EVALUATION OF HEAVY METAL BIOREMEDIATION POTENTIAL USING MICROBES FROM CONTAMINATED LANDFILL SOIL

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FACULTY OF SCIENCE
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ABSTRACT

Chemical evaluations and characterization had often served as the commonly adopted options for assessing the potential impact of pollutants, which at the same time provide insight into the possible remediation technologies. However, heterogeneous substances may not be best studied in aforementioned forms because of the varied characteristics and concentrations of discrete components. Considering the high distribution of waste disposal site in Malaysia, the study was designed to isolate and identify bacterial species from leachate polluted soil. This study also aimed to generate a blend of microbial inoculum with high heavy metal resistance to serve as potential combination for optimal bioremoval of heavy metal from contaminated sites. The study also was undertaken to assess heavy metal removal performance in monometal and polymetal systems. Last but not least was to evaluate the behavioral changes of microorganisms due to metal pollution. Various methods adopted in the study ranged from soil samples collection from selected contaminated soil, microbial isolation, microbial identification, microbial inoculum build up, preparation of heavy metal standard solutions and designing experiment based on monometal system and polymetal system. Microsoft Excel and SPSS were statistical tools used in the study. Results were recorded based on 2- and 8-Day incubation period for both monometal and polymetal system. Different treatments displayed varying capacity in heavy metal removal. In monometal system, the highest rate constant value for Treatment A was Pb (K= 0.370 day-1) while Treatment B is Fe (K= 0.338 day-1) and Treatment AB is Fe (K= 0.376 day-1) in two days. Meanwhile in

eight days, the highest rate constant value for Treatment A is Mn (K= 0.178 day^{-1}) while Treatment B is Fe (K= 0.095 day^{-1}) and Treatment AB is Fe (K= 0.167 day^{-1}). In polymetal system, the highest rate constant value for Treatment A is Pb (K= 0.550 day^{-1}) and Treatment B also is Pb (K= 0.251 day^{-1}) while Treatment AB is Ni (K= 1.242 day^{-1}) in two days. Meanwhile in eight days, the highest rate constant value for Treatment A is Cr (K= 0.320 day^{-1}) while Treatment B is Pb (K= 0.188 day^{-1}) and Treatment AB is Pb (K= 0.067 day^{-1}). These results suggested that there were complex interactions exist within the bacteria. The removal of heavy metal was also found to be dependent with exposure duration and metal complexity. In general, Gram-positive bacteria displayed a better heavy metal removal performance than Gram-negative bacteria. In the presence of heavy metals, Gram-positive and Gram-negative bacteria have different threshold of tolerance as reflected by their bacterial count and the final pH condition. Therefore, it can be concluded that different microbial blends have different optimal conditions to achieve the best heavy metal removal performance.

Keywords: monometal, polymetal, Gram-positive, Gram-negative, rate constant

PENILAIAN POTENSI BIOREMEDIASI LOGAM BERAT MENGGUNAKAN MIKROB DARI TANAH PELUPUSAN YANG TERCEMAR

ABSTRAK

Penilaian dan pencirian kimia sering menjadi pilihan utama dalam mengkaji kesan terhadap pencemaran, pada masa yang sama juga memberikan pandangan mengenai teknologi pemulihan yang sesuai. Walau bagaimanapun, bahan heterogen berkemungkinan tidak dapat dikaji dengan kaedah ini disebabkan oleh ciri dan kepekatan bahan yang berbeza. Dengan peningkatan taburan kawasan pelupusan sampah di Malaysia, kajian ini dijalankan untuk mengenal pasti dan mengasingkan spesies bakteria dari tanah yang tercemar dengan bahan larut lesap Kajian ini juga bertujuan untuk menghasilkan gabungan inokulum mikroorganisma yang berketahanan tinggi terhadap logam berat, justeru berfungsi sebagai suatu kombinasi yang berpotensi untuk menyingkiran logam berat secara optimum dari kawasan yang tercemar. Kajian ini juga dijalankan untuk menilai prestasi penyingkiran logam berat dalam sistem monometal dan polimetal. Akhir sekali adalah untuk menilai perubahan tingkah laku mikroorganisma akibat pencemaran logam berat. Pelbagai kaedah telah digunakan dalam kajian ini meliputi pengumpulan sampel tanah dari tanah yang tercemar, pengasingan mikroorganisma, mengenalpastian mikroorganisma, penyediaan inokulum mikroorganisma, penyediaan larutan piawai logam berat dan merancang eksperimen berdasarkan sistem monometal dan sistem polimetal. Microsoft Excel dan SPSS adalah alat statistik yang digunakan dalam kajian ini. Keputusan kajian direkod berdasarkan tempoh inkubasi 2- dan 8-hari untuk kedua-dua sistem monometal dan polimetal. Rawatan yang berbeza menunjukkan keupayaan yang berbeza dalam penyingkiran

logam berat. Dalam sistem monometal, kadar tertinggi bagi nilai pemalar untuk Rawatan A ialah Pb (K = 0.370/hari) sementara Rawatan B adalah Fe (K = 0.338/hari) dan Rawatan AB adalah Fe (K = 0.376/hari) dalam masa dua hari. Sementara dalam masa lapan hari, kadar tertinggi bagi nilai pemalar untuk Rawatan A ialah Mn (K = 0.178/hari) sementara Rawatan B adalah Fe (K = 0.095/hari) dan Rawatan AB adalah Fe (K = 0.167/hari). Dalam sistem polimetal, kadar tertinggi bagi nilai pemalar untuk Rawatan A adalah Pb (K = 0.550/hari) dan Rawatan B juga Pb (K = 0.251/hari) sementara Rawatan AB adalah Ni (K = 1.24/hari) dalam masa dua hari. Sementara dalam masa lapan hari, kadar tertinggi bagi nilai pemalar untuk Rawatan A adalah Cr (K = 0.320/hari) sementara Rawatan B adalah Pb (K = 0.188/hari) dan Rawatan AB adalah Pb (K = 0.067/hari). Keputusan ini menunjukkan bahawa terdapat interaksi yang kompleks di antara bakteria tersebut. Penyingkiran logam berat juga bergantung kepada jangka masa pendedahan terhadap logam dan juga unsur yang terdapat di dalam logam. Secara amnya, bakteria Gram-positif menunjukkan prestasi penyingkiran logam berat yang lebih baik berbanding bakteria Gram-negatif. Kehadiran logam berat menyebabkan bakteria Gram-positif dan Gram-negatif mengalami kadar toleransi yang berbeza seperti yang ditunjukkan oleh kiraan bakteria dan keadaan pH pada akhir kajian. Kesimpulannya, gabungan mikroorganisma yang berlainan mempunyai keadaan optimum yang berbeza untuk mencapai prestasi penyingkiran logam berat terbaik.

Kata kunci: monometal, polimetal, Gram-positif, Gram-negatif, nilai pemalar

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Ag	:	Silver
Al	:	Aluminium
As	:	Arsenic
C	:	concentration of residual metal in NB
C_0	:	initial concentration of metal in NB
$C_{0(x)}$	:	initial concentration of metal x in the NB at the start of experiment
Cd	:	Cadmium
$C_{F(x)}$	:	final concentration of metal x at the end of the experiment
Cr	:	Chromium
Cu	:	Copper
Fe	:	Iron
$Fe(OH)_3$	:	Iron (III) hydroxide
$FeSO_4$	:	Ferrous sulphate
H_2O	:	Water
H_2SO_4	:	Sulphuric acid
Hg	:	Mercury
K	:	First order rate constant for metal uptake per day
Mn	:	Manganese
Ni	:	Nickel
O_2	:	Oxygen
Pb	:	Lead

t	:	Time
Zn	:	Zinc
AIDS	:	acquired immune deficiency syndrome
ATSDR	:	Agency for Toxic Substances and Disease Registry
CFU	:	Colony forming unit
DNA	:	Deoxyribonucleic acid
MHLG	:	Ministry of Housing and Local Government
MSW	:	Municipal solid waste
PAH	:	polycyclic aromatic hydrocarbons
ppm	:	Part per million
RNA	:	Ribonucleic acid
WHO	:	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background

Municipal solid waste (MSW) generation is influenced by the global population growth, urbanization, economic condition and changes in lifestyle (Vonck, 2009). As the population increases, the demand for goods and services also increases which leads to the introduction of various products to meet the need of the consumers (Odum & Odum, 2006). According to Hoornweg & Bhada-Tata (2012), 1.3 billon tonnes of MSW are generated annually for the whole world and this amount is expected to rise to 2.2 billion tonnes by 2025.

As a consequence of the rise in goods and services production, the amount of MSW generated and disposed also increases in Asia (United Nations, 2009). Even though urbanization plays a significant role in environment and social sustainability, the amount of land area required for food supply, energy and waste disposal eventually will pose a greater problem in the future (Saheri *et al.,* 2009).

According to reports, Malaysia generates 38, 563 tonnes of MSW every day since 2015 and is expected to increase by 5.19% in 2020 to 49,670 tonne per day (Yong *et al.,* 2020). In Malaysia, solid waste issues were not strongly emphasized by the government until the late 1970s. The solid waste management in the country started with street cleaning, collection and transportation of municipal solid waste to already established disposal sites (Fauziah & Agamuthu, 2012). Ministry of Housing and Local Government Malaysia (MHLG, 2015) reported that average solid waste generation per capita is 1.17 kg/day. The capital of Malaysia, Kuala Lumpur, has an average generation rate of 1.35 kg/person/day (MHLG, 2015).

MSW management is one of the major challenges to be addressed by Vision 2020 (United Nations Development Programme, 2008). The importance of waste management issues signified after the event of landfill leachate contamination to Klang Valley drinking water supply in 2006 (Fauziah *et al.,* 2009). As the government has put a major involvement in waste management system, more sanitary landfills have been built while establishment of new dumps are strictly prohibited (Fauziah *et al.,* 2009).

Landfill is the most widely used method for solid waste disposal in the world because it is the least expensive option (Theng *et al.,* 2004). In Malaysia, landfilling has become difficult because the existing landfill sites are reaching their capacity while constructing new landfill sites have been very challenging due to the shortage of land (Manaf *et al.,* 2009). According to Zin *et al.* (2012), there are 261 landfills in Malaysia where 111 of them had been closed, while the remaining are operating and undergoing upgrading into controlled landfills.

Table 1.1 lists the landfills in Malaysia as of 2015. The biggest challenge in landfilling practice is the environmental pollution caused by the production of landfill leachate (Aderemi *et al.,* 2011). Leachate from non-sanitary MSW landfills polluted the groundwater and surface water (Ismail & Manaf, 2013).

Table 1.1: Landfills in Malaysia (MHLG, 2015)

State	Landfills in Operation		Landfills not in Operation	Total
	Sanitary	Non- Sanitary		
Johor	1	13	23	37
Kedah	1	7	7	15
Kelantan	-	13	6	19
Melaka	1	2	5	8
N. Sembilan	-	7	11	18
Pahang	-	16	16	32
Perak	-	17	12	29
Perlis	-	1	1	2
P. Pinang	1	2	1	3
Sabah	-	19	2	21
Sarawak	3	46	14	63
Selangor	3	5	14	22
Terengganu	-	8	12	20
WP KL	-	0	7	7
WP Labuan	-	1	0	1
Total	**10**	**156**	**131**	**297**

According to Kamarudzaman *et al.* (2011), landfill leachate usually consists of heavy metals which is one of the most hazardous components to the environment. Other organic compounds and inorganic matters such as ammonia, sulphate and cationic metals are also present in the leachate (Kamarudzaman *et al.,* 2011). Landfill leachate characteristics vary from one landfill to another depending on the operation type, the

age of the landfill, solid waste composition, climate, hydrological condition, chemical and biological activities, moisture content, pH, temperature and degree of stabilization (Al Raisi *et al.,* 2014).

Improper landfill leachate treatments may promote health problems and environmental pollutions. According to the report by Öman and Junestedt (2008), heaviest metals that has been deposited remain inside the landfills and only less than 0.02% has been leached out. However, the concentration of heavy metals that has been leached out might differ according to the process of precipitation, dissolution adsorption, mobilization and immobilization (Bijaksana & Huliselan, 2010). High concentrations of heavy metals can be found in food waste, plastics, coal cinders, glass, dust and textile (He *et al.,* 2006).

As reported by Liu and Sang (2010), concentration of most heavy metals that seep out from the waste depends on the leaching amounts. From the study, heavy metals from leachate can lead to secondary pollution. Heavy metal pollution is a major concern because it can contribute to ecosystem disturbance whereas exposure to heavy metals such as mercury, lead, cadmium can lead to serious health problem throughout the world (Meena *et al.,* 2005). Table 1.2 shows the sources of heavy metals in the environment.

Table 1.2: Sources of heavy metals in landfill leachate (Verma & Dwivedi, 2013)

Heavy metals	Sources
Chromium	Mining, industrial coolants, chromium salts manufacturing, leather tanning
Lead	Lead acid batteries, paints, E-waste, Smelting operations, coal- based thermal power plants, ceramics, bangle industry
Mercury	Chloralkali plants, thermal power plants, fluorescent lamps, hospital waste, electrical appliances
Arsenic	Smelting operations, thermal power plants, fuel
Copper	Mining, electroplating, smelting operations
Nickel	Smelting operations, thermal power plants, battery industry
Cadmium	Zinc smelting, waste batteries, e-waste, paint sludge, incineration and fuel combustion
Zinc	Smelting, electroplating

According to Jaishankar *et al.* (2014), the most commonly found heavy metals are zinc, copper, lead, nickel, chromium, cadmium and arsenic. Heavy metals pollution might cause adverse health effect to humans because these toxic metals might lead to bioaccumulation and biomagnification in the human body (Pawan, 2012). These toxic materials might enter human body through food and water; inhalation of polluted air; cosmetics usage and drugs. Meanwhile, the excess intake of trace metal elements might enhance oxidative damage, which is the key component of chronic inflammatory disease and initiