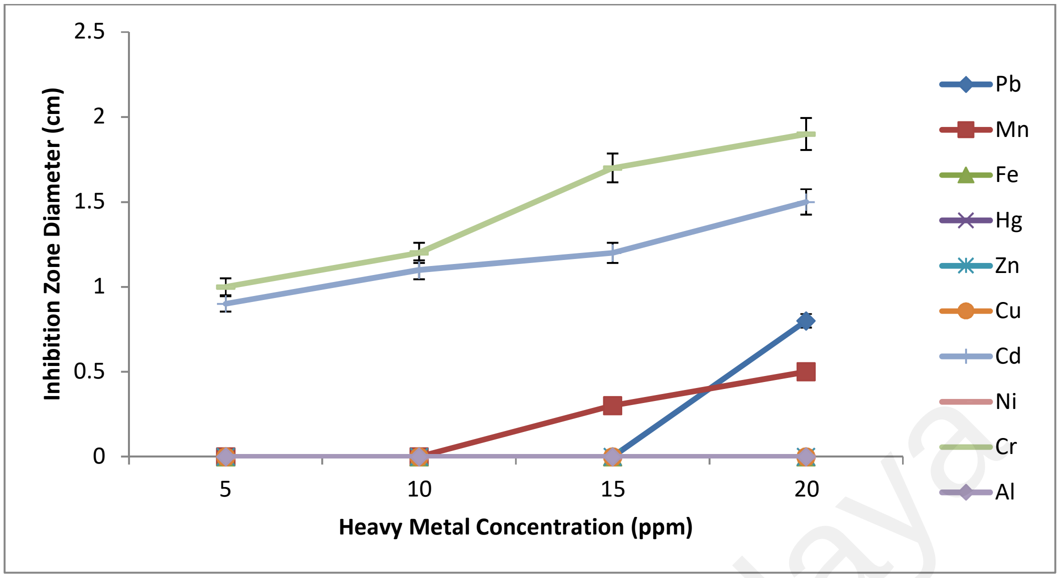

Figure 4.5: Measured inhibition zone diameter (IZD) of *O. intermedium* exposed to different metals

Figure 4.6 shows the measured inhibition zone diameter of *S. acidaminiphilia* exposed to different metals. The highest measured inhibition zone diameter (IZD) was when the microbe was exposed to Fe. The IZD value was 1.9cm. Besides that, *S. acidaminiphilia* also been observed to shows inhibition when exposed to Ni and Cr. For Ni, the IZD ranged between 0.5 cm to 1.3 cm while it was in range of 0.2 cm to 1.0 cm for Cr. From this study, resistance of this microbe towards different types of metal and at different concentration implies that the same bacteria can resist to high concentration of a particular metal but may show opposite reaction when exposed to another type of heavy metal at the same concentration.

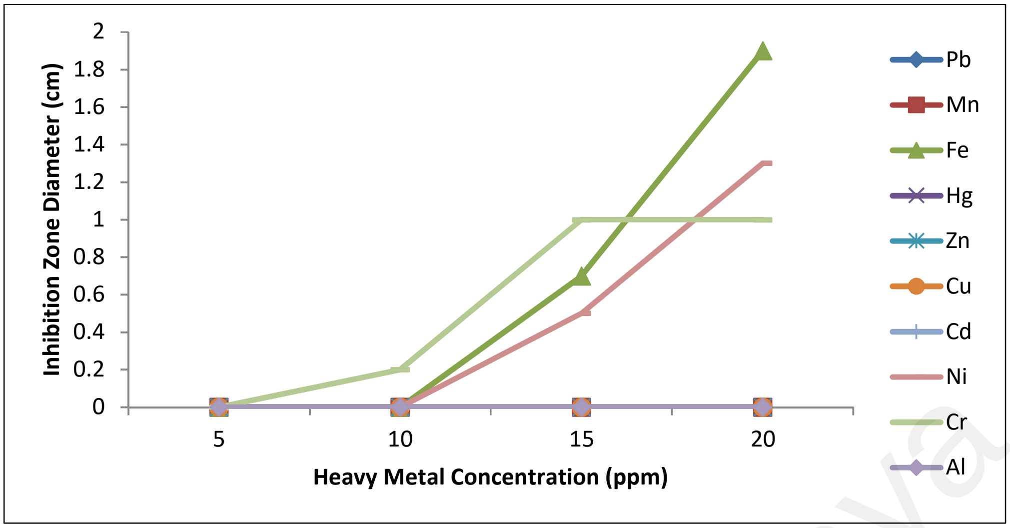

Figure 4.6: Measured inhibition zone diameter (IZD) of *S. acidaminiphilia* exposed to different metals

For *B. cereus* (Figure 4.7), maximum IZD value of 2.0 cm was observed when exposed to 20 ppm of Cd while at 5 ppm the value was 1.4 cm. Besides Cd, *B. cereus* was observed to have inhibition zone when exposed to 20 ppm of Al with IZD of 0.4cm. The extent of *B. cereus* to sustain and tolerate different heavy metals except for Cd can be further recommended as agent for bioremediation and supported by previous researchers on the ability of *Bacillus sp* for removal of heavy metal from contaminated soil (Emenike *et al*., 2016).

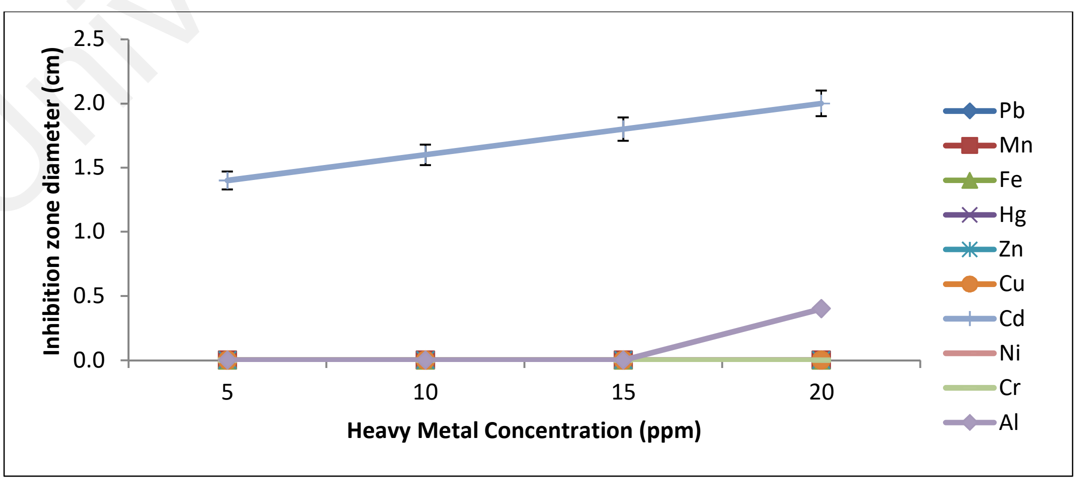

Figure 4.7: Measured inhibition zone diameter (IZD) of *B. cereus* exposed to different metals

D. tsuruhatensis was one of the isolates among the eighteen isolates that were showing less tolerance towards heavy metal. Figure 4.8 shows the measured IZD of *D. tsuruhatensis* to different heavy metal. Maximum IZD value of 4.5cm were observed when *D. tsuruhatensis* was exposed to Pb, Zn, Cd, Ni and Cr, even at low concentration

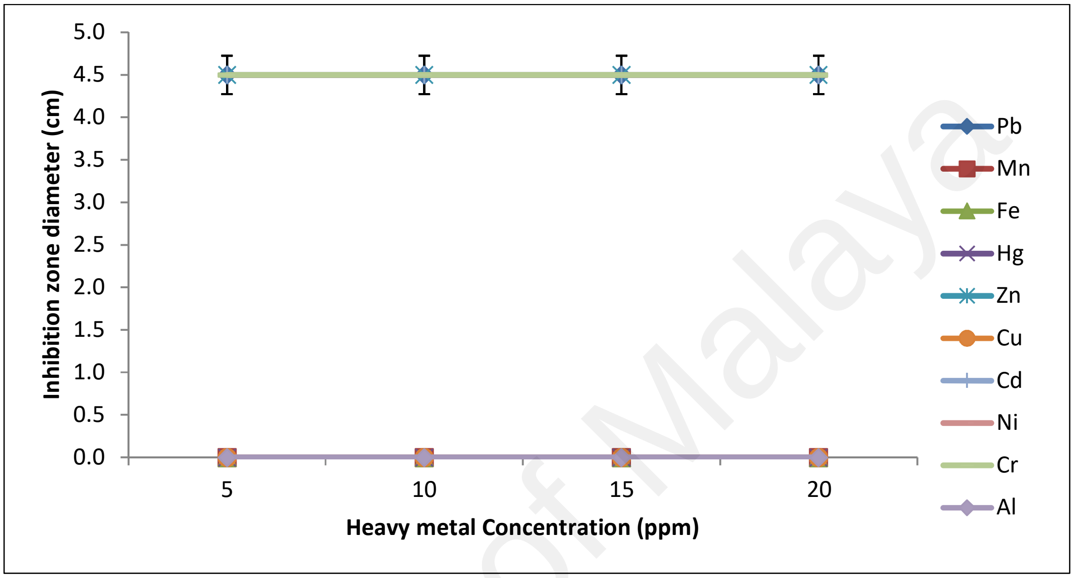

Figure 4.8: Measured inhibition zone diameter (IZD) of *D. tsuruhatensis* exposed to different metals

of 5 ppm. No growth was observed when *D. tsuruhatensis* was introduced to Pb, Zn, Cd, Ni and Cr. However for other metals (Mn, Fe, Hg, Cu, and Al) the microbes were not showing any sign of inhibition even at concentration above 20 ppm. The response of microbial communities to heavy metals depends on the concentration and availability of metals and is dependent on the actions of complex processes, controlled by multiple factors such as the type of metal, the nature of medium and microbial species and supported by De Rore *et al*. (1994), Goblenz *et al*. (1994), Hashemi *et al*. (1994), Olasupo *et al*. (1993) and Tomioka *et al*. (1994). This could be the reason for such results obtained.

Beside *D. tsuruhatensis*, *C. gleum* also showed lower tolerance towards heavy metals based on the sensitivity test. The measured IZD is shown in Figure 4.9. *C. gleum* demonstrated maximum IZD of 4.5 cm when exposed to Pb, Mn, Fe, Hg, Cr and Al at 5

- 20 ppm. The isolates were also showing inhibition when exposed to Cd above 5 ppm with maximum IZD of 2.0 cm. However, *C. gleum* was highly resistant towards metals such as Zn, Cu, and Ni where inhibition zone were observed. *C. gleum* has never been isolated from metal contaminated soil previously but its presence in the leachate contaminated soil can be associated with its tolerance towards selective heavy metals.

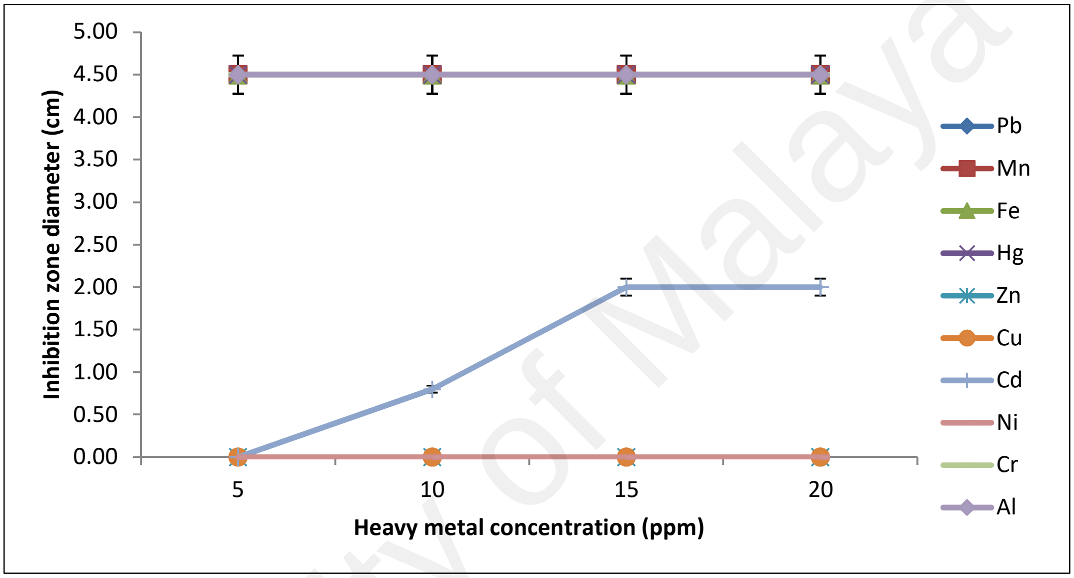

Figure 4.9: Measured inhibition zone diameter (IZD) of *C. gleum* exposed to different metals

For *S. marcescens* (Figure 4.10), highest IZD was 0.8 cm from Cd exposure. The size of inhibition zone, increased as the concentration of metal increases. *S. marcescens* showed no IZD for most of the metals studies which indicates that this isolates was highly resistant to the exposed metals.

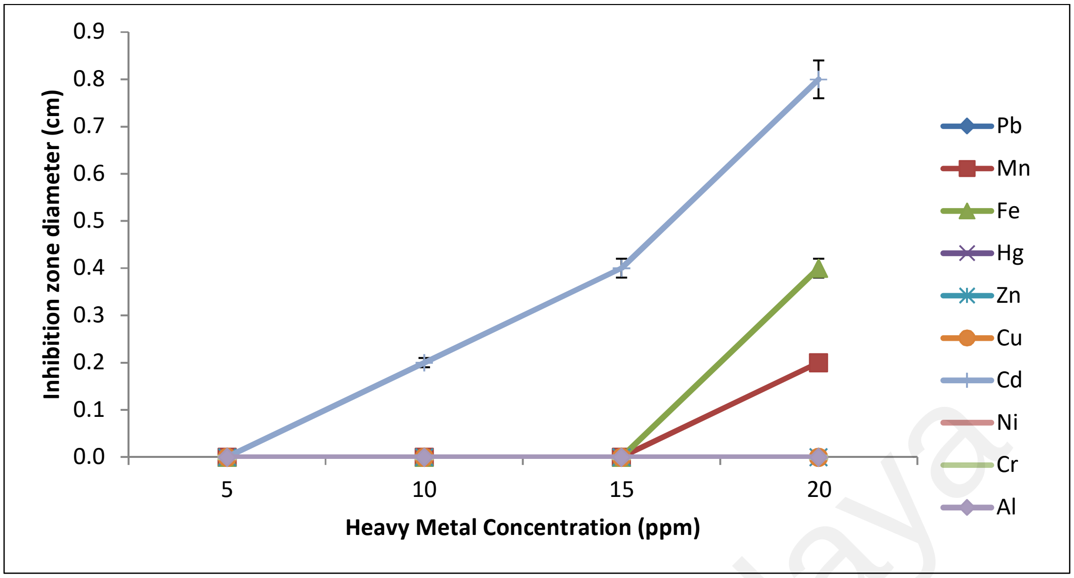

Figure 4.10: Measured inhibition zone diameter (IZD) of *S. marcescens* exposed to different metals

The IZD for *B.vietnamiensis* is shown in Figure 4.11. *B. vietnamiensis* is the most tolerant microbe observed in this study. The IZD at 20 ppm for Cr exposure was 1.1 cm. This microbe was highly tolerant towards all other metals.

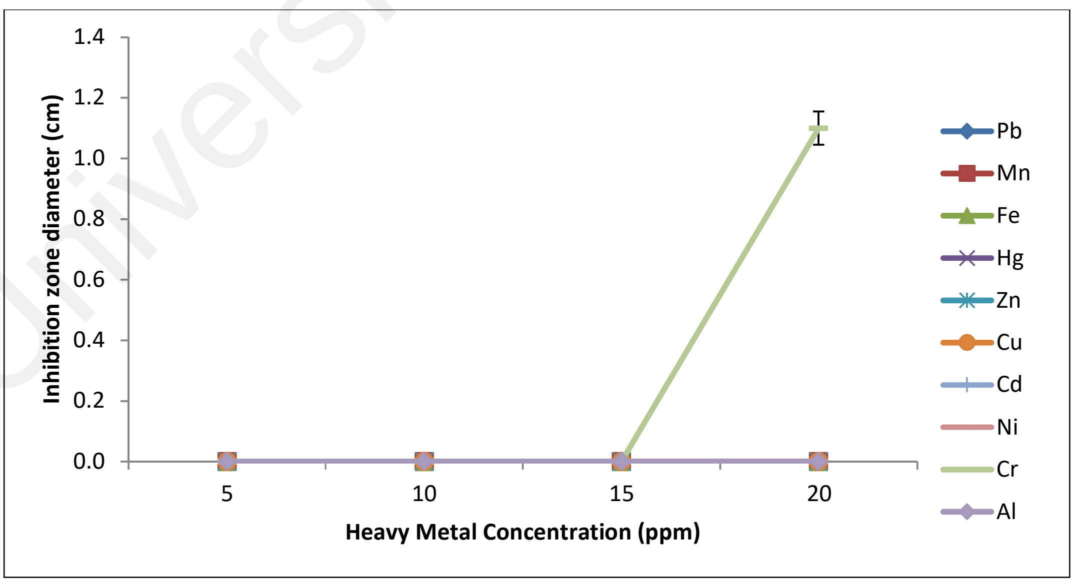

Figure 4.11: Measured inhibition zone diameter (IZD) of *B. vietnamiensis* exposed to different metals

The presence of gene involved in response towards heavy metal in this microbes may contribute highly for its resistance mechanisms towards heavy metal (Jayanthi *et al*., 2016).

The IZD measured for *A. ebreus* is shown in Figure 4.12. The bacteria was not inhibited when exposed to Pb, Fe, Zn, Mn, Hg, Cu, Ni and Al but has low tolerance towards Cd and Cr. The IZD was 1.2 cm and 1.6 cm for 15 ppm and 20 ppm of Cr, respectively. IZD value of 4.5cm was recorded when the microbe was exposed to 10 ppm, 15 ppm and 20 ppm of Cr. The response of microbes varied which may be due to specific characteristics of the bacterial species that influenced the metal binding, bio-sorption or bio-immobilization of metal ions in aggregate state (Jayanthi *et al*., 2016).

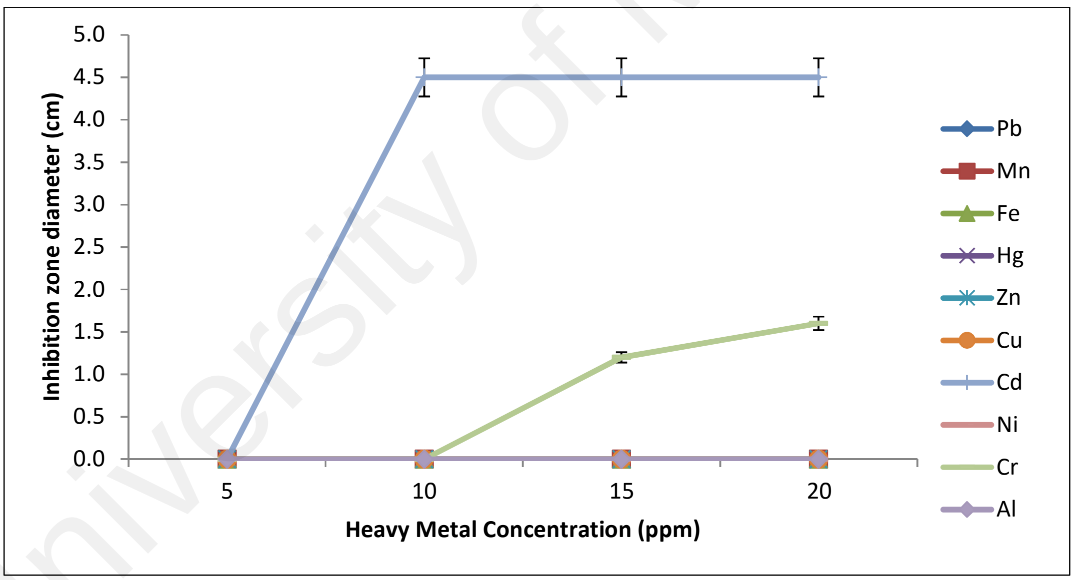

Figure 4.12: Measured inhibition zone diameter (IZD) of *A. ebreus* exposed to different metals

In case of *B. diminuta* (Figure 4.13), exposure to all metals has IZD value of 0.6 cm to 2.80 cm except for Pb, Mn and Al. This indicates that this microbe has least tolerance towards metals even at lower concentration.

For Hg, same rate of IZD was observed when *B. diminuta* was exposed to 5ppm to 20 ppm of Hg concentration, the IZD was 0.8 cm. At 5ppm Zn, the IZD of *B. diminuta* was 1.0 cm and with the increase in Zn concentration IZD was 2.0 cm. When *B. diminuta* was exposed to different concentrations of Fe, IZD of 0.6 cm was measured at 20 ppm. The exposure of *B. diminuta* to different concentrations of Cu demonstrated minimum IZD of 1.4 cm for 5 and 10 ppm exposure of Cu and maximum value of 2.0cm when exposed to 15 and 20 ppm of Cu. The measured zone of inhibition increased as the concentration of Cu increased. The microbe becomes more sensitive to Cu as the concentration increased indicating that it is not able to resist the toxicity of Cu.

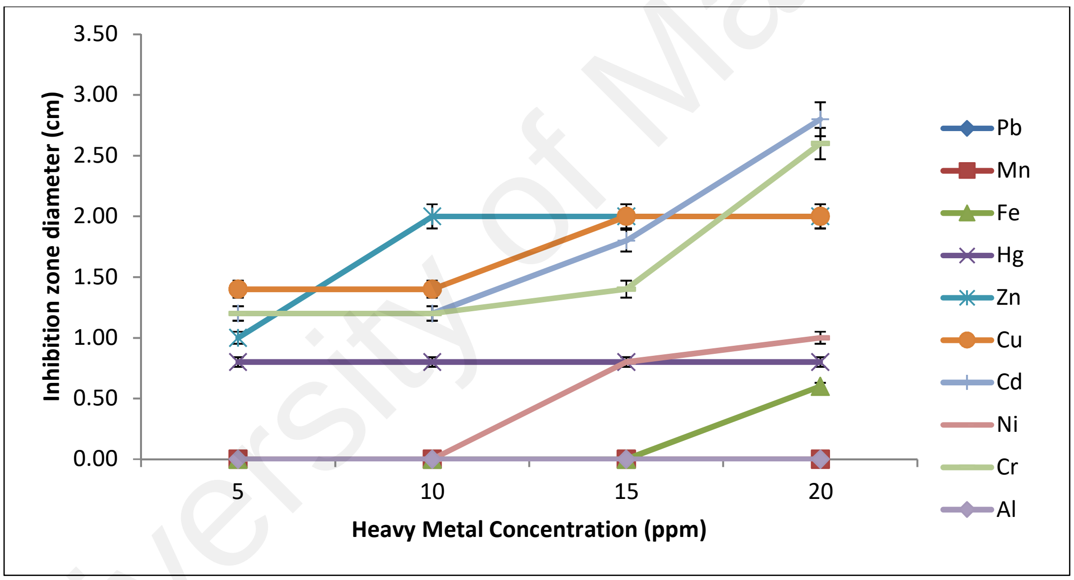

Figure 4.13: Measured inhibition zone diameter (IZD) of *B. diminuta* exposed to different metals

B. diminuta also showed zone of inhibition when exposed to Cd, Ni and Cr. For Cd, the IZD value ranged between 1.2cm to 2.8cm and even at low concentration of Cd (5 ppm). In case of Ni, IZD were 0.8 cm and 1.0 cm when exposed to 15 and 20 ppm, respectively. Lastly, Cr showed inhibition even at low concentration (5 ppm), and the IZDs ranged between 1.2 cm to 2.6 cm.

Cloacibacterium sp. was also tested for sensitivity towards heavy metal and the measured IZD is showed in Figure 4.14. The microbe was showing less tolerance towards Cu, Cd, Ni and Cr with IZD of 2.2 cm at 20 ppm Cu. However there was no inhibition below 20 ppm Cu. *Cloacibacterium* sp. also showed inhibition when exposed to Cd at different concentrations (5 - 20 ppm). Increase in Cd concentration further increased the IZD whereby at 5 ppm, the IZD was 1.0cm and it increased to 1.46cm, 1.86 cm and 2.2 cm for 10 ppm, 15 ppm and 20 ppm of Cd, respectively.

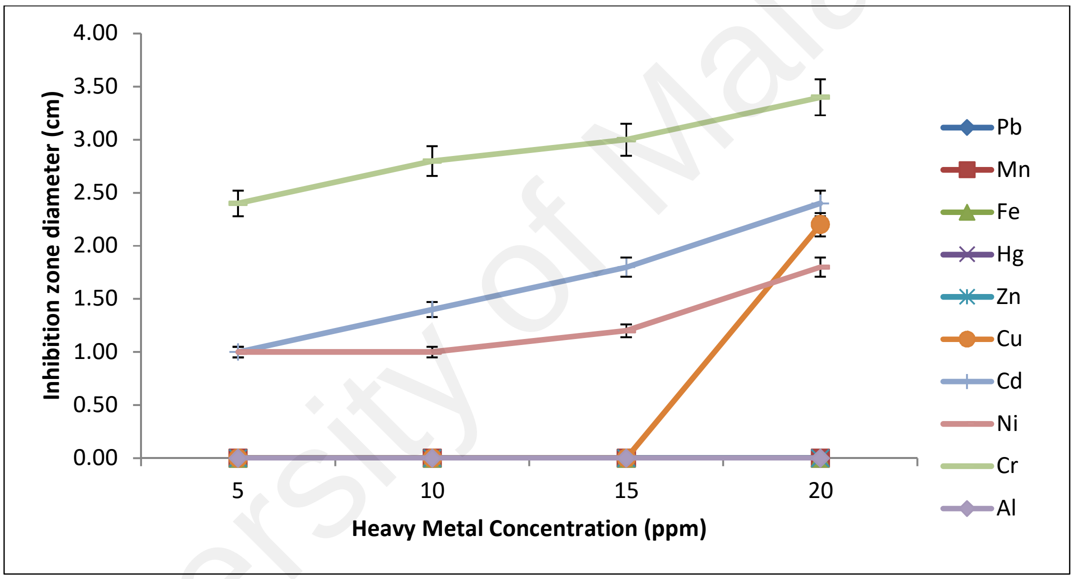

Figure 4.14: Measured inhibition zone diameter (IZD) of *Cloacibacterium* sp. exposed to different metals

Low concentration of Cd can cause severe toxicity to organisms (Lucious *et al*., 2013; Wei *et al*., 2009; Karnachuck *et al*., 2003). Similar to Cd, *Cloacibacterium* sp. also showed inhibition at low concentration of Ni and Cr. For Ni, the IZD ranged between 1 cm to 2.2 cm whereas for Cr the IZD ranged from 2.4 cm to 3.4 cm. The response of microbes to different pollutants (metals) may differ due to nature of pollutants and the concentration/level of toxicity of the pollutants (Jayanthi *et al*., 2016).

Figure 4.15 illustrates the measured IZD for *R. ruber* exposed to different metals. The microbe showed zone of inhibition when exposed to Cd and Cr. The IZD for Cd ranged between 1.7 cm to 3.1 cm for 5 ppm, 10 ppm, 15 ppm and 20 ppm. For Cr, the IZD was 0.2 cm to 3.6 cm at 10 ppm. 10 ppm and above and IZD ranged within 0.2 cm to 3.6 cm. The high value of IZD may be associated with the resistance of microbes towards a particular metal. The less tolerance is the microbe the higher is the zone of inhibition.

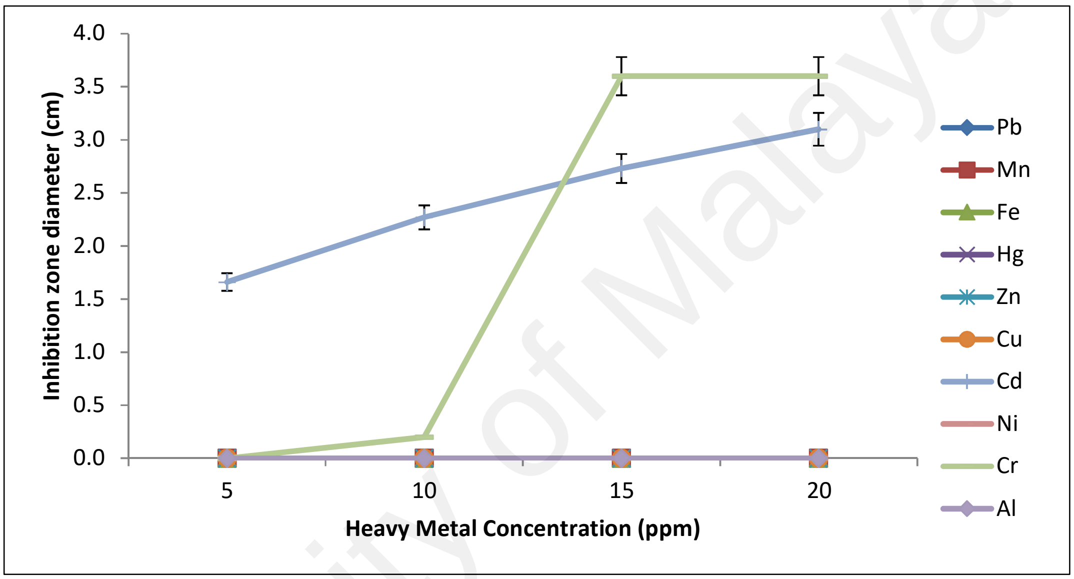

Figure 4.15: Measured inhibition zone diameter (IZD) of *R. ruber* exposed to different metals

The measured zone of inhibition for *B. aryabhattai* is shown in Figure 4.16. Zone of inhibition occurred when the microbe was exposed to Zn, Cu, Cd and Cr. The IZD for ranged between 2.4 cm to 4.5cm and for Cd it ranged between 1.0 cm to 2.4 cm.

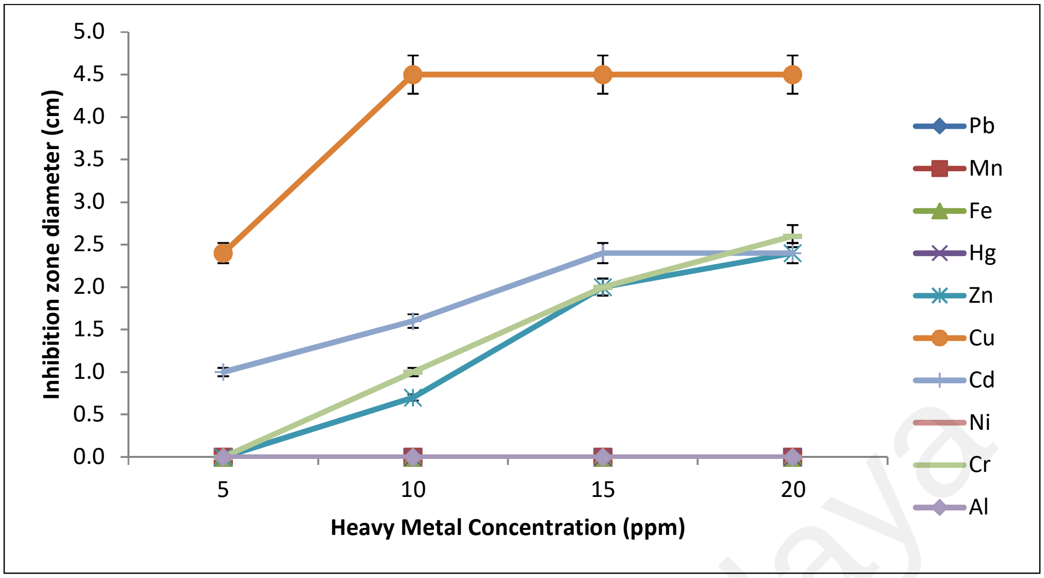

Figure 4.16: Measured inhibition zone diameter (IZD) of *B. aryabhattai* exposed to different metals

Zone of inhibition for *B. kochii* exposed to different metals is depicted in Figure 4.17. *B. kochii* showed zone of inhibition when exposed to Mn, Fe, Zn, Cu, Cd, Ni and Cr. For Mn, Fe and Cu, the measured IZD was at 20 ppm and for Cr it was at 10 ppm and above. However for Zn, Cd and Ni, IZD was measured even low concentration (5ppm). *B. kochii* tends to be less tolerant towards most of the metals. The microbe was not able to resist even at low concentration of Zn and Ni that 4.5 cm IZD was obtained.

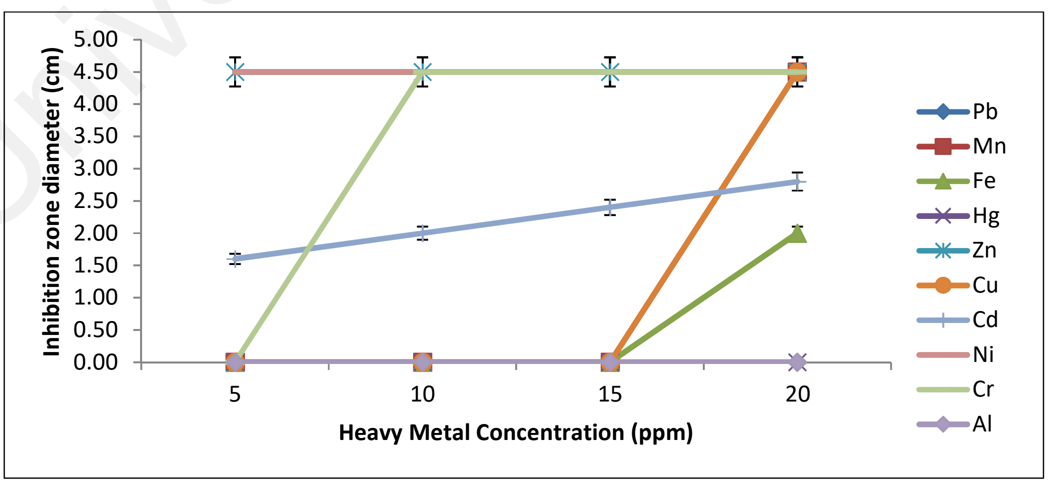

Figure 4.17: Measured inhibition zone diameter (IZD) of *B. kochii* exposed to different metals

Among eighteen isolated, *J. hoylei* (Figure 4.18) shows the highest tolerance after *B. vietnamiensis* towards all metal except for Zn. The maximum IZD value were 1.9cm at 20ppm Zn. Besides that, *J. hoylei* was also showing inhibition at 5 ppm to 15 ppm and the IZD value ranged between 0.8 - 1.5cm. This implies that the reduced IZD in the measured IZD after exposing to various heavy metal indicates its high tolerance behaviour to heavy metal contamination (Jayanthi *et al.*, 2016). This bacteria has not been previously studied for heavy metals however the positive tolerance towards different heavy metal compared to other microbes shows its potential for remediation in a contaminated soil.

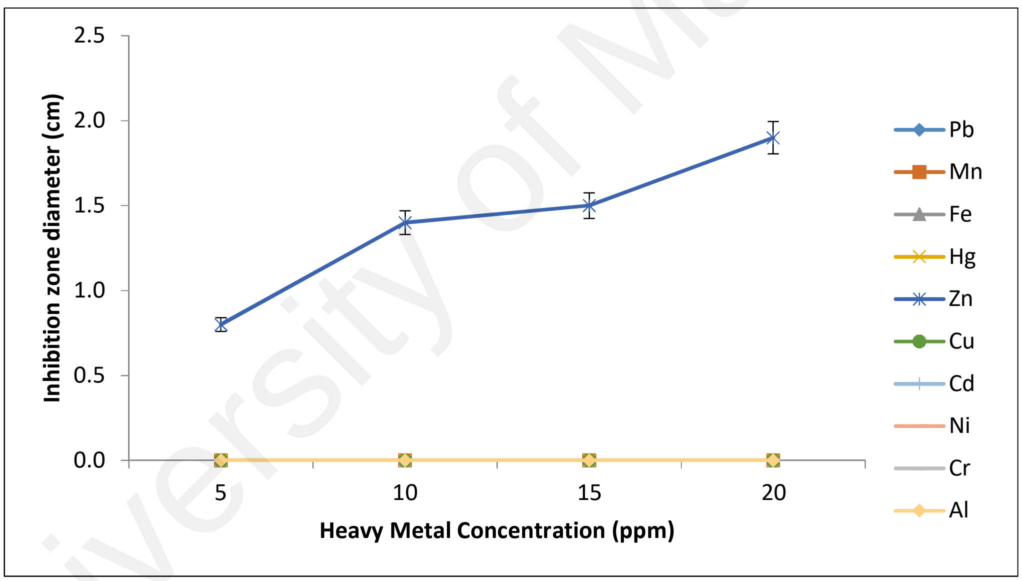

Figure 4.18: Measured inhibition zone diameter (IZD) of *J. hoylei* exposed todifferent metals

Most of the microbes were gave low or zero IZD value which indicates that the microbes are highly resistant to the metal. This is probably because most of the microbes in the landfill either contains genes that develop resistance towards heavy metals or can passively uptake heavy metals through different mechanisms. The potential microbes can be further grouped according to its specific group and studied for remediation of contaminated soil.

4.5 Formulation of potential microbial cocktails to remove heavy metals from soil contaminated with leachate

The identified microbes from Taman Beringin and Bukit Beruntung landfill soil were further grouped into seven different treatments as shown in Table 4.8 to study the influence of bacterial group in reduction/removal of heavy metals from contaminated soil.

The formulated treatments are groups that contained all isolated bacteria; gram negative bacteria; gram positive bacteria; highly sensitivity bacteria (based on sensitivity test whereby microbes that showed high tolerance towards different metal above 20ppm); medium/ low sensitivity bacteria (based on sensitivity test whereby microbes that showed mild inhibition and complete inhibitions towards different metal); proteo-bacteria; non-proteo bacteria. The grouping of microbes is expected to help determine the optimal metal reduction/removal potential of the isolates (Emenike *et al*., 2016). Bacterial mixtures can perform more complex tasks and survive in more changeable environments than a single culture (Brenner *et al*., 2008).

Table 4.8: Bacterial formulation for bioaugmentation experiment

Bacteria	Control	A	B	C	D	E	F	G
O. intermedium	NB	√	√		√		√	
B. vietnamiensis	NB	√		√	√		√	
S. acidaminiphilia	NB	√	√		√		√	
A.ebreus	NB	√	√		√		√	
B.diminuta	NB	√	√			√	√	
D.tsuruhatensis	NB	√	√			√	√	
A. caviea DNA 4	NB	√	√		√		√	
P. mendocina	NB	√	√		√		√	
S. marcescens marcescens	NB	√	√		√		√	
P. alcaligenes	NB	√	√			√	√	
Cloacibacterium sp.	NB	√	√		√			√
B. aryabhattai	NB	√		√		√		√
R. ruber	NB	√		√	√			√
B. pumilus	NB	√		√	√			√
B. kochii	NB	√		√		√		√
J. hoylei	NB	√		√	√			√
B. cereus	NB	√		√	√			√
C. gleum	NB	√	√			√		√

NB = No bacteria addition, A =All microbes, B= Gram negative, C =Gram positive, D= High Sensitivity, E=Medium and Low Sensitivity, F =Proteobacteria, G= Nonproteo bacteria

4.6 Bioreduction/ bioremoval of heavy metal contaminated soil

This section will discuss the impact of the microbial diversity/treatment formulated in previous section and utilized for the bioreduction/removal of metals from the leachate contaminated soil taken from two non-sanitary landfill namely Taman Beringin landfill and Bukit Beruntung landfill.

4.6.1 Bioaugmentation experiment of Taman Beringin leachate contaminated soil with different bacterial treatment at 10% v/w

4.6.1.1 Lead (Pb)

Figure 4.19 depicts Pb concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. Initial concentration of Pb was 18 mg/kg and after 100 days of remediation, Treatment C showed highest metal reduction (43%) as compared to the other treatments used in this study as illustrated in Figure 4.20. The residual concentration of Pb in Treatment C after 100 days of remediation was 10.33 mg/kg. Second highest reduction of Pb from the soil is by Treatment G, 41%.

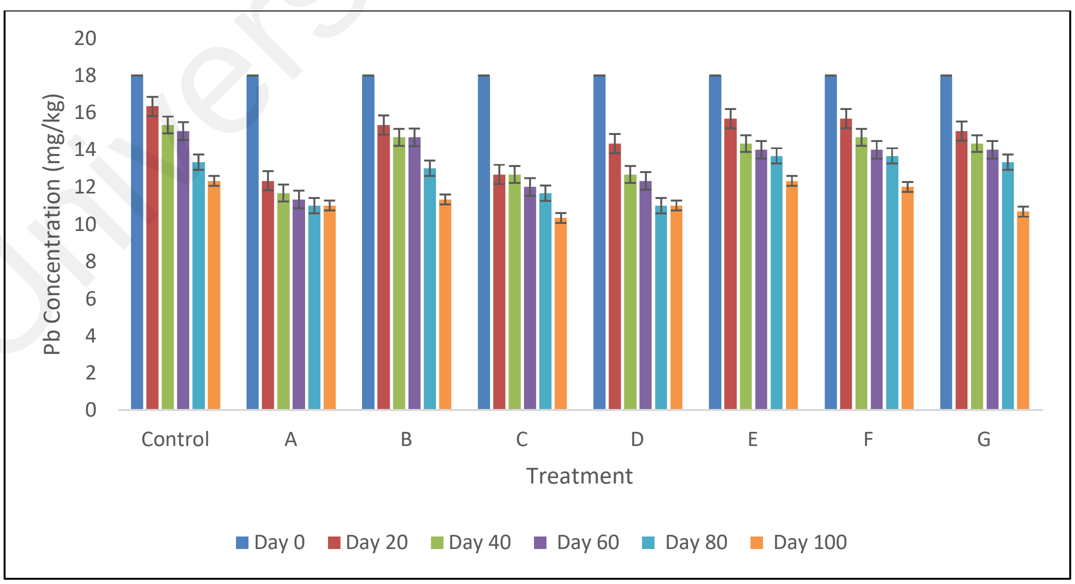

Figure 4.19: Pb concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

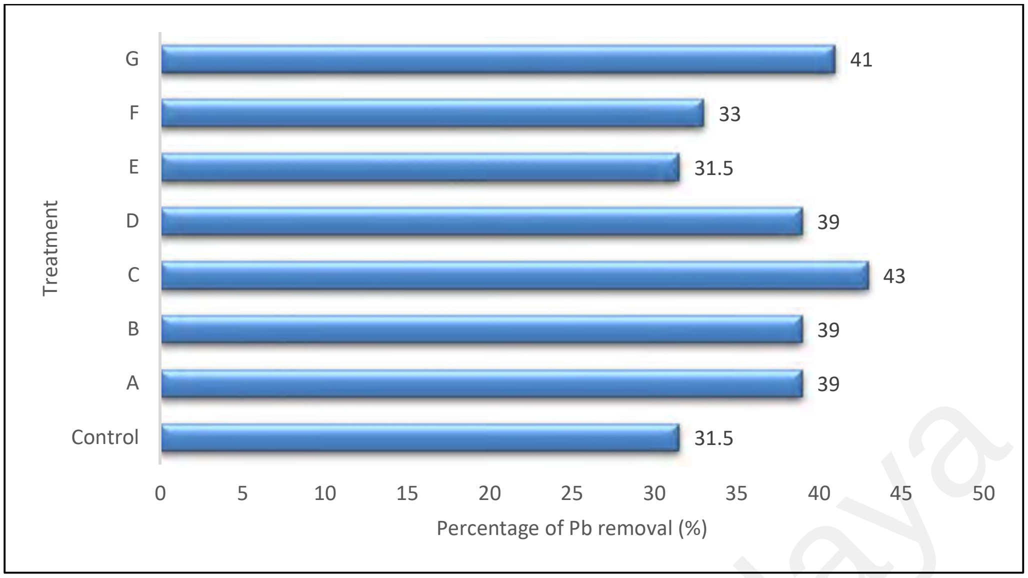

Figure 4.20: Percentage of Pb removed in soil from Taman Beringin Landfill during bioaugmentation experiment

In Control experiment only 31% of Pb were reduced from the soil and the residual concentration was 12.33mg/kg. Reduction of Pb in the soil ranged from 31.5% to 39% in Treatment A, B, D, E and F. The order of Pb removal among the eight treatments tested is Treatment C < G < A, B, D < F < E, Control.

Statistical analysis indicates significant different between the initial and final days of monitoring for all treatment (A-G) (p=0.00). The resistance of microbes to heavy metals is an important factor to be considered in the study of remediation because it is directly related to the survival and growth of the bacteria being used to treat contaminated sites (Li & Ramakrishna, 2011). Furthermore statistical analysis also showed significant difference between the best treatment (C) and Control (p=0.039). Therefore, the introduction of Treatment C showed a significant reduction of Pb in the contaminated soil. Similar studies by Emenike (2013) with different group of microbes also revealed higher reduction of Pb to leachate contaminated soil. Mechanism involved for higher reduction of Pb by gram positive bacteria may probably because of the chemical composition of the cell wall of the bacteria as suggested by Kaewchai and Praseitan

(2002). Gram-positive bacteria use the high teichoic acid content in the glycoprotein cell wall (Ruttiya *et al*., 2016). Hence, this may be the reason for for enhanced reduction of Pb by the Treatment C compared to other treatments.

4.6.1.2 Arsenic (As)

Figure 4.21 illustrates As concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. The initial concentration of As was 103 mg/kg. By addition of different treatments to the contaminated soil, reduction in the As concentration was observed. Soil amended with Treatment F showed highest As removal (61%) (Figure 4.22). Treatment D showed 60% removal of As in the contaminated soil. Both Treatment F and D showed greater potential for As reduction as compared to other treatments. This probably was because both treatment A and D contained 60% similar microbes in their group which resulted in similar As removal percentage. This in agreeable to previous findings whereby some of treatment augmented with different group of microbes possess similar rate of metal reduction for specific metals (Emenike, 2013).

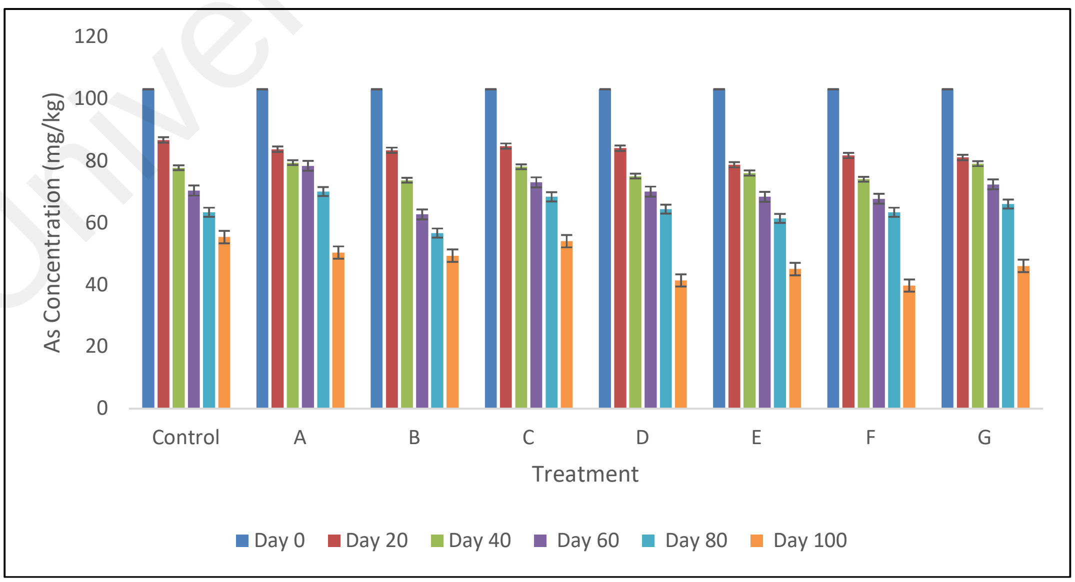

Figure 4.21: As concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

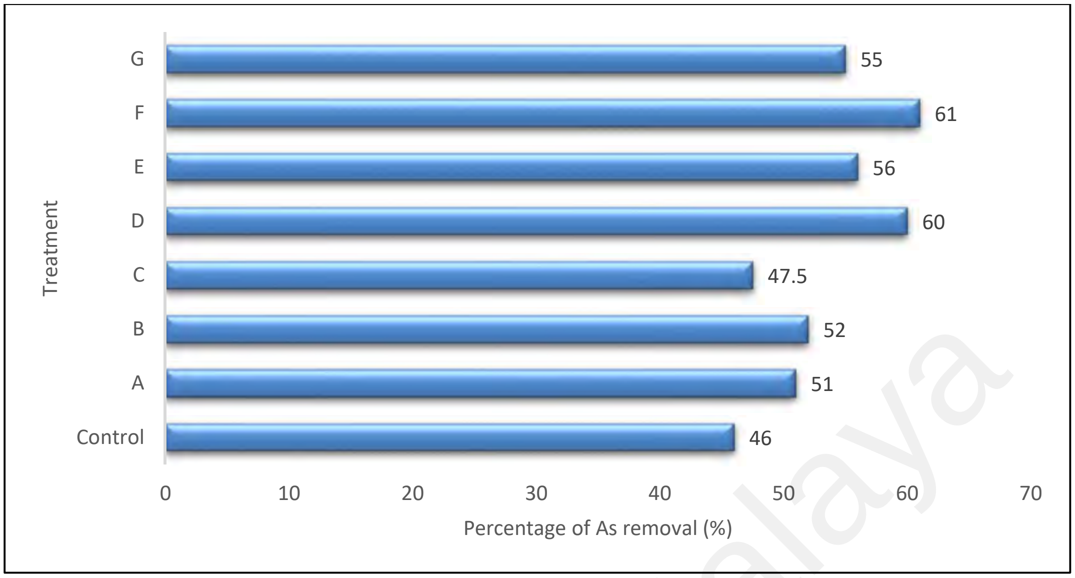

Figure 4.22: Percentage of As removed in soil from Taman Beringin Landfill during bioaugmentation experiment

The order of As reduction among the treatments was Treatment F (61%) < D (60%) < E (56%) < G (55%) < B (52%) < A (51%) < C (47.5%) < Control (46%). Statistical analysis revealed significant differences between Control & D (p= 0.03) and Control & F (p= 0.01).

Therefore, the higher As reduction by Treatment F can be associated with the selection and optimization of the microbes that increased the reduction of As. Treatment F contained *Aeromonas* sp. and *Pseudomonas* sp. that possibly induced higher removal of As from the contaminated soil and in congruence with Pepi *et al*. (2007) and Yamamura *et al*.(2007) who confirmed that *Aeromonas* sp. and *Pseudomonas* sp. are highly tolerant bacteria to As. The best interactions between the microbes can result in better and optimum As removal.

4.6.1.3 Aluminium (Al)

Figure 4.23 demonstrates Al concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. Initial concentration of Al found in soil was 49600 mg/kg. Reduction in the Al concentration (Figure 4.24) after remediation period followed the order of Treatment F (87%) < A (86.5%) < C &G (86%) < B (82%) < D (81.5%) < E (79%) < Control (34%). Most treatments, except for Control showed reduction above 75% Significant difference between the treatments (A-G) and Control was observed (p= 0.00). The removal activities for Al was very high as compared to other heavy metals studied. Most of the treatments amended with microbes showed reduction above 80% and the reason that could contribute to such reduction is due to the imbued interactions that exist among the introduced microbes upon manipulation of cell diversity (Emenike *et al.*, 2016). Previous study by Kuddus *et al.* (2013) revealed that a bioremediation activity successfully occurs when 65% or more metals were removed from the system.

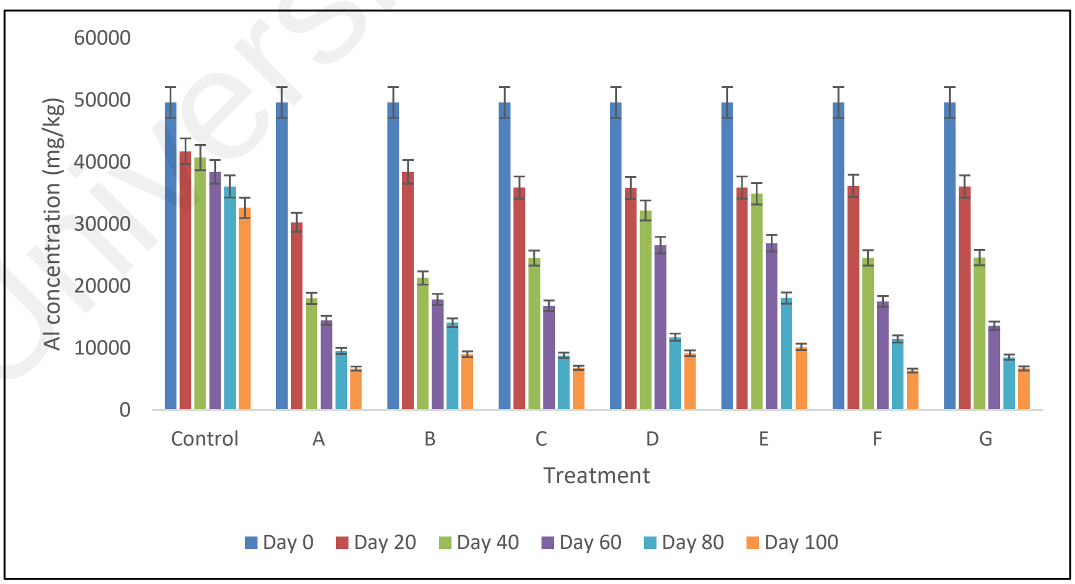

Figure 4.23: Al concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

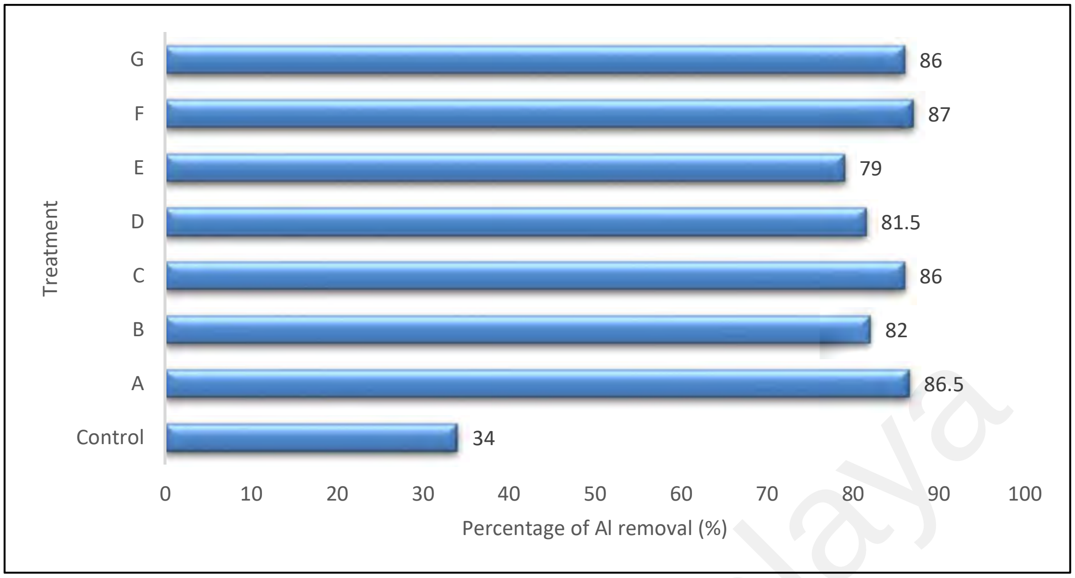

Figure 4.24: Percentage of Al removed in soil from Taman Beringin Landfill during bioaugmentation experiment

The reduction of Al with amended treatments (A-G) was double as compared to Control. This implies that addition of bacterial group consortia for remediation of Al in the contaminated soil shows a significant removal of Al no matter what group of microbes were added. The microbes were able to synergistically reduce Al concentration in the contaminated soil. Since no difference was observed between the treatments, one can assume that each treatment contained at least a minimum of three types of microbes that could significantly reduce the Al concentration and supported by findings of Emenike *et al*. (2017) with amendment of different group of microbes. The influence of microbes as introduced into the soil correlated with the fact that enhancement of metal reduction can be achieved with introduction of microbes as reported by Emenike *et al*. (2013) and Sprocati *et al*. (2011).

4.6.1.4 Manganese (Mn)

Figure 4.25 shows Mn concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. In this study, the highest reduction of Mn was by soil amended with Treatment F. The residual concentration of Mn for Treatment F was 149.33 mg/kg and percentage of removal was 47%. The reduction of Mn by addition of Treatment F exhibited four (4) times higher removal as compared to Control. It is clear that addition of inoculum played significant role in the removal of Mn from the contaminated soil. This was probably because the organisms in Treatment F were capable of initiating metabolic reactions that enhanced the degradation or uptake of Mn and therefore resulted in higher Mn removal by Treatment F. The results between the initial and final day of monitoring showed significant difference for all treatment (A-G) and the degree of significant was p=0.00. While Treatment F soil recorded highest percentage of reduction as compared to other treatments, the percentage of Mn removal (Figure 4.26) among the amendment when compared to Control gave the order of reduction as Treatment F (47%) < B, E (37%) < C (33%) < G (30%) < D (27%) < A (19%) < Control (11%). Significant differences between Treatment F and among treatments was recorded (p=0.00). The variations in Mn reduction among the treatments can be possibly linked with the bacterial strains in each treatments.

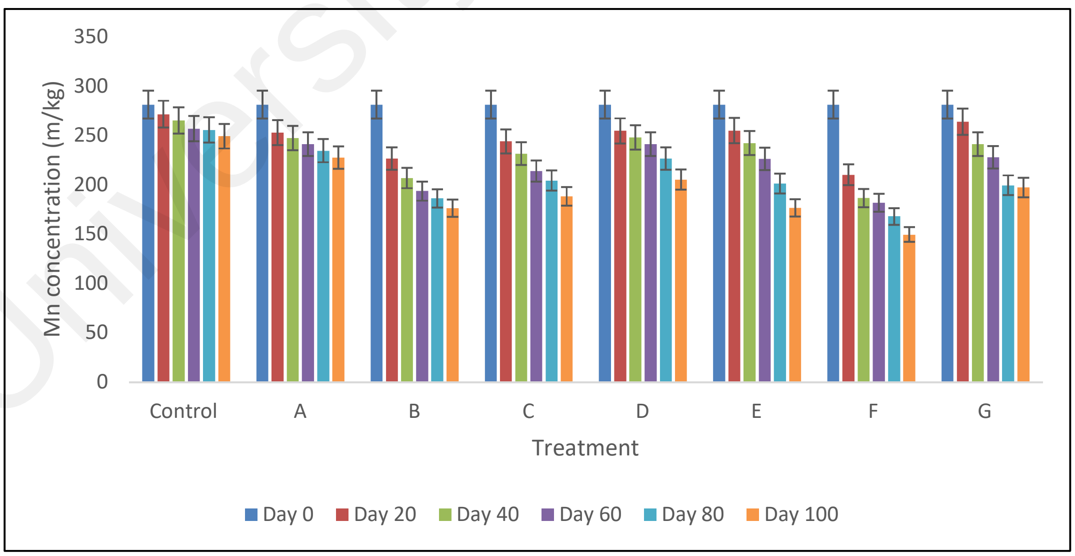

Figure 4.25: Mn concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

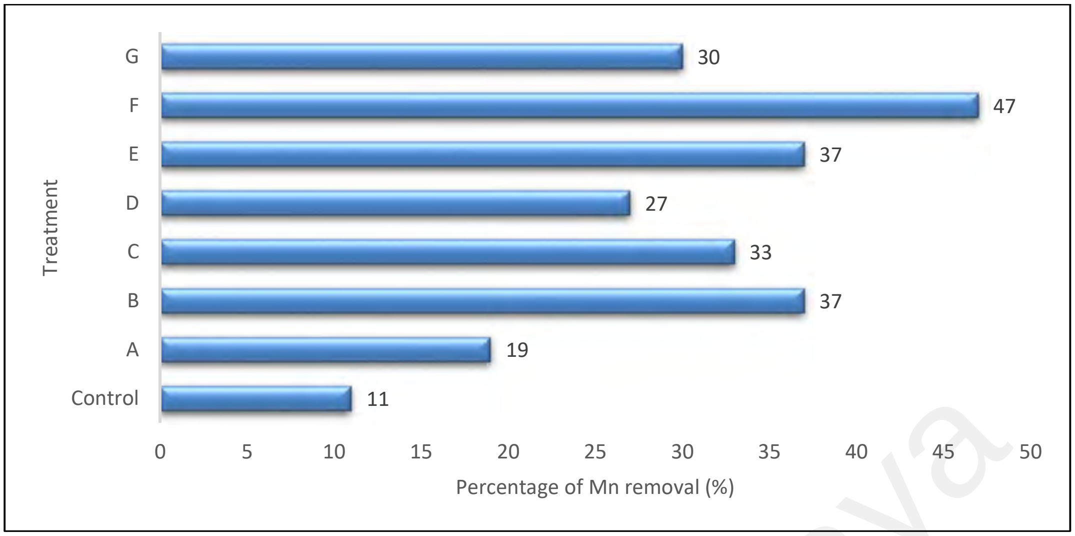

Figure 4.26: Percentage of Mn removed in soil from Taman Beringin Landfill during bioaugmentation experiment

The combination of *Burkholderia* sp., *Ochrobacterium* sp. and *Pseudomonas* sp. may have played major role in higher Mn reduction in Treatment F. This is probably because *Burkholderia* sp. has Multiple Nramp isoforms (Kehres & Maguire, 2003) which enabled Mn uptake in bacteria using transporter, Nramp (MntH). The transporter comprise both high and low affinity Mn uptake systems and the transporter utilized depends on the concentration of Mn in the environment. Similarly, *Ochrobacterium* sp. strain also been previously reported to be capable of reducing Mn from contaminated environment by Lebuhn *et al*. (2006) and Ozdemir *et al*. (2003). *Pseudomonas* sp. was also identified to be a good agent for Mn remediation (Santelli *et al*., 2012). Therefore, the special form of intraction among three organism may probably the reason behind the optimal reduction of Mn by Treatment F as compared to other treatments.

4.6.1.5 Copper (Cu)

Figure 4.27 indicates Cu concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. The mean residual concentration of Cu in Treatment A, B, C, D, E, F, G and control were 20 mg/kg, 22 mg/kg, 21.67 mg/kg, 19.67

mg/kg, 23 mg/kg, 21.33 mg/kg and 23.67 mg/kg, respectively. All treatments augmented with microbial isolate showed 60 - 67% reduction in the Cu (Figure 4.28). Treatment D demonstrated the highest removal of Cu (67%) while Treatment A removed 66% of Cu from the leachate contaminated soil.

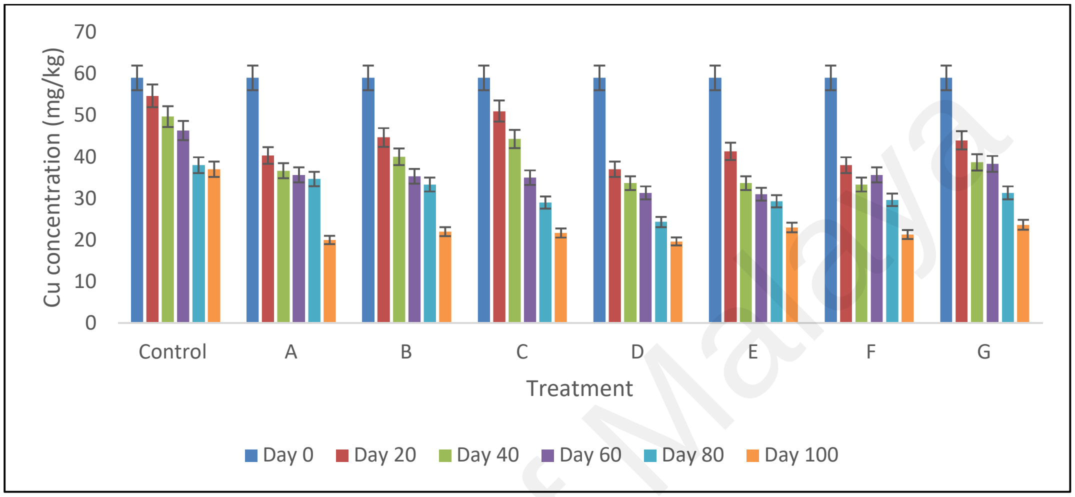

Figure 4.27: Cu concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

However, control treatment showed 37% of Cu reduction. Significant difference was observed among treatments (A-G) and Control ($p = 0.00$). This may imply that some microbes tend to be more sensitive to specific metals but can have high tolerance to another metal and this study agree with Nieto *et al*. (1989). The responses of microbes to pollution may vary from one environment to another or may vary among species because of the nature of pollutants and the varying concentrations. The reduction of Cu by the introduced treatments reflects the strength of the microbes and suggest that most of the isolated bacteria were able to reduce Cu immatter of the group. The reduced Cu concentration can be associated with the type of metabolites produced by the species during the experiment compared to Control. The *Bacillus* sp., *Rhodococcus* sp., *Brevundimonas* sp. and *Stenotrophmonas* sp. has been previously associated with Cu removal from contaminated systems (Emenike *et al*., 2017; Choudary & Sar, 2009;

Plociniczak *et al*., 2013) which is probably the reason for higher reduction in treatments that contained those microbes compared to control.

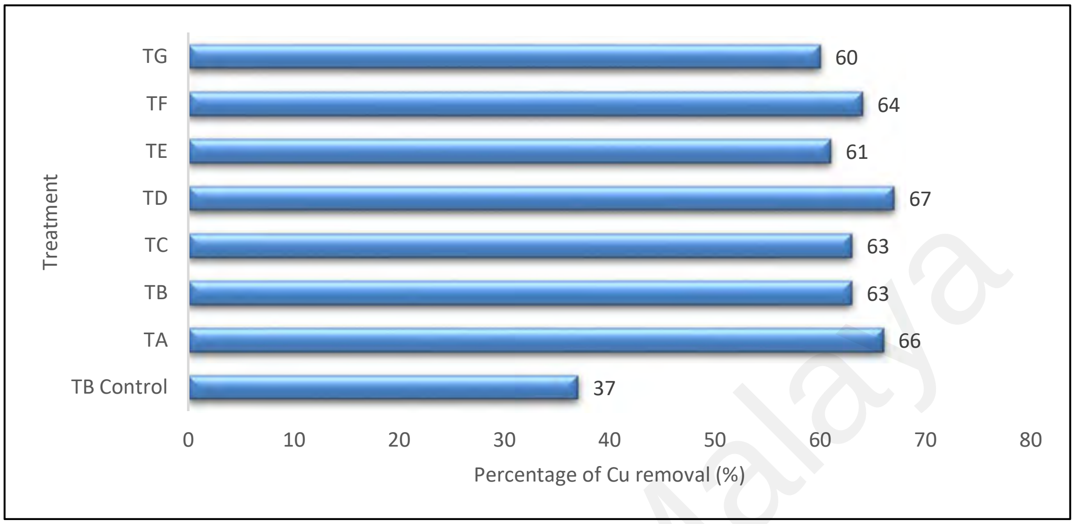

Figure 4.28: Percentage of Cu removed in soil from Taman Beringin Landfill during bioaugmentation experiment

4.6.1.6 Zinc (Zn)

Zn concentration in soil from Taman Beringin Landfill across time with different bacterial treatments is shown in Figure 4.29 and percentage of removal is illustrated in Figure 4.30. At Day 0, the concentration of Zn was 49 mg/kg and after 100 days the residual concentration of Treatment B was 18 mg/kg highest removal (63%). In Treatment B. the concentration of Zn rapidly decreased in first 20 days but gradually reduced till Day 80. After Day 80 the reduction was quite rapid which may have resulted in higher removal of Zn. The reason for such reduction to occur can be explained by rapid uptake of Zn by the group bacteria at the beginning of remediation because the initial population of microbes will be definitely high and uptake mechanisms was more rapid unlike after day 20 the depletion in microbial pollution may occur.

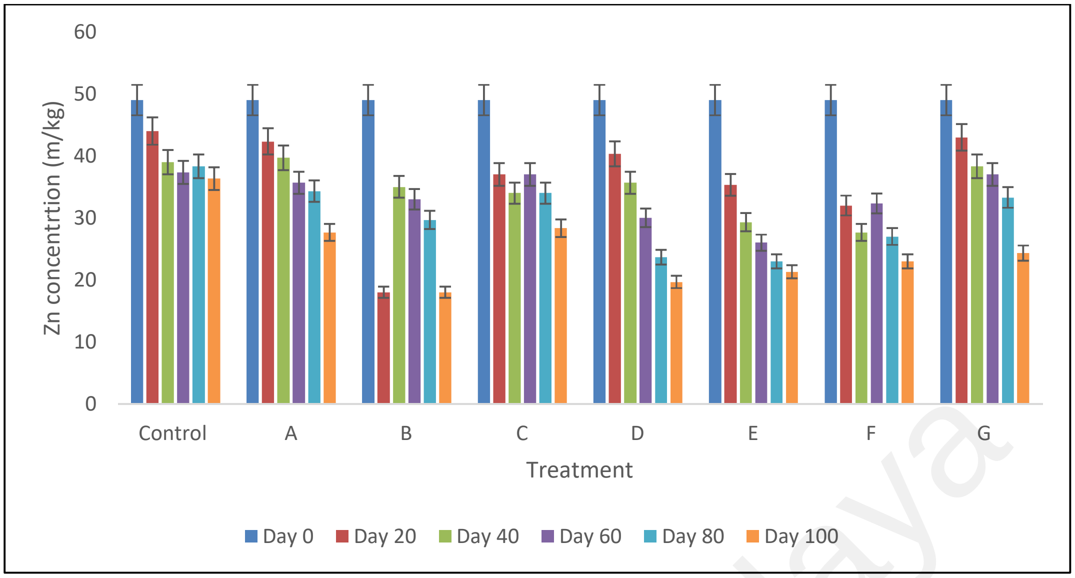

Figure 4.29: Zn concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

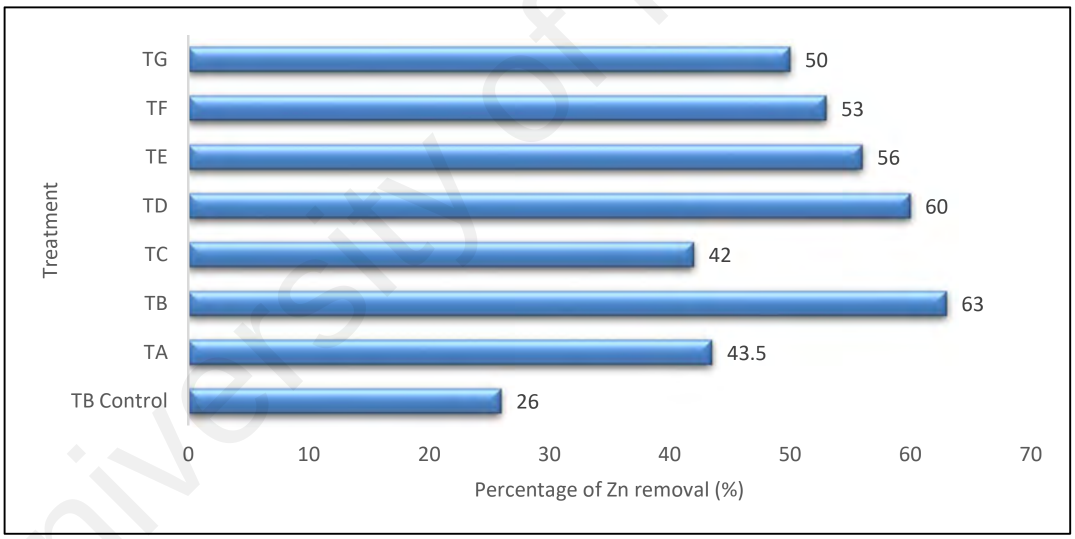

Figure 4.30: Percentage of Zn removed in soil from Taman Beringin Landfill during bioaugmentation experiment

The possible presence of effective metal uptake enzymes in some of microbes used for Treatment B may contribute to higher percentage of Zn removal. The existence of *Pseudomonas* sp., *Ochrobacterium* sp., *Stenotrophmonas* sp., .*P.mendocina* and *Serratia* sp. in Treatment B may associated with higher reduction of Zn. *Pseudomonas* sp. has been reported for Zn removal by Green *et al.*(2008). Similarly, *Ochrobacterium* sp., *Stenotrophmonas* sp., *P.mendocina* and *Serratia* sp. has been previously reported for Zn remediation when used as single culture (Pandey *et al*., 2013; Pages *et al*., 2008; Chien *et al*., 2007; Sahar, 2012).

Besides that Treatment D, E, F and G also exhibited high removal efficiency of Zn from the contaminated soil and the removal of Zn was above 50%. For control, the percentage of removal was only 26%. This clearly indicates that addition of different microbes into the contaminated soil can reduce Zn concentration by almost two-folds.

Higher removal of Zn by Treatment B which represents gram negative bacteria may contain enzymes, glycoproteins, lipopolysaccharides, lipoproteins and phospholipids which are the active sites involved in the metal binding process and this is agreeable to the findings of Fomina and Gadd (2014) and Gupta *et al*. (2015). Similarly, Lucious *et al*. (2013) also reported that gram negative bacteria are capable of remediating high concentration of heavy metal. Significant difference between Treatment B and control ($p= 0.00$), thus proving the positive removal of Zn from the leachate contaminated soil. Besides that, Treatment D, E, F and G as well showed significant difference in Zn reduction when compared with control at $P < 0.05$. The resistance of microbes towards toxic levels of zinc can be due to extracellular accumulation sequestration by metallothioneins (MT) as reported by Olafson *et al*. (1998), Morby *et al*. (1993), Robinson (1998), Paulsen (1997) and Nies (1999) in gram negative bacteria.

4.6.1.7 Iron (Fe)

Figure 4.31 and Figure 4.32 illustrate Fe concentration in soil from Taman Beringin landfill across time with different bacterial treatments and percentage of removal. Initial concentration of Fe was 42,900 mg/kg and after 100 days remediation the residual concentration was 27,378 mg/kg, 24,860 mg/kg, 10,366 mg/kg, 13,257 mg/kg, 13,600 mg/kg, 12,533 mg/kg, 10,763 mg/kg, 21,238 mg/kg for Treatment Control, A, B, C, D, E, F and G, respectively. The highest reduction of Fe concentration was by Treatment F (75%). With exception to Treatment A, B and Control, all treatments showed reduction in Fe concentration of more than 60%. The reduction of Fe may probably be associated to enhanced metabolic activities in such treatments from strengthened microbial concentration and diversity. 36% of reduction was recorded in the Control treatment and this could be due to natural remediation carried out by the soil indigenous microbes. With exception to Treatment A, B and Control, all treatments showed linear drop in the Fe

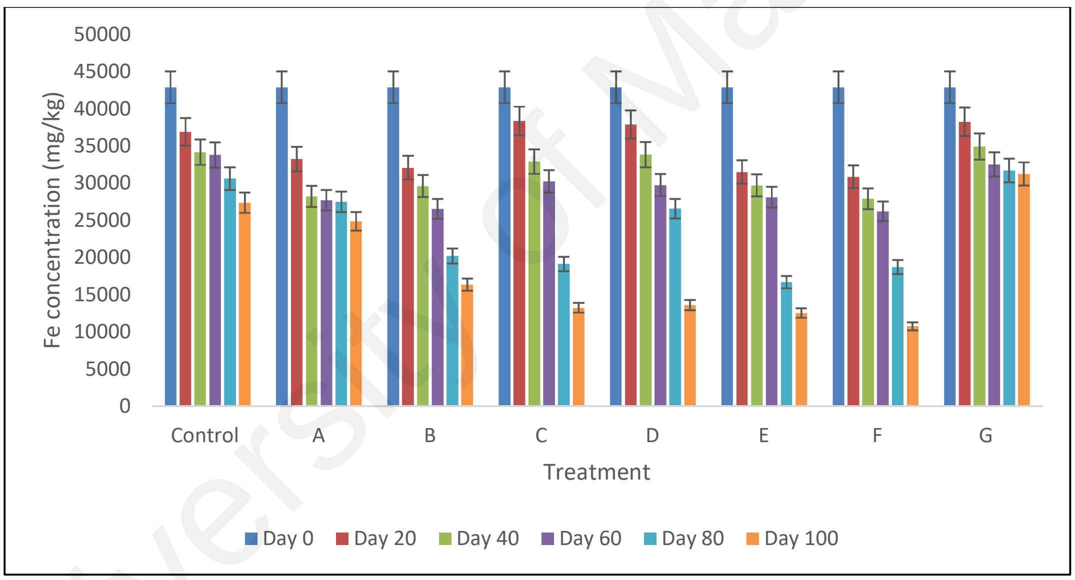

Figure 4.31: Fe concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

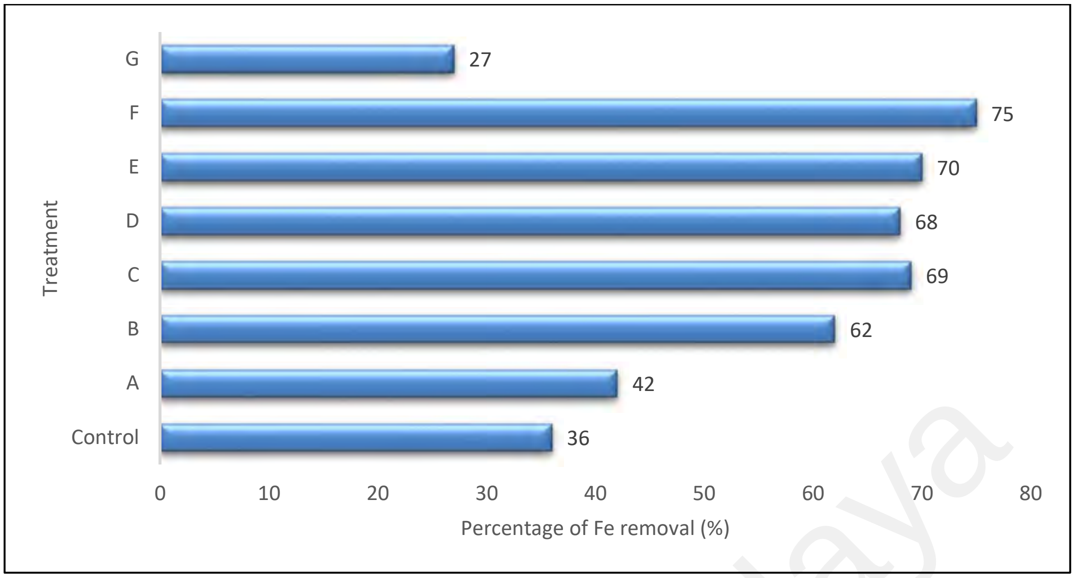

Figure 4.32: Percentage of Fe removed in soil from Taman Beringin Landfill during bioaugmentation experiment

concentration across the monitoring days. Emenike *et al*. (2016) also reported similar trend of reduction in studied metal concentrations but different type of bacteria treatment augmentation.

Treatments B, C, D, E and F also exhibited significant differences in the reduction of Fe as compared to Treatment Control, A and G. Treatment G recorded the lowest percentage of Fe removal as compared to other treatments and this may because the particular grouping of bacteria was not effective for Fe reduction.

The highest reduction by Treatment F may possibly because that group contains highest number of microbes (90%) that may enhanced several aspects of the and this is agreeable to Emenike *et al*. (2017), who reported that the diversity of microbes plays an important role in metal removal in soil remediation. The tolerance of microbes towards the heavy metal play a very critical for metal reduction in the soil. The association of *A. caviae* DNA 4, *P. mendocina* and *O. intermedium and S. marcescens* in Treatment F (proteo bacteria) could be the reason for higher reduction of Fe in the contaminated soil. This is supported by studies by Sahar (2012) who reported 40% of reduction in Fe concentration with single strain of *Serratia* sp and preliminary study conducted by Jayanthi *et al*. (2016) also showed that *A. caviae* DNA 4, *P. mendocina* and *O. intermedium* were among the microbes which has highest tolerance towards Fe whereby absolute growth of microbes were observed in the plates inoculated with different concentration of Fe. Furthermore, the findings are also in congruence with Fauziah *et al*. (2017) who reported that addition of microbes, namely proteo bacteria to leachate contaminated soil can reduce the heavy metal content at a significant rate.

4.6.1.8 Nickel

Figure 4.33 represents Ni concentration in soil from Taman Beringin Landfill across time with different bacterial treatments. Soil amended with Treatment F (59%) showed highest Ni reduction as compared to other treatments (Figure 4.34). This indicates the potential of Treatment F for bioreduction of Ni from leachate contaminated soil. Initial concentration of Ni in soil was 21 mg/kg and the residual concentration of Ni after 100 days remediation with Treatment F was 8.67 mg/kg. From the results obtained Treatment F possess greater potential for Ni reduction as compared to other treatments amended with microbes. Treatments A-E and G was also able to reduce 49-52.3% of Ni concentration in the soil when control only reduce it by 44%.

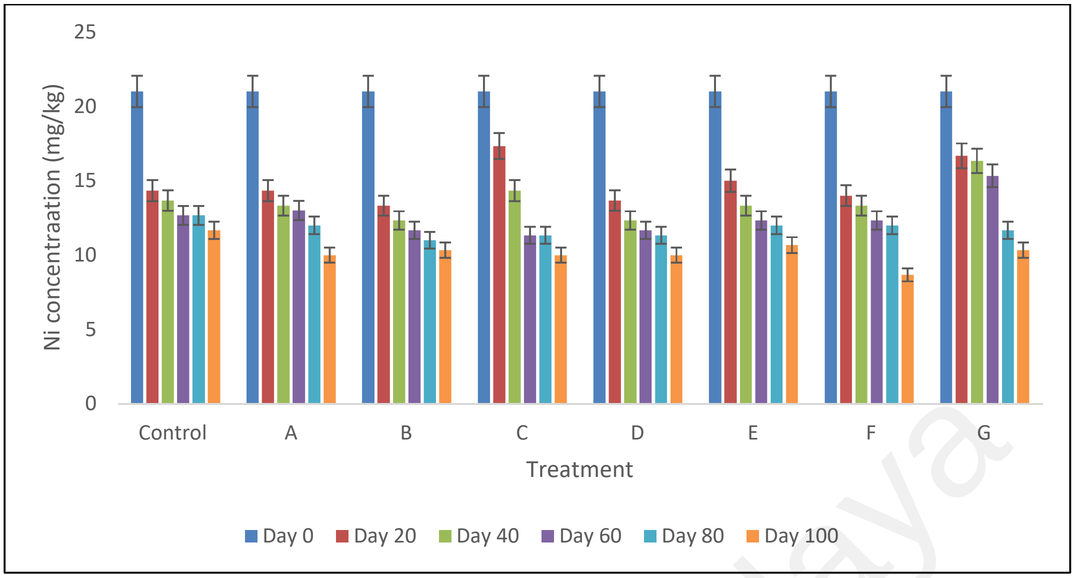

Figure 4.33: Ni concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

Treatment F, proteo bacteria was able to remediate high concentration of Ni as compared to other treatments which may probably be due to some of the microbes in this group that are highly resistant microbes towards Ni. *Proteobacteria* group appeared to be associated positively with heavy metal removal. It associated with *in situ* microbial community and suggests possible roles in Ni reduction by proteo bacteria (Kirpichtchikova *et al*., 2006). *Brevundimonas* sp. is one of microbes from the group of proteo bacteria that have been reported by Singh and Gadi (2012) that successfully reduced 52% of Ni from the Ni contaminated soil. Even though this microbes is present in the other group of microbes, the microbial interaction mechanism from the same group were able to enhance higher Ni removal. Interaction between the microbes may be the

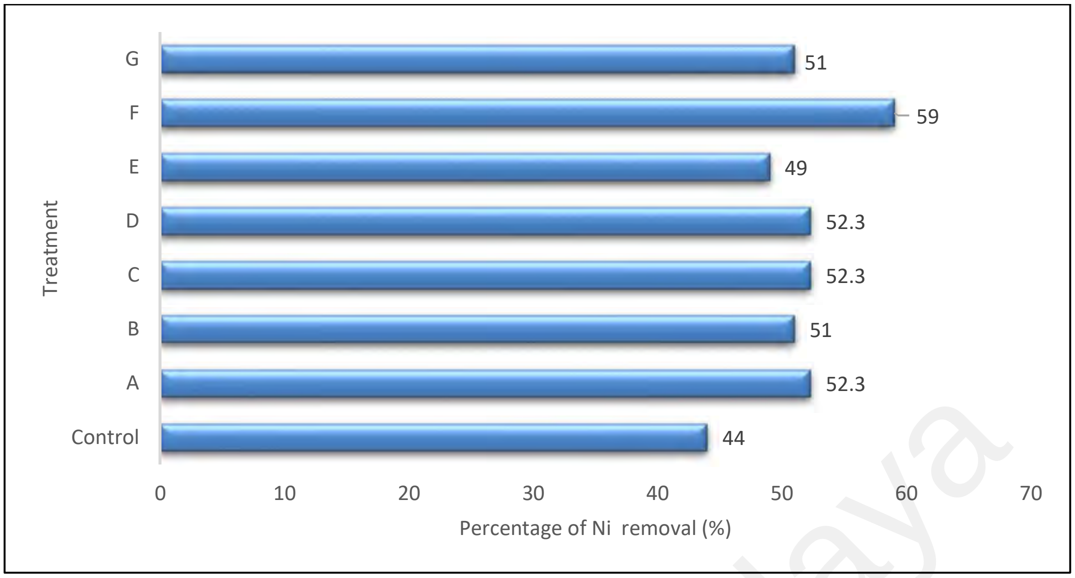

Figure 4.34: Percentage of Ni removed in soil from Taman Beringin Landfill during bioaugmentation experiment

reason for higher removal of heavy metal by selected treatment. Emenike *et al*. (2016) reported that the manipulation on the microbial diversity as well as the concentration promotes better metal reduction in the soil. The tolerance and survival of bacteria in metal toxic environment evolved several types of mechanisms. This includes by efflux of metal ions outside the cell, accumulation of the metal ions inside the cell and reduction of the heavy metal ions to a less toxic state as suggested Laila *et al*. (2011).

Most of the treatments except for Treatment E and control exhibits more than 50% reduction in Ni concentration and according to bioremediation law successful remediation is achieved when reduction is above 50%. Statistical analysis shows a significant difference between the best treatment (Treatment F) and control (p=0.017). The higher reduction of Ni by Treatment F reflects the strength of treatment compared to other treatment and according supported by Emenike *et al*. (2017), who stated that combination of treatment poses a peculiar interaction that synergize the metal reduction in the soil.

4.6.1.9 Chromium (Cr)

Figure 4.35 illustrates Cr concentration in soil from Taman Beringin landfill across time with different bacterial treatments. The most efficient reduction of Cr was by Treatment F. Cr was reduced from 46 mg/kg to 18 mg/kg with addition of Treatment F with 61% of removal (Figure 4.36). This indicates that addition of Treatment F enhanced the removal of Cr from the contaminated soil. The residual concentration of other treatments were, Treatment A (23.33 mg/kg), B (26 mg/kg), C (24.67 mg/kg), D (23.67 mg/kg), E (24.67 mg/kg), G (25 mg/kg) and Control (26.67 mg/kg) with the percentage of removal ranged of 41% to 50%. Similar studies by Emenike *et al*. (2017b) and Emenike (2013), who recorded higher percentage of removal of Cr with amendment of microbial consortia of different group of treatments as compared to the natural remediation.

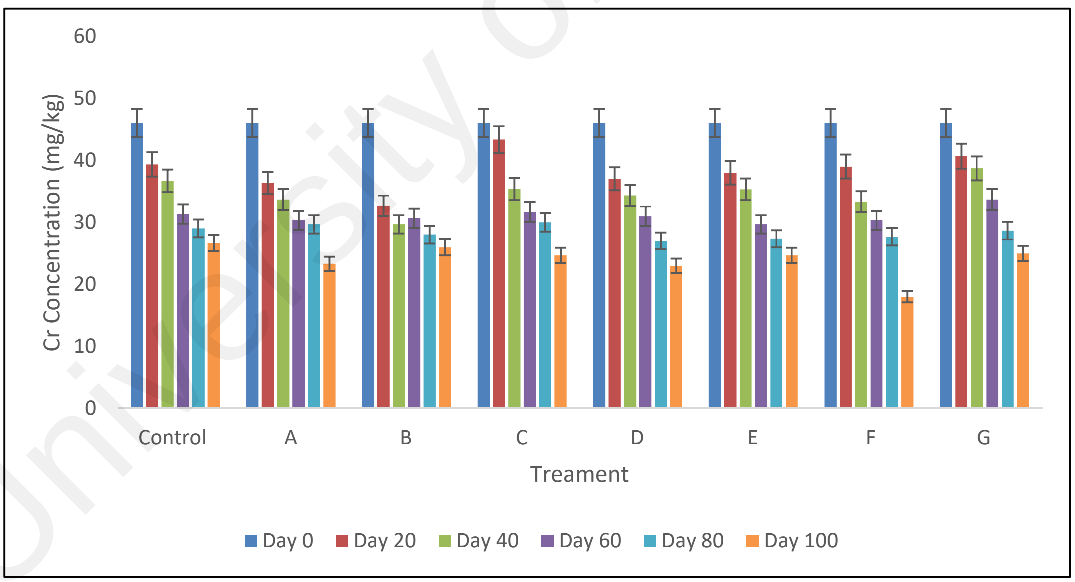

Figure 4.35: Cr concentration in soil from Taman Beringin Landfill across time with different bacterial treatments

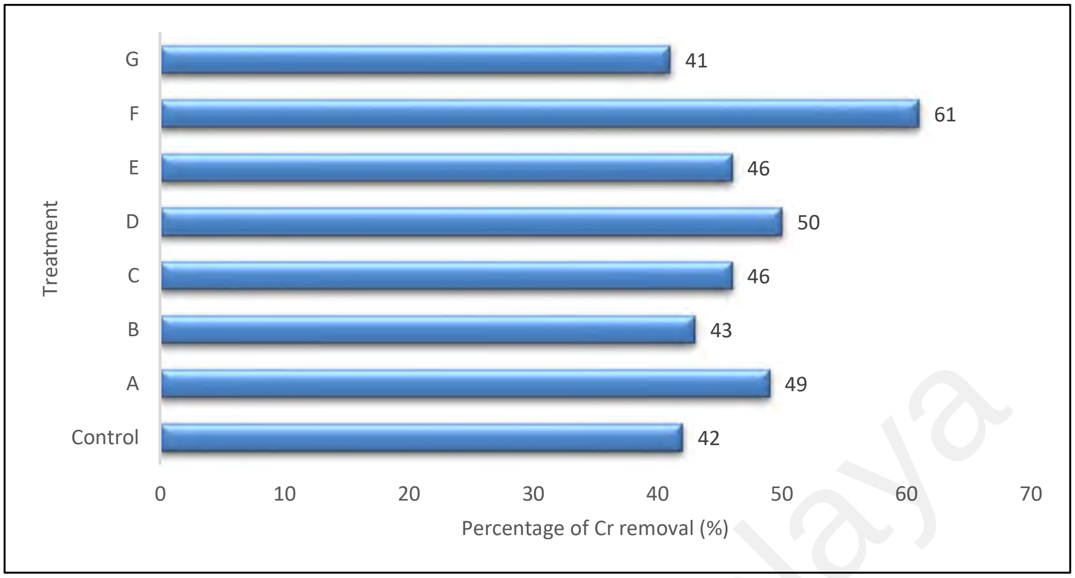

Figure 4.36: Percentage of Cr removed in soil from Taman Beringin Landfill during bioaugmentation experiment

Statistical analysis shows significant different between Treatment F and control (p=0.002). Treatment G shows the lowest reduction with 1% difference as compared to Control. This probably occurs due to mineralization of microbes in Treatment G possibly due to some unidentified reason that peculiar to Cr metabolisms. Contaminated soil amended with Treatment F shows an enhancement in Cr removal as compared to other treatments.

The reason that could be associated with higher reduction of Cr is that the microbe in Treatment F may be because the proteo bacteria is dominant species in landfill it is reported by Flavio *et al*. (2005), that the most dominant species isolated from Cr contaminated soil is belongs to proteo group which may reason for higher Cr removal by this group of bacteria. The involvement of *Pseudomonas* sp. could be reason associated with higher removal of Cr by Treatment F. Previous studies on enhanced reduction of Cr from contaminated soil with addition of *Pseudomonas* sp. has been reported by Hassan *et al*. (1998).

The difference in reduction by the different treatments may probably because the removal of specific metal ions varies among microorganisms because of differences in affinity and electronegativity of the metal ions as suggested by Ajay Kumar *et al*. (2009). Besides that, strain selection, concentration of bacteria and inoculum heterogeneity are important for successful bioremediation as also established by Sprocati *et al*. (2012).

4.6.1.10 First order rate constant and half-life of heavy metals for bioaugmentation experiment of soil from Taman Beringin Landfill

Table 4.9 shows the first order rate constant of the heavy metals for bioaugmentation experiment of soil from Taman Beringin Landfill when treated with different treatment at 10% v/w bacterial concentration. The rate constant was calculated using the first order kinetic model. The calculation is aimed to estimate the daily ability of the microbes to uptake or bio-reduce metals while comparing with control. Treatment A, Treatment C, Treatment F and Treatment G showed the highest removal rate for Al (0.020 day^{-1}) as compared to other metals in this study.

For As, Mn, Fe, Ni and Cr the highest first order rate constant was with Treatment F and value was 0.0095 day^{-1}, 0.0063 day^{-1}, 0.014 day^{-1}, 0.0088 day^{-1} and 0.0094 day^{-1} respectively. Considering the fact that no previous bioaugmentation experiment research had utilized the similar pattern of microbe treatments especially from group of Proteo, however the profound reduction of metal by this treatment may be associated with blending of microbes and while some microbes were found to be previously tested as single culture

Table 4.9: First order rate constant of heavy metals for bioaugmentation experiment of soil from Taman Beringin Landfill

Metal	First order rate constant (k) day^{-1}							
	Control	**Treatment A**	**Treatment B**	**Treatment C**	**Treatment D**	**Treatment E**	**Treatment F**	**Treatment G**
Pb	0.0037	0.0049	0.0046	0.0055	0.0049	0.0038	0.0045	0.0052
As	0.0062	0.0071	0.0073	0.0064	0.0091	0.0082	0.0095	0.008
Al	0.0041	0.02	0.017	0.020	0.017	0.016	0.020	0.020
Mn	0.0012	0.0021	0.0047	0.004	0.0031	0.0046	0.0063	0.0035
Cu	0.0047	0.0108	0.0099	0.01	0.011	0.0094	0.010	0.0094
Zn	0.003	0.0057	0.01	0.0055	0.0091	0.0083	0.0076	0.007
Fe	0.0045	0.0055	0.0096	0.0117	0.115	0.012	0.014	0.0031
Ni	0.0059	0.0074	0.007	0.0074	0.0074	0.0067	0.0088	0.007
Cr	0.0054	0.0068	0.0057	0.0062	0.0069	0.0062	0.0094	0.0061

for metal removal from contaminants. Emenike (2013) reported that blending the microbes gives an optimal removal of heavy metals. Highest first order rate constant for Cu was at 0.011 day^{-}1 when augmented with Treatment D. Furthermore for Pb and Zn the highest first order rate constant were recorded in Treatment C and B and rate of metal uptake.was 0.0055 day^{-1} and 0.01 day^{-1} respectively. The treated soil showed higher first order rate constant as compared to control, where Treatment F (Proteo bacteria) was the most dominant group that showed higher metal reduction namely for As, Al, Mn, Fe, Ni, and Cr. Blending of microbes gives an optimal removal of heavy metal as compared to control.

The half-life is a function of bioremoval rate constant. Table 4.10 shows the half-life value of heavy metals for bioaugmentation experiment of soil from Taman Beringin landfill inoculated with 10 % v/w of microbial treatment. Hence, the results revealed that, the shortest half-life was recorded for Al by Treatments A, C, F and G. The removal of Al to be half was 34.65 days. For metals such as As, Mn, Fe, Ni and Cr the shortest half-life was recorded when the polluted soil was augmented inoculum F and the half-life was 72.96 days, 110.02 days, 49.5 days, 78.76 and 73.73, respectively. The difference between the treatments based on their half-life value is due to the concentration of heavy metals removed by the particular treatment. The highest half-life value was recorded for Control for all studied metals and similarly Emenike *et al*. (2016) and Auta *et al*. (2017) reported highest half-life value for Control.

Table 4.10: Half- life value for bioaugmentation experiment of soil from Taman Beringin Landfill

Metal	Half-life t½ (days)							
	Control	**Treatment A**	**Treatment B**	**Treatment C**	**Treatment D**	**Treatment E**	**Treatment F**	**Treatment G**
Pb	187.33	141.46	150.68	126.02	141.46	182.4	154	133.29
As	111.8	97.62	94.95	108.30	76.17	84.53	72.96	86.64
Al	169.06	34.65	40.77	34.65	40.77	43.32	34.65	34.65
Mn	577.62	330.07	147.48	173.29	223.59	150.68	110.02	198.04
Cu	147.48	64.18	70	69.3	63	73.74	69.3	76.71
Zn	231	121.60	69.3	126.02	76.17	83.51	91.2	99.02
Fe	154	126	72.2	59.24	60.27	57.76	49.5	223.6
Ni	117.48	93.67	99.02	93.67	93.67	103.45	78.76	99.02
Cr	127.18	101.93	121.60	111.79	100.45	111.79	73.73	113.63

4.6.1.11 Bacterial count for bioaugmentation experiment of soil from Taman Beringin Landfill

Bacterial count was also taken into account during the 100 days remediation period and Figure 4.37 shows the bacterial count across time with different treatments for remediation of soil from Taman Beringin Landfill. It is important to understand the distribution of microbes in a remediation set up because the count represents the survival of microbes in metal contaminated soil. The count was taken every 20 days. At Day 0, the average bacterial count for all treatment was 1.4 $\times 10^8$ CFU/g as compared to 2.5 $\times 10^7$ CFU/g was in control. The bacterial count was showing a fluctuating trend during the 100 days. Emenike (2013) and Lin *et al*. (2010) also observed similar trend of bacterial count for remediation study of heavy metal with inoculation of different group of microbes.

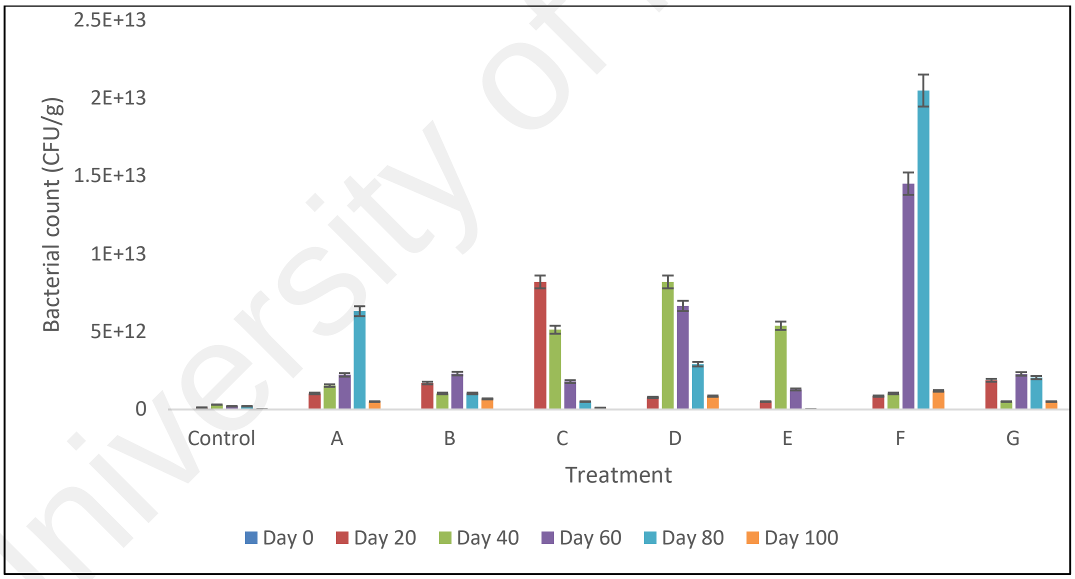

Figure 4.37: Bacterial count across time with different bacterial treatments for remediation of soil from Taman Beringin Landfill

At Day 20, increase in the bacterial count was observed for all treatment. This was expected due to the introduction of inoculum at the beginning of the experiment. The highest count was in Treatment C with 8.19 $\times 10^{12}$ CFU/g. The group that contained all microbes (Treatment A) was showing lower value of bacterial count while it is expected to contain higher count because it is loaded with all the eighteen microbes.

At Day 40, Control, Treatment A, Treatment B, Treatment E and Treatment F showed increased in the bacterial count. The increases in the number of bacteria can be due to availability of nutrients in the soil. The conditions that were very favourable for the cell duplication might be available occurred therefore increase in bacterial count observed. Treatment F was showing the highest count among all the treatments (1.45 X 10^{13} CFU/g). At Day 80, decrease in the bacterial count for almost all the treatments was observed with the exception of Treatment A and F. Other treatments showed decrease in the bacterial count which may probably due to the fact that the cell was no longer duplicating and may undergo stress associated with the continued metal toxicity. Additionally, the remediation process may limit the availability of nutrients at this stage.

At the end of experiment (Day 100), significant reduction in the bacterial count was observed for all the treatments. The reduction is most likely be due to the highly reduced nutrient availability in the microcosms and the microbes were stressed out due to the metabolic process for removal or transformation of heavy metals in the soil. The highest bacterial count was in Treatment F. Bacterial count for Treatment F was 1.19 X 10^{12} CFU/g while the lowest was the Control. Treatment F had the highest microbial count and also has highest heavy metal removal for As (61%), Al (87%), Mn (47%), Fe (75%), Ni (59%) and Cr (61%).

4.6.1.12 Soil redox potential for bioaugmentation experiment of soil from Taman Beringin Landfill

The soil redox potential was also measured across the remediation period and is depicted in Figure 4.38. The variation of the redox potential across the treatments shows the solubility of the heavy metal in the soil. Comparison between the treatments showed that Treatment F (294.96 mV) had the highest redox potential value at end of the 100 days. Emenike *et al*. (2017), reported similar observation towards the end of bioaugmentation experiment study on heavy metal removal from contaminated soil with different group of microbial consortia and all the treatment were having higher redox potential value. Strong correlation of $y = -0.482x + 187.86$ with R^2 value of 0.94 existed between the redox potential and As concentration. Overall observation of the redox potential value showed that most of the treatments have an increasing trend. This indicates that metal transformation was taking place because increase in redox potential is a reflection of decrease in solubility of metals in contaminated soil. With the increased redox potential, it can be assumed that the extractable concentration of metals in leachate contaminated soil decreased while undergoing bioremediation and is supported by findings of Emenike *et al*. (2017); Chuan *et al*. (1995); Yamaguchi *et al*. (2011).

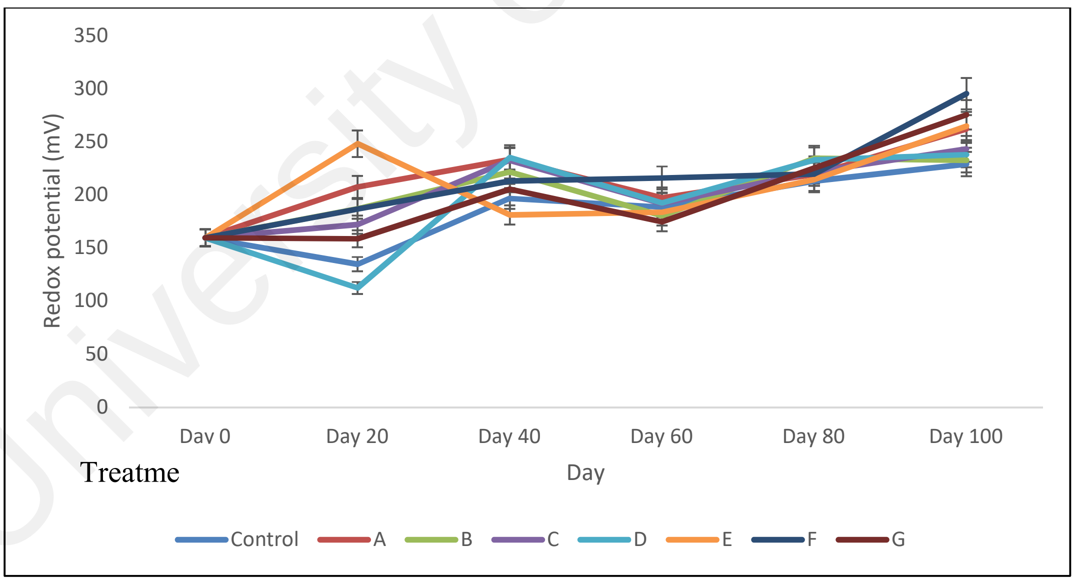

Figure 4.38: Soil redox potential across time with different treatments for bioaugmentation experiment of soil from Taman Beringin Landfill

4.6.1.13 Soil pH for bioaugmentation experiment of soil from Taman Beringin Landfill

pH of soil from Taman Beringin Landfill was also measured and is illustrated in Figure 4.39. A pH of 8.02 was recorded at the initial stage of the bioaugmentation experiment studies. The initial soil pH was suitable for the growth of microbes. However, the pH varied from slightly acidic to neutral pH when remediation took place. Treatment F and Treatment G showed slightly acidic pH at Day 20, but turned neutral thereafter until Day 100. The reason for the changes in pH maybe attributed to the immobilization of metal upon the introduction of bacterial inoculum. Similarly, Abioye (2012); Emenike *et al*. (2017b) and Krishna *et al*. (2013) also reported reduction of heavy metals at neutral pH.

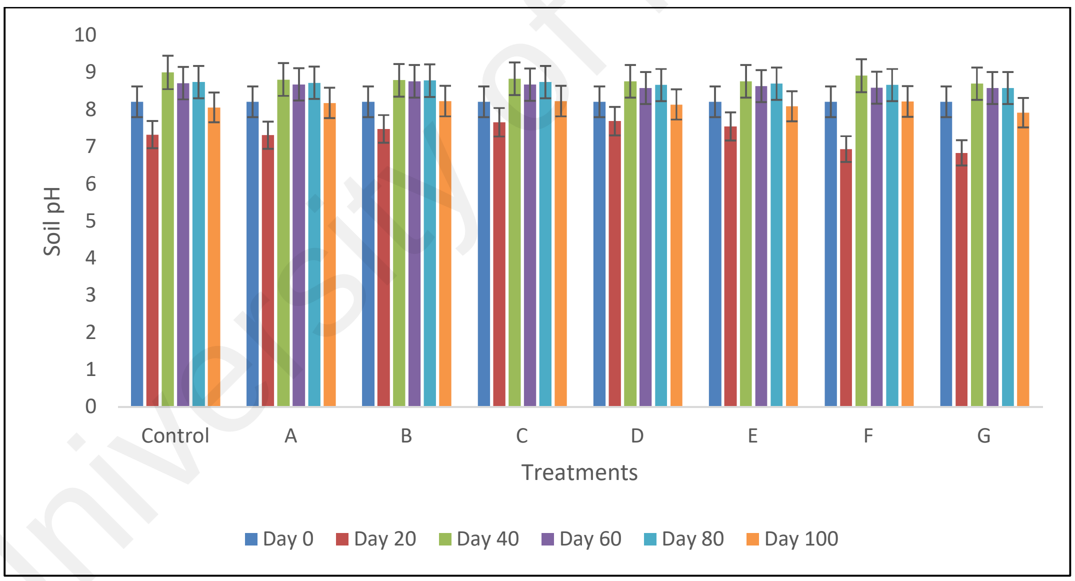

Figure 4.39: Soil pH across time with different treatments for remediation of Taman Beringin Landfill soil

4.6.2 Bioaugmentation experiment of Bukit Beruntung leachate contaminated soil with different bacterial treatment at 10% v/w

Leachate contaminated soil from Bukit Beruntung Landfill was also inoculated with different microbial combination at concentration of 10% v/w and remediation was allowed to occur for 100 days. The concentration of heavy metal varied and almost all metal present in the soil before treatment were above the prescribed limit.

4.6.2.1 Lead (Pb)

Pb concentration in soil from Bukit Beruntung Landfill across time with different treatments is illustrated in Figure 4.40. Amending the contaminated soil with Treatment C resulted with the highest reduction of Pb at 37% (Figure 4.41) where the concentration of Pb was reduced from 67 mg/kg to 36 mg/kg. Treatment D was also able to reduce the concentration of Pb removal was 36%. Other treatments amended with microbes (A, B, E, F and G) reduced 29% to 34 %. Statistical analysis shows significant difference between Treatment (B, C, D and G) and Control. The reduction of Pb by Control was only 18% while reduction by Treatment C and D was two times higher.

However, addition of inoculums to the contaminated soil did not achieve more than 50% of removal as it should for an ideal remediation. This probably was because the microbes may need longer than 100 days to achieve more than 50% removal capacity or it may require continuous addition more inoculum. The resistance of bacteria towards Pb may probably be related to the genes present in the microbes which resulted in higher uptake by Treatment C. Previous findings by Apell (2004), Jaroslawiecka and Piotrowska (2014) and Naik and Dubey (2013) revealed that the tolerance of bacteria may probably be because Pb resistant bacteria carry the genes encoding the P-type ATPases and phosphatase. P-type ATPases belong to the family of transmembrane transporters, which are responsible for the transport of ions and small organic molecules across the

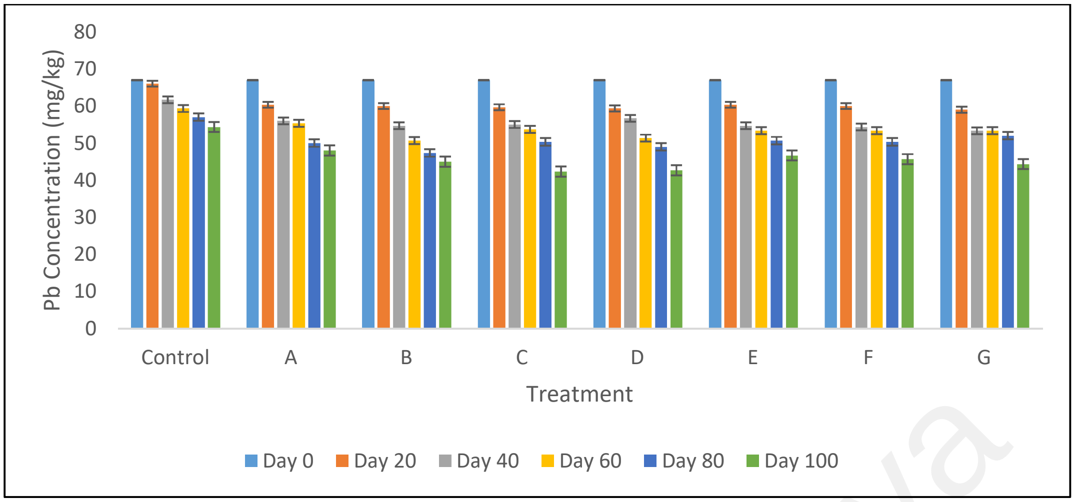

Figure 4.40: Pb concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

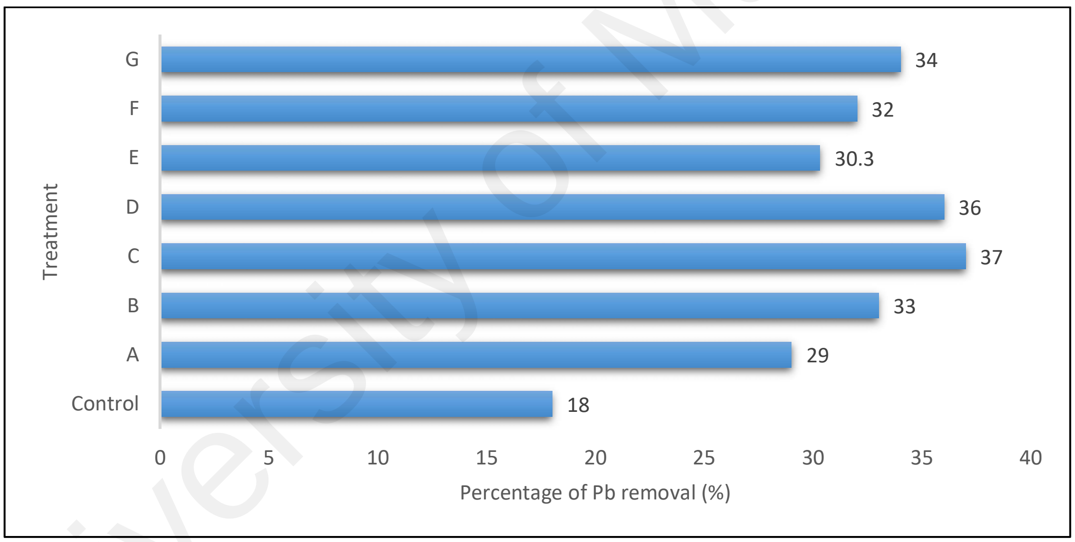

Figure 4.41: Percentage of Pb removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

cytoplasmic membrane using ATP as the energy source. Treatment C consists of gram positive. The peptidoglycan layer of cell wall components of Gram-positive bacteria, which contains alanine, glutamic acid, meso-di-aminopimelic acid, polymer of glycerol and teichoic acid are the active sites involved in metal binding processes which could have contributed to high removal of Pb in this study and in congruence with findings of Fomina and Gadd (2014). The findings of this study is also agreeable with findings of

Chelliah *et al*. (2006) and Banerjee *et al*. (2016) who reported extensive use of gram positive bacteria for removal of Pb from contaminated site.

4.6.2.2 Arsenic (As)

Figure 4.42 depicts As concentration in soil from Bukit Beruntung Landfill across time with different treatments. As concentration in all the treatment amended with microbes (A-G) were reduced from 7 mg/kg to 1 mg/kg towards end of the experiment with 86% removal capacity (Figure 4.43). As concentration in the control was only reduced to 5.33 mg/kg removal percentage was 29%. Similarly Adams *et al*. (2014) reported reduction in the As with the introduction of microbial consortia while control showed lower reduction of As. Significant difference between the treatments (A-G) and the control (p=0.00) was obtained. Analysis on the As reduction across the biomonitoring days showed rapid decrease in As concentration for all treatment (A-G). The results revealed no variation in the percentage of As removal towards the end of experiment and this probably was because the initial concentration was considerably low and microcosms may contains microbes that was able to reduce the As concentration at similar rate. the residence microbes that are in the contaminated soil has the capability to degrade the metal (As) in equal form.

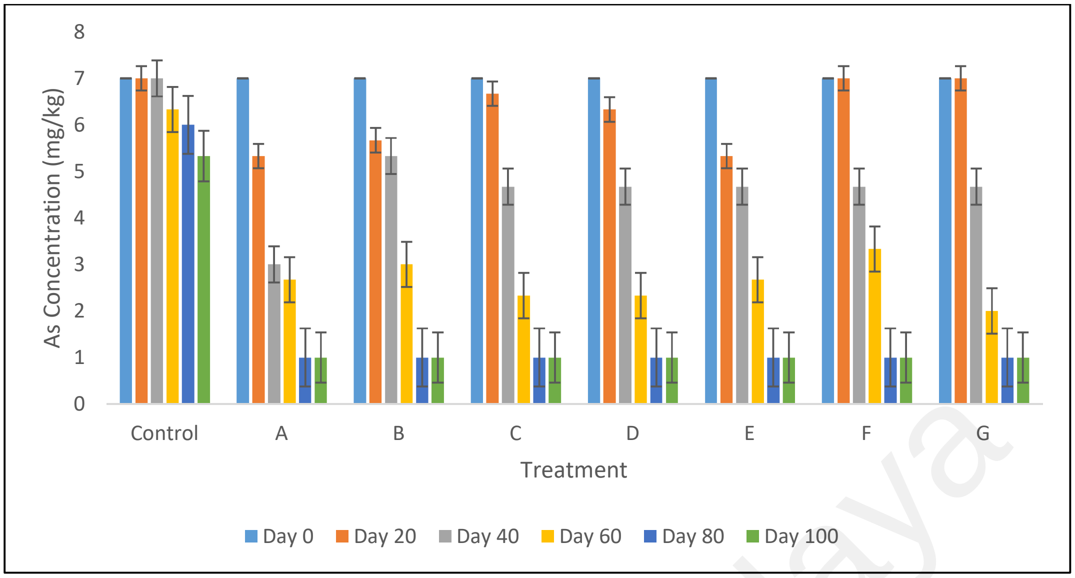

Figure 4.42: As concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

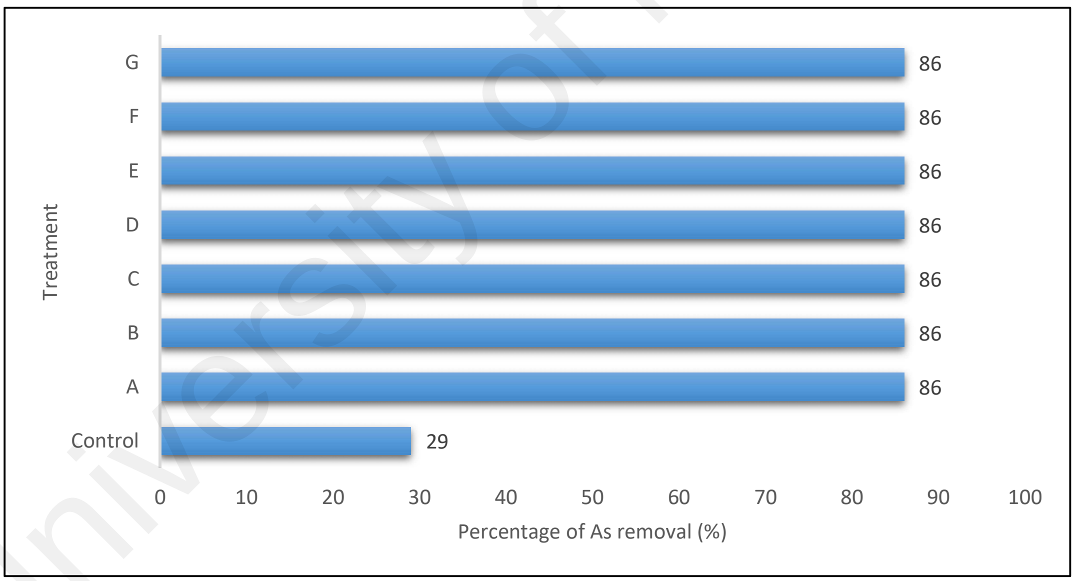

Figure 4.43: Percentage of As removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

The introduction of consortia to As contaminated soil enhanced the removal of As unlike Control which only recorded 29%. The findings of Maheswari and Murugesan (2009), also revealed that As can be significantly removed from contaminated soil with the addition of microbes and the removal of As was similar to results obtained in this study however the group of microbes was differ. Each treatment group may probably contain at least more than three type of bacteria which have resistance towards heavy metal, hence able to reduce 86% of As from the soil and as supported by findings of Luo *et al*. (2011) and Adams *et al*. (2014).

4.6.2.3 Aluminium (Al)

Figure 4.44 shows Al concentration in soil from Bukit Beruntung Landfill across time with different treatments. In the first 20 days, the results show rapid removal of Al concentration for all the treatment added with microbes. This may indicate the rapid uptake of Al by the introduced microbes which utilized it in their active metabolic

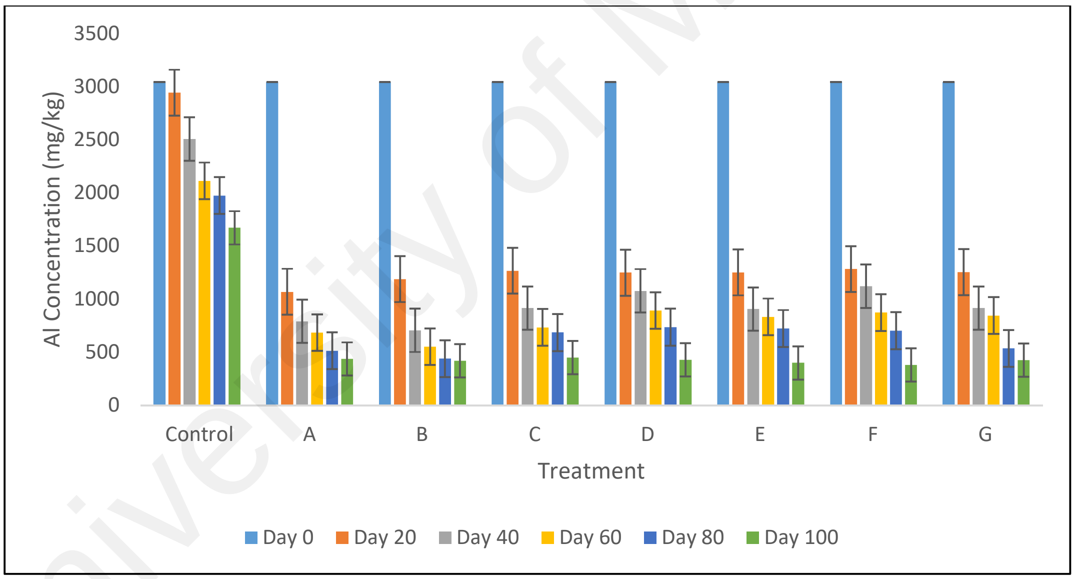

Figure 4.44: Al concentration in soil from Bukit Beruntung Landfill across time with different treatments

activities. At the end of remediation, Treatment F reduced to 378.67 mg/kg and percentage of reduction was 87.5% (Figure 4.45). Similarly, all other treatments with exception of control as well reduced Al and percentage of removal was 85- 87.5% and Control showed 45% of Al reduction. This indicates a that the study has achieved the

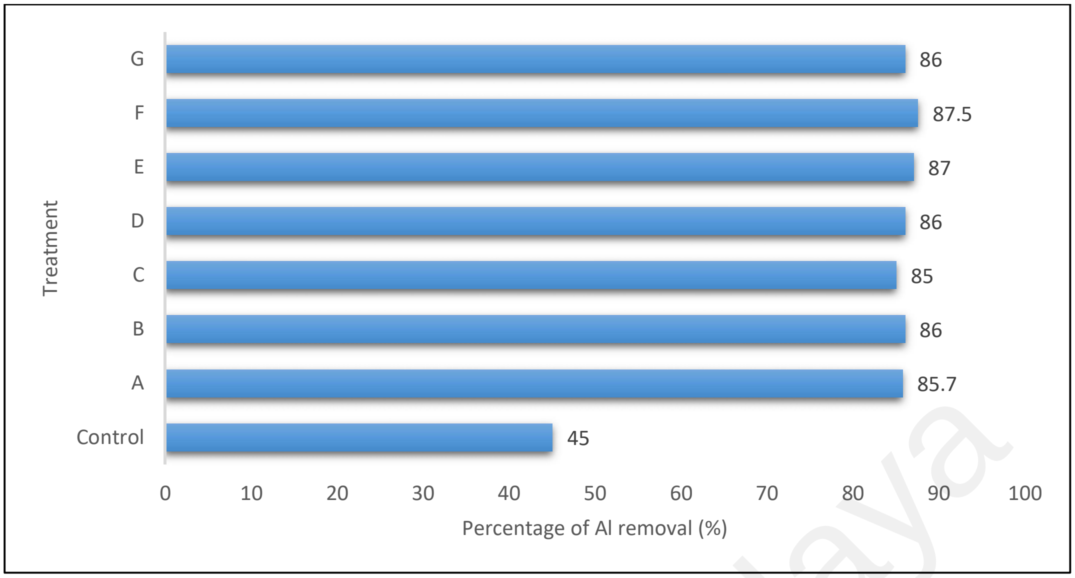

Figure 4.45: Percentage of Al removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

successful remediation as proposed by Babu *et al.*(2013), that reported successful bioaugmentation activity occurs when 65% or more metal removal was achieved. Emenike (2013) also reported similar reduction of Al by the introduced microbes and the removal may probably be because of the influence of microbe immobilization of Al in the microcosm. The degradation of Al was shows two-fold higher removal with the amendment of microbes compared to control. The interaction of introduced microbes regardless of the number of strain or species showed enhanced Al reduction in the soil microcosm.

The presence of *Bacillus* sp. and *Pseudomonas* sp. may further enhance the removal of Al in the study. *B. vietnamiensis* could metabolize significantly in the presence of metals just as it is common to discover that substrate degraders easily adapt to the discrete substrate environment and become dominant microbe. These microbes could adopt metabolically mediated or physicochemical pathways of uptake when introduced into a contaminated system (Jayanthi *et al.*, 2016). All the treatment (A-G) showed significant difference when compared with control and (p=0.00).

4.6.2.4 Manganese (Mn)

Figure 4.46 shows Mn concentration in soil from Bukit Beruntung Landfill across time with different treatments. Highest reduction of Mn (49%) was recorded for Treatment F as illustrated in Figure 4.47, followed by Treatment B (46 %) while other treatments augmented with microbes showed Mn reduction that ranged from 34% to 45%. The Control experiment only showed 26.7 %. Treatment F has 1.8 times higher reduction capacity as compared to Control. There is significant difference at $P < 0.05$ between Treatment F and control ($p=0.00$). This probably is because the microbes in Treatment F was able to undergo metabolic reaction to uptake or degrade Mn from the contaminated soil. Variance in the heavy metal removal by different groups can be due to some

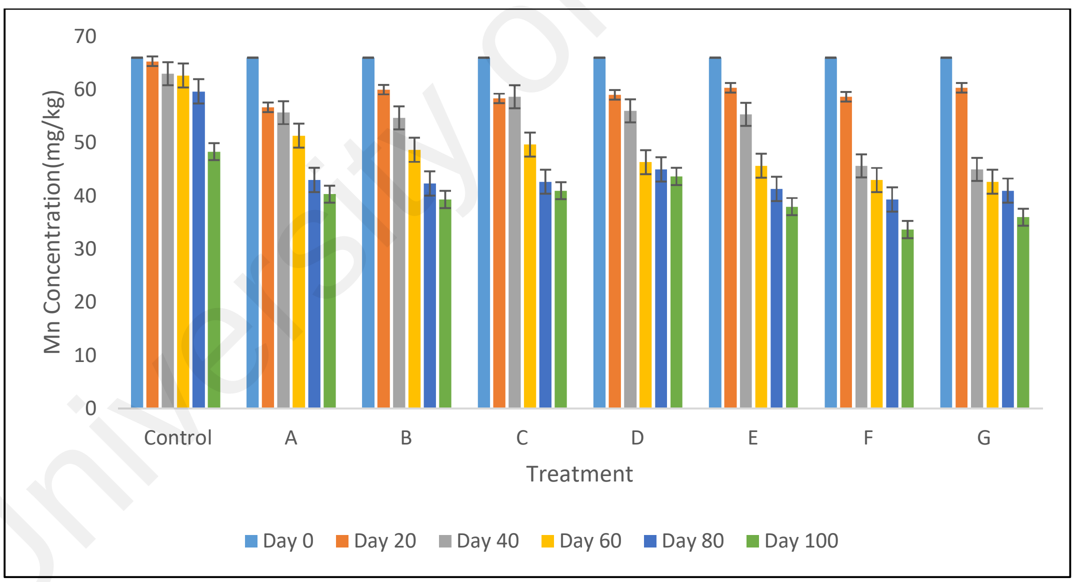

Figure 4.46: Mn concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

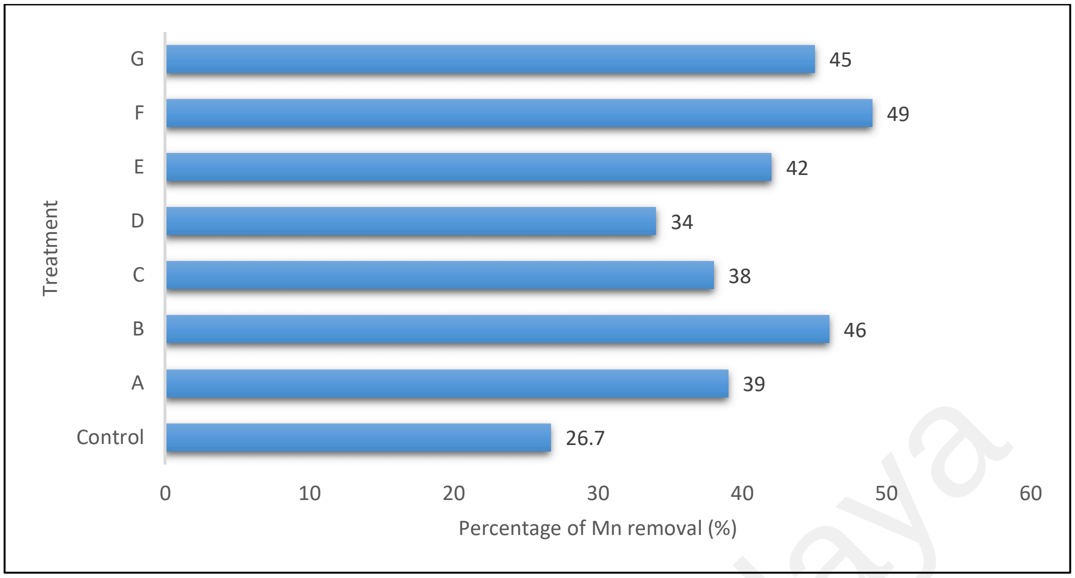

Figure 4.47: Percentage of Mn removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

microbes that tends to be more specific and sensitive to one metal but have higher tolerance to another metals and this is supported by findings of Nieto *et al*. (1989). The synergistic effect that occurs among the microbes in Treatment F (proteo-bacteria) for metal binding and Mn reduction may further enhance the removal rate compared to other treatments. The association of *A. caviea* DNA 4, *P. alcaligenes*, *P. mendocina*, *S. acidaminiphilia and O. intermedium* in proteo bacteria could be reason for higher reduction of Mn by Treatment F. *Stenotrophmonas* sp. was previously used to remove Mn from wastewater and 70% of reduction in the Mn concentration by Natalia *et al*. (2015). Jayanthi *et al*. (2016) reported microbes such as *A. caviea* DNA 4, *P. alcaligenes*, *P. mendocina*, *S. acidaminiphilia and O. intermedium* shows high tolerance towards Mn and can be good remediation agent.

4.6.2.5 Copper (Cu)

Cu concentration in soil from Bukit Beruntung Landfill across time with different treatments is illustrated in Figure 4.48. In this study, the highest Cu reduction was observed when the soil were amended with Treatment F and the percentage of Cu removal were 65% (Figure 4.49).

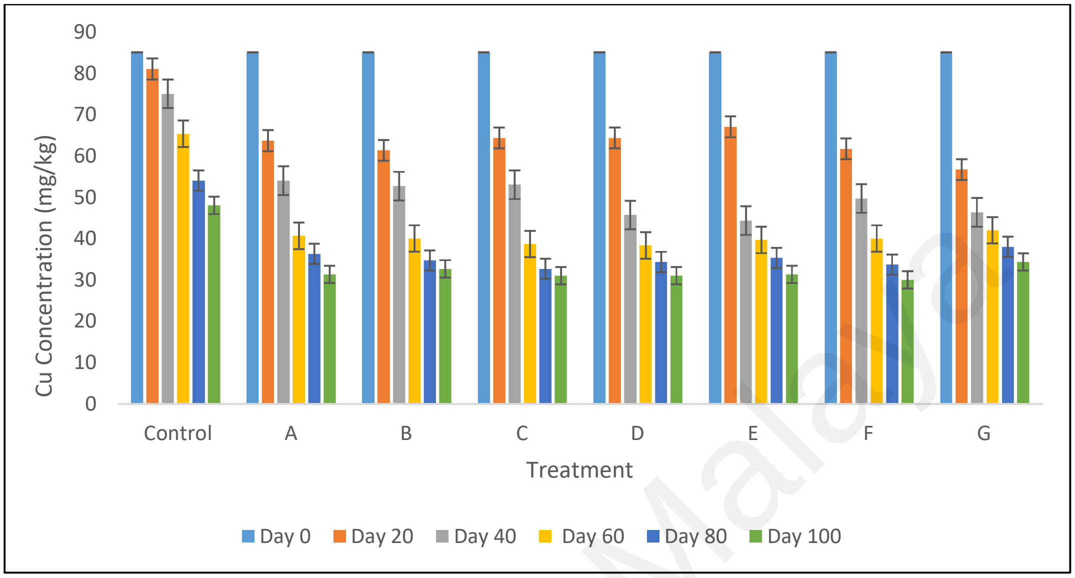

Figure 4.48: Cu concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

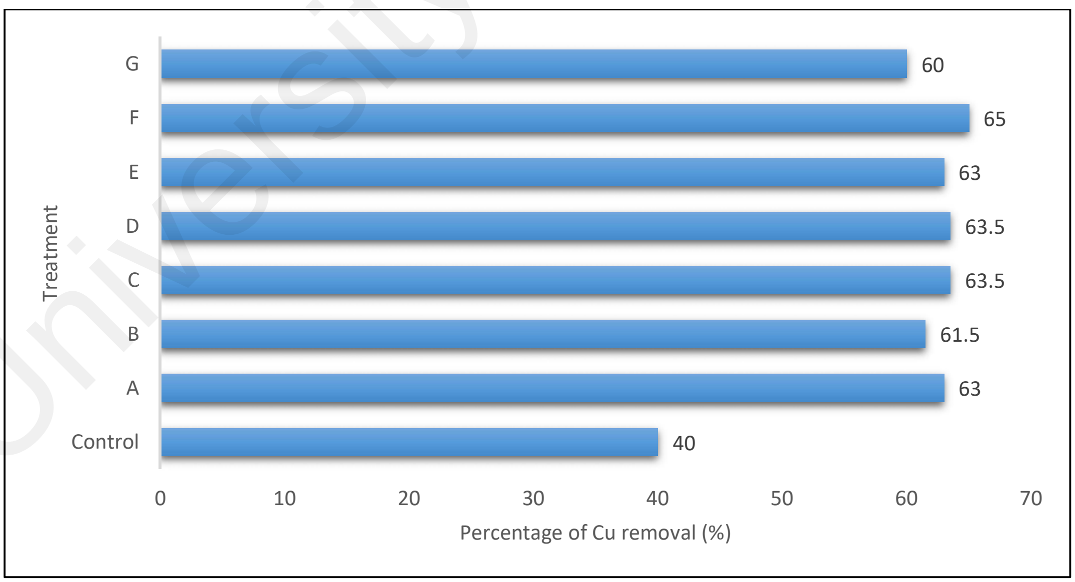

Figure 4.49: Percentage of Cu removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

Furthermore, other treatments able to reduce Cu to a significant level and the order of reduction were: Treatment F > D, C > A, E > B > G > control. The percentage of reduction by the treatments were ranged from 60 % to 65 % whereas control only showed 40% of Cu removal.

The bioaugmentation of microbes of contaminated soil showed significant Cu reduction. Similarly Ashok *et al*. (2011) also reported that addition of microbial consortia to metal contaminated soil can enhance Cu removal. The detoxification of metal by microbes in soil may possibly occur by metal binding involving chelators, such as metallothein, glutathione-derived-peptides called and metal binding peptides. These chelators bind to heavy metals and facilitate microbial absorption and the transportation of metal ions as suggested by Ayangnearo and Bababola (2017). *Stenotrophmonas* sp. and *Pseudomonas* sp. may have played major role in higher reduction in Treatment F because these organisms have been previously applied for Cu removal from wastewater and polluted effluents by Ghosha *et al*. (2012) and Elif *et al*. (2012).

4.6.2.6 Zinc (Zn)

Zn concentration in soil from Bukit Beruntung Landfill across time with different treatments is illustrated in Figure 4.50. Results showed the highest reduction was by Treatment D and removal percentage was 50% (Figure 4.51). It was a decrease in the concentration of Zn to 159.33 mg/kg from the initial of 319 mg/kg. Treatment D may have enhanced the soil microcosm to promote the removal of Zn. Significant difference were obtained between the concentrations of Zn initial and final of treatments. Other treatment showed reduction of Zn in ranged of 41 to 50% and control showed 15% of reduction. Statistical analysis showed significant difference at between the treatments (A-G) and control.

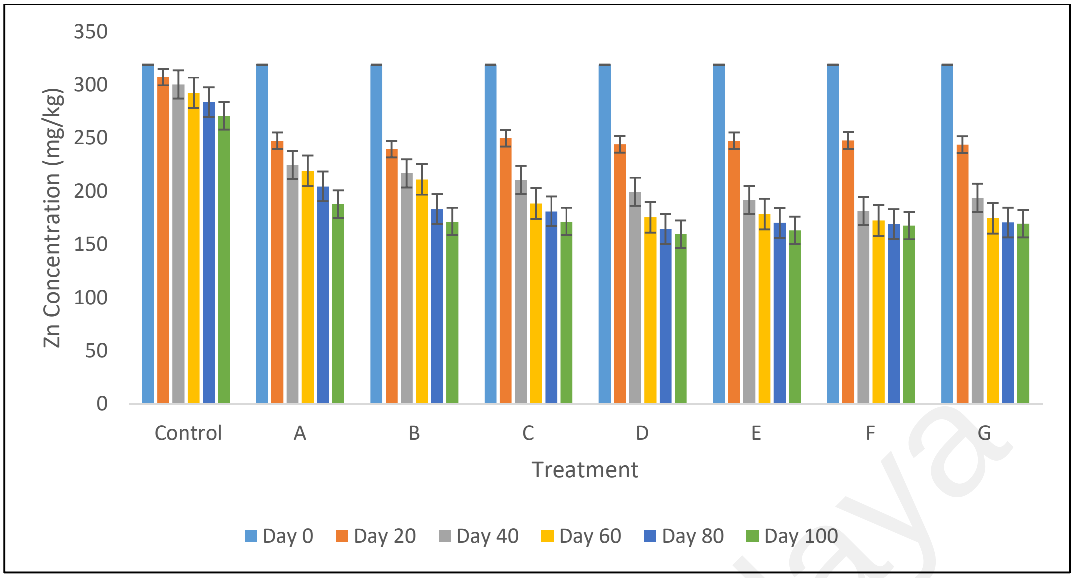

Figure 4.50: Zn concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

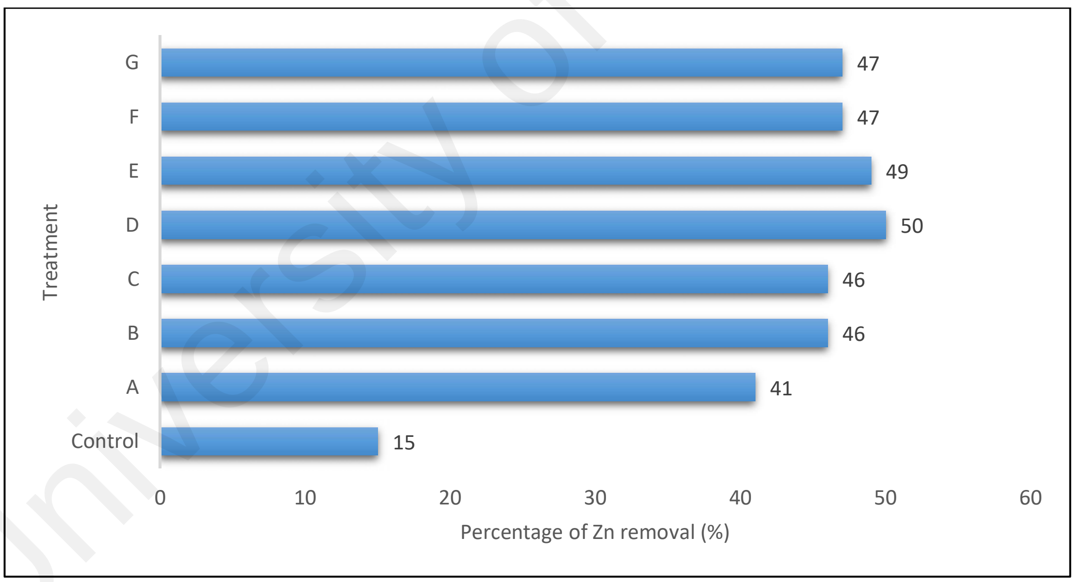

Figure 4.51: Percentage of Zn removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

Explaining the reason that caused higher Zn reduction by Treatment D is because the group contains the blending of microbes which was highly tolerant to metals. Therefore, the microbes were showing high tolerance towards metal such as Zn. The other treatments as well showed higher Zn reduction with the exception of control. The introduction of inoculum into contaminated soil played an important role for Zn removal in the soil. Each group of treatment contained one or more than one type of microbe that actively uptakes Zn into their cell or transform them and reduces the concentration of Zn in the soil and strains such as *Bacillus* sp. and *Pseudomonas* sp. were also previously reported to uptake Zn at higher rate (Aniszewski *et al*., 2010; Plociniczak *et al*., 2013; Babu *et al*., 2013).

4.6.2.7 Iron (Fe)

Fe concentration in soil from Bukit Beruntung Landfill across time with different treatments is shown in Figure 4.52. The residual concentration of Fe in Treatment F were less than levels found in other treatments. Treatment F showed an enhanced Fe removal and percentage of Fe removal 86%. Further analysis on the results also revealed that other treatments reduced Fe in range of 74.5% to 86% and control showed 27.5% of reduction. Significant differences between Treatment (A-G) when compared with control (p=0.00). The bioavailability of heavy metal to microbes could be the reason for the rapid removal of Fe from the soil because by microbes amended treatment (A-G) uptake the metal for their metabolic activities and in congruence with findings of Abioye (2013) and Jorgensen *et al*. (2000).

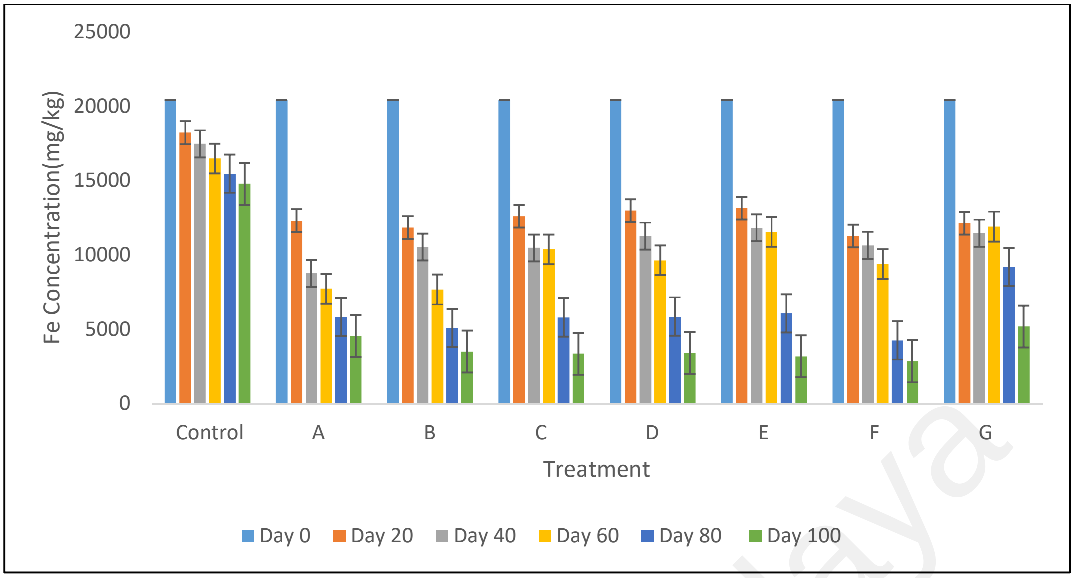

Figure 4.52: Fe concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

Comparison between the different treatments revealed that Treatment F demonstrated highest removal of Fe towards end of experiment. Treatment F was able to actively removed 86% (Figure 4.53) of Fe from the BB soil. Furthermore other treatments also remove Fe from BB soil between the ranges of 74.5% to 86%. The reduction of metal in soil by microbe is probably through immobilization mechanisms, and thereby reduce the bioavailability of metals. These biotransformation is an important component of biogeochemical cycles of metals exploited in bioremediation of metal contaminated soils as suggested by Gadd (2000). The higher reduction of Fe by Treatment F may probably be due to enhanced metabolic activity with augmentation of proteo bacteria. Proteobacteria group appeared to be associated positively with heavy metal removal and supported by findings of Tatiana *et al*. (2011). Significant differences between Treatment (A-G) when compared with control and p=0.00.

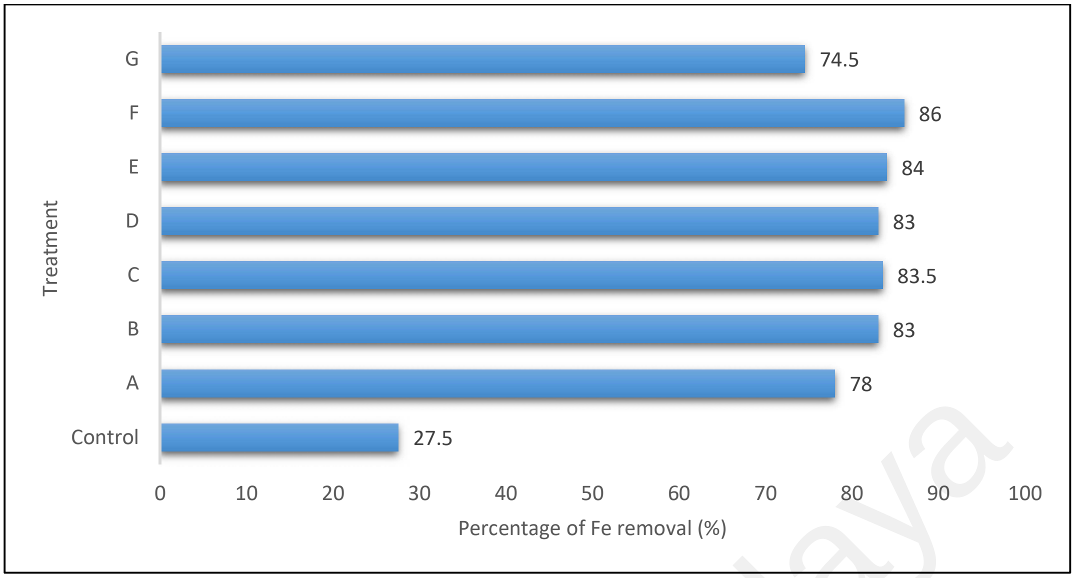

Figure 4.53: Percentage of Fe removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

4.6.2.8 Nickel (Ni)

Ni concentration in soil from Bukit Beruntung Landfill across time with different treatments is illustrated Figure 4.54. The highest reduction of Ni was by Treatment F. The initial concentration of Ni was 9 mg/kg and at end of 100 days experiment it was reduced to 2 mg/kg. The percentage of Ni reduction is illustrated in Figure 4.55 and the reduction of Ni follow the order of Treatment F (77.7%) > A, D, G (70%) > C, E (66.67%) > B (59%) > Control (40.7%). Significant reduction in the metal concentration was observed when the contaminated soil was amended with microbes. Treatments amended with microbes showed reduction of more than 50% when the Control gave only 40.7%.

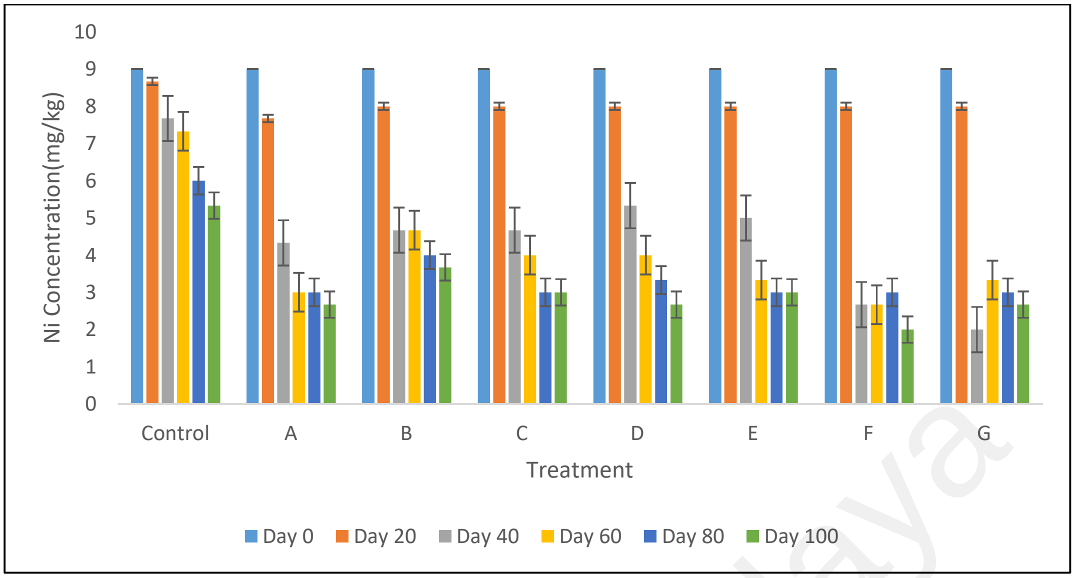

Figure 4.54: Ni concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

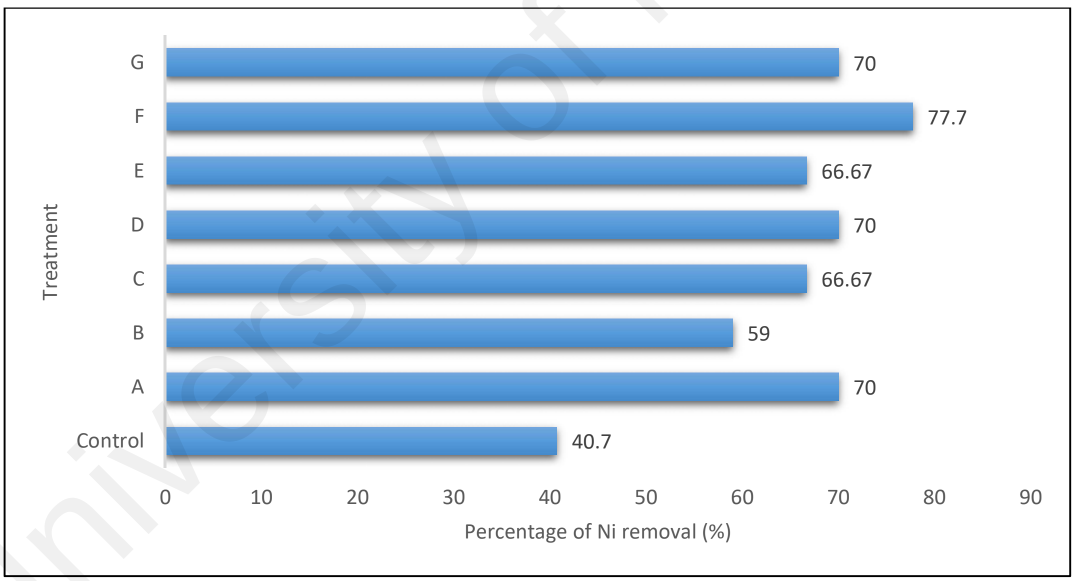

Figure 4.55: Percentage of Ni removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

The higher reduction by microbes amended treatment may probably be because the cell surface of all microorganisms are negatively charged owing to the presence of various anionic structures and its gives bacteria the ability to bind metal cations which can be either extracellular or intracellular accumulation (Ahalya *et al*., 2011; Renita *et al*., 2015).

Furthermore, blending of microbes used in study suggest that the combination possess a peculiar relations among the microbes that synergize Ni reduction especially by Treatment F. Selection of microbes is ideal for optimal removal of heavy metal and supported by Jeyasingh and Philip (2005) who stated that reduction of metal can take place with addition of indigenous microbes isolated from an existing contaminated site. Reduction of heavy metal by bacteria is mainly through the resistance of bacteria to heavy metals which is conferred by products of genes simulated on plasmids rendering genetic manipulations for strain improvement and supported by findings of Silver *et al*. (2001). Presence of *B. vietnamiensis* and *P. mendocina* may probably be related to highest reduction of Ni by Treatment F and these microbes were previously studied for Ni removal by Idris *et al*. (2004) and Chong *et al*. (2012). Statistical analysis showed that they are significant differences at $P < 0.05$ in the concentration of Ni between control and treatments (A-G).

4.6.2.9 Chromium (Cr)

Figure 4.56 illustrates the Cr concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments. The highest reduction in the Cr concentration after Day 100 was 67% by Treatment F (Figure 4.57). Treatment A and G also reduced 59% of Cr concentration in the contaminated soil. Control only shows 36% of Cr reduction. Besides that Treatment B, C and D, showed 49% reduction of Cr. The reason for higher removal

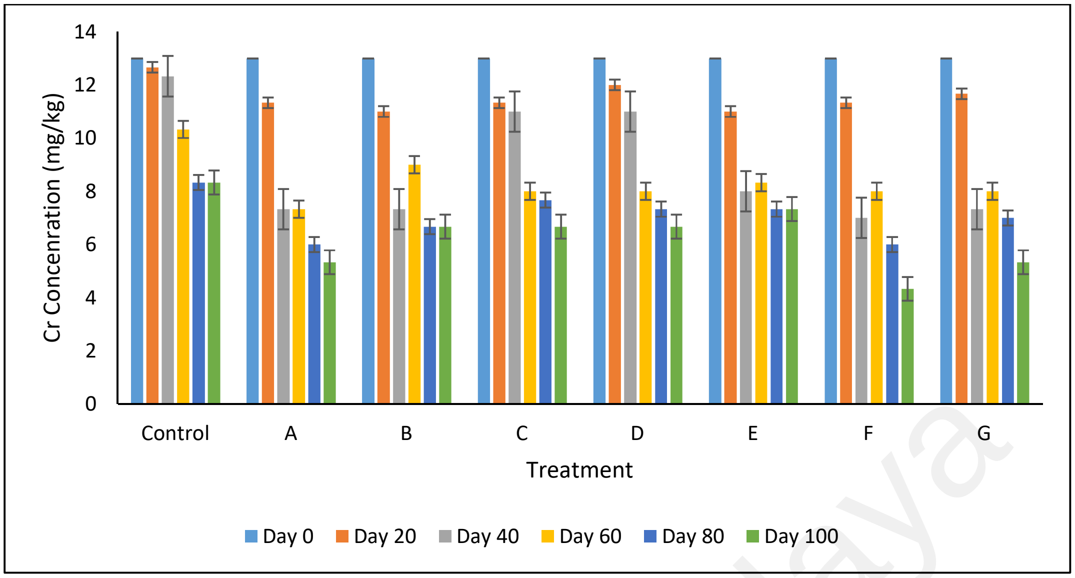

Figure 4.56: Cr concentration in soil from Bukit Beruntung Landfill across time with different bacterial treatments

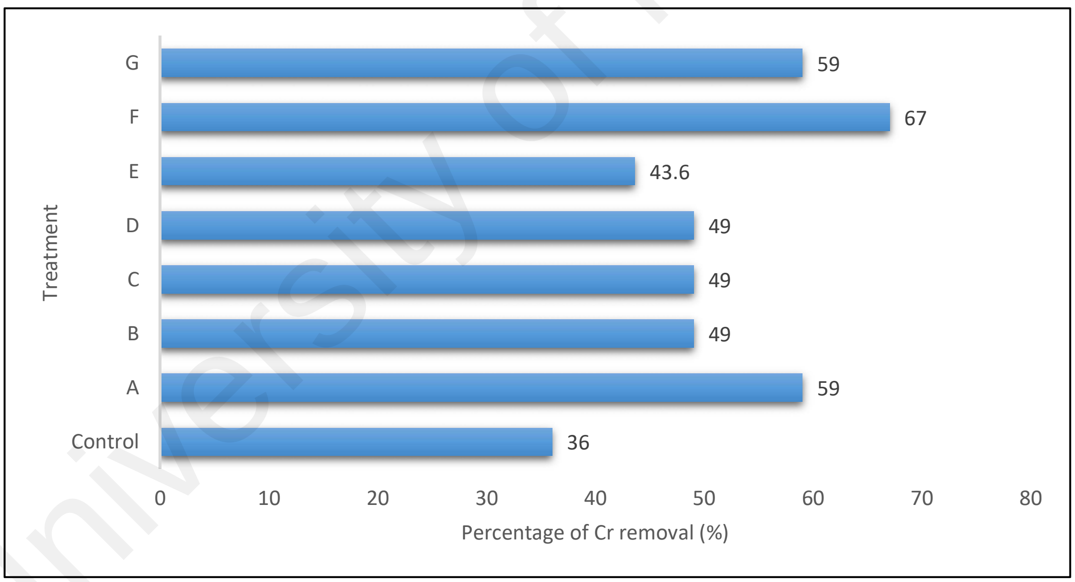

Figure 4.57: Percentage of Cr removed in soil from Bukit Beruntung Landfill during bioaugmentation experiment

of Cr by Treatment F can be attributed to the types of microbes present in the treatment which was able to actively uptake Cr from the soil using different strategies such as cell binding precipitation or any other method. Microbial transformations of metals are often the result of metal resistance mechanisms that include complexation and precipitation mechanisms as well as solubilization mechanisms that offer bioremediation strategies (Kumar *et al*., 2011). The reduction of Cr relies on the reduction of soluble and mobile hexavalent Cr (VI) to reduce form Cr (III) and the reduction occurs if the growth of microbes is stimulated via addition of carbon source (Adams *et al*., 2014).

There is a significant difference between Treatment (F) with control (p=0.007). Microbial reduction of Cr by Treatment F also can be due to presence of *Pseudomonas* sp. which catalyzed the metal by using soluble enzymes. Microbes demonstrated various types of resistance mechanisms in response to heavy metal exposure and this encoded by the chromosal genes, but the most usual loci conferring resistance are located on the plasmid of the microbes (Raja *et al*., 2006).

4.6.2.10 First order rate constant and half-life of heavy metals for bioaugmentation experiment of soil from Bukit Beruntung Landfill

Table 4.11 shows the first order rate constant of the heavy metals for bioaugmentation experiment of soil from Bukit Beruntung Landfill treated with different treatment at 10% v/w concentration. Between the eight different treatments, Treatment E and F have the highest first order rate constant for Al, at 0.02 day^{-1}. This implies that the two groups have higher tendency to convert Al in contaminated soil. This finding is agreeable to the results reported by previous bioaugmentation experiment set-up, for heavy metal contaminated sites (Alvarez *et al*., 2017). This is also similar to first order rate constant of Al from soil collected from Taman Beringin Landfill.

Besides that, Treatment F recorded the first order rate constant for Cr (0.01 day^{-1}), Ni (0.015 day^{-1}), Fe (0.0198 day^{-1}), Cu (0.01 day^{-1}) and Mn (0.0067 day^{-1}). This maybe because Treatment F contained the best microbial diversity for the maximum reduction of metals to occur. All treatments (A-G) was showed similar first order rate constant (0.019 day^{-1}) for As. For Pb and Zn the highest first order rate constant was by Treatment

C and Treatment D while Control has the lowest removal for all nine metals. Therefore, the augmentation of different microbial group and its combination may significantly increase the rate of removal constant of metal in soil.

Table 4.11: First order rate constant of heavy metals for bioaugmentation experiment of soil from Bukit Beruntung Landfill

Metal	Removal rate constant (k) day^{-1}							
	Control	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F	Treatment G
Pb	0.0021	0.0033	0.004	0.0046	0.0045	0.0036	0.0038	0.0041
As	0.0027	0.019	0.019	0.019	0.019	0.019	0.019	0.019
Al	0.006	0.019	0.019	0.019	0.019	0.02	0.02	0.019
Mn	0.0031	0.0049	0.0051	0.0047	0.0041	0.0055	0.0067	0.006
Cu	0.0057	0.01	0.0095	0.01	0.01	0.01	0.01	0.009
Zn	0.0016	0.0053	0.0062	0.0062	0.0069	0.0067	0.0064	0.0063
Fe	0.0032	0.015	0.017	0.018	0.0179	0.0185	0.0196	0.0137
Ni	0.0052	0.012	0.009	0.01	0.012	0.01	0.015	0.012
Cr	0.0045	0.0089	0.0067	0.0067	0.0067	0.0067	0.01	0.009

Half-life value of heavy metals for bioaugmentation experiment of soil from Bukit Beruntung Landfill amended with 10% v/w microbial treatment is given in Table 4.12. Among the metals tested, Al has the lowest half-life (34.65 day $^{-1}$) when treated with Treatment E and Treatment F, respectively. The removal of Al to be half are 34.65 day $^{-1}$ in average for both treatments. For metal such as Cr, Ni, Fe, Cr and Mn, the shortest half-time was with addition of Treatment F. Control shows the highest half-life compared to other treatments. Low removal rate and subsequent higher half-life in control treatment can due to reduction of metal by the indigenous soil microbes and this is agreeable with Adesodun and Mbagwu (2008).

Table 4.12: Half-life value of heavy metals for bioaugmentation experiment of soil from Bukit Beruntung Landfill

Metal	Half-life t½ (days)							
	Control	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F	Treatment G
Pb	331.65	210	173.25	150.65	154	192.5	182.37	169
As	256.67	36.47	36.47	36.47	36.47	34.65	34.65	36.47
Al	115.5	36.47	36.47	36.47	36.47	34.65	34.65	36.47
Mn	223.54	141.43	135.88	147.44	169.02	126	103.43	115.5
Cu	121.57	69.3	72.94	69.3	69.3	69.3	69.3	77
Zn	433.12	130.75	111.77	111.77	100.43	103.43	108.28	110
Fe	216.56	46.2	40.76	38.5	38.71	37.46	35.35	50.58
Ni	133.27	57.75	77.86	69.3	57.75	69.3	46.2	57.75
Cr	155.73	77.8	103.43	103.43	103.43	121.57	63	77.86

4.6.2.11 Bacterial count for bioaugmentation experiment of Bukit Beruntung Landfill soil

Bacterial count across time with different bacterial treatments for remediation of soil from Bukit Beruntung Landfill soil is depicted in Figure 4.58. Bacterial count during the bioaugmentation experiment of Bukit Beruntung Landfill soil showed a fluctuated throughout the 100 days At Day 0, the average count in the treatments was 6.6×10^9 CFU/g and control accounts for 5.6×10^9 CFU/g. Similarly, Abioye (2012) also reported higher count of bacteria in the amended soil compared to Control. At Day 20, increase in the bacterial population was observed for all the treatments including control compared to the initial concentrations. The increase of the microbial population is because microbial cells were duplicating with the available nutrients therefore increase in the population is expected.

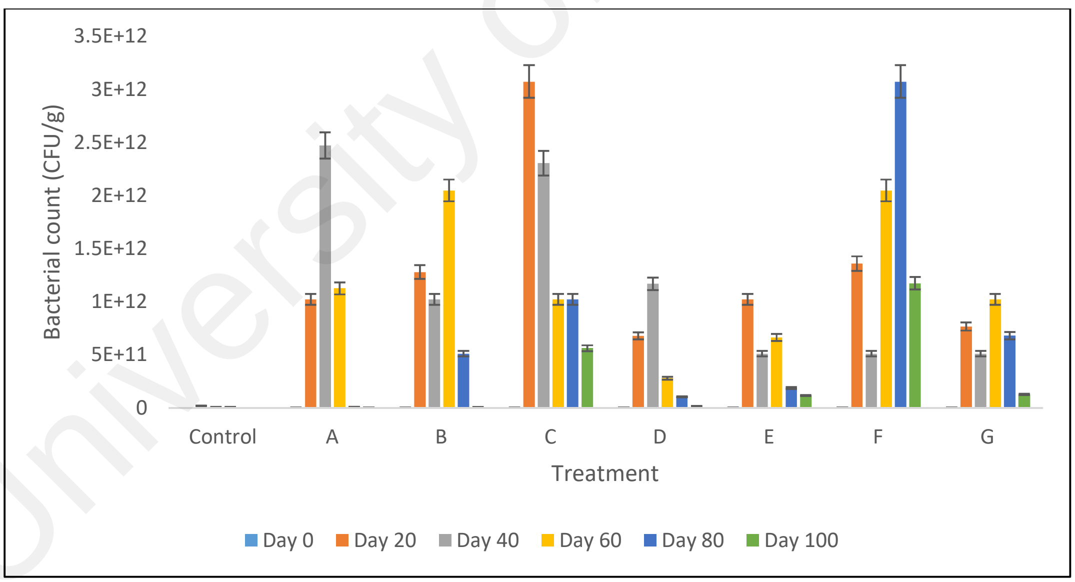

Figure 4.58: Bacterial count across time with different bacterial treatments for remediation soil from Bukit Beruntung Landfill

At Day 40, increase in the bacterial count was observed for Treatment A and C while all other treatments were showed decreasing trend in the count of bacteria. The condition was conducive enough for both of treatment to have increased count. At Day 60, the population were increasing for Treatment B, D, E, F and G while the remaining treatments shows decreased in bacterial count. Difference in microbial ecology of the treatments can contribute to bacterial proliferation which shows different in counting of the bacteria and as supported by Antai and Mgbomo (1989).

Towards the 100 days of remediation, Treatment F showed higher microbial count as compared to other treatments which was also significantly difference between Treatment F and other treatments. It could be because it was one of the treatments that has highest metal reduction as compared to other treatments. For all treatments decrease in the counting of bacteria is expected because the bacteria cell are no longer duplicating due to lack of nutrients towards end of experiment and confirmed by Emenike (2013).

4.6.2.12 Soil redox potential for remediation soil from Bukit Beruntung landfill

Soil redox potential for bioaugmentation experiment of soil from Bukit Beruntung Landfill is illustrated in Figure 4.59. The variation across the biomonitoring days was observed for all the treatments indicating the solubility of the heavy metals in the soil. Similar to results obtained for the soil remediation of soil collected from TBL, highest value of redox potential was observed in Treatment F (280.2 mV) at end of the biomonitoring day. This indicates that metal reduction is taking place and this is in agreement of Emenike *et al*. (2017) who also reported similar increase in the soil redox potential value for bioremediation studies. All treatments showed increase in the soil redox potential value at Day 100 even though at Day 40, redox potential value decreased in some treatments. The increase in soil redox potential value showed that the concentration of metal decreased with the decrease in the solubility of metal.

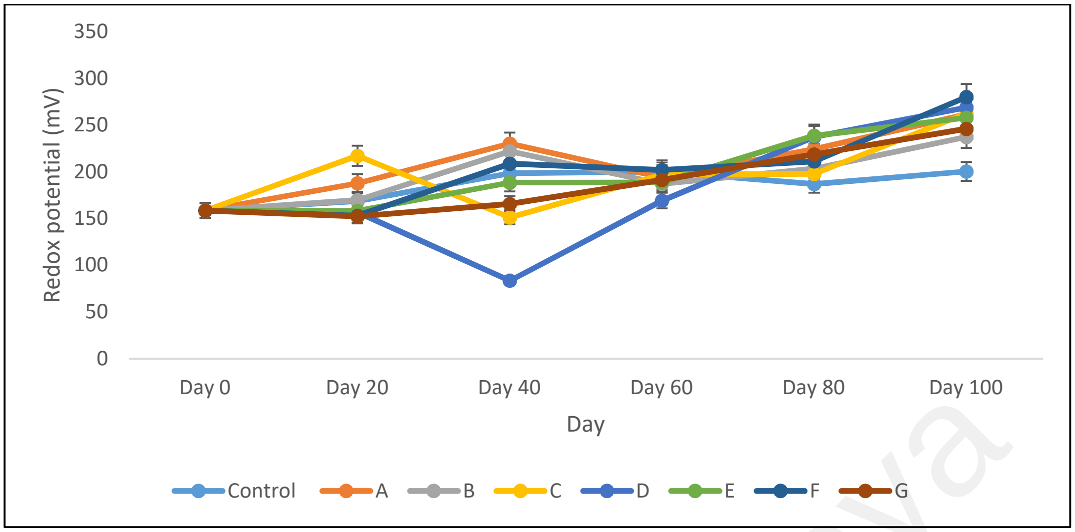

Figure 4.59: Soil redox potential across time with different treatments for remediation of soil from Bukit Beruntung Landfill

4.6.2.13 Soil pH for remediation of soil from Bukit Beruntung Landfill

The soil pH for bioaugmentation experiment of soil from Bukit Beruntung Landfill is illustrated in Figure 4.60. The initial pH of the soil used for bioremediation studies was pH 8.14. Throughout, the remediation the pH changed from pH 7 to 9. The pH range was appropriate for microbes to carry out biological activities. All treatments including Control showed slightly lower pH (7.42-7.7) at Day 20, however after Day 20 it returned back to neutral pH until end of remediation. The biological activities are regulated by enzymes that operate within a fairly stringent range of pH. When the extremes of this range are exceeded then microbial growth will be disrupted. Boonchan (2000) and Joanne *et al*. (2008) reported that optimum pH for bioremediation is between 6.0 and 8.9. From the results obtained it indicates that pH level fell within a suitable range to support microbial growth in all the treatment categories and also control.

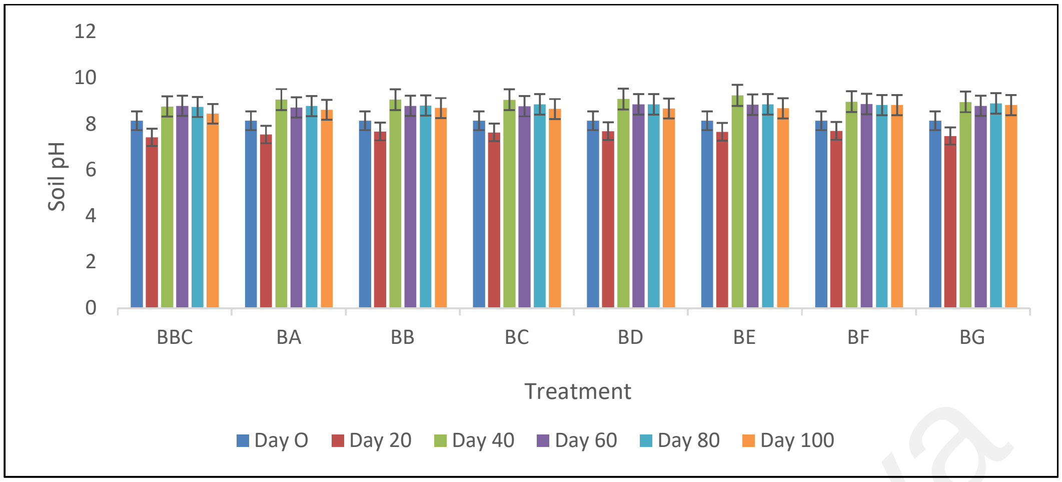

Figure 4.60: Soil pH across time with different treatments for remediation of soil from Bukit Beruntung Landfill

4.7 Effect of inoculum concentration on remediation of heavy contaminated soil (Taman Beringin landfill and Bukit Beruntung landfill)

Previous section discussed the impact of the microbial diversity utilized for the bioreduction/ removal of metals from the polluted soil and results showed significant change in the metal concentrations across the eight treatments, wherein Treatment F was the most promising soil amendment. Therefore, the study deemed necessary to investigate further on the optimization of the metal removal potentials of the treatments with respect to varying the inoculum concentrations in the soil microcosm. The present study hypothesized that increasing the inoculum concentration from 10% v/w to 20% v/w and 30% v/w, respectively will significantly remove the metals from the soil microcosm than the use of 10% v/w. Therefore Table 4.13 and Table 4.14 illustrates the metal reduction in Taman Beringin and Bukit Beruntung landfill soil across the induced concentration with respective to treatments characterized of varying microbial diversity.

Table 4.13: Percentage of heavy metal removal with addition of 10, 20 and 30% v/w of bacterial inoculum for remediation of soil fromTaman Beringin Landfill

		Percentage of heavy metal removal (%)								
Treatments	**Concentration of treatment(% v/w)**	**Pb**	**As**	**Al**	**Mn**	**Cu**	**Zn**	**Fe**	**Ni**	**Cr**
Control	**10**	31.5	46	34	11	37	26	36	44	42
	20	31.5	44.1	34	9.45	35	25.7	36	44	42
	30	31.5	44.1	34	9.45	35	25.7	36	44	42
A	**10**	39	51	86.5	19	66	43.5	42	52.3	49
	20	35.2	55.6	44.5	28.5	66	32.65	57.1	52.4	65.2
	30	35.2	64.4	53	34.5	68.9	38.1	59.6	54.8	59.2
B	**10**	39	52	82	37	63	63	62	51	43
	20	42.6	60.2	45.8	43	59.3	55.1	62	47.6	57.2
	30	42.6	65.6	49.7	49.8	59.3	56.6	62.8	57.5	47.4
C	**10**	43	47.5	86	33	63	42	69	52.3	46
	20	44.4	70.2	47.2	38.9	69.4	30.6	65	52.4	63
	30	46.3	61.4	52.9	42	70	34.2	66	54.8	44.7
D	**10**	39	60	81.5	27	67	60	68	52.3	50
	20	37	69.6	52.8	35.6	73.4	48.9	63.17	52.4	65.9
	30	38.9	73.5	50.7	40.2	73.4	49.3	66.3	60.3	48.7
E	**10**	31.5	56	79	37	61	56	70	49	46
	20	35	63.4	53.9	39.8	55.9	48.9	70	55.5	63
	30	35.2	73.25	42.5	49.1	57.1	46.7	69	58.9	59.9
F	**10**	33	61	87	47	64	53	75	59	61
	20	33	74.4	55.2	48.7	62.7	40.8	76	57.1	56.5
	30	37	75.1	55.1	53.9	60.5	43.4	76	63	64.5
G	**10**	41	55	86	30	60	50	27	51	41
	20	42.6	67	48	36.6	54.2	36.7	57.10	47.6	60.8
	30	42.6	69	53.2	41.9	54.8	43.2	59.4	58.9	46.7

Table 4.14: Percentage of heavy metal removal with addition of 10, 20 and 30% v/w of bacterial inoculum for remediation of soil from Bukit Beruntung Landfill

		Percentage of heavy metal removal (%)								
Treatments	**Concentration of treatment(% v/w)**	**Pb**	**As**	**Al**	**Mn**	**Cu**	**Zn**	**Fe**	**Ni**	**Cr**
Control	10	18	29	45	26.7	40	15	27.5	40.7	36
	20	18	29	45	26.7	40	15	27.5	40.7	36
	30	18	29	45	26.7	40	15	27.5	40.7	36
A	10	29	86	85.7	39	63	41	78	70	59
	20	29	86.67	85.9	38.9	63.2	43.1	81.7	72	60.4
	30	29	86.67	87.7	38.9	63.2	44.5	82.9	73.3	61.5
B	10	33	86	86	46	61.5	46	83	59	49
	20	33.9	86.67	87.1	46.55	62.84	46.5	83.6	60	56.3
	30	35.5	86.67	88.5	47.11	63.9	46.7	84.8	60	58.33
C	10	37	86	85	38	63.5	46	83.5	66.67	49
	20	37.2	86.67	87.9	38.33	65.3	48.1	84.2	68	50
	30	38.3	86.67	88	39.44	65.6	49.9	86.7	68	50
D	10	36	86	86	34	63.5	50	83	70	49
	20	37.2	86.67	85.9	40	65.6	50.5	86.3	70.7	49
	30	37.8	86.67	87.2	40	66.6	50.9	85.8	74.7	50
E	10	30.3	86	87	42	63	49	84	66.67	43.6
	20	30.5	86.67	87.5	43.33	63.54	49.9	85.7	66.7	50
	30	32.8	86.67	88	44	63.9	50	87.1	68	52.1
F	10	32	86	87.5	49	65	47	86	77	67
	20	33.3	90	88.5	56	65.6	51.5	86.6	78.7	67.7
	30	34.4	90	89.5	56.11	68.4	59.0	90	80	77.10
G	10	34	86	86	45	60	47	74.5	70	59
	20	34	86.67	87.7	46.67	60	48.9	82.1	70	59.3
	30	34	86.67	87.9	49.44	60.41	49.4	84.90	74.67	61.5

4.7.1 Lead (Pb)

The effect of inoculum concentration on soil bioaugmentation experiment of Taman Beringin Landfill revealed increased reduction of Pb with the amendment of Treatment B, C, E, F and G with increase in the inoculum concentration from 10% to 30% v/w. Amendment of Treatment C with different inoculum concentration showed increase in percentage of reduction whereby at 10% v/w the reduction was 43% and it further increased to 44.4 % and 46.3% respectively for 20 % and 30% v/w inoculum concentration. Other treatments as well showed increase in the Pb reduction with about 2% when the inoculum concentration was decreased. Similarly, this also congruence with findings of Kathiravan *et al*. (2011) reported that increase in the inoculum size showed an enhancement in bio removal of heavy metals from contaminated system.

Amending the soil from Bukit Beruntung landfill with 20% and 30% v/w of the inoculum also recorded variations to the degree of metal reduction across the microbial diversity. The effect of inoculum concentration on Pb reduction showed that treatment amended with 30% v/w inoculum concentration possess higher Pb reduction compared to 10 % v/w and 20% v/w concentration for all treatments (A-G). At 10 % v/w, all the treatments were showing least Pb reduction however by increasing the inoculum concentration to 20% and 30% v/w concentration increased in the reduction was observed. Soil amended with Treatment C showed highest Pb reduction at all three concentration (10-30% v/w) compared to other amended treatments. This result also concurred with study by Ronald (1993), who reported that the rate of degradation increased linearly as the concentration of cell increased. It can be observed that from this study, higher the number of microbe cell the higher the metal reduction.

4.7.2 Arsenic (As)

Soil collected from Taman Beringin Landfill showed increased As reduction with increased inoculum concentration (20- 30% v/w) where at 10% v/w, the reduction was ranged at 47.5 % to 61% while at 20 % and 30% v/w increased As reduction in the range of 55.6 % to 74.4 % and 61.4 % to 75.1%, respectively by Treatment (A-G). Significant difference in the As removal among the three concentrations (10- 30%v/w) was observed for all the treatments amended with microbes. Treatment C showed the least removal with 10% v/w amendment, but when the concentration of inoculum was increased to 20% v/w, higher reduction were recorded. Therefore, the reduction of As can be further increased with increasing concentration of enhance the enhance the metal uptake and transformation by the microbes. Wang *et al*. (1990) also reported increased reduction of heavy metals with increase in the cell density.

Increase in concentrations of inoculum to 20% v/w showed enhanced As reduction for all the treatments (A-G) with the highest reduction possessed by soil amended with Treatment F for remediation of soil collected from Bukit Beruntung Landfill. However, increase in the inoculum concentration (30% v/w) showed no change in percentage of reduction for all the treatments (A-G). Therefore optimization of inoculum concentration is necessary to obtain optimal metal reduction. This directly conform with study by Sabu *et al.* (2005) who reported that it is important to optimize size of inoculum to obtain optimal metal reduction. Quantity of inoculum plays an important role in the enzyme/ metabolic activity of heavy metal. At low concentration of inoculum the metabolic activity can be slow and with higher concentration competitive between the microbial populations can occur therefore optimization of inoculum is necessary.

4.7.3 Aluminium (Al)

Increase in the inoculum concentration (20 -30% v/w) decreased Al reduction for all treatments (A-G) for soil from Taman Beringin Landfill. When only 10% v/w inoculum were added to the soil microcosm, the reduction by all treatments were above 75% whereas 20% and 30% v/w inoculum recorded below 60% reduction for extractable Al. Redzwan *et al*. (2015) using *Clostridium* sp. at different inoculum concentration (5-15%v/v), where the highest rate of reduction was recorded at the least inoculum concentration (5% v/w). In soil collected from Bukit Beruntung Landfill, increased concentration of inoculum resulted in higher reduction of Al at 30% v/w for all treatments (A-G). The overall reduction of Al was also above 80% for all treatments (A-G). Similarly, Pal and Paul (2004) also observed increased reduction of metals using increased cell concentration of *Bacillus* sp.

4.7.4 Manganese (Mn)

Observing the effect of inoculum amendment of Mn reduction, the study showed that all the treatments (A-G) have increased Mn reduction with the increase in the inoculum concentration (20- 30% v/w). When only 10% v/w of inoculum was introduced into the soil, the least Mn removal was recorded in Treatment A (19%), but at increased concentration, 20 % and 30% v/w the reduction increased to 28.5% and 34.5%, respectively. There are significant differences between the three concentrations for Treatment A. Similarly, treatments (B-G) also showed significant differences when the inoculum were increased from 10% to 20% and 30% v/w. Hence, it implied that at 30% v/w amendment increase the Mn removal was two fold increase. This directly conforms with Mujeeb *et al*. (2006), who reported that the increase in the inoculum size provided a higher number of bacterial cells and resulted in higher metal reduction. Recall that Treatment F was the most significant amendment when 10% v/w inoculum was used, yet the overall Mn reduction was less than 50% in all treatments. Approximately 54% of Mn was eventually removed when the polluted soil was amended with a higher concentration (30% v/w). It is possible that the interaction between the microbial diversity from Treatment F and dissociated Mn from the leachate polluted soil required increase in the cell density per unit area to enhance ion immobilization and transformation into metal complexes.

Reduction of Mn in soil from Bukit Beruntung Landfill also increased with increase in the inoculum concentration for all treatments (A-G). Higher reduction of Mn was observed at 30% v/w inoculum concentration where at 10% v/w of inoculum removal recorded by Treatment F was 49%, but increasing the concentration, 20 % and 30% v/w gave 56% and 56.1% reduction, respectively. Statistical analysis also showed significant difference among the three concentrations of Treatment F (10% v/w, 20% v/w and 30%). Hence, it implied that at 30% v/w amendment increased the Mn reduction in the soil. This directly conforms with findings of Sharifzadeh and Hossein (2015).

4.7.5 Copper (Cu)

The results from Taman Beringin (Table 4.13) revealed a variation where the increase in the inoculum concentration (20% and 30% v/w) showed increase in the Cu reduction for Treatment A, C and D while other treatments (B, E, F and G) showed a decreased in the reduction of Cu. The findings are consistent with previous reports on the increase in the reduction percentage with increase in the inoculum size however its also depends on the group of microbes (Pattanapipitpaisal *et al.*, 2001; Pal & Paul, 2004; Sultan & Hasnain, 2007). This development could be due to some complex interactions and behaviour of microbes with respect to cellular responses when cell density is high hence possible mineralization and solubilisation could have influenced ionic dissociation when

20% - 30% v/w were used. Therefore Cu reduction may not fully dependent on the inoculum concentration rather bacterial diversity.

Study on effect of inoculum concentration on soil from Bukit Beruntung Landfill revealed increased Cu reduction with increased inoculum (20-30% v/w). This also concurred with study by Ezaka (2012), who highlighted that increase in inoculum concentration of *Bacillus* sp., *Pseudomonas* sp., *E. coli* and *Staphylococcus* sp. increased the reduction of metal. Highest reduction of Cu was obtained in Treatment F. At 10%, 20% and 30% v/w concentration, Treatment F recorded 65%, 65.6% and 68.4% of Cu reduction respectively.

4.7.6 Zinc (Zn)

The effect of inoculum concentration on Zn reduction in soil from Taman Beringin Landfill, showed that all the treatments (A-G) showed decreased in Zn reduction as the inoculum concentration increases (20-30% v/w). 10% v/w of inoculum has the best results for Zn reduction as compared to 20-30% v/w. Hence for Zn removal from the contaminated soil the optimal reduction of Zn was observed at 10% v/w of inoculum concentration and therefore any further increased in the inoculum concentration did not enhance Zn reduction. This results also concurred with study by Basu *et al*. (2014), who reported that introduction of larger volume of inoculum in media did not affect removal of heavy metals. This may be due to the fact that higher number of bacterial cells reduces the probability of contact between bacteria cells and Zn and it's not necessarily increase the removal capability with increased inoculum concentration. Similarly, observation was made by another group of researchers who reported higher biotransformation of metal at lower cell density (Philips *et al*., 1998; Debasmita & Rajasimman, 2013).

Increase in the inoculum size (20% - 30% v/w) increased the reduction of Zn and the maximum reduction of Zn was recorded at 30% v/w inoculum concentrations for all treatment (A-G) in soil of Bukit Beruntung Landfill. Significant increase in the reduction of Zn possess by soil amended with Treatment F. Enhancement in the Zn reduction was observed for soil amended with high concentration of inoculum and the results from this study also concurred with Brinda and Velan (2011).

4.7.7 Iron (Fe)

The results showed that almost all treatments except Treatment C and D have increased abilities in Fe reduction with the increase in the inoculum concentration (10-30% v/w) for soil from Taman Beringin Landfill. Among the treatments, Treatment G recorded two-fold increased Fe reduction, where 27% removal was recorded under the influence of 10% v/w, 57.1% and 59.4% were recorded under 20% and 30% v/w, respectively. This indicates that amendments with 20% and 30% v/w inoculum to the soil reduced the Fe in Treatment G. This conforms to findings by Mona (2008) and McLean *et al*. (2000) significant difference at between the inoculum concentrations of Treatment G (10% v/w, 20% v/w and 30% v/w).

Similarly, reduction of Fe increased in the soil from Bukit Beruntung for all treatments (A-G) as concentration of the inoculum was increased (20-30% v/w). The highest reduction of Fe was observed with amendment of Treatment F. When the microcosm was amended with only 10% v/w of inoculum the reduction of Fe was 86% and with further increase of inoculum concentration at 20% and 30% v/w showed 86.6% and 90% of Fe reduction, respectively. In as much as the differences with respect to percentage of reduction may appear little, but statistically, the distribution showed significant differences.

4.7.8 Nickel (Ni)

Ni reduction was increased in all treatment (A-G) for soil from Taman Beringin Landfill when the inoculum concentration was increased (20-30% v/w). The higher removal of Ni was for Treatment D and E. When 10% v/w concentration was introduced, the reduction of Ni concentration by Treatment D was 52.3 %, but it reached 60.3% when 30% v/w of inoculum was used. Hence, this implies that at 30% v/w concentration of inoculum higher removal of Ni can be obtained. Statistical analysis also revealed that the increase in the inoculum concentration shows significant difference in the concentration of Ni (p=0.00). Similar increase was also observed for Treatment E, from 49% at 10% v/w concentration to 55.5% and 58.9% at 20% v/w and 30% v/w concentration, respectively. This study also agrees with the findings by Mujeeb *et al*. (2006) which reported higher rate of metal reduction at higher inoculum size.

Ni reduction increased for all treatment (A-G) for soil from Bukit Beruntung Landfill. The most increased was by Treatment D and G. When 10% v/w inoculum of Treatment D were added, the reduction of Ni was 70% but further increased to 74% at 30% v/w. Similarly, Treatment G also has increase in Ni reduction when soil were amended with 30% v/w inoculum concentration. There is a significant difference in Treatment D and Treatment G.

4.7.9 Chromium (Cr)

The results showed an increase in the concentration of Cr reduced as the inoculum size increased from 10% to 20% v/w for all treatment except Control and Treatment F for soil collected from Taman Beringin Landfill. There a significant difference in the percentage of Cr reduced between 10% and 20% v/w. At 10% v/w most of the treatments showed reductions of between 41% to 50% with exception of Treatment F but at 20% v/w reduction to 55% to 66%. However, further increase in the inoculum to 30% v/w resulted in decrease in the Cr removal for all treatments. This may due to the fact that, 20% v/w is the most optimal concentration required for Cr removal. This is agreeable with Muhammad and Shahida (2003) reported that the increase the inoculum size from of 2.4 x 10^7 cells/ml to 9.6 x 10^7 cells/ml increased the rate of Cr reduction.

As for contaminated soil from Bukit Beruntung all treatments (A-G) showed increased in reduction of Cr with the increased in the inoculum concentration (20-30%v/w). The highest reduction was by Treatment B, E and F and there are significant differences between the different concentrations. Hence it implies that at 30% v/w, higher reduction of Cr was observed. The overall comparison showed that Treatment F reduction above 75% at 30% v/w concentration compared to lower inoculum concentration amendment. This study also concurred with the study by Emadzadeh *et al.* (2016) who reported that increase of *B.cereus* inoculum concentrations from 5ml to 20 ml showed that increasing the concentration resulted in an increased rate of Cr removal.

4.7.10 Bacterial count in soil of Taman Beringin Landill and Bukit Beruntung landfill with inoculum amendment at 20% and 30% v/w amendment

Figure 4.61 and Figure 4.62 show the bacterial count in soil Taman Beringin and Bukit Beruntung landfill, respectively when treated with 20% inoculum fluctuated throughout the 100 days experiment. Initial bacterial count for the treatment of soil from TBL ranged from 2.99×10^{11} CFU/g to 2.4×10^{12} CFU/g while the control was 2.3×10^8 CFU/g. At Day 60, the count increase in Treatment A, F and G while it was decreasing in other

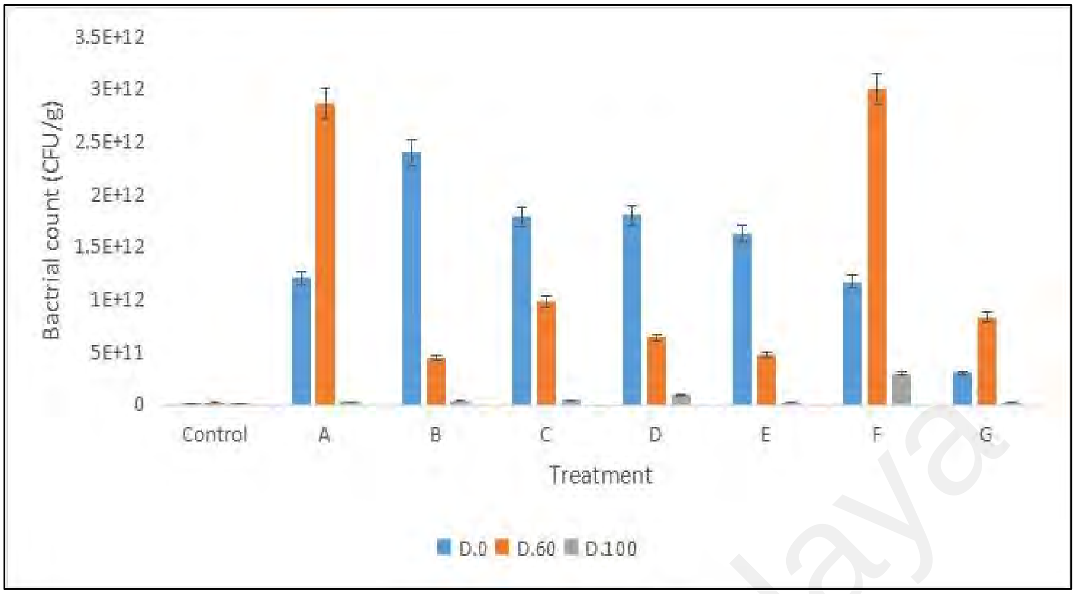

Figure 4.61: Bacterial count across time with different treatments for remediation of soil from Taman Beringin Landfill amended with 20% of inoculum concentration

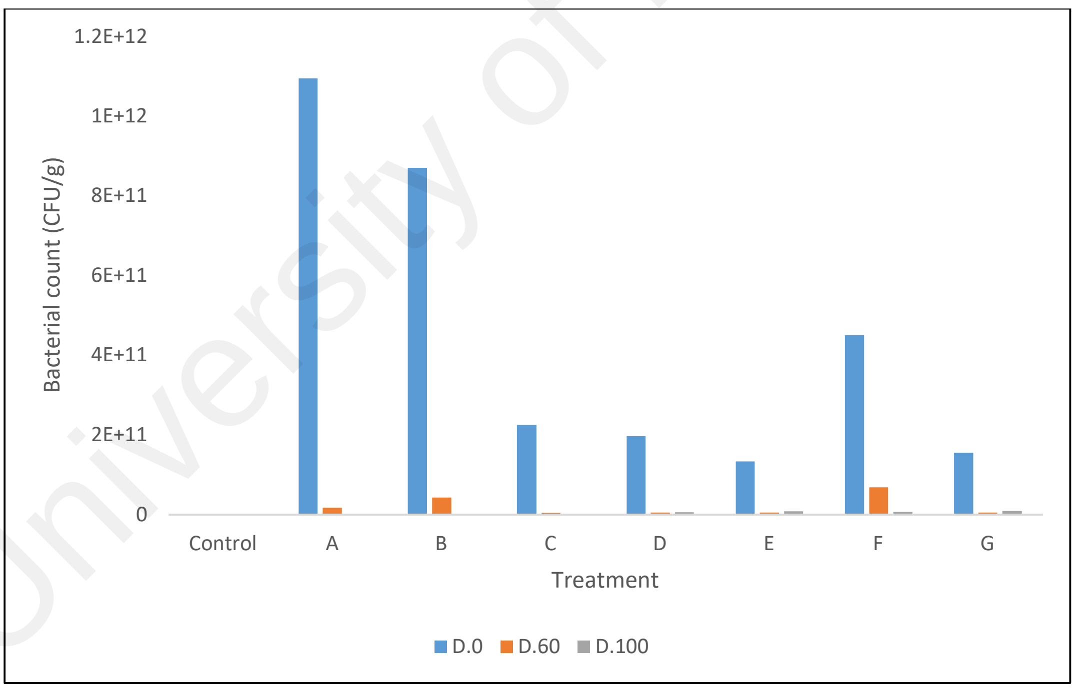

Figure 4.62: Bacterial count across time with different treatments for remediation of soil from Bukit Beruntung Landfill amended with 20% of inoculum concentration

treatments. The decrease in the microbial population may be due to the drop in available nutrients that discourage rapid multiplication. Similar decrease in the population growth was observed with experiment using 10% v/w inoculum. Bacterial count at Day 100 was reduced in all treatments as compared to Day 0. Such reduction can occur because cell duplication is hindered due to nutrients depletion and this changes was significant difference for all treatments (A-G)

For soil from Bukit Beruntung Landfill, the initial bacterial count applied with 20 % v/w inoculum, ranged between 1.33×10^{11} CFU/g to 1.094×10^{12} CFU/g while control was 6×10^{8} CFU/g. At Day 60, decrease in the bacterial counts was recorded for all treatments (A-G). The decrease may due to depletion of nutrient for microbial survival. The bacterial count continue to drop at Day 100.

Bacterial count in the remediation of soil collected from Taman Beringin Landfill and Bukit Beruntung landfill using 30% inoculum are illustrated in Figure 4.63 and Figure 4.64, respectively. There is a decreasing trends in bacterial count throughout the 100 days where it was 3×10^{9} CFU/g to 3.64×10^{13} CFU/g at Day 0. At Day 60, almost all the treatments showed decrease in bacterial count and further decreased at Day 100 to 2.56×10^{9} CFU/g to 2.8×10^{11} CFU/g. There is a significant difference bacterial count between Day 0, 60, and 100 for all treatments (A-G).

Bacterial count for Bukit Beruntung using 30% v/w concentration recorded a decrease in bacterial count across the monitoring days. Initial bacterial count was 1.33×10^{12} CFU/g to 3.65×10^{12} CFU/g and it decreases to 3.84×10^{9} CFU/g to 6.82×10^{10} CFU/g at Day 60. Similarly at Day 100, the count decreased to 1.28×10^{7} CFU/g to 8.57×10^{9} CFU/g. The decrease in the bacterial count may be probably due depletion of nutrient for microbial survival.

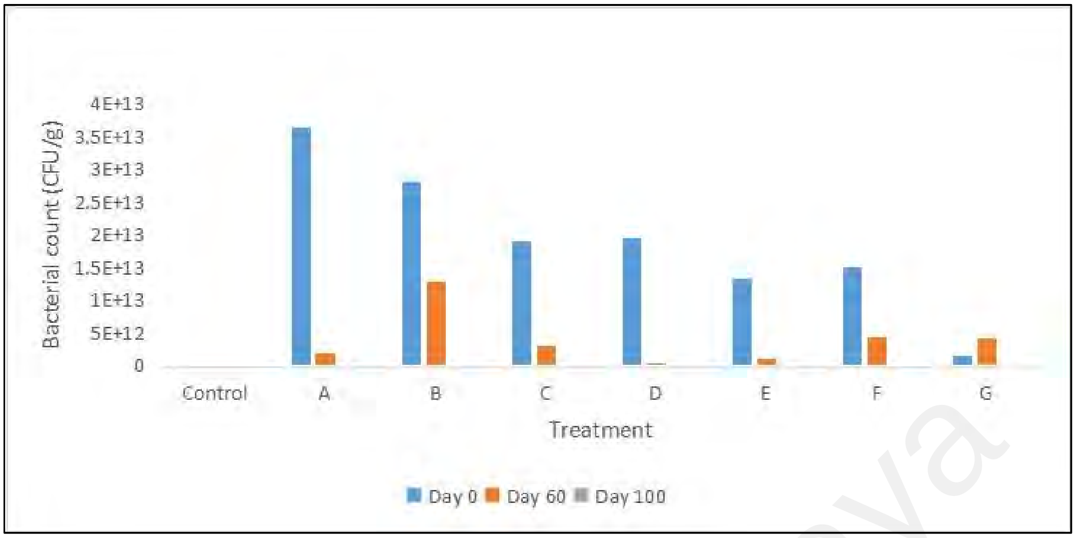

Figure 4.63: Bacterial count across time with different treatments for remediation of soil from Taman Beringin Landfill soil amended with 30% of inoculum concentration

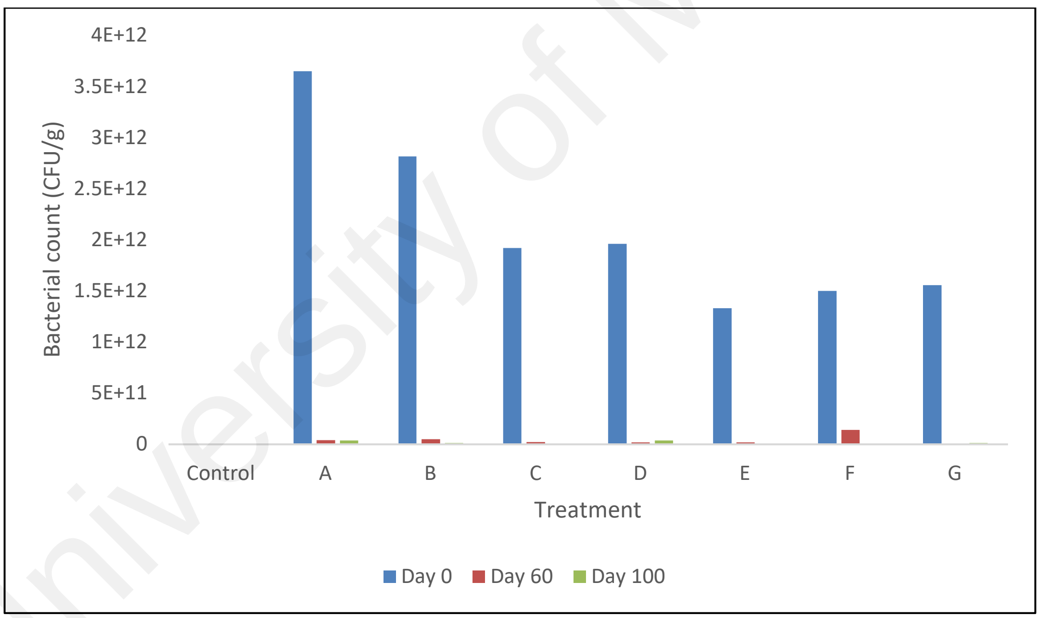

Figure 4.64: Bacterial count across time with different treatments for remediation of soil from Bukit Beruntung Landfill amended with 30% of inoculum concentration

4.8 *In situ* bioaugmentation experiment study

From the laboratory study, the best treatment namely Treatment F (consist of *O.intermedium*, *S. acidaminiphilia*, *A. ebreus*, *B. diminuta*, *A. caviae* DNA 4, *D. tsuruhatensis*, *P. alcaligenes*, *P. mendocina*, *S. marcescens marcescens* and *B. vietnamiensis)* was tested for application in Taman Beringin Landfill.

4.8.1 Lead (Pb)

Figure 4.65 shows the concentrations of Pb during the *in situ* study in Taman Beringin Landfill. As shown in Figure 4.65, fluctuation in the concentration of Pb was observed across the remediation period in both un-amended (control) portion and microbe-amended (proteo-bacteria) portions of the landfill soil. This may be due to lateral flow of leachate within the soil matrix of the landfill.

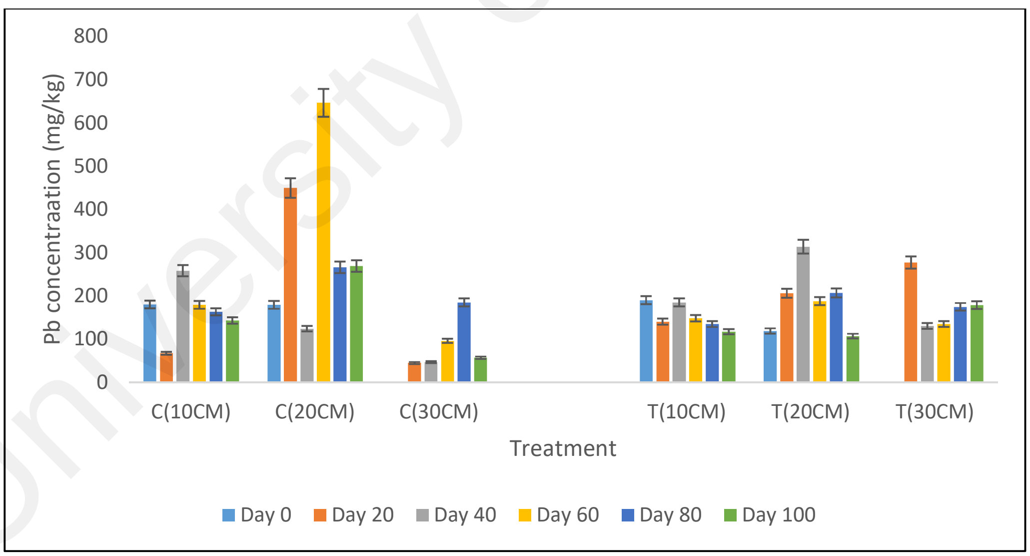

Figure 4.65: Pb concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

After 100 days, soil analysis showed reduction in Pb concentration at 10cm depth for both control and soil amended with microbes. For instance, Treatment T showed higher reduction of Pb (38.24%) while control showed 20.55% of Pb reduction. However, Pb concentration increased in control at 20cm depth in contrast to the reduction of Pb at 10cm depth. While, Treatment T (proteo-bacteria) portion showed 10% of Pb reduction at 20cm depth, a slight decrease in reduction of Pb compared to 10cm depth. Besides, an increase in Pb concentration in both control and microbe-amended portion was observed at 30cm depth of soil. It is possible that limited availability of oxygen at 30cm reduced the activities of the microbes at deeper part of the soil, therefore reduction of metal was limited. Hence, aerating the lower depth of the soil could increase the Pb reduction. This may be supported by the bacterial population found across the measured depths (Figure 4.66); the highest count was recorded at 10cm depth when compared with 20 and 30cm depths. There is a significant difference in Pb reduction between the un-amended and microbe-amended portions of the landfill ($p = 0.00$ at 10cm and 20cm depth). Microorganisms respond in various ways that lead to metal contamination, such as compartmentalization, exclusion, synthesis of the binding protein like metallothioneins, and formation of complex products.

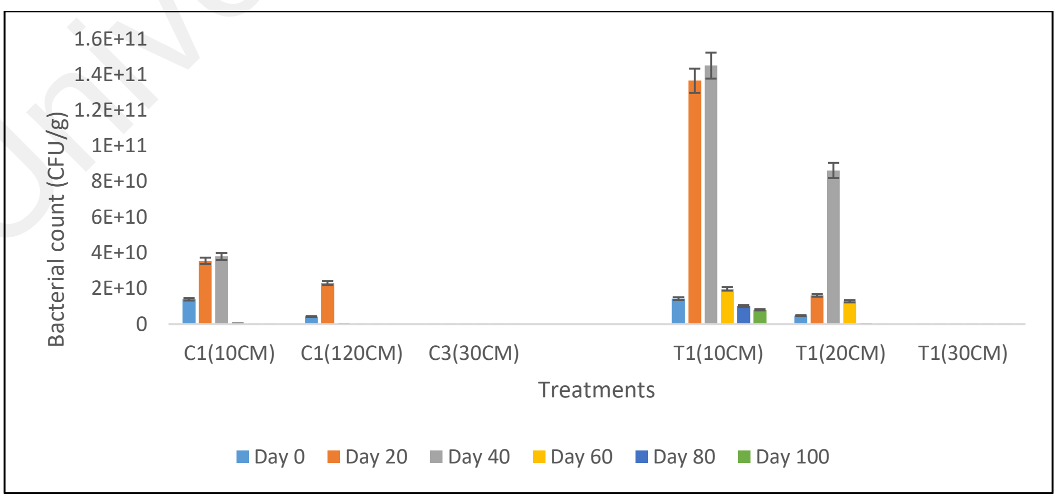

Figure 4.66: Bacterial count across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.2 Aluminium (Al)

Concentrations of Al during the *in situ* study in Taman Beringin Landfill is illustrated in Figure 4.67. The concentration of Al for control and soil amended with Treatment F showed variability due to continuous lateral flow of leachate into the soil.

The concentration of Al was decreased in microbe amended soil unlike Control which showed increase in concentration of metal at 10cm depth at the end of the study. Even though, the reduction was not high as recorded under the lab scale, the introduction of Treatment F showed 5.2% of Al reduction in the landfill. However, Al reduction (21.11%) was higher in soil amended with microbes at 20cm depth than at 10cm depth. After 100 days, the redox potential value was also higher at 20cm depth than to 10cm depth (Figure 4.68). This confirms that the maximum reduction of Al occurred at 20cm. As reduction of Al was lower at both 10cm depth and 30cm depth. The concentration of Al increased at 30cm depth in contrast to concentration of Al at 10cm depth and 20cm depth, where Al concentration was reduced.

Reduction in the concentration of Al in microbe amended soil occurred due to the injection of proteobacteria into the contaminated soil. Hence during the study, microbes in treatment F have shown the potential to reduce Al metal. Similarly, microorganisms isolated from the landfill have been reported to remediate heavy metal elements in the environment (Atkinson *et al*., 1996; Jayanthi *et al*., 2016).

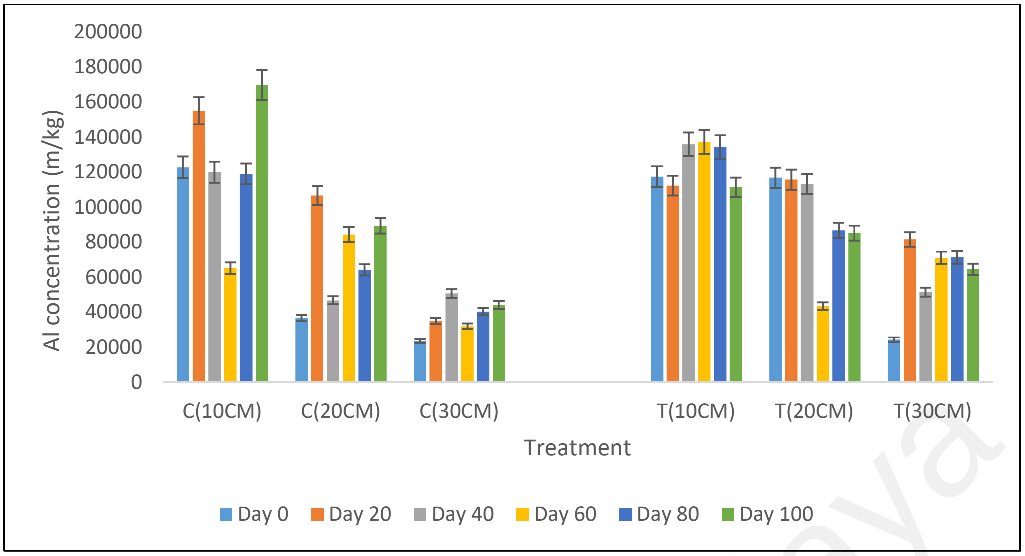

Figure 4.67: Al concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

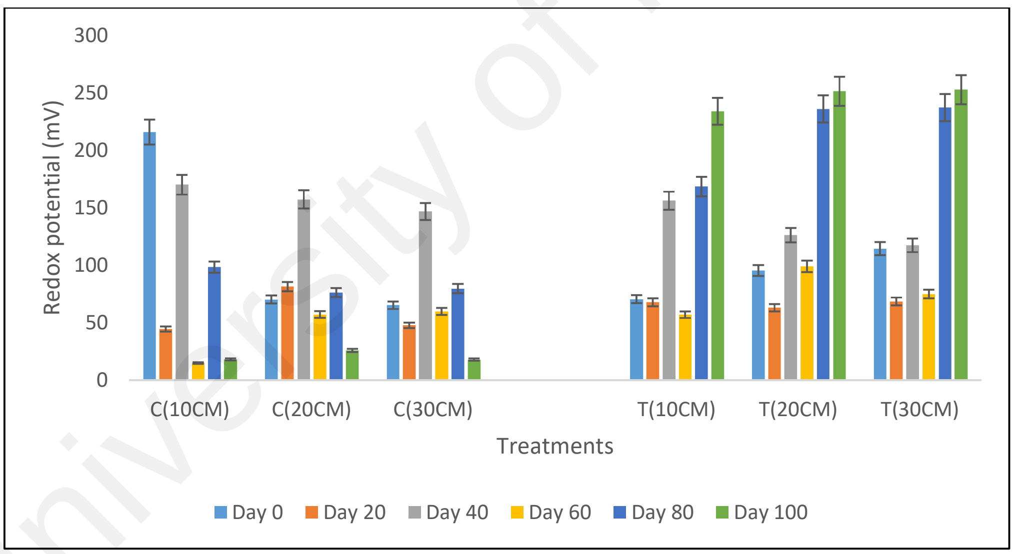

Figure 4.68: Soil redox potential across time for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.3 Manganese (Mn)

Concentrations of Mn during the *in situ* study in Taman Beringin Landfill is shown in Figure 4.69. The overall comparison of the Mn concentration revealed fluctuation in the metal concentration. Soil analysis for 10cm depth revealed a significant reduction in concentration of Mn in microbe amended soil (64.54 %). Although 44.7% reduction in concentration of Mn was also observed in control. It may be due to microbes present in the landfill soil, as bacteria from phyla Proteobacteria have been reported to possess the potential of remediation of a wide spectrum of heavy metal contamination (Karelova *et al*., 2011) and they are also abundant in the landfill environment.

The results of soil analysis on the concentration of Mn at 20cm and 30cm depth showed increase in concentration of metal for both microbe amended soil and control soil instead of reduction.

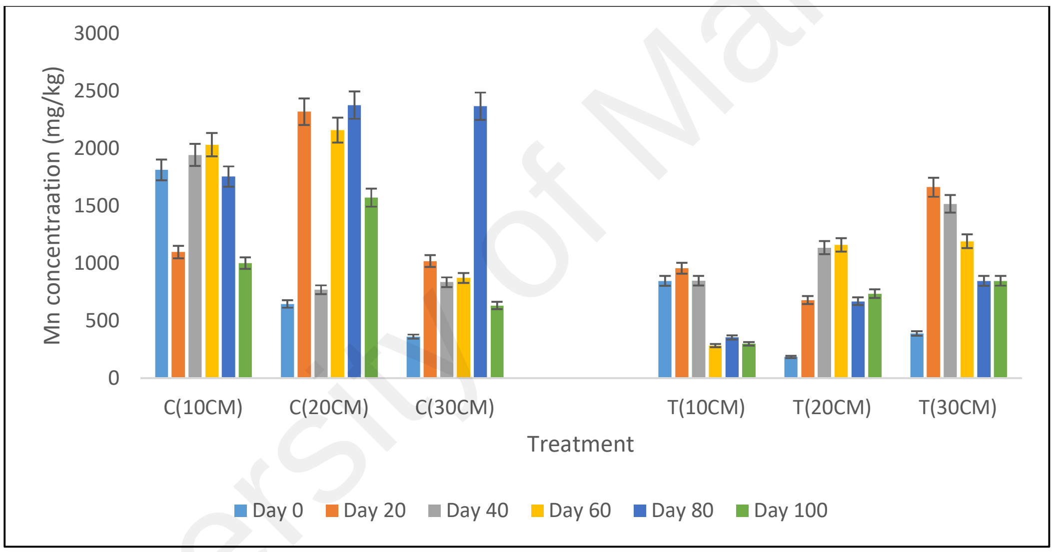

Figure 4.69: Mn concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.4 Copper (Cu)

Figure 4.70 illustrates the concentrations of Cu during the *in situ* study in Taman Beringin Landfill. The concentration of Cu showed variability across the monitoring days in the study area. However, microbe amended soil showed reduction in Cu concentration.

At 10cm depth, microbe amended soil showed 63.52% of Cu reduction whereas non-amended soil showed increase in concentration of Cu. Increase in concentration of Cu may be due to continuous flow of leachate into the soil that possibly increased the metal concentration in the soil. Although leachate flowed into both sides; the microbe amended and control area, but it infers that normal microflora within the un-amended side could not reduce metal in the soil as compared to the bioaugmented side. The microbes amended soil showed significant reduction in the Cu reduction and statistical analysis also revealed significant differences at p=0.00 between the initial and final concentration of Cu in microbe amended soil. The inoculum might actively absorb the metal from the soil through different mechanisms that contributed to 63.52% of Cu reduction at end of the experiment. Mohamad and Khanom (2017) also recorded abundance of phylum Proteobacteria in both active and closed landfills. So, the existence of proteobacteria in the studied landfill and the addition of the inoculum could have enhanced the reduction of metal in the landfill study.

Reduction in the concentration of Cu was not favourable at 20cm and 30cm depth. The concentration of Cu at 20cm and 30cm depth soil increased instead of reducing the Cu concentration by both microbe amended soil and non-amended soil (control). Cu reduction at depth 20cm and 30 did not occur due to continuous leachate contamination in the soil and limited availability of oxygen for the microbial survival.

Figure 4.71 shows the monitoring of soil pH across the monitoring days of experiment. The results reveal that there was slight change in pH of soil across the monitoring days that was between pH (6-7). Soil pH ranged of 6-7 indicating the occurrence of microbial degradation. Slightly acidic condition is the most conducive pH level range for metal reduction to take place by the microbes.

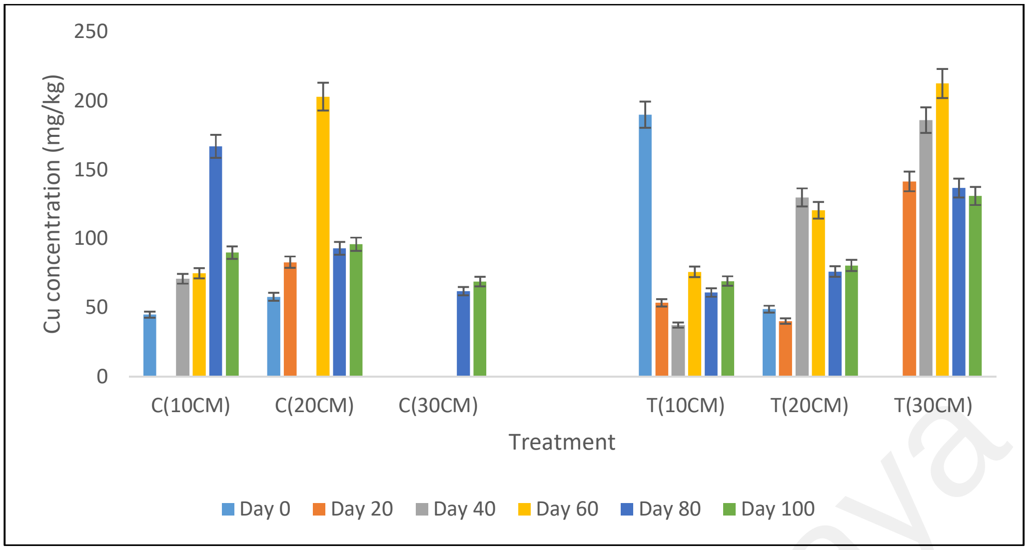

Figure 4.70: Cu concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

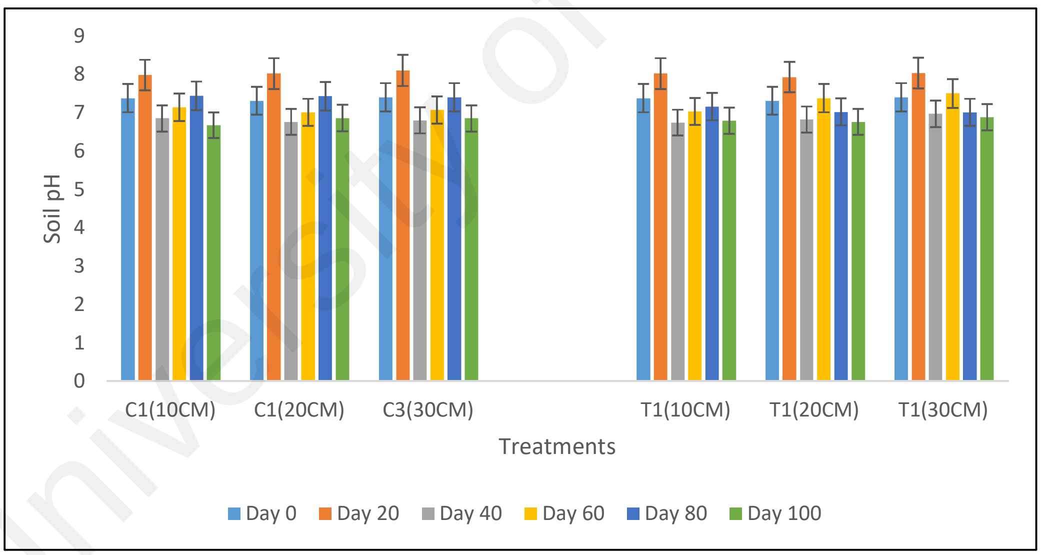

Figure 4.71: Soil pH across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.5 Zinc (Zn)

Concentrations of Zn during the *in situ* study in Taman Beringin Landfill is demonstrated in Figure 4.72. Throughout the study period of 100 days, concentration of Zn showed fluctuation.

At 10cm depth, microbe amended soil showed maximum Zn reduction among the three depth intervals of 10cm, 20cm and 20cm. The percentage of Zn reduction in microbe amended soil at 10cm depth was 10.38%. While control showed increase in concentration of Zn at 10cm depth. This indicates that addition of microbes increased the Zn reduction. On the contrary, the results recorded for 20cm and 30cm depth showed increase in concentration of Zn for microbe amended soil, in addition to increase in concentration of Zn in non-amended soil. This may be due to decrease in the population of bacteria as observed in Figure 4.66 with increasing depth of soil. Fierer *et al*. (2003) recorded decrease in the population count when the soil depth increased.

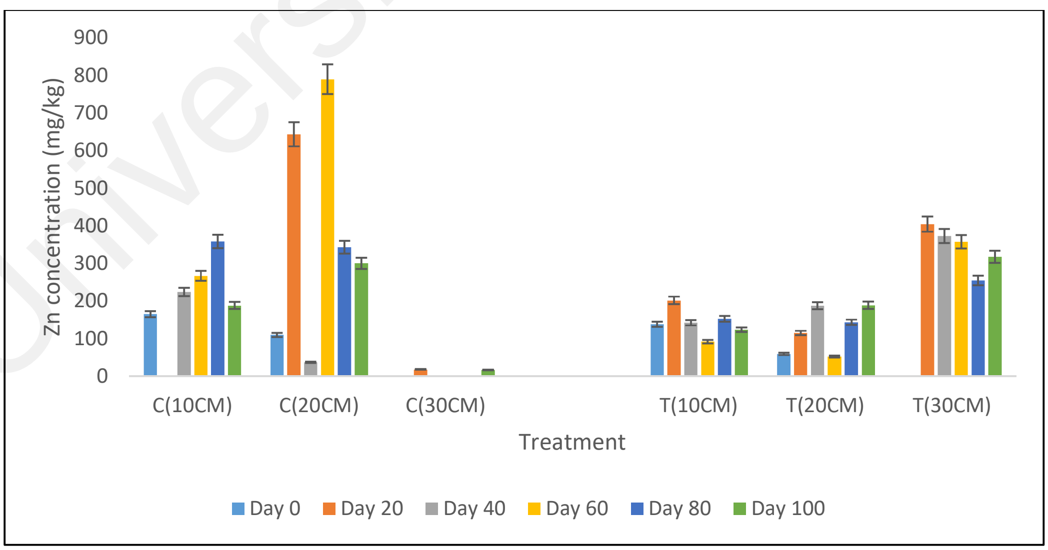

Figure 4.72: Zn concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.6 Iron (Fe)

Concentrations of Fe during the *in situ* study in Taman Beringin Landfill is illustrated in Figure 4.73. The overall distribution of Fe concentration showed fluctuation throughout the study period.

At 10cm depth, the results of soil analysis revealed a significant Fe reduction in microbe amended soil while the non-amended soil showed increase in concentration of Fe. The microbe amended soil showed 18.75% reduction in concentration of Fe at the end of experimental set up. The addition of microbes in the amended soil significantly increased the reduction of Fe. Addition of microbes, namely from proteo-bacteria to leachate contaminated soil can significantly reduce the metal contaminant from soil as reported by Fauziah *et al*. (2017). On the other hand, the increase in concentration of Fe may be due to increase in metal concentration from leachate flow, hence the continuous addition of metals to the soil.

The result of soil analysis from 20cm depth also revealed significant reduction in the concentration of Fe for microbe amended soil and the percentage of reduction was considerably high (59%). However, control soil showed increase in concentration of Fe. The higher reduction of Fe concentration by microbe amended soil may be due to enhanced microbial activity that takes place in the soil.

The result of soil analysis from 30cm depth revealed similar results as recorded for Pb, Al, Mn, Zn and Cu. The reduction in concentration of Fe was negative reduction for both, microbe amended soil and non-amended soil. Survival of microbes is limited at this depth and could have mitigated the metal reduction. According to Archana *et al.* (2015), low population of microorganism is found at the deeper strata of soil. Similarly, Hoorman and Islam (2010), reported that bacterial population varied with the depth. The results of population count (Figure 4.66) also concurred with the Archana *et al.* (2015) and Hoorman and Islam (2010) where the population decreased with increase in soil depth.

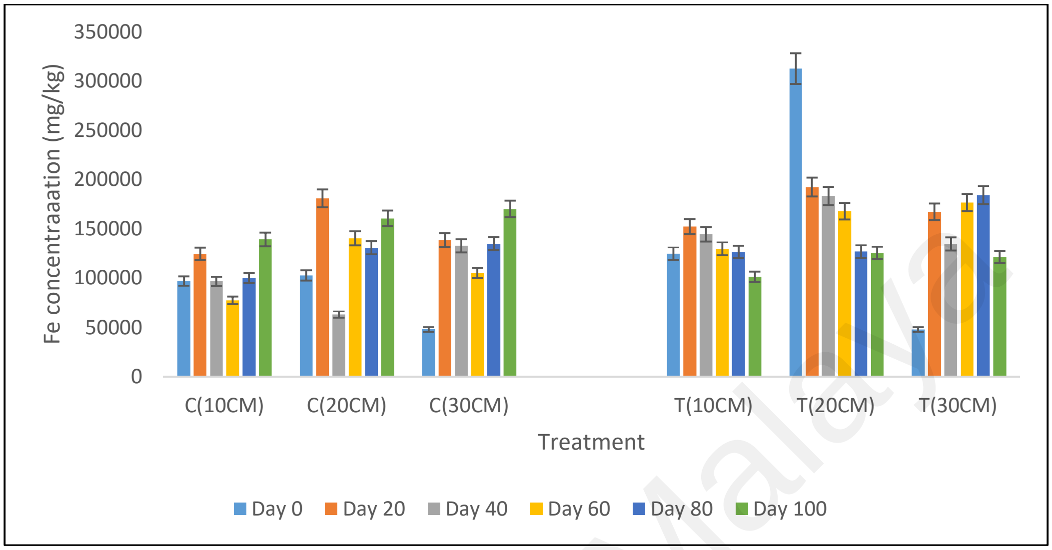

Figure 4.73: Fe concentration across days for *in situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.7 Chromium (Cr)

Concentrations of Cr during the *in situ* study in Taman Beringin Landfill is shown in Figure 4.74. The results of soil analysis from 10cm depth revealed reduction for the concentration of Cr in both microbe amended soil and un-amended soil. The reduction in Cr concentration in microbe amended soil and un-amended soil was 89.49% and 69.72% of reduction in Cr concentration respectively. The reduction in concentration of Cr was significantly different between microbe amended soil and un-amended soil ($P < 0.05$). Similarly, soil analysis from 20cm depth, showed reduction in Cr concentration both portions of the soil; microbe amended soil and un-amended soil. The percentage of reduction in concentration of Cr was 66.89 % and 50% for microbe amended soil and un-amended soil respectively. The higher Cr reduction in microbe amended soil may be due to the presence of discrete potential of proteobacteria for metal reduction. Proteobacteria may have the main role in degrading the organic and inorganic substances, that also includes heavy metal in leachate or metal contaminated soil (Kochling *et al.*, 2015).

At 30cm depth of soil, increase in Cr concentration was observed for both unamended soil and microbe amended soil. The growth of microbes is limited at lower depths of soil due to insufficient oxygen, as most of proteobacteria are aerobic bacteria. Similar findings were presented by McNabb and Startser (2009), who reported that microbes' population is higher in aerobic condition compared to anaerobic condition i.e. at shallower depths than at deeper depth respectively.

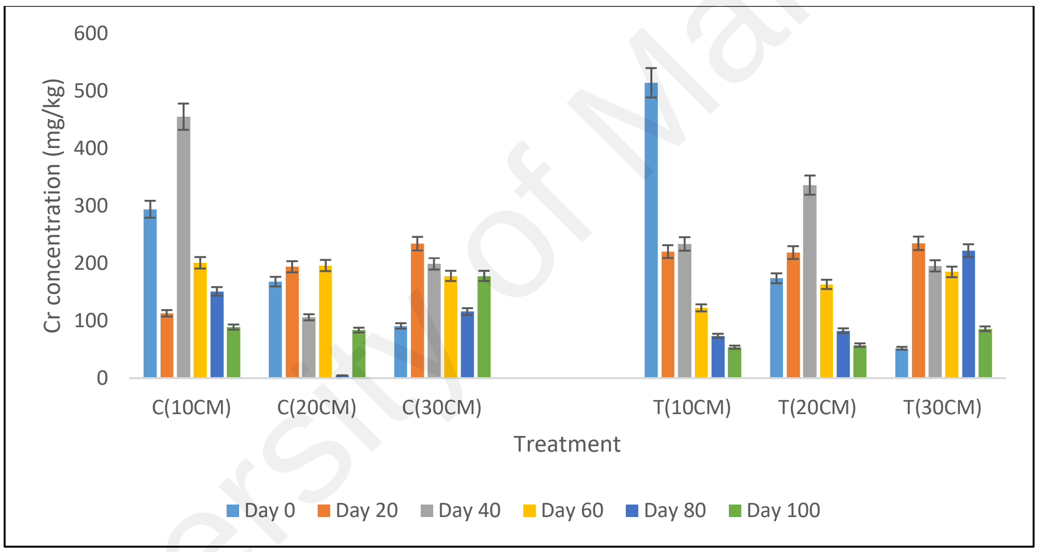

Figure 4.74: Cr concentration across days for *in-situ* bioaugmentation of metal-contaminated soil of Taman Beringin landfill (C represents un-amended soil and T represents microbes amended soil)

4.8.8 First order rate constant and half-life value for the *in situ* study at Taman Beringin Landfill

The first order rate constant for *in situ* study of soil from Taman Beringin Landfill is tabulated in Table 4.15. The estimation of removal rate is necessary to calculate as it determines the removal capability of treatment soil; amended with microbes because it pertains each metal. It is also important to estimate the rate of removal per day for comparing natural remediation process exhibited by control with remediation processes shown by treatment (Emenike, 2013). The first order rate constant for all metals studied recorded higher for microbe amended soil treatment. The first order rate constant by bioaugmentation for Pb, Cr, Zn, Mn and Cu at 10cm depth of soil, Treatment T (10cm), recorded higher than other depths i.e. 20cm depth and 20cm depth. The rate first order rate constant for Pb, Cr, Zn, Mn and Cu was 0.0048 day^{-1}, 0.022 day^{-1}, 0.001 day^{-1}, 0.01 day^{-1} and 0.01 day^{-1} respectively. However, for Al and Fe, the rate of metal removal was recorded higher at 20cm depth of soil, Treatment T (20cm), than at other depths of soil; 10cm and 30cm. The first order rate constant of Al and Fe was 0.0031 day^{-1} and 0.009 day^{-1} respectively. The microbe amended soil reduced the metal concentration at higher rate compared to un-amended soil, which indicates the ability of microbes i.e. proteobacteria, to carry out bio-reduction of metal. Moreover, blending or addition of microbes to soil increases the rate of metal reduction. However, it must be noted that selection of bacteria treatment is necessary to achieve optimum reduction.

Table 4.15: First order rate constant for *in situ* bioaugmentation of soil from Taman Beringin Landfill

	Removal rate (day^{-1})					
Metals	**C (10cm)**	**C(20cm)**	**C(30cm)**	**T (10cm)**	**T (20cm)**	**T(30cm)**
Pb	0.0023	-	-	0.0048	0.001	-
Al	-	-	-	0.0005	0.0031	-
Cr	0.011	0.007	-	0.022	0.011	-
Zn	-	-	-	0.001	-	-
Fe	-	-	-	0.002	0.009	-
Mn	0.006	-	-	0.01	-	-
Cu	-	-	-	0.01	-	-

The half-life value is the estimation of the time taken for the metal to be reduced to half shown in Table 4.16. At 10cm depth of microbe amended soil (T 10cm), gave the shortest half-life was recorded for Pb, Cr, Zn, Mn and Cu and the half-life was 144.40 days, 31.5 days, 693.1 days, 69.3 days and 69.3 days respectively. Whereas at 20cm depth of microbe amended soil (T 20cm), the shortest half-life was recorded for Al and Fe and the half-life were 223.59 days and 77.01 days respectively. On the other hand, longest half-life was recorded for Pb, Cr and Mn in control. All other metals showed increased concentration in Control. The variation in half-life between the treatment and control and also variation in half life between several metals indicates the different rate of metal uptake by the microbes. Among the metals studied, Cr was the metal that took the shortest time, 31.5 days, with the amendment of microbes.

Table 4.16: Half-life value for *in situ* bioaugmentation of soil from Taman Beringin Landfill

	Half-life t½ (day)					
Metals	**C (10cm)**	**C(20cm)**	**C(30cm)**	**T (10cm)**	**T (20cm)**	**T(30cm)**
Pb	301.36	-	-	144.40	693.14	-
Al	-	-	-	1386.29	223.59	-
Cr	63.01	99.02	-	31.5	63	-
Zn	-	-	-	693.1	-	-
Fe	-	-	-	346.57	77.01	-
Mn	115.52	-	-	69.3	-	-
Cu	-	-	-	69.3	-	-

4.9 Phytoremediation of heavy metal contaminated soil under greenhouse conditions

The potential of four plants to uptake of metals from contaminated soil will be discussed in the following section. The phytoremediation study is divided into two sections which represents two landfills (operating and non-operating status). Taman Beringin landfill is non –operating landfill while Bukit Beruntung landfill represents operating landfill. The plants have not been previously studied for its ability to uptake nine metals therefore the study is necessary to investigate further on the ability of these plants for accumulation or uptake of metals from soil. However *T. spatachea* and *C.comosum* was previously investigated for its tolerance towards Pb by Melania and Myrna (2017) and Wang *et al*. (2011). The four plants studied were *Cordyline* sp., *D. variegated*, *T. spatachea* and *C. comosum*.

4.9.1 Uptake of heavy metals by different plants in soil from Taman Beringin Landfill

4.9.2 Response of plants

The plants used in this study was monitored for 120 days. No plant death was recorded during this period. However *Cordyline* sp. showed sign of leaf yellowing as shown in Plate 4.1. All other plants were recorded healthy and no visible changes in the appearance was observed.

Plates 4.1: The appearance of studied plants for phytoremediation of soil from Taman Beringin Landfill (A: *Cordyline* sp.; B: *D.variegated*; C: *T. spatachea*; D: *C.comosum*)

Table 4.17 shows the height of plants before and after remediation of soil from Taman Beringin Landfill, and the results revealed the height of plants in control soil was much higher than those grown in contaminated soil. The height of plants shows an increase in the contaminated soil however the height of the plants grown in control soil (no metal pollution) shows higher growth rate. The toxicity of metal in the polluted soil may probably affect the growth of plants and similarly the findings of this study are also in agreement with Meera (2013) and Dimitriou *et al*. (2006). Their findings revealed significant reduction in the growth of plants when the plant were treated using leachate compared to control.

Table 4.17: Height of plants before and after phytoremediation of heavy metal in soil from Taman Beringin Landfill

Treatment	Initial (cm)	Final height (cm)	Control (cm)
Cordyline sp.	25±1	44.67±0.57	52±3
D. variegated	23±2.08	28.5±0.5	32±2
T. spatachea	25±1	30.5±0.5	32±1
C. comosum	32±2	59.67±0.57	65±2

Figure 4.75 and Figure 4.76 showed the fresh weight of the studied plants after the completion of 120 days of phytoremediation experiment. The fresh weight and dry weight of plant grown in contaminated soil were higher compared to plant grown in control soil. This may due to accumulation of heavy metals which increased the weight for plant grown in contaminated soil. Meera (2012) also recorded similar results for the plant grown with leachate application compared to control. This may due to accumulation of heavy metals which increased the weight for plant grown in contaminated soil. Meera (2012) also recorded similar result for the plant grown in leachate compared to control.

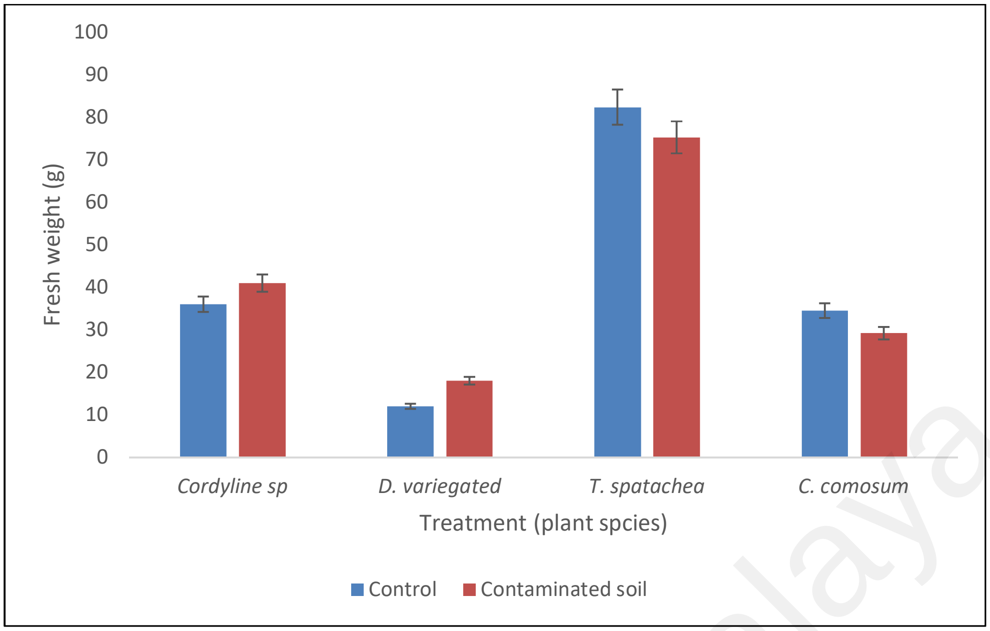

Figure 4.75: Fresh weights of plants grown in soil from Taman Beringin Landfill

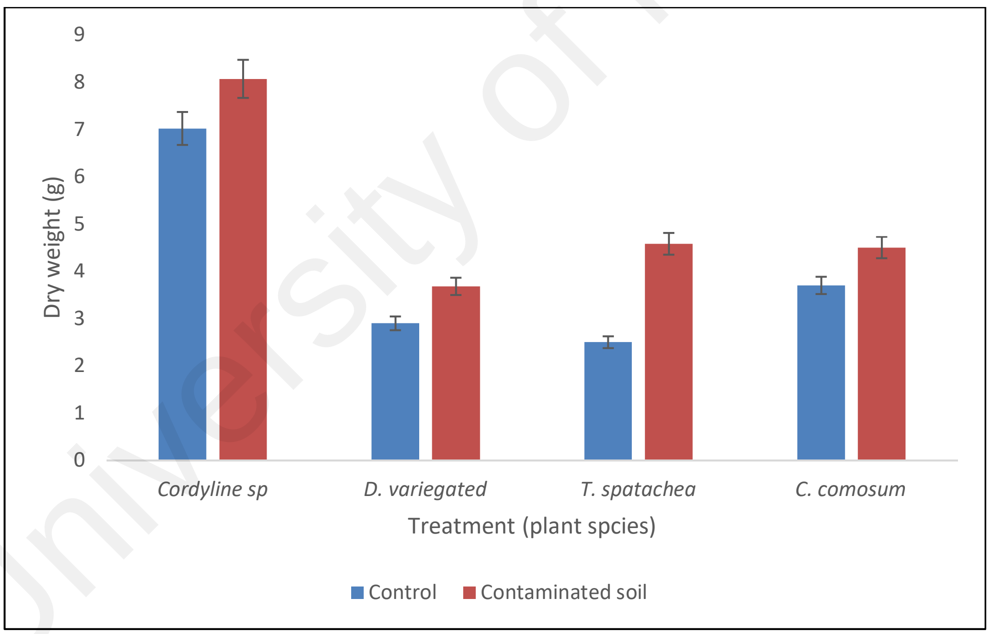

Figure 4.76: Dry weight of plants grown in soil from Taman Beringin Landfill

4.9.3 : Phytoremediation of heavy metal in soil from Taman Beringin Landfill using different plants

4.9.3.1 Lead (Pb)

Table 4.18 shows concentration of Pb in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants. The results revealed variance in the metal removal by the different plants and removal percentage of Pb ranged from 55% to 63% and Control recorded 33%. *Cordyline* sp. showed highest removal (63%) of Pb while other studied plants showed 55% of removal. The uptake of metal by the plants were above 50% which can be good indicator for metal removal especially Pb.

The four plants showed significant differences (p=0.00) when compared with control. Comparison among the studied plants revealed that *Cordyline* sp. showed higher percentage of Pb removal from soil as compared to other studied plants. Similarly, Alaribe and Agamuthu (2015) also reported that some plants has the ability to reduce high concentration of Pb from contaminated soil.

Table 4.18: Concentration of Pb in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Pb removal (%)
Cordyline sp.	18	6.66±1.15	63
D. variegated	18	8±0	55
T. spatachea	18	8±0	55
C. comosum	18	8±1	55
Control	18	12.00±1	33

Figure 4.77 shows concentration of Pb in shoot and root of different plants grown in soil from Taman Beringin Landfill. The highest accumulation of Pb were found to be in

the root of *Cordyline* sp. The uptake of Pb in the root was 6.33 mg/kg and 2.33 mg/kg in the shoot of *Cordyline* sp. Similarly, *T. spatachea* and *C. comosum* also accumulated higher concentration of Pb in root as compared to the shoot of the plant. Significant differences in the concentration of Pb accumulated in root and shoot *Cordyline* sp. (p=0.008) and *C. comosum* (p=0.011) was obtained.

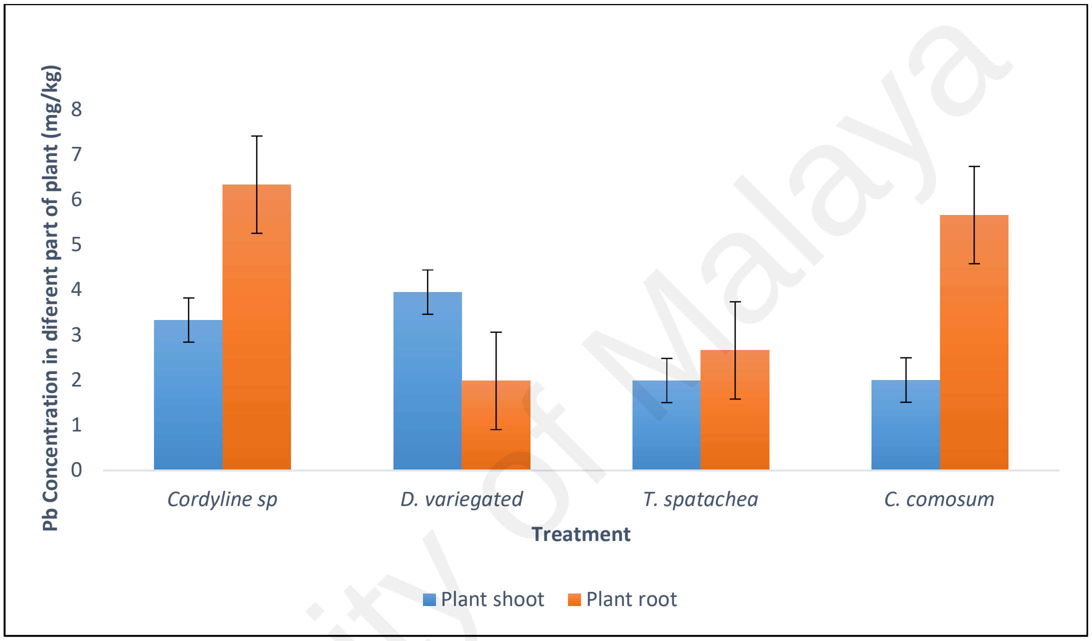

Figure 4.77: Concentration of Pb in shoot and root of different plants grown in soil from Taman Beringin Landfill

The study concurred with findings of Stephen *et al*. (2013) who reported that the higher Pb concentration was accumulated in the root of *Medicago sativum* plant compared to other parts of the plant. Blaylock and Huang (2002) also reported similar results whereby most of Pb absorbed from the contaminated soil remained in the root as the first barrier in Pb translocation to the above ground part of plant. The higher accumulation may probably be because the absorption of metal from soil is mainly the roots and according to Brajes *et al*. (2017) the transport of Pb is mainly through the absorption of lead by roots which occurs via the apoplastic pathway or via Ca^{2+}-permeable channels.

4.9.3.2 Arsenic (As)

Concentration of As in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants is tabulated in Table 4.19. *Cordyline* sp. showed the highest As removal from the soil (85%).The concentration reduced to 15.67 mg/kg from 103 mg/kg. The second highest removal of As was recorded with *D. variegated* and *C. comosum* and removal percentage was 80%. Control recorded the lowest removal (49.5%) There is a significant difference between the three plants and control (p=0.00). The high removal of As from contaminated soil may probably be related to the metal detoxifying enzymes called phytochelatins in plants. Similarly, Evelyn (2016) also reported high reduction of As in soil by using T. *accuminata* plant compared to control.

Table 4.19: Concentration of As in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of As removal (%)
Cordyline sp.	103	15.67±0.57	85
D. variegated	103	19	81.5
T. spatachea	103	26.33±6.35	74.4
C. comosum	103	20.33±0.57	80.2
Control	103	52±6.08	49.5

Concentration of As in shoot and root of different plants grown in soil from Taman Beringin Landfill is illustrated in Figure 4.78. The result revealed highest accumulation of As in *Cordyline* sp. in both root and shoot of the plant. However, root showed 61.7 % higher accumulation of As as compared shoot of *Cordyline* sp. As accumulation in the root was 80.95 mg/kg and 30.97 mg/kg in the shoot. Other plants accumulated As in the range of 61.99 mg/kg - 70.9 mg/kg in root and 20.95 mg/kg – 25.95 mg/kg in the

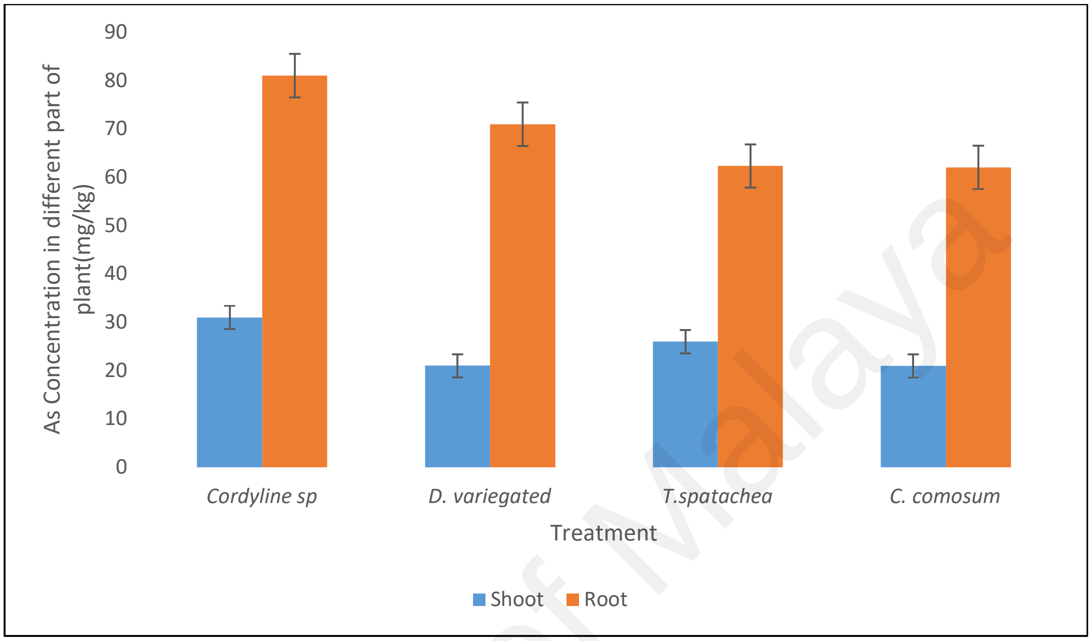

Figure 4.78: Concentration of As in shoot and root of different plants grown in soil from Taman Beringin Landfill

shoot of the plant. The results revealed variance in the As uptake by plants and according to the Nosheen *et al.* (2013), the transportation of As from root to shoot varies considerably among the four plants which may under the genetic control. The findings also agreeable with Oliveira *et al*. (2014) who reported that most of the plants accumulate As concentration in the roots and few plants were able to translocate As from roots to shoots. Though, the plants accumulated high concentration of As in the root compared to shoot, the uptake of As from soil was considerably high (74%-85% of As accumulation) therefore it can be good phytoextraction plant.

4.9.3.3 Aluminium (Al)

Concentration of Al in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants is illustrated in Table 4.20. The percentage removal of Al by studied by plants was ranged between 65.5 % - 67.5%. No variance in removal of Al was observed among the plants rather it was able to reduce the concentration of Al almost two-fold higher compared to control. There are significant difference between plants and control (p = 0.00).

Table 4.20: Concentration of Al in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Al removal (%)
Cordyline sp.	49600	16116.67	67.5
D. variegated	49600	17083.33	65.5
T. spatachea	49600	16866.67	66
C. comosum	49600	16740	66
Control	49600	31600	36

Concentration of Al in shoot and root of different plants grown in soil from Taman Beringin Landfill is depicted in Figure 4.79. The highest accumulation of Al was in the root of the *Cordyline* sp. The order of Al accumulation by different plants are: *Cordyline* sp. (13826 mg/kg) < *C. comosum* (13380 mg/kg) < *T. spatachea* (12053 mg/kg) < *D. variegated* (11146.67 mg/kg). However, it is observed that the accumulation of Al in the shoot of the plants was reduced four-fold as compared to the Al accumulated in the root. This is probably because roots are directly exposed to soil for the primary metal extraction and similar findings were also reported by Yuebing *et al*. (2011). There are significant differences in the concentration of Al accumulated in plant root and shoot for the plant treatments and the difference were, *Cordyline* sp. (root & shoot), p=0.001; *D.* variegated (root & shoot), p = 0.001; *T. spatachea* (root & shoot), p =0.00 and *C. comosum* (root & shoot), p = 0.00.

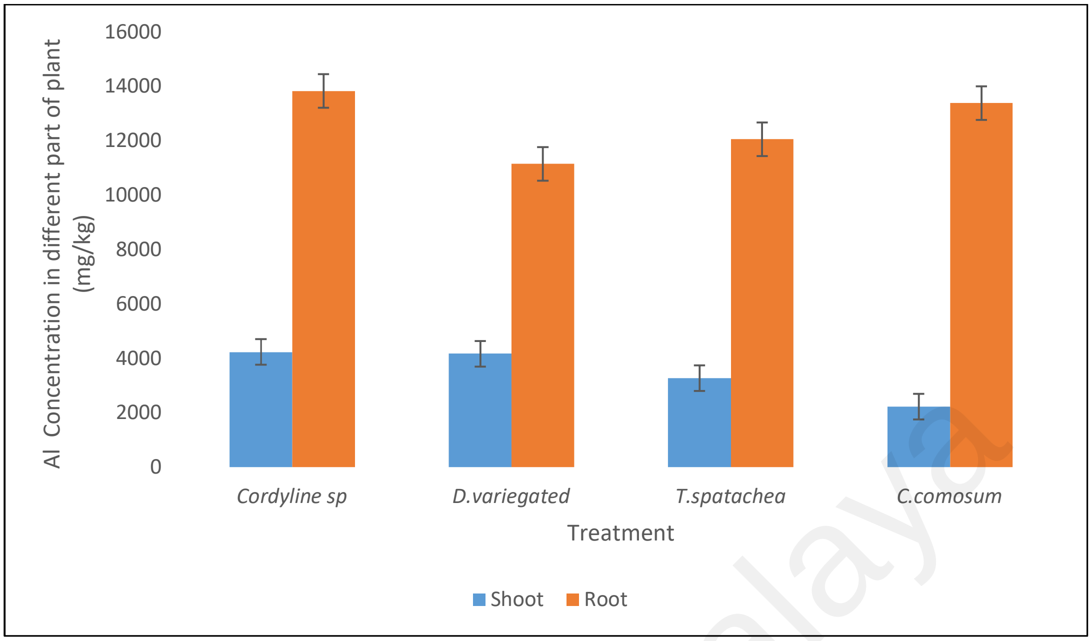

Figure 4.79: Concentration of Al in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.4 Manganese (Mn)

Concentration of Mn in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants is shown in Table 4.21. The highest percentage of Mn removal from the contaminated soil was by *Cordyline* sp. The percentage of removal was 78.8 %. Removal of Mn from the soil by *D. variegated*, *T. spatachea* and *C. comosum* was 70.4%, 69.6% and 75.3%, respectively. There are significant difference between plants and Control (p=0.00). The higher uptake by *Cordyline* sp. could be due to differences in physiology of the plants and similar to findings of Jayanthi *et al*. (2017). The four plants showed increased removal of Mn in contaminated soil and the removal four times higher as compared to control. This indicates the strength of plants for uptake metal from soil.

Table 4.21: Concentration of Mn in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Mn removal (%)
Cordyline sp.	281	59.33±12.70	78.8
D. variegated	281	83	70.4
T. spatachea	281	85.33±12.89	69.6
C. comosum	281	69.33±1.15	75.3
Control	281	235±17.89	16

Figure 4.80 illustrated the concentration of Mn in shoot and root of different plants grown in soil from Taman Beringin Landfill. Comparison between the four plants revealed the highest accumulation of Mn was in the shoot of *Cordyline* sp. The accumulation was 3.4 fold higher than the concentration of Mn in the root of *Cordyline* sp. Similarly, *D. variegated*, *T. spatachea* and *C. comosum* also accumulated high concentration of Mn in the shoot of the plant as compared to the Mn accumulated in the root. This indicates that the plant was able transport of metal from the root to shoot and therefore it can be good hyper accumulator plant. The transport of metals from the roots to the shoots involved long distance transfer and translocation in the xylem and storage in the vacuole of leaf cells. The nature of plant species to transport metal from root to shoot such as Mn could be main factors that contribute to such accumulation and this findings was supported by Okieimen *et al*. (2011) and Nazir *et al*. (2011).

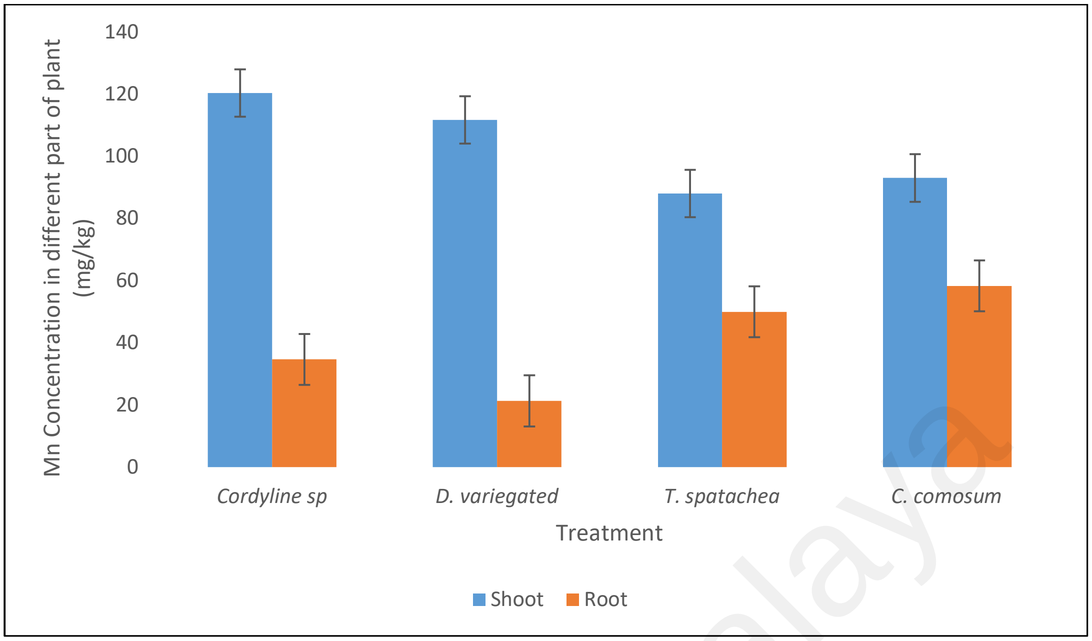

Figure 4.80: Concentration of Mn in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.5 Copper (Cu)

Concentration of Cu in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants is shown in Table 4.22. Rapid removal of Cu from the contaminated soil by *Cordyline* sp. Similarly, *D. variegated*, *T. spatachea* and *C. comosum* also showed high removal of Cu. The removal percentage was in the range of 90.3% - 94.35%. The Cu concentration reduced in range of 3.33 to 5.67 mg/kg from the initial concentration. However the removal of Cu by control was only 28% and it was 3.2 times lower than the Cu removal by plants. The higher removal of Cu by the plants indicates its ability for Cu removal from the contaminated soil. The higher removal of Cu from soil may probably be governed by the nature of the metals and the nature of plant for Cu accumulation and this is supported by Saadia and Azka (2016). There are significant difference in metal removal among the four plants and Control (p=0.00).

Table 4.22: Concentration of Cu in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Cu removal (%)
Cordyline sp.	59	3.33±0.57	94.35
D. variegated	59	4.67±0.57	92
T. spatachea	59	5.67±1.15	90.3
C. comosum	59	4.67±0.57	92
Control	59	42±2.0	28

Concentration of Cu in shoot and root of different plants grown in soil from Taman Beringin Landfill is depicted in Figure 4.81. The concentration of metals in plants differed among the four plants however, all the plants showed highest Cu accumulation in the root as compared to shoot. The uptake of Cu in roots of the studied ranged between 26.33 mg/kg to 28.67 mg/kg. The highest concentration of Cu in root was by *D.*variegated and the accumulation was 28.67 mg/kg. Similar results was recorded by different researchers. Scucz (2014) and Coupe *et al*. (2013) reported that Cu concentration in root was higher than the concentration of Cu in shoot of studied plants. Considerable amount of Cu also accumulated in the shoot of plants. This probably was because translocation of Cu might have occurred.

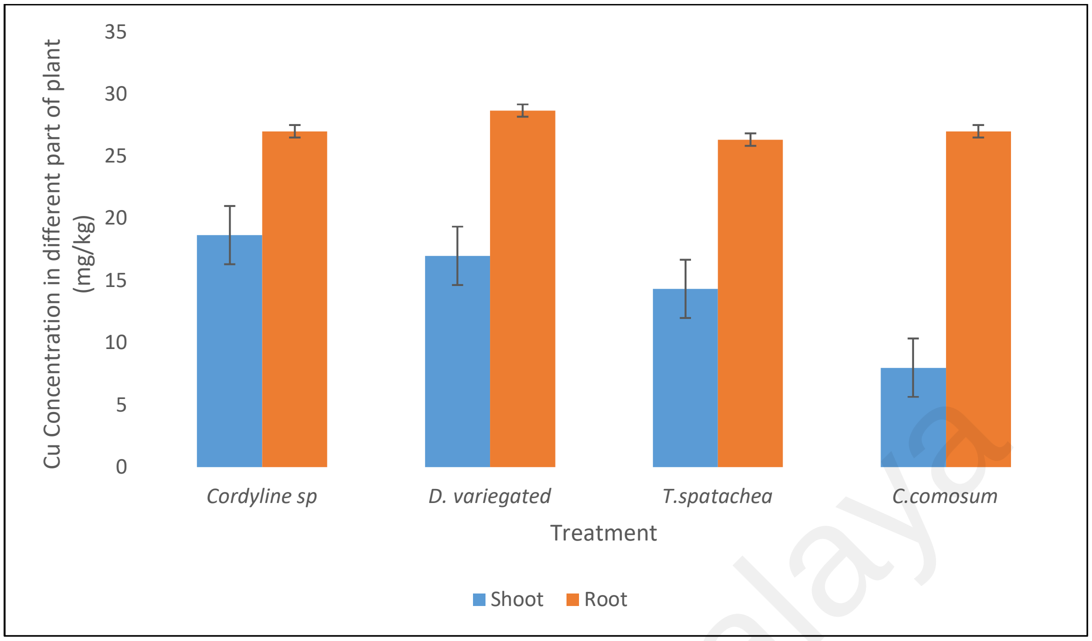

Figure 4.81: Concentration of Cu in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.6 Zinc (Zn)

Table 4.23 depicts concentration of Zn in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants. The highest removal of Zn from the contaminated soil was by *Cordyline* sp. The concentration of Zn reduced to 11 mg/kg and the removal percentage was 77.55 %. The removal of Zn by other plants was in ranged of 70% -75.5% and overall order removal by the treatments are *Cordyline* sp. > *C. comosum*> *D. variegated* > *T. spatachea* > Control. The removal of Zn in plant amended soil amended was two-fold higher as compared to non- amended soil. This probably was because plant are known to have several members of the Zn-regulated transporters in the iron (Fe)-regulated transporter-like protein (ZIP) gene family (Guerinot, 2000) which was characterized and shown to be involved in metal uptake and transport in plants and supported by findings of Eide *et al.* (1996), Korshunova *et al.* (1999), Vert *et al.* (2001) and Connolly *et al.* (2002). The ZIP proteins are predicted to have eight transmembrane domains, with their amino- and carboxyl-terminal ends situated on the outer surface of the plasma membrane (Guerinot, 2000). These proteins vary considerably in overall length due to a variable region between the transmembrane domains (TM) TM-3 and TM-4, which is predicted to be on the cytoplasmic side, providing a potential metal-binding domain rich in histidine residues. There are significant differences between plants and control (p= 0.00). This indicates that all four plant were able to reduce considerable amount of Zn in soil as compared to natural remediation that occurred in Control.

Table 4.23: Concentration of Zn in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Zn removal (%)
Cordyline sp.	49	11	77.55
D. variegated	49	14±1	71
T. spatachea	49	14.67±0.57	70
C. comosum	49	12±1.73	75.5
Control	49	32±1.73	35

Concentration of Zn in shoot and root of different plants grown in soil from Taman Beringin Landfill is illustrated in Figure 4.82. The results revealed higher accumulation of Zn in the shoot of the plants as compared to root and accumulation was two-fold higher. The highest accumulation of Zn was by *Cordyline* sp. The order of Zn accumulated in the plant shoot was *Cordyline* sp. (10.33 mg/kg) > *C. comosum* (8 mg/kg) > *D. variegated* (7 mg/kg) > *T. spatachea* (6.33 mg/kg. Looking into, Zn accumulation in root revealed highest accumulation by *Cordyline* sp. The accumulation of Zn in different part of the plants indicates that the plants are tolerant to Zn therefore it was able to accumulate and transport the metal to different part of the plants. This findings was agreeable to Baker and Brooks (1989) and Boyd (1998) who reported that the tolerant of plants is basically involved two mechanisms which is through exclusion or accumulation of metals. The higher accumulation of Zn in the shoot of the plants was also similar to findings of Renides *et al*. (2014) who reported higher accumulation of Zn in the shoot compared to root of lettuce plant. The study reported increase in the Zn concentration in the shoot of the plant compared to the root whereby translocation from roots to shoots increased.

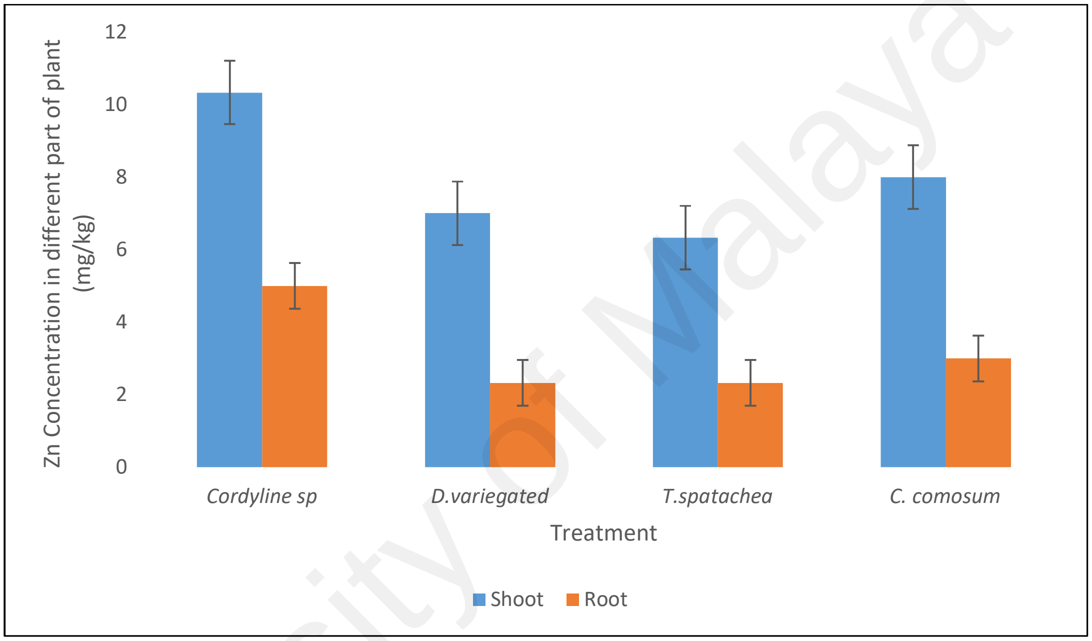

Figure 4.82: Concentration of Zn in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.7 Iron (Fe)

Table 4.24 shows the concentration of Fe in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants. After 120 days of phytoremediation, the highest reduction of Fe was demonstrated by *C. comosum* and the removal percentage was 56.4%. Other plants showed removal of Fe from the contaminated soil in the range of 30.7% - 53%. It is observed all plant except *T. spatachea* recorded Fe removal of 50% and the removal was significantly difference compared to Control.

Table 4.24: Concentration of Fe in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Fe removal (%)
Cordyline sp.	42900	20500±2240	52
*D.*variegated	42900	19766±2367	53
T. spatachea	42900	29700±866	30.7
C. comosum	42900	18700±3290	56.4
Control	42900	26533.33±923.7	38

Though, Fe are metal with low solubility and can be easily mobilized from the soil or translocation within the plant, *T. spatachea* showed low removal of Zn. This may probably be because each plant has its own threshold value for different type of metal where it trigger its toxicity and inhibit the uptake of metal from the soil. The higher removal of metal by *Cordyline* sp, *D. variegated* and *C. comosum* may probably related with its higher Fe uptake from the soil and the removal above 50% indicates it's a good phytoremediation indicator plant. The higher uptake of Fe by plants can also because the Fe is one the major elements required for plant growth as macronutrients and similar to findings of Ashton (2016).

Concentration of Fe in shoot and root of different plants grown in soil from Taman Beringin Landfill is illustrated in Figure 4.83. The accumulation of Fe in the root was higher in all plants as compared to in the shoot. The accumulation of Fe in the root was four times higher than shoot for all four plants. Among the four plants studied, the highest removal of Fe was in the root of *C. comosum* and the Fe accumulation was 15500 mg/kg. Other plants accumulated Fe in the root which range of 7000 mg/kg - 12100 mg/kg. Appreciable amount of Fe also recorded in the shoots of the studied plants with the highest accumulation by *C. comosum* (4500 mg/kg). Plants basically evolved two strategies to uptake Fe from the soil. Non-grasses activate a reduction-based Strategy I when starved for Fe whereas Strategy II is that grasses activate a chelation-based strategy as suggested by Sun *et al*. (2007).

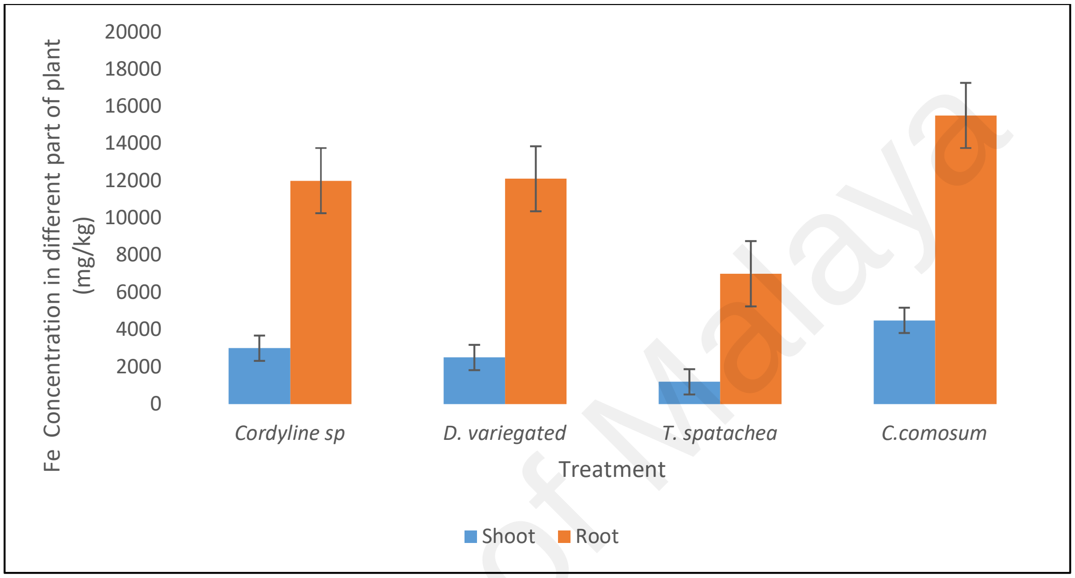

Figure 4.83: Concentration of Fe in shoot and root of different plants grown in soil from Taman Beringin Landfil

4.9.3.8 Nickel (Ni)

Table 4.25 represents shows the concentration of Ni in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants. Highest removal of Ni was by *Cordyline* sp. and *C. comosum* and the percentage of Ni removal was 88.9%. Besides that, *D. variegated* and *T. spatachea* also showed 85.7% of Ni removal from contaminated soil. However, the removal of Ni by Control was only 46%. This implies that, the four plants were able to reduce considerable amount of Ni and can be a good Ni removal plant. There are significant difference between plants and Control (p=0.00). The mechanism of metal removal by plants are by passive diffusion or active transport mechanisms and similar to findings of Jing *et al*. (2015).

Table 4.25: Concentration of Ni in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Ni removal (%)
Cordyline sp.	21	2.33±0.57	88.9
D. variegated	21	3	85.7
T. spatachea	21	3	85.7
C.comosum	21	2.33±0.57	88.9
Control	21	11.33±1.67	46

Concentration of Ni in shoot and root of different plants grown in soil from Taman Beringin Landfill is shown in Figure 4.84. Highest Ni removal from contaminated soil was by *Cordyline* sp in the plant root (3 mg/kg). Other plants recorded accumulation of Ni in the root ranged of 1.66 mg/kg to 2.65 mg/kg. The accumulation of Ni in the root was two-fold higher as compared to shoot. The Ni by the four plants was in the range of 0.94 to 0.97 mg/kg. Sharman & Dhiman (2013) also reported similar findings, where the plant accumulated high concentration of Ni in the root and less in the shoot.

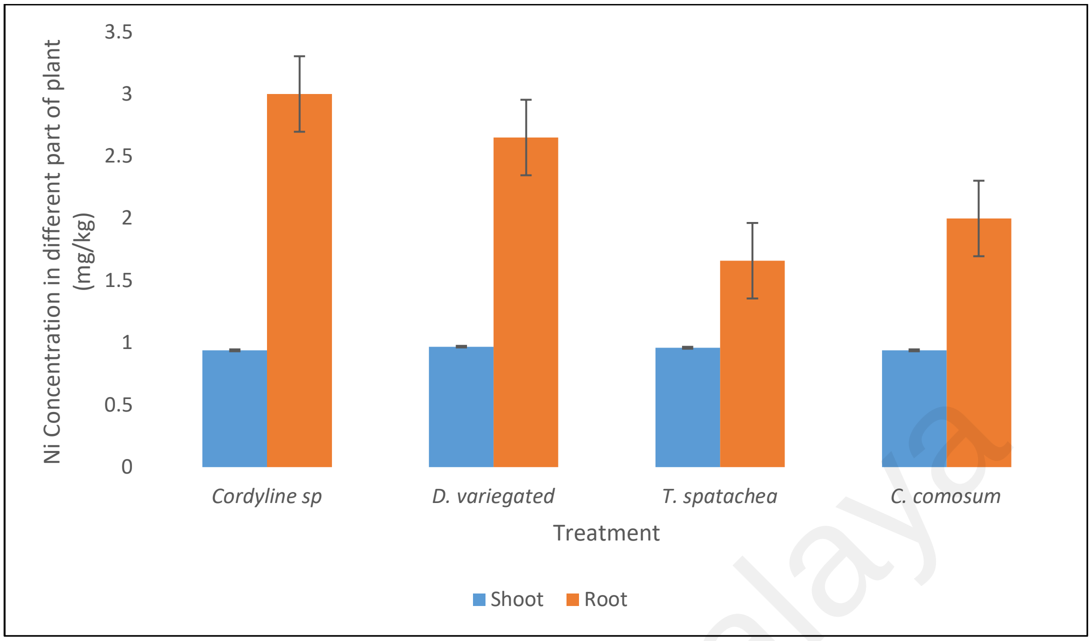

Figure 4.84: Concentration of Ni in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.9 Chromium (Cr)

Concentration of Cr in contaminated soil from Taman Beringin Landfill before and after phytoremediation using different plants is tabulated in Table 4.26. The result revealed that highest Cr accumulation was by *Cordyline* sp. The concentration of Cr reduced to 11.67 mg/kg from 46 mg/kg and the removal percentage was 75%. *D. variegated* also demonstrated 72.4 % of Cr removal from the contaminated soil. The overall order of Cr possess by different plants follow the order of: *Cordyline* sp. > *D. variegated* > *C. comosum* > *T. spatachea*. Control showed 45.6 % of Cr removal from contaminated soil. The higher Cr removal in soil treated with plants may probably be due the metal homeostasis mechanisms in plants which allow the uptake of metal from contaminated soil or water and distribute the metal into different part of the plant tissue. Jayanthi *et al.* (2017) also reported the potential of *Cordyline* sp. to remove the Cr metal in soil showed its ecological importance (Alaribe & Agamuthu, 2015) and supported by another findings by Perumal (2010) reported that the *Cyperus rotundus* and *Ludwigea* sp. able to removed

60% of Cr from a contaminated soil without supplements of organic wastes. Though, no specific Cr transporters has been identified in plants, it is likely that the metal is transported by carriers of essential element (Coelho *et al*., 2017) which resulted in high Cr removal by the four plants.

Table 4.26: Concentration of Cr in contaminated soil from Taman Beringin Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Cr removal (%)
Cordyline sp.	46	11.67±1.52	75
D. variegated	46	12.67±1.15	72.4
T. spatachea	46	15.67±2.33	66
C. comosum	46	14.67±3.78	68.1
Control	46	25±1	45.6

Figure 4.85 illustrates the concentration of Cr in shoot and root of different plants grown in soil from Taman Beringin Landfill. The accumulation of Cr differed among the four plant species. The root of the plants accumulated higher concentration as compared to the shoot. Cr accumulation in the root among the four plants ranged of 4.67 mg/kg to 15 mg/kg. Among the plant species, *D. variegated* accumulated highest concentration of Cr in the root and the concentration accumulated was 15 mg/kg. The accumulation of Cr in the shoot of plants was in the range of 0.99 mg/kg – 1.66 mg/kg. The higher accumulation of Cr in root as compared to shoot may probably be because the plants are metal tolerant plant therefore it limit soil to root and root to shoot transport and similar findings was reported by Yoon *et al*. (2006).

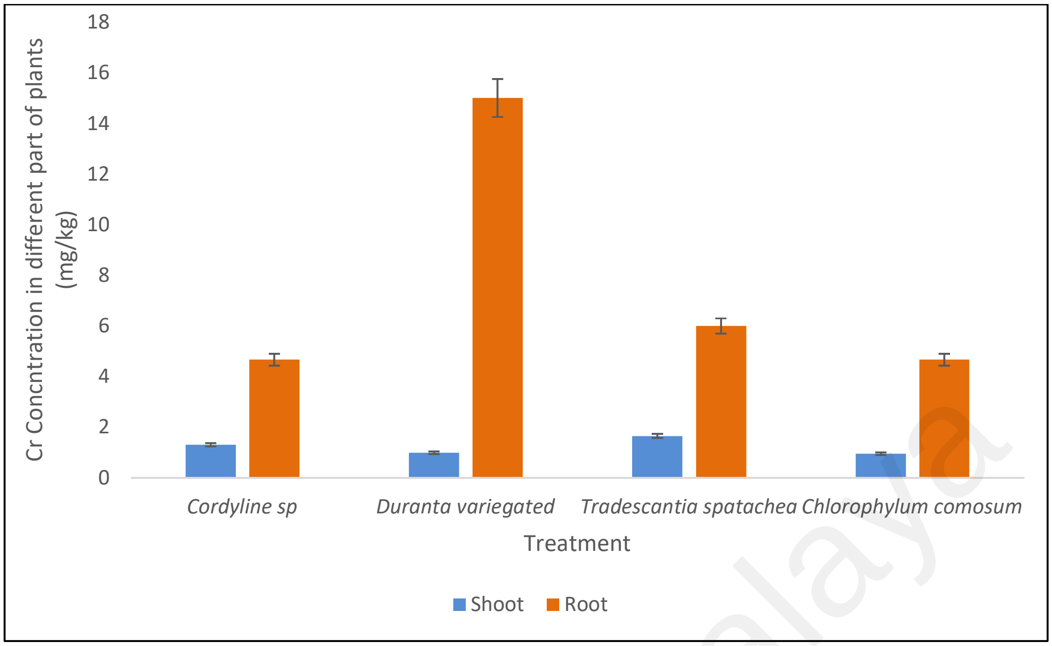

Figure 4.85: Concentration of Cr in shoot and root of different plants grown in soil from Taman Beringin Landfill

4.9.3.10 Bacterial count for phytoremediation of soil from Taman Beringin Landfill

Figure 4.86 illustrated the bacterial count across time for phytoremediation of soil from Taman Beringin landfill using different plants. Fluctuation in the bacterial count for all treatment was observed across the monitoring days however overall distribution revealed higher count in soil treated with *Cordyline* sp as compared to other plants. This probably because, *Cordyline* sp exhibited highest metal removal (8 out 9 metal) as compared to other plants. So this directly confirm that the increase in the population count may be due to rhizosphere metal accumulation might be taking rapidly by *Cordyline* sp.

At Day 0, the bacterial count was in the range of 1.86×10^9 CFU/g for all plants and it increases at Day 30 in the range of 7.25×10^9 CFU/g to 1.4×10^9 CFU/g. However, the microbial count showed decreased number for all plants with exception of *Cordyline* sp at Day 60. Monitoring at Day 90, however showed increased in the bacterial count and at the final day of monitoring (Day 120), the bacterial count decreased in the range of1.62×10^{11} CFU/g to 3.46×10^{11} CFU/g for all plants. The overall distribution of bacterial count was also similar to findings of Abioye (2010) and Arezoo (2013) who also reported fluctuation in the bacterial count for phytoremediation of heavy metal with addition of organic waste across the monitoring days.

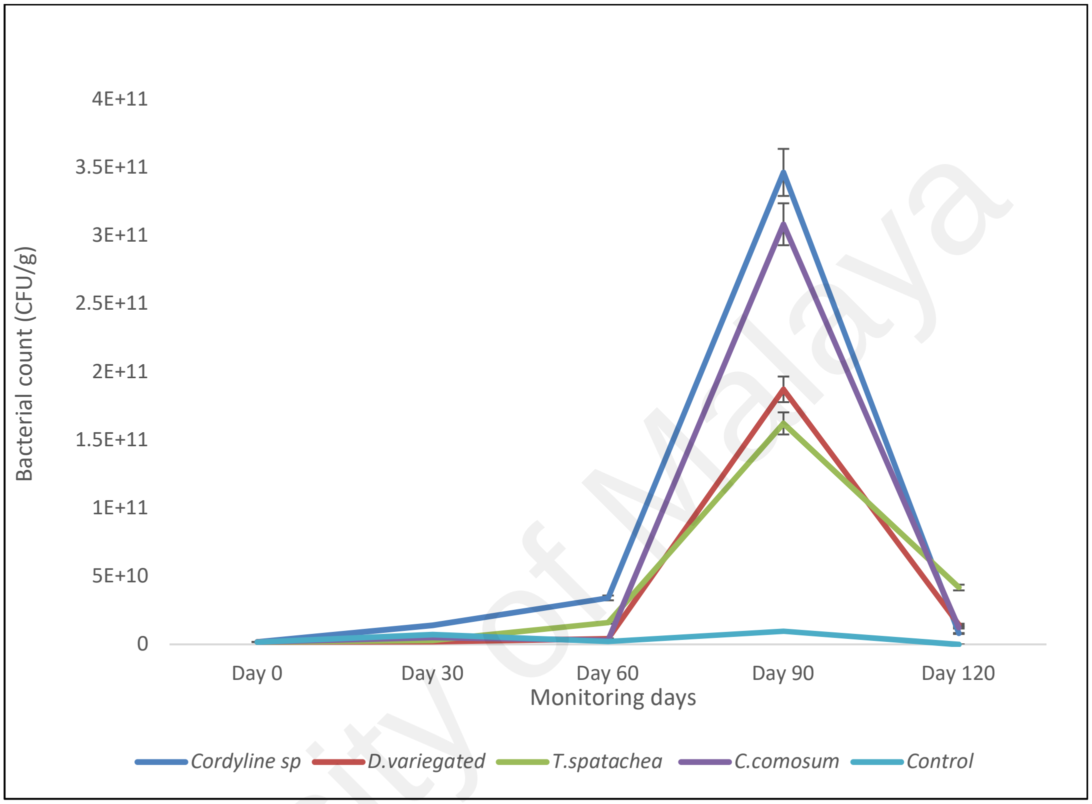

Figure 4.86: Bacterial count across time for phytoremediation of soil fromTaman Beringin Landfill using different plants

4.9.3.11 : Changes in soil pH for phytoremediation of soil from Taman Beringin Landfill using different plants

Figure 4.87 below shows the soil pH across the monitoring days for phytoremediation of soil from Taman Beringin Landfill using different plants. The initial soil pH was 7.57 and soil pH across monitoring days revealed no significant changes and ranged of pH 7.57 to 8.63 was recorded. The pH of soil was slightly alkaline. Similarly, the findings also concurred with Abioye (2011) who also reported slightly alkaline pH during the phytoremediation of Zn and Fe by *Jatropha curcas*.

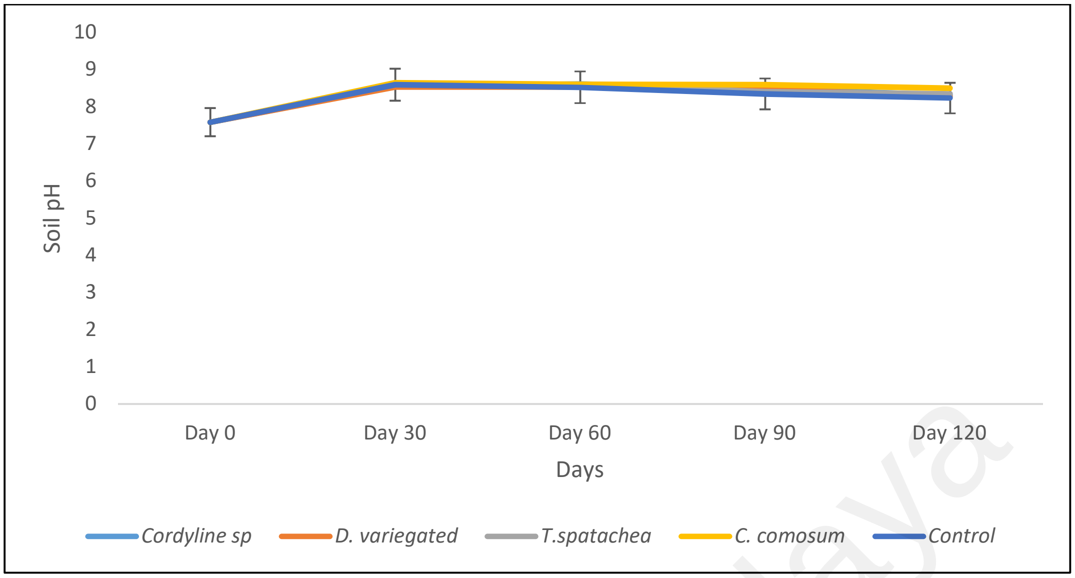

Figure 4.87: Changes in soil pH across time with different plant species for phytoremediation of Taman Beringin landfill soil

4.9.3.12 : Bioconcentration factor and Translocation factor of metal in plants for phytoremediation of soil from Taman Beringin Landfill using different plants

The potential of plants for phytoremediation of heavy metals can be assessed through Bioconcentration Factor (BCF) and Translocation Factor (TF). Table 4.27 illustrates the BCF of different plants used in phytoremediation of soil from Taman Beringin Landfill. Among the four plants, the highest BCF was recorded in *Cordyline* sp. where the BCF was 13.7 for Cu uptake. Similarly, *Cordyline* sp. also recorded the highest BCF for Pb, As, Al, Mn, Cu and Zn uptake with BCF of 1.6, 7.14, 1.12, 2.61, 13.7 and 1.39 respectively. Yet, *C. comosum* recorded the highest (1.06) for Fe uptake while *T. spatachea* and *D. variegated* have the highest BCF (1.71) for Ni uptake and BCF (1.26) for Cr uptake, respectively. Comparison among the four studied plants revealed that *Cordyline* sp. has high BCF for most of the metals.

Table 4.27: Bioconcentration Factor of metal uptakes for phytoremediation of soil fromTaman Beringin Landfill using different plants

Heavy Metal	***Cordyline* sp.**	***D. variegated***	***T. spatachea***	***C. comosum***
Pb	1.6	0.74	0.58	0.95
As	7.14	4.83	3.35	4.32
Al	1.12	0.89	0.90	0.93
Mn	2.61	1.66	1.61	2.18
Cu	13.7	9.77	7.17	7.49
Zn	1.39	0.66	0.59	0.91
Fe	0.73	0.73	0.27	1.06
Ni	1.49	0.87	1.71	1.26
Cr	0.51	1.26	0.48	0.38

Table 4.28 illustrates the TF of different plants used in phytoremediation of soil from Taman Beringin Landfill. The TF value greater than one (1) was recorded for *Cordyline* sp. for Mn (3.47) and Zn (2.06); *D.variegated* for Pb (1.99), Mn (5.46) and Zn (2.10); *T. spatachea* for Mn (1.76) and Zn (2.76); *C. comosum* for Mn (1.59) and Zn (3.43). The results revealed that all four plants shows higher translocation from root to shoot for Mn and Zn as compared to other metals. Therefore it can be concluded that the four plants are Mn and Zn accumulator but excluder for other metal studied.

Table 4.28: Translocation factor of metal uptakes for phytoremediation of Taman Beringin landfill soil

Heavy Metal	*Cordyline* sp.	*D. variegated*	*T. spatachea*	*C. comosum*
Pb	0.53	1.99	0.25	0.35
As	0.38	0.29	0.41	0.33
Al	0.30	0.37	0.27	0.16
Mn	3.47	5.46	1.76	1.59
Cu	0.69	0.59	0.54	0.29
Zn	2.06	2.10	2.76	3.43
Fe	0.40	0.36	0.57	0.47
Ni	0.40	0.36	0.57	0.47
Cr	0.28	0.06	0.27	0.20

4.9.3.13 : First order rate constant for phytoremediation of soil from Taman Beringin Landfill using different plants

Table 4.29 shows the first order rate constant for phytoremediation of Taman Beringin landfill soil using four different plants. Between the four plants, the highest first order rate constant was recorded for *Cordyline* sp. for Cu removal with removal rate constant of 0.023 day $^{-1}$. *Cordyline* sp. recorded highest first order rate constant for Pb (0.0082 day $^{-1}$), Ni (0.018 day $^{-1}$), As (0.015 day $^{-1}$), Zn (0.012 day $^{-1}$), Cr (0.011 day $^{-1}$) and Al (0.0093 day $^{-1}$). This result revealed that *Cordyline* sp. is the most promising plant heavy metal from removal for Taman Beringin as compared to other plants. For Mn, the highest first order rate constant was in *D. variegated* and *C. comosum* (0.021 day $^{-1}$). *C. comosum* has the highest removal rate constant for Fe (0.0068 day $^{-1}$). Control recorded the lowest removal rate for all metal. The overall order of the uptake of nine metals followed the order of *Cordyline* sp. < *D. variegated* and *C. comosum* < *T. spatachea* < Control.

Cordyline sp. with highest metal removal capacity indicated that this plant can be a good phytoextraction plant.

Table 4.29: First order rate constant for phytoremediation of soil from Taman Beringin Landfill (day $^{-1}$)

Heavy Metal	*Cordyline* sp.	*D. variegated*	*T. spatachea*	*C. comosum*	Control
Pb	0.0082	0.0067	0.0067	0.0067	0.0033
Cu	0.023	0.021	0.019	0.021	0.0028
Ni	0.018	0.016	0.016	0.018	0.0051
As	0.015	0.014	0.011	0.013	0.0056
Mn	0.013	0.021	0.019	0.021	0.0028
Zn	0.012	0.010	0.010	0.011	0.0035
Cr	0.011	0.010	0.0089	0.0095	0.005
Fe	0.0061	0.0064	0.003	0.0068	0.004
Al	0.0093	0.0088	0.0089	0.0090	0.0037

4.9.4 Uptake of heavy metal by different plants in soil from Bukit Beruntung Landfill

4.9.5 Response of plants

The response of plant grown in soil from Bukit Beruntung Landfill are illustrated in Plate 4.2. All the plant were showing healthy and with no visible changes. Table 4.30 shows the height of plant before and after phytoremediation of heavy metal in soil from Bukit Beruntung Landfill. Variation in the plant height was observed between the four plants. All plants showed increased height when grown in control soil as compared to plant grown in contaminated soil. This findings is also agreeable with findings of Kibra (2008).

Plates 4.2: The appearance of studied plants for phytoremediation of soil from Bukit Beruntung Landfill (A: *Cordyline* sp.; B: *D. variegated*; C: *T. spatachea*; D: *C. comosum*)

Table 4.30: Height of plants before and after phytoremediation of heavy metal in soil from Bukit Beruntung Landfill

Treatment	Initial (cm)	Final height (cm)	Control(cm)
Cordyline sp.	25±1	46±3	52±3
D. variegated	23±2.08	30±1	32±2
T. spatachea	25±1	29±2	32±1
C. comosum	32±2	73±3	65±2

The fresh weight and dry weight of the plants is recorded and illustrated in Figure 4.88 and Figure 4.89, respectively. The fresh weight and dry weight of the plants grown in contaminated soil was higher than that of plant grown in control soil. This is congruence with findings of Frank and Agamuthu (2015) who also recorded higher plant weight when grown in contaminated soil as compared to control soil.

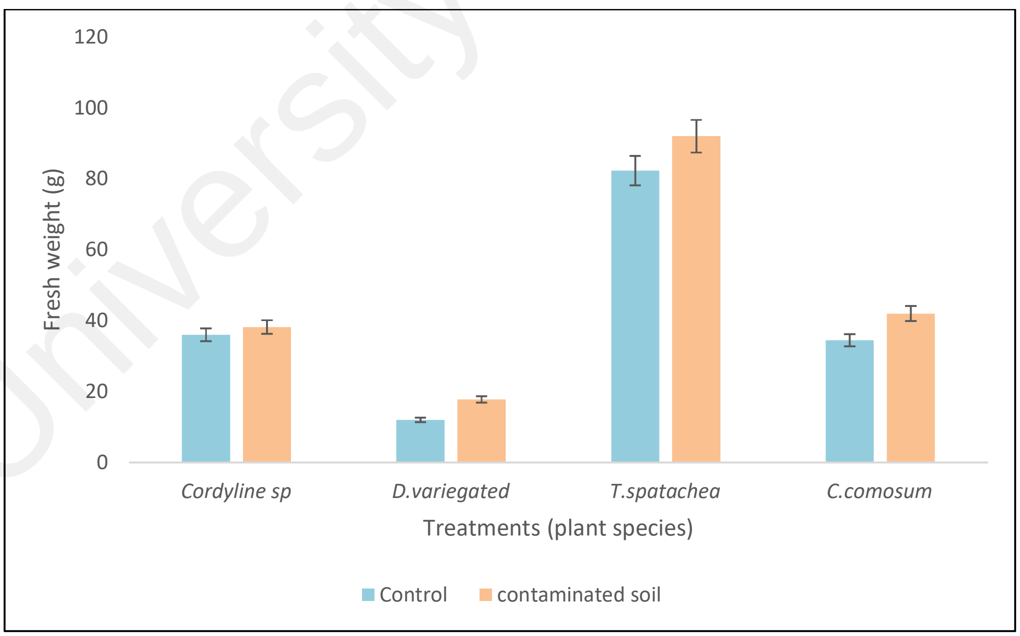

Figure 4.88: Fresh weight of plants grown in soil from Bukit Beruntung Landfill

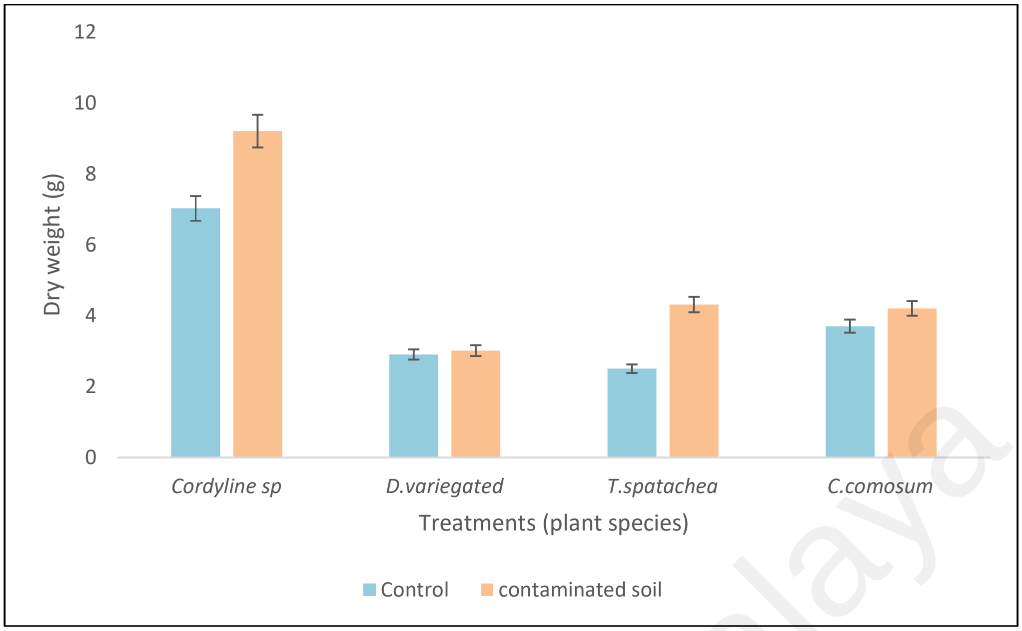

Figure 4.89: Dry weight of plants grown in soil from Bukit Beruntung Landfill

4.9.6 Phytoremediation of heavy metal in soil from Bukit Beruntung Landfill using different plants

4.9.6.1 Lead (Pb)

Table 4.31 depicts the concentrations of Pb in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. The results revealed that all four plants showed 78% of Pb removal from the contaminated soil and Control recorded 24% of removal. There are significant difference reduction in the Pb concentration between the plant and control (p= 0.00). Similarly, Amir *et al*. (2014) and Zaki (2015) also reported high accumulation of Pb by the plants. The metal tolerance ability is an indispensable property to the plant exposed to metal contaminated soil. *C. comosum* and *T. spatachea* was also previously studied by Melania and Myrna (2017) and Wang *et al*. (2011) for Pb removal from soil and the plants was able to remove high amount of Pb from contaminated soil.

Table 4.31: Concentration of Pb in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Pb removal
Cordyline sp.	67	14.66±0.57	78
D. variegated	67	14.33±0.57	78.6
T. spatachea	67	14.60±0.57	78
C. comosum	67	14.33±0.57	78.6
Control	67	52±1.73	22.4

Figure 4.90 shows concentration of Pb in shoot and root of different plant grown in soil from Bukit Beruntung Landfill. At final harvest, *Cordyline* sp. showed the highest accumulation of Pb in the root (20 mg/kg).Other plants accumulated Pb ranged of 11.65 mg/kg to 15.33 mg/kg in the plant root. Pb accumulated in the shoot of plants was in the range of 10.99 mg/kg to 12 mg/kg. Higher accumulation of Pb was found in the root as compared to the shoot of the plants and this findings also concurred with Mellem (2008) and Spirochova (2002), who also reported higher Pb accumulation in roots as compared to shoot of the plants. The high concentration of metal in the root than shoot may possibly because the plants has low mobility from plant root to shoot and agreerable with findings of Nazir *et al*. (2011).

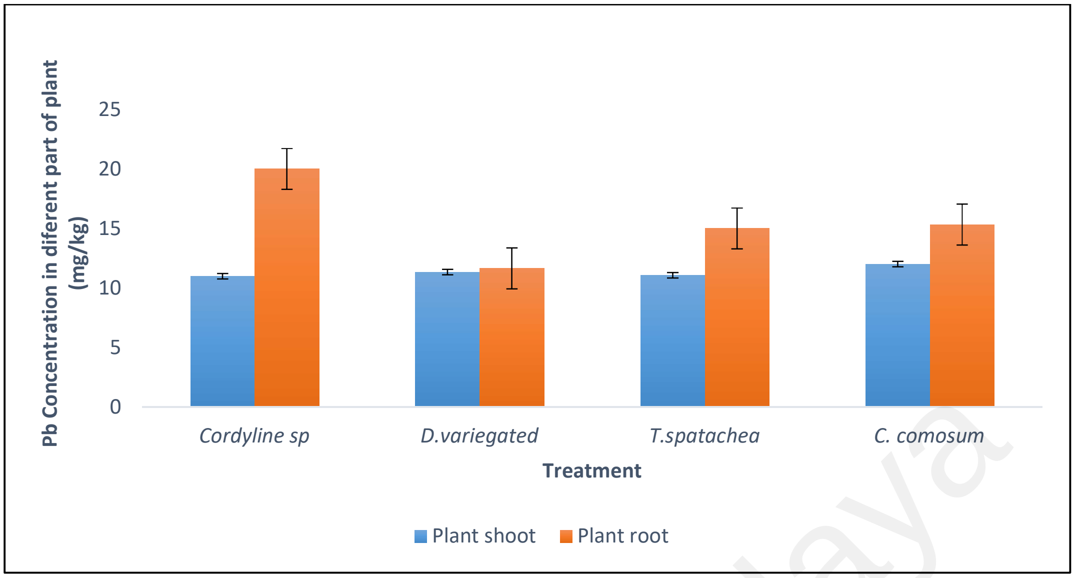

Figure 4.90: Concentration of Pb in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.2 Arsenic (As)

Table 4.32 shows the concentrations of As in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. All four plants possessed high removal of As in the range of 86% to 87.7% of removal and Control showed 24% of removal. The reduction of As by the plants was three- fold higher than control. There are significant difference between plants and Control (p=0.00). The uptake of As from contaminated soil to plant involved mass flow and diffusion of As from soil to plant root.

Concentration of As in shoot and root of different plant grown in soil from Bukit Beruntung Landfill is illustrated in Figure 4.91. The ratio of As accumulation in both plant root and shoot was almost same for all plants and in the range of 0.95 - 0.98 m/kg. This probably because As removal by all plants was almost similar. Though, not significant *D. variegated* and *C. comosum* showed slightly higher accumulation of As in the root as compared to shoot. This findings also congruence with findings of Chintakovid *et al.*(2008) and Nosheen *et al.* (2014), who also recorded higher accumulation of As in the root.

Table 4.32: Concentration of As in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of As removal (%)
Cordyline sp.	7	0.95±0.05	86.4
D. variegated	7	0.91±0.02	87
T. spatachea	7	0.86±0.05	87.7
C. comosum	7	0.91±0.02	87
Control	7	5.33±0.07	24

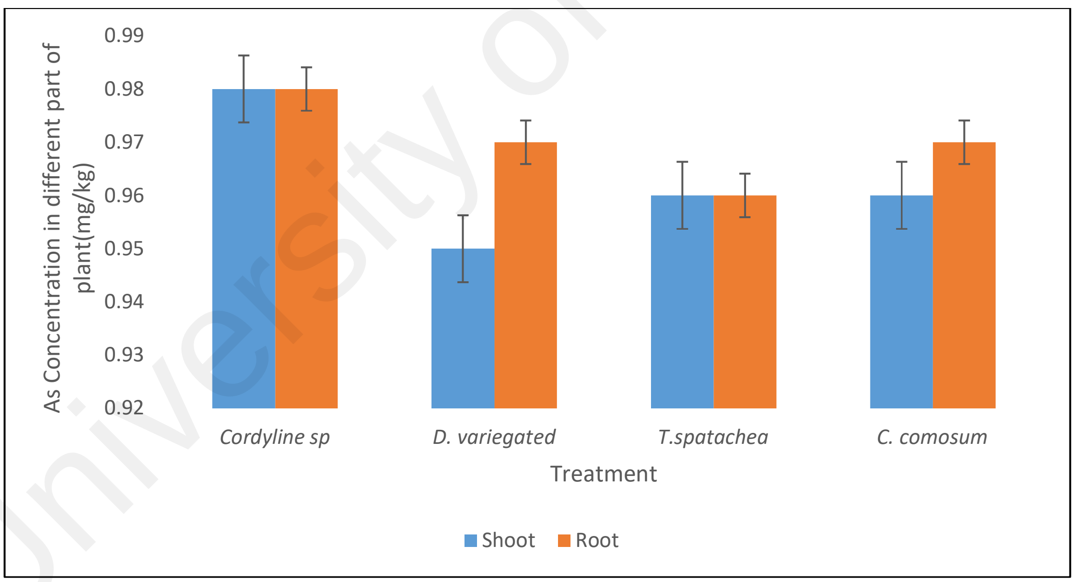

Figure 4.91: Concentration of As in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.3 Aluminium (Al)

Table 4.33 shows the concentrations of Al in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. *C. comosum* showed the highest removal of Al and the removal percentage was 60%. Other plants also showed reduction in the Al concentration in the soil and follow the order of *C.comosum* (60%) < *T. spatachea* (54%) < *D. variegated* (45%) < *Cordyline* sp. (43%) < Control (42%). The higher removal of Al by *C. comosum* showed that this plant is capable of for Al removal from contaminated soil and can be good tolerant plants towards Al.

Table 4.33: Concentration of Al in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Al removal (%)
Cordyline sp.	3040	1723.33±22.73	43
D. variegated	3040	1670.60±20.67	45
T. spatachea	3040	1400.2±14.33	54
C. comosum	3040	1200±21	60
Control	3040	1752±42.3	42

Figure 4.92 illustrated the concentration of Al in shoot and root of different plant grown in soil from Bukit Beruntung Landfill. At final harvest, highest concentration of Al accumulation was in root of *C. comosum* and Al accumulated was 925 mg/kg.

The concentration of Al accumulated in plant in the root was in the range of 780 mg/kg to 925 mg/kg. Accumulation of Al in the shoot of the plants are three times lower than Al accumulated in root and concentration of Al accumulated was in the range of 151 mg/kg - 344 mg/kg. There are significance difference between Al accumulated in plant root and shoot for all plants. The translocation of Al ion was very slow to the upper part of the plants and this was in congruence with findings of Ma *et al*. (1997). Root tissues accumulate higher concentrations of metals than shoots, which indicated greater plant availability of the substrate metals.

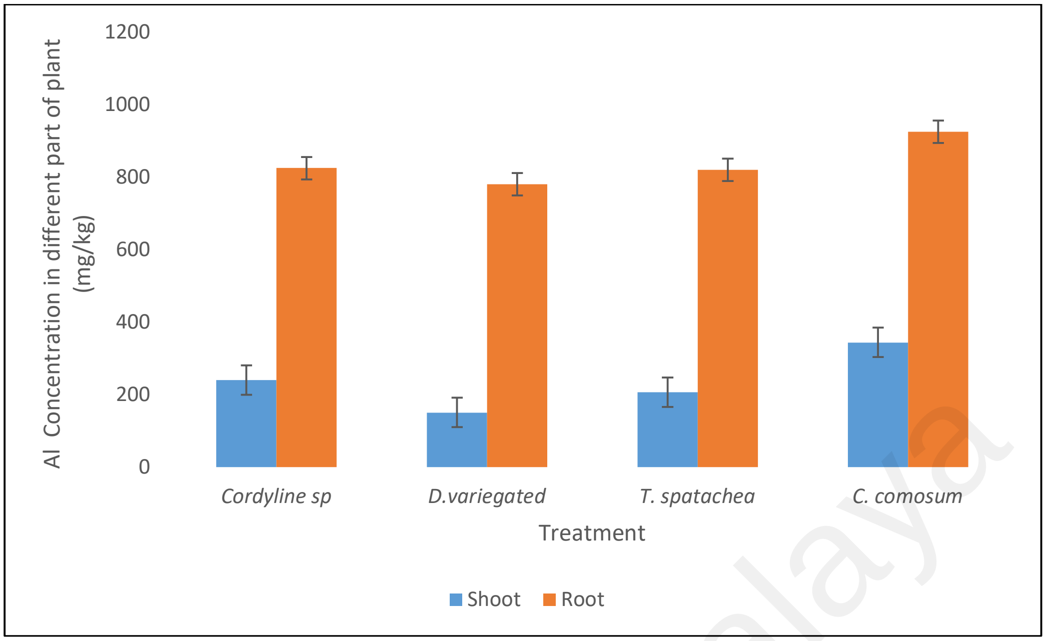

Figure 4.92: Concentration of Al in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.4 Manganese (Mn)

Table 4.34 indicates the concentrations of Mn in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. The results revealed highest removal of Mn was by *Cordyline* sp. (40%). Other plants showed removal of Mn from soil in the range of 29 - 37% and Control recorded 27% of removal. The tolerance of plant to metal is one of important that contributes higher removal of metal from soil. The four plants showed removal below 50% and this probably because the plants are not much tolerable to Mn.

Table 4.34: Concentration of Mn in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Mn removal (%)
Cordyline sp.	66	39.33±0.57	40
D. variegated	66	47±1.73	29
T. spatachea	66	44.33±8.08	33
C. comosum	66	41.33±12.70	37
Control	66	48.33±15.56	27

Figure 4.93 shows the concentration of Mn in shoot and root of different plant grown in soil from Bukit Beruntung Landfill. The accumulation of Mn in the plant parts varied among the different plant species. The results revealed higher accumulation of Mn in the shoot as compared to the root of *Cordyline* sp. and *C. comosum*. The concentration of Mn accumulated in *Cordyline* sp. and *C. comosum* was 12.67 mg/kg and 12 mg/kg, respectively. While, *D. variegated* and *T. spatachea* showed higher accumulation of Mn in the root and the concentration accumulated was 10.33 mg/kg and 12.33 mg/kg, respectively. The higher concentration of Mn accumulated in shoot of *Cordyline* sp and *C. comosum* is possibly because the translocation of metal from root to the shoot of the plant. This shows that the plant can be good hyper accumulator plant. This findings also concurred with study by Kehui *et al*. (2015) who reported higher accumulation of Mn in the above ground part of *Polygonum lapathifolium L.* as compared to the root. Similarly, findings of Hexing *et al.* (2011) also showed higher concentration of Mn accumulated in the aerial part of the Mn hyperaccumulator plant, *Phytoalcca acinosa Roxb* plant.

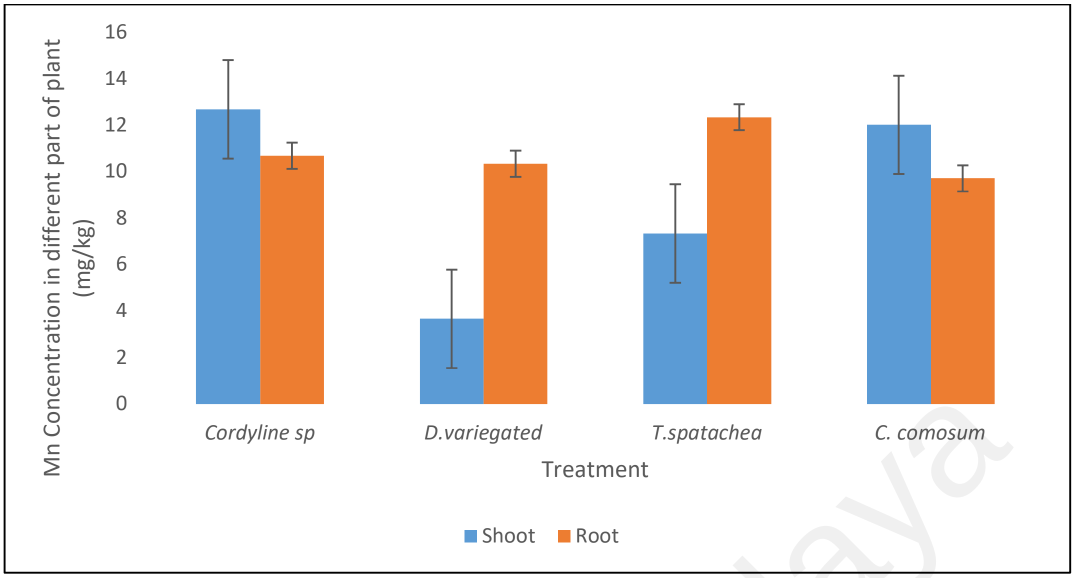

Figure 4.93: Concentration of Mn in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.5 Copper (Cu)

Table 4.35 shows the concentrations of Cu in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. Considerable amount of Cu was able to be reduced from the contaminated soil with the highest Cu uptake *T. spatachea* and the percentage of Cu removal was 81.5%. *Cordyline* sp., *D. variegated* and *C. comosum* showed 77%, 78% and 79% of Cu removal, respectively. Control showed 50.5% of Cu removal. There are significant difference between plants and control (p=0.00). The reduction of Cu by control can be due natural bio attenuation process take place in the soil. Uptake of heavy metal from soil is carried out by plants through root cells and the metal are translocated by membrane metal transporters and metal binding proteins to their destination. The process involved the specific proteins that are responsible to carried out different functions for balancing the essential metals and to avoid deficiency as well while the excess metal is accumulated in the place where there is less harm to plant cellular processes. The role of metallothioneins and phytochelatins are metal chelating molecules that also have role for Cu tolerance by plants (Zhou & Goldsbrough, 1995; Rauser, 1995; Cobbet & Goldsbrough, 2002).

Table 4.35: Concentration of Cu in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Cu removal (%)
Cordyline sp.	85	19.33±5.13	77
D. variegated	85	18.67±1.15	78
T. spatachea	85	15.67±2.30	81.5
C. comosum	85	18±2	79
Control	85	42±0.57	50.5

Concentration of Cu in shoot and root of different plant grown in soil from Bukit Beruntung Landfill is shown in Figure 4.94. The accumulation of Cu was higher in root as compared to shoot for all four plants. The order of Cu accumulation in the root among the studied plants was *T. spatachea* (36.33 mg/kg) < *C. comosum* (26 mg/kg) < *Cordyline* sp. (23.73 mg/kg) < *D. variegated* (17.33 mg/kg). The accumulation of Cu in the shoot of the four plants was in the range of 13.67 mg/kg - 21 mg/kg. The amount of Cu accumulated by plants from soil is depends on plant ability in metal transportation at soil and root interface and also on the total amount of Cu in the soil. Similar study was conducted by Maryam *et al*. (2015) on phytoremediation of Cu contaminated sludge using tropical plants; *Jatropha curcas*, *Acacia mangium* and *Hopea odorata* also showed a higher accumulation of Cu in the root compared other parts of the plants. Highest Cu absorption in roots of studied plants was also recorded by Majid *et al*. (2011).

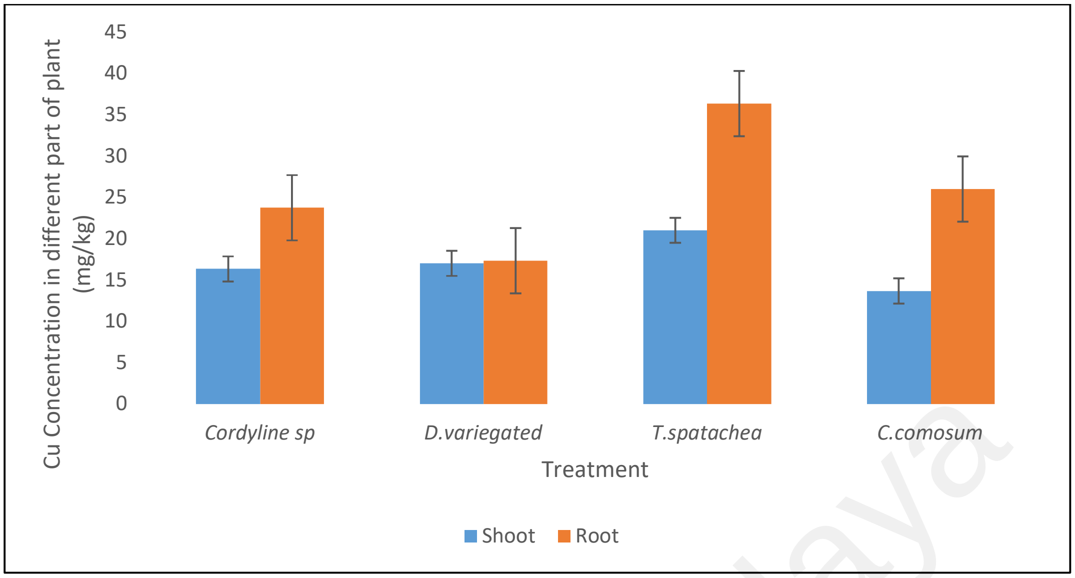

Figure 4.94: Concentration of Cu in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.6 Zinc (Zn)

Table 4.36 shows the concentrations of Zn in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants. *C.comosum* showed the highest removal of Zn and percentage of removal was 73%. Other plants showed removal of Zn in the range of 62 - 72%. Removal of Zn from the contaminated soil by Control was 16%. The four plants showed four times higher removal of Zn as compared to Control and this indicates the ability of the plants for Zn removal from compared to soil without plant. Statistical analysis also revealed significant differences between four plants and Control ($p=0.00$). Metal removal by plants can be greatly enhanced by the judicious selection of plant species and this is agreeable with Nouri *et al*. (2009).

Table 4.36: Concentration of Zn in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Zn removal (%)
Cordyline sp.	319	90±4	72
D. variegated	319	120±6	62
T. spatachea	319	119±1.73	63
C. comosum	319	87.33±3.73	73
Control	319	267±0.57	16

Figure 4.95 illustrates the concentration of Zn in shoot and root of different plant grown in soil from Bukit Beruntung Landfill. Accumulation of Zn in the root and shoot was differ among the plants however root showed higher accumulation of Zn as compared to the shoot. Zn accumulated in the root of plants was in the range 70 – 120 mg/kg while the concentration accumulated in the shoot of the plants was in the range of 26 – 75.67 mg/kg. Among the four plants, *C. comosum* recorded the highest accumulation of Zn in the root (120 mg/kg) and *Cordyline* sp. recorded the highest accumulation Zn in the shoot (75.67 mg/kg). The accumulation of high concentration of Zn in the root of the plants indicates that the plants is tolerant plant for Zn and the translocation of metal to above ground part are limited.

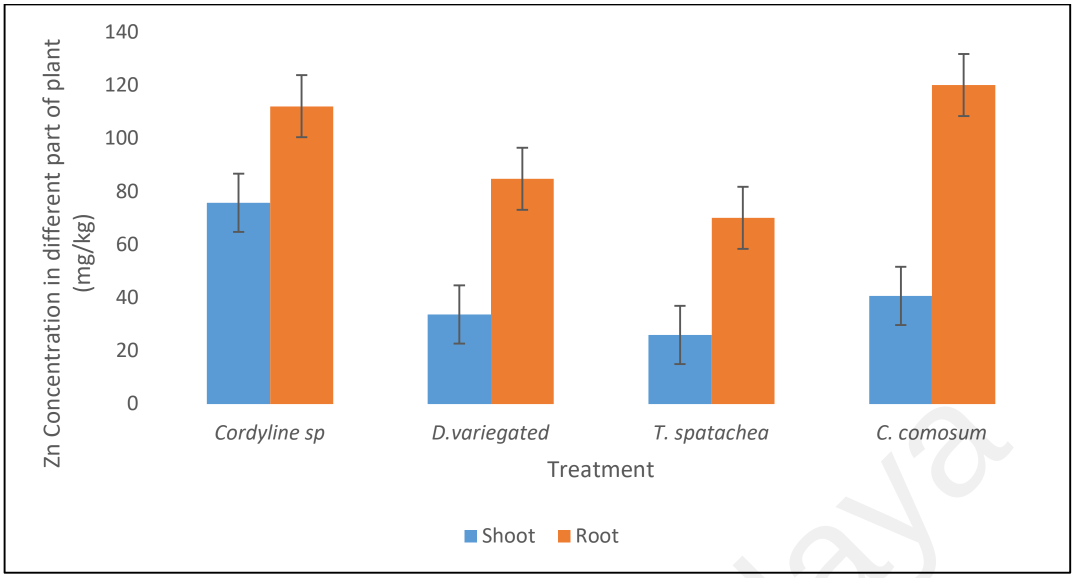

Figure 4.95: Concentration of Zn in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.7 Iron (Fe)

Concentrations of Fe in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants is shown in Table 4.37. The highest Fe removal was by *T. spatachea* and the removal percentage was 48.5%. *Cordyline* sp., *D. variegated* and *C. comosum* showed 37%, 44% and 41% of Fe removal and Control showed 28.5% of removal. The removal by the plants was below 50% and this probably was because the initial concentration of Fe was considerably high (21400 mg/kg). The plants may possibly require longer period for the optimal removal of Fe from soil to occur. However, the higher removal of Fe from soil by plants as compared to control showed that the plants are tolerant towards and Fe it can grow in soil with high concentration of Fe and this was also congruence with study conducted by Mahtab and Fatemeh (2013) who reported the considerable amount of Fe removal by studied plants exposed to high concentration of Fe.

Table 4.37: Concentration of Fe in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Fe removal (%)
Cordyline sp.	20400	12908±422	37
D. variegated	20400	11500±519.61	44
T. spatachea	20400	10500±618.33	48.5
C. comosum	20400	12100±3637.30	41
Control	20400	14567±3521	28.5

Concentration of Fe in shoot and root of different plant grown in soil from Bukit Beruntung Landfill is shown in Figure 4.96. Concentration of Fe accumulated by the plants was in ranged of 1106.67 mg/kg - 4610 mg/kg in the root and 1299 mg/kg - 2119.33 mg/kg found in the shoot of the plants. *T. spatachea* showed the highest accumulation of Fe in the root (4610 mg/kg). The concentration of Fe accumulated was differed among the plants species similarly as recorded by Mahtab & Fatemeh (2013). The accumulation of Fe by plants involved two mechanisms. Strategy I is the acidify the rhizosphere of the plant to increase the Fe solubility and use a ferric-reductase to reduce Fe^{3+} to Fe^{2+} which is transported into roots via an Fe^{2+} transporter while Strategy II metal binding ligand and mugineic acid is synthesized enzymatically from three molecules of S-adenosyl methionine and secreted from roots to bind Fe^{3+} in the rhizosphere. The Fe (III) – Ma then enters the root via a specific transporter. There are significant differences between the Fe accumulated in the root and shoot of *T. spatachea* (p= 0.038).

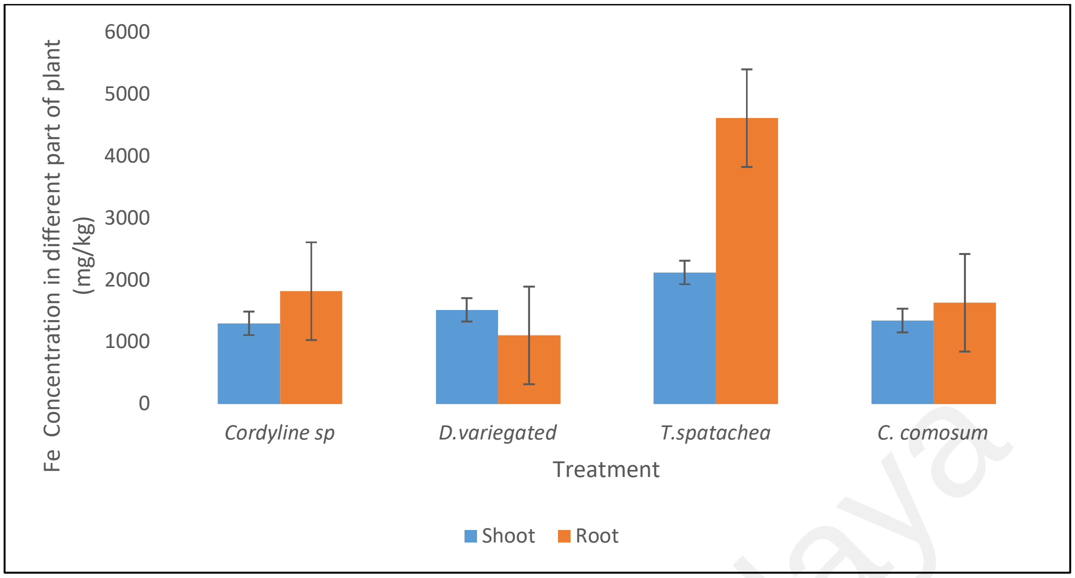

Figure 4.96: Concentration of Fe in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.8 Nickel (Ni)

Concentrations of Ni in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants is tabulated in Table 4.38. The highest removal of Ni was recorded by *Cordyline* sp. and the percentage of Ni removal was 48.22%. The removal of Ni by other plants was in the range of 22.2 % to 44.4%. The reduction of Ni was below 50% for all treatments however Cordyline sp., *D. variegated* and *T. spatachea* showed two times higher removal as compared to Control. The uptake of metal is basically depends on the species of plants and type of contaminants and this is agreeable with Biljana *et al*. (2015). In this study, the selection of species shows any important factor for removal of Ni because the plants uptake of Ni was varied among the species. The ratio of uptake of Ni between active and passive transport varies with the species, form of Ni and concentration in the soil or nutrient solution (Dan *et al*., 2002; Vogel *et al*., 2005). The ability of plants to uptake is Ni mainly use the phytoextraction mechanisms whereby the absorbed metal is transported to different parts of the plants.

Table 4.38: Concentration of Ni in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Ni removal (%)
Cordyline sp.	9	4.66±1.15	48.22
D. variegated	9	5.33±0.57	40.7
T. spatachea	9	5±1	44.4
C. comosum	9	7±1.73	22.2
Control	9	7±1.73	22.2

Figure 4.97 illustrated the concentration of Ni in shoot and root of different plant grown in soil from Bukit Beruntung Landfill. The results shows that metal concentrations in the plant tissue differs among the plants indicating the different ability of Ni uptake of the plants. The highest accumulation was in root of *T. spatachea* (3 mg/kg) while *Cordyline* sp. recorded the highest accumulation in the shoot of the plants (2 mg/kg). The Ni accumulation by plants may possible be due the presence of Nicotinamine as metal chelator. Nicotinamine (NA) is important metal chelator found in plants that forms strong complexes with most of the transition metal ions. The role for NA was proposed in Ni hyperaccumulation after the identification of Ni-NA complexes in Ni-exposed roots of *T. caerulescens* (Vacchina *et al*., 2003; Mari *et al*., 2006). The metal tolerance by plants could be attributed to the accumulation ability for the particular metal. Root of the plant in the most important sink for metal accumulation and the similar findings was also reported by Arezoo (2013).

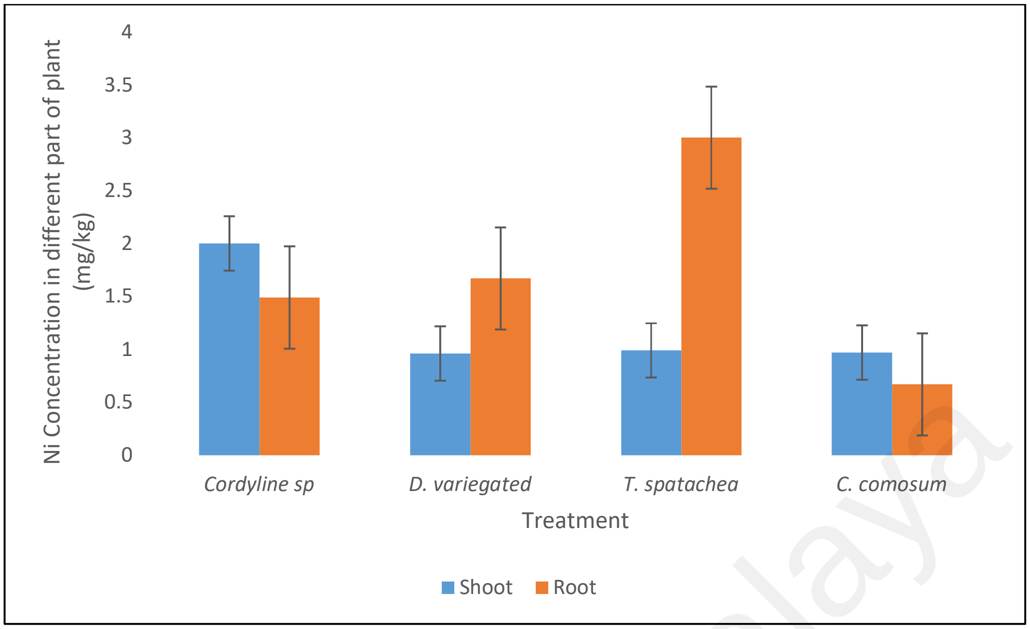

Figure 4.97: Concentration of Ni in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.9 Chromium (Cr)

Concentrations of Cr in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation using different plants is tabulated in Table 4.39. At final day of experiment set up, the highest percentage of Cr removal was recorded by *C. comosum* and percentage of removal was 54%. *Cordyline* sp., *D. variegated* and *T. spatachea* showed removal of Cr in the range of 43.6 – 49% and Control reduced showed 36% of removal. Comparison with Control showed than the reduction of Cr was higher for soil amended with plants though only *C. comosum* showed removal above 50%.

Table 4.39: Concentration of Cr in contaminated soil from Bukit Beruntung Landfill before and after phytoremediation

Treatment	Initial Concentration of heavy metal in soil(mg/kg)	Residual Concentration of heavy metal in soil(mg/kg)	Percentage of Cr removal (%)
Cordyline sp.	13	7.33±0.57	43.6
D. variegated	13	6.67±0.57	49
T. spatachea	13	7±1.73	46
C. comosum	13	6±1	54
Control	13	8.33±0.57	36

Concentration of Ni in shoot and root of different plant grown in soil from Bukit Beruntung Landfill is illustrated in Figure 4.98. The results showed higher accumulation of Cr in the plant root compared to the shoot. The Cr accumulated in the root of studied plants ranged 1 to 3.33 mg/kg while the Cr accumulated in the shoot at range of 0.98 to 1.3 mg/kg. Though the difference were not significant the findings was contrast with study conducted by Coelho *et al*. (2017), the Cr bioaccumulation was up to 11-fold greater in roots than in the aerial parts. This does not happen in this study however the metal in the root was higher than shoot. Root tissues play an essential role in the differential tolerance of a species to heavy metals, since they are able to regulate absorption from the rhizosphere and the subsequent sequestration and/or translocation to aerial parts.

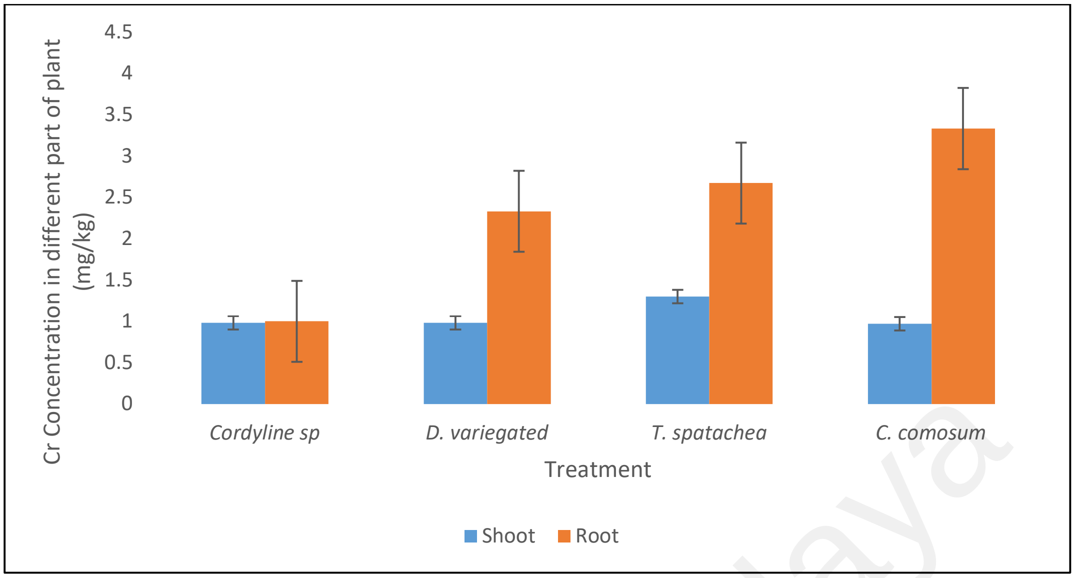

Figure 4.98: Concentration of Cr in shoot and root of different plants grown in soil from Bukit Beruntung Landfill

4.9.6.10 : Bacterial count for phytoremediation of soil from Bukit Beruntung Landfill soil

Bacterial count across time for phytoremediation of soil from Bukit Beruntung Landfill using different plant revealed a fluctuation distribution across the monitoring days for all treatment as illustrated in Figure 4.99. The average bacterial count was 3.71 × 10^9 CFU/g. At Day 30, increase in the bacterial count was recorded for all treatments and the highest count was for *T. spatachea* (2.56 × 10^{12} CFU/g) and Control showed the least count (1.84 × 10^9 CFU/g). The increase in the population may possible be due to uptake of heavy metal by plants in the rhizosphere zone. At Day 60, the bacterial count continued to accelerate for all treatments and the count was in the range of 1.81 × 10^{10} CFU/g and 5.12 × 10^{12} CFU/g. However at Day 90 and 120, decrease in the population count was recorded all the studied treatments. The bacterial count varied across the monitoring days in the soil microcosm that can be due to changes in the concentration of metal due to uptake by plants. *T. spatachea* and *C. comosum* recorded higher count across the monitoring days as compared to other treatment.

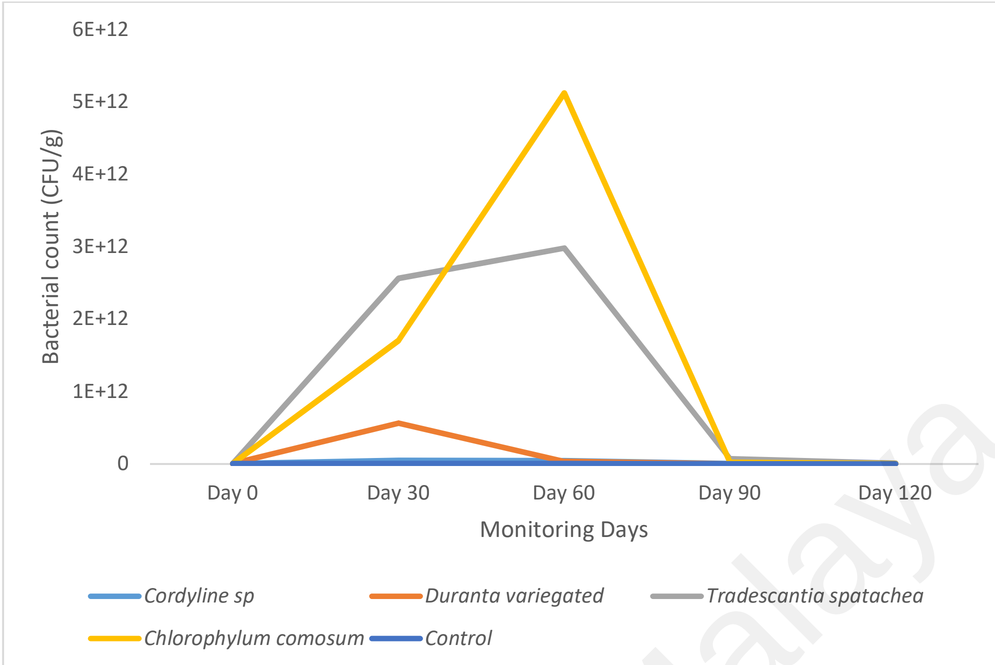

Figure 4.99: Bacterial count across time with different plant for phytoremediation of soil from Bukit Beruntung Landfill

4.9.6.11 : Changes in soil pH for phytoremediation of soil from Bukit Beruntung Landfill using different plants

The changes in soil pH for phytoremediation of soil from Bukit Beruntung Landfill is illustrated in Figure 4. 100. The figure clearly indicates no significant changes in the soil pH across the monitoring days among the plant species. The initial soil pH was 8.35 and it remains alkaline during the phytoremediation experiment. Abioye (2010) also recorded similar soil pH for phytoremediation of using metals *Jatropha curcas* plant.

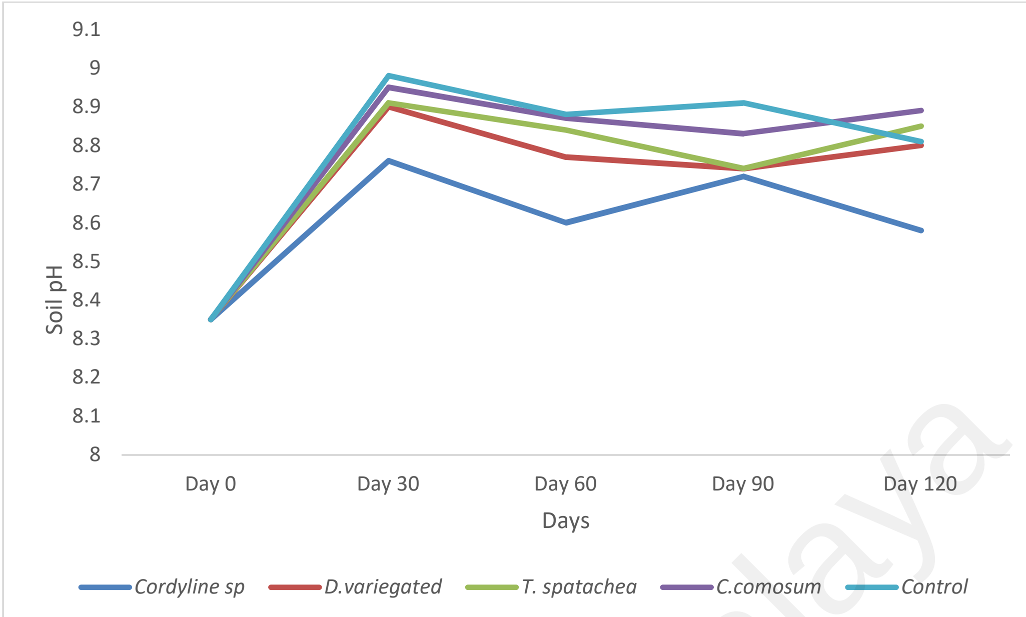

Figure 4.100: Changes in soil pH across time with different plant for phytoremediation of soil from Bukit Beruntung Landfill

4.9.6.12 : Bioconcentration factor (BCF) and Translocation factor (TF) for phytoremediation of soil from Bukit Beruntung Landfill

The BCF and TF for phytoremediation of soil from Bukit Beruntung Landfill using different plants is tabulated in Table 4.40 and Table 4.41, respectively. The BCF of heavy metal by the studied plants ranged between 0.22 to 3.65. *T. spatachea* recorded highest BCF for Cu (3.65), As (2.23), Ni (0.79), Cr (0.86) and Fe (0.64). For Pb (2.11), Mn (0.59) and Zn (2.08), the highest BCF value was by *Cordyline* sp. and for Al, *C. comosum* recorded highest BCF (1.05). The BCF varied between the studied plants across different metals. Differences in the BCF value could possibly due to type of metals and the plants metal accumulation factor that shows the variance.

Table 4.40: Bioconcentration Factor of metal uptakes for phytoremediation of soil from Bukit Beruntung Landfill using different plants

Heavy Metal	*Cordyline* sp.	*D. variegated*	*T. spatachea*	*C. comosum*
Pb	2.11	1.60	1.78	1.90
Cu	2.07	1.83	3.65	2.2
Ni	0.74	0.49	0.79	0.23
As	2.06	2.1	2.23	2.12
Mn	0.59	0.29	0.44	0.44
Zn	2.08	0.98	0.80	1.83
Cr	0.27	0.49	0.86	0.71
Fe	0.24	0.22	0.64	0.24
Al	0.61	0.55	0.73	1.05

TF greater than one (1) indicates the potential of the plant to be used in phytoextraction of heavy metal. *Cordyline* sp. has higher TF value for Ni (1.34), As (1.0), and Mn (1.18). Secondly, *D. variegated* showed TF value of 1.4 for Fe, *T. spatachea* for As and TF value was 1 and *C. comosum* showed TF greater than 1 for Ni (1.44) and Mn (1.23). Therefore the four plants can be a good phytoextraction for selected heavy metals.

Table 4.41: Translocation factor of metal uptakes for phytoremediation of soil from Bukit Beruntung Landfill using different plants

Heavy Metal	*Cordyline* sp.	*D. variegated*	*T. spatachea*	*C. comosum*
Pb	0.54	0.97	0.73	0.78
Cu	0.69	0.98	0.57	0.52
Ni	1.34	0.57	0.33	1.44
As	1	0.97	1	0.99
Mn	1.18	0.35	0.59	1.23
Zn	0.67	0.39	0.37	0.33
Cr	0.98	0.42	0.48	0.29
Fe	0.71	1.4	0.46	0.82
Al	0.29	0.19	0.25	0.37

4.9.6.13 : First order rate constant for phytoremediation of soil from Bukit Beruntung Landfill using different plants

Table 4.42 shows the removal rate constant calculation using first order kinetic modelling for phytoremediation of soil from Bukit Beruntung Landfill using four different plants. The results revealed that among the plant species, the highest first order rate constant was recorded for As with *T. spatachea* and the value was 0.017 day^{-1}. For Pb, the highest first order rate constant (0.0128 day^{-1}) was recorded for *D. variegated* and *C. comosum*.

Table 4.42: First order rate constant for phytoremediation of soil from Bukit Beruntung Landfill using different plants (day^{-1})

Heavy Metal	*Cordyline* sp.	*D. variegated*	*T. spatachea*	*C. comosum*	Control
Pb	0.0126	0.0128	0.0126	0.0128	0.0021
Cu	0.012	0.0125	0.014	0.0128	0.0058
Ni	0.0054	0.0043	0.0048	0.002	0.002
As	0.016	0.016	0.017	0.016	0.0022
Mn	0.0043	0.0028	0.0033	0.0039	0.0026
Zn	0.010	0.008	0.008	0.0010	0.00015
Cr	0.0047	0.0055	0.0051	0.0064	0.0037
Fe	0.0038	0.0047	0.0055	0.0043	0.0028
Al	0.0047	0.0049	0.0064	0.0077	0.0045

For Cu and Fe the best first order rate constant was recorded by *T. spatachea* at 0.014 day^{-1}, and 0.0055 day^{-1}, respectively. *Cordyline* sp., recorded the higher first order rate constant of Ni (0.0054 day^{-1}) and Mn (0.0043 day^{-1}) than that of other plants. For Zn, the best first order rate constant (0.010 day^{-1}) was by *Cordyline* sp. and and *C. comosum*. *C. comosum* as well showed best first order rate constant (0.0064 day^{-1}) for Cr. Variation in first order rate constant value was observed among the plant species for all the studied metals. Comparison among the plant species showed that *T. spatachea* and *C. comosum* was the most prominent species for metal removal/ uptake for phytoremediation of soil from Bukit Beruntung Landfill. The order of removal rate constant of metal removal by the plants was in order of *T. spatachea* < <*C. comosum* < *Cordyline* sp. < *D. variegated*. The four plants were able to uptake the metal from the contaminated soil at varying concentration and this indicated their ability in soil remediation.

4.10 General Summary

Summaries of salient features from bioaugmentation approach and phytoremediation under laboratory conditions are given in Table 4.43 and Table 4.44.

The concentration of metals in soil from Taman Beringin Landfill and Bukit Beruntung Landfill varied. Treatment F (proteo) works best in both soil. Higher removal of Al, As, Mn, Fe, Cr and Ni was recorded for Bukit Beruntung Landfill as compared to Taman Beringin Landfill via bioaugmentation approach. This possibly because lower concentration of metal was present in Bukit Beruntung Landfill than in soil. Therefore the microbes tends to remediate higher percentage of heavy metals which means the toxicity of metal is lower in Bukit Beruntung Landfill.

For phytoremediation experiment, higher metals removal was for Taman Beringin Landfill soil whereby *Cordyline* sp showed higher metal removal as compared to other studied plants while phytoremediation of Bukit Beruntung Landfill soil showed higher removal of metal with *Tradescantia spatachea* and *Chlorophylum comosum*. When look into the percentage of metal removal, Taman Beringin Landfill recorded higher metal removal namely for Fe, Mn, Cu, Zn, Cr, Ni and Al as compared to Bukit Beruntung Landfill.

Comparison of results to compare bioaugmentation and phytoremediation method revealed that metals reductions (Pb, As, Mn, Cu, Cr, Zn and Ni) of Taman Beringin Landfill soil is better with phytoremediation technique. However, for Bukit Beruntung landfill soil showed best removal was by bioaugmentation approach especially to Al, Mn, Fe, Cr and Ni. Table 4.45 indicates the economic value for both method adopted in this study which can be further considered for real application of the remediation.

Table 4.43: Summary of Salient findings of Bioaugmentation experiment and phytoremediation under laboratory condition

Activity	Taman Beringin Landfill	Bukit Beruntung Landfill
Status of landfill	Closed operation in year 2005	Active (started operation in year 2001)
Amount of Waste received daily	1800-2000 tonnage	1500 tonnage
Heavy Metal found in leachate	Fe, Mn, Cu, Zn, Cd, Hg, Cr, Ni and Al	As, Fe, Mn, Cu, Zn, Pb, Cd, Hg, Cr, Ni and Al
Heavy Metal found in soil	As, Fe, Mn, Cu, Zn, Pb, Cr, Ni and Al	As, Fe, Mn, Cu, Zn, Pb, Cd, Cr, Ni and Al
Bioaugmentation experiment		
i) Best treatment for bioaugmentationexperiment with 10% v/w cocktail inoculum amendment	Treatment F (Proteo-bacteria- consist of *O.intermedium*, *S.acidaminiphilia*, *A. ebreus*, *B. diminuta*, *A.caviae* DNA 4, *D.tsuruhatensis*, *P.alcaligenes*, *P. mendocina*, *S. marcescens marcescens* and *B.vietnamiensis*)	Treatment F (Proteo-bacteria- consist of *O.intermedium*, *S.acidaminiphilia*, *A. ebreus*, *B. diminuta*, *A.caviae* DNA 4, *D.tsuruhatensis*, *P.alcaligenes*, *P. mendocina*, *S. marcescens marcescens* and *B.vietnamiensis*)
Top heavy metal removed with bacterial cocktail	As, Fe, Mn, Cr, Ni and Al	Fe, Mn, Cu, Cr, Ni and AL
Highest first order rate constant	Al (0.020 day $^{-1}$)	Al (0.020 day $^{-1}$)
Shortest half life	Al (34.65 days)	Al (34.65 days)
ii) Best Inoculum concentration	30% v/w concentration	30% v/w concentration
Phytoremediation experiment		
Best plant	*Cordyline* sp	*T.spatachea* & *C.comosum*
Top Heavy metal removed	As, Mn, Cu, Zn, Pb, Cr, Ni and Al	*T.spatachea* (As, Fe and Cu) *C.comosum* (Zn, Pb, Cr and Al)
Highest Bioconcentration Factor	Cu (13.7)	Cu (3.65)
Highest Translocation Tactor	Mn (3.47)	Ni (1.44)
Highest first order rate constant	Cu (0.023 day $^{-1}$)	As (0.017 day $^{-1}$)

Table 4.44: Summary of *in- situ* soil bioaugmentation experiment at Taman Beringin Landfill

Activity	**Findings**
Treatment used	F (Proteo-bacteria- consist of *O.intermedium*, *S.acidaminiphilia*, *A. ebreus*, *B. diminuta*, *A.caviae* DNA 4, *D.tsuruhatensis*, *P.alcaligenes*, *P. mendocina*, *S. marcescens marcescens* and *B.vietnamiensis*)
Soil depth with best removal	10cm
Heavy metal removal at 10cm depth	Pb (38.24%), Al (5.2%), Mn (64.54%), Cu (63.52%), Zn (10.38%), Fe (18.75%) and Cr (89.49%)
Highest first order rate constant	Cr (0.011 day^{-1}) with amendment of Treatment F
Shortest half-life	31.5 days with amendment of Treatment F

Table 4.45: Economic value of bioaugmentation and phytoremediation method

Method	**Weight of soil**	**Economic value**
Bioaugmentation	2kg	200ml of inoculum cost about RM 4.50
Phytoremediation	2.25kg	Each plants cost about RM 8.30

4.11 Research Novelty

1. The formulated treatment especially those belonging to Proteo-group augmented to the leachate contaminated soil showed enhanced heavy metal reduction in both laboratory and actual landfill condition.

2. Studied plants namely *Cordyline* sp, *Tradescantia spatachea* and *Chlorophylum comosum* showed significant removal of metal from contaminated soil. No studies has been carried out using these plants except for lead.

3. Both techniques can be adopted and practically applied by landfill management especially in Malaysia and tropical countries to reduce the metal pollution in landfill because metals are highly toxic even at very low concentrations.

4.12 Future Recommendation

1. Future studies maybe further carried out using different combination/formulation of microbial treatments/cocktail to further increase the metal reduction in leachate contaminated soil.

2. More detailed studies on actual landfill condition should be carried out to further increase the metal reduction in leachate contaminated soil with different combination of microbes. Aeration and mixing of soil during the experiment set up is also necessary because the results obtained reported very limited reduction of metals at lower depth of soil.

3. Further studies should be carried out with higher inoculum concentration of the treatments to increase and achieve optimal heavy metal removal.

CHAPTER 5: CONCLUSION

Characterization of soil and leachate from Taman Beringin Landfill and Bukit Beruntung Landfill revealed the presence of heavy metals (Pb, As, Cu, Al, Fe, Cr, Ni, Mn, and Zn) and the metal were higher than prescribed Malaysian limit.

Eighteen bacterial species were isolated from the leachate contaminated soil from TBL and BBL which were identified as *O. intermedium*, *S. acidaminiphilia*, *A. ebreus*, *B. diminuta*, *Cloacibacterium* sp, *A. caviae* DNA 4, *D. tsuruhatensis*, *P. alcaligenes*, *C. gleum*, *P. mendocina* and *S. marcescens marcescens, B. vietnamiensis, B. aryabhattai, R.ruber, B.pumilus, B.kochii, J.hoylei* and *B.cereus.*

Among the 18 isolates, *B. vietnamiensis* demonstrated the highest tolerance for the metals (>20ppm) during the sensitivity assessment, though other microbes also showed different levels of tolerance towards the heavy metals studied.

Microbial cocktail formulated using eighteen isolated were treatments that contained all isolated microbes or gram negative bacteria or gram positive bacteria or highly sensitivity isolates (based on sensitivity test) or medium/ low sensitivity isolates or proteo-bacteria and non-proteo bacteria.

The best remediation results on soil collected from Taman Beringin landfill showed the reduction of 61%, 87%, 47%, 75%, 59% and 61% for As, Al, Mn, Fe, Ni and Cr, respectively by Proteo-bacteria group. Similarly, Proteo-bacteria also removed Al (87%), Mn (49%), Cu (65%), Fe (86%), Ni (78.7%) and Cr (67%) from Bukit Beruntung Landfill contaminated soil. The first order rate constant revealed highest removal rate constant for Al (0.02 day^{-1}). Removal rate constant with the highest value for TBL experiment was for, As, Mn, Fe, Ni, Al and Cr by Treatment F, and the removal rate constant was 0.0095 day^{-1}, 0.0063 day^{-1}, 0.014 day^{-1}, 0.0088 day^{-1}, 0.020 day^{-1} and 0.0094 day^{-1} respectively. Besides that, Treatment F recorded the highest removal rate constant for Cr (0.01 day^{-1}), Ni (0.015 day^{-1}), Fe (0.0198 day^{-1}), Cu (0.01 day^{-1}) and Mn (0.0067 day^{-1}) and Al (0.020 day^{-1}) for bioaugmentation studies of soil from BBL. Similarly the shortest half-life, highest bacterial count and highest soil redox potential value was recorded in soil amended with treatment F for both landfills.

The effect of inoculum concentration on metal removal in the contaminated soil using different concentrations of inoculum (10% to 30% v/w) revealed that reduction of metals increased with increase in the inoculum concentrations. The study also concludes that proteo-bacteria group still remained the best treatment for reduction of heavy metal in landfill leachate contaminated soil with different inoculum concentration amendment.

The in-situ/field trials at Taman Beringin landfill for a duration of 100 days also revealed significant reduction of some of the metals (Pb, Mn, Fe, Al, Cu, Cr and Zn) especially at sub-surface soil (10 cm) with the introduction of proteo-bacteria consortia.. The metal concentration was much lower in the microbe amended plots as compared to control plots. Microbe amended soil at 10cm depth recorded higher removal rate than other depths of 20cm and 30cm. The percentage of removal for microbe amended soil at 10cm depth for Pb, Al, Mn, Cu, Zn, Fe and Cr was 38.24%, 5.2%, 64.54%, 63.52%, 10.38%, 18.75% and 89.49% respectively. The removal rate constant using first order kinetic model for Pb, Cr, Zn, Mn and Cu was 0.0048 day^{-1}, 0.022 day^{-1}, 0.001 day^{-1}, 0.01 day^{-1} and 0.01 day^{-1} respectively at 10cm depth of microbe amended plot.

Lastly, Phytoremediation studies with four different plant species namely *Cordyline* sp, *D. variegated*, *T. spatachea* and *C. comosum* revealed that *Cordyline* sp. was the most promising plant for the removal of Cu , Pb, Ni, As. Zn, Cr and Al from the soil collected from Taman Beringin Landfill. The highest percentage of metal removal for phytoremediation of soil from Taman Beringin landfill was for Cu (94.35 %), Pb (63%), Ni (88.9%), As (85%), Zn (77.55%), Cr (75%) and Al (67.5%) by *Cordyline* sp. The highest removal rate constant using first order kinetic model was recorded for *Cordyline* sp for Cu (0.023 day $^{-1}$) for phytoremediation of soil from Taman Beringin Landfill. *Cordyline* sp. also recorded higher removal rate constant for Pb (0.0082 day $^{-1}$), Ni (0.018 day $^{-1}$), As (0.015 day $^{-1}$), Zn (0.012 day $^{-1}$), Cr (0.011 day $^{-1}$) and Al (0.0093 day $^{-1}$). For phytoremediation of Bukit Beruntung contaminated soil, *T. spatachea* was the most prominent species for metal removal, especially for As (87.7%), Cu (81.5%) and Fe (48.5%) while *C. comosum* showed higher removal for Pb (78.6%), Al (60%), Zn (73%) and Cr (54%) . The results revealed that among the plant species, the highest removal rate constant using first order kinetic model was recorded for As (0.017 day^{-1}) with *T. spatachea*. *T. spatachea* also recorded removal rate constant for Cu (0.014 day^{-1}) and Fe (0.0055 day^{-1}) and *C. comosum* showed high removal rate constant for Pb (0.0128 day^{-1}), Al (0.0077 day^{-1}), Zn (0.0010 day^{-1}) and Cr (0.0064 day^{-1}). Significant amount of metal was also observed to accumulate in the plant parts especially in the root of studied plants. Therefore, it can be established that *Cordyline* sp, *T. spatachea* and *C. comosum* have the ability to remove heavy metal from contaminated soil.

Hence the study conclude that bacterial isolates, especially those that belong to Proteo bacteria showed higher metal removal from contaminated soil, while *Cordyline* sp, *T. spatachea* and *C. comosum* have high potential to be used for phytoremediation of heavy metal.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

ISI Publications

1. Jayanthi, B., Emenike, C.U., Agamuthu, P., Khanom, S., Sharifah, M., Fauziah, S.H. (2016). Selected microbial diversity of contaminated landfill soil of Peninsular. Malaysia and the behavior towards heavy metal exposure. *Catena*, *147*, 25-31.

2. Jayanthi, B., Emenike, C.U., S.H Auta., Agamuthu, P., Fauziah, S.H. (2016). Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia. *International Biodeterioration & Biodegradation*. http://dx.doi.org/10.1016/j.ibiod.2016.10.053.

3. Emenike, C.U., Jayanthi, B., Agamuthu, P., Fauziah, S.H. (2018). Biotransfrmation and removal of Heavy metals: A review of phyto and microbial remediation assessment of contaminated soil. *Environmental reviews*. https://doi.org/10.1139/er-2017-0045.

Scopus Publications

1. Jayanthi, B., Emenike C.U., Agamuthu, P., Fauziah, S.H. (2017). Potential of Cordyline sp plant for remediation of metal-leachate contaminated soil. *International Journal of Chemical Engineering and Applications*, *8*(3), 199 - 202.

2. Fauziah S.H, Jayanthi B, Emenike C.U, Agamuthu, P. (2017). Remediation of Heavy Metal Contaminated Soil Using Potential Microbes Isolated from a Closed Disposal Site. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *7*(4), 230 -237.

Conference Paper

1. Jayanthi, B., Emenike, C.U., Agamuthu, P and Fauziah, S.H. (2016). Remediation of metal from leachate contaminated soil using isolates from landfill. 6th International Conference on Solid Waste Management (IconSWM 2016), Kolkata, India.

2. Jayanthi, B., Emenike, C.U., Agamuthu, P., Fauziah, S.H. (2015). Metal Pollution Sensitivity of Microbial Isolates from Open Non-Sanitary Landfill Soil. 14th APRU Doctoral Students Conference (DSC). Zhejiang University (ZJU) in Hangzhou, China.23-27 Nov 2015.
3. Jayanthi, B., Emenike, C.U., Agamuthu, P., Fauziah, S.H. (2016). Profiling and Optimization of Bacterial Species for Bioreduction of Heavy Metal Pollution. Workshop on Evaluation and Prediction of nutrients availability from biowaste using sensor and cloud technology to meet crop demands in Malaysia. 19th February 2016. University of Malaya.
4. Jayanthi, B., Emenike, C.U., Agamuthu, P., Khanom, S., Sharifah, M., Fauziah, S.H. (2015). Microbial diversity of contaminated landfill soil of Peninsular Malaysia and the behaviour towards heavy metal exposure. 2015 International Conference on Water Resource and Environment (WRE2015). July 25-28, 2015 Beijing, China.
5. Jayanthi, B., Emenike, C.U., Agamuthu, P., Fauziah, S.H. (2017). Potential of *Cordyline* sp for metal remediation in contaminated soil. 2017 4th International Conference on Chemical and Biological Sciences (ICCBS 2017).March 13-15, 2017 Prague, Czech Republic.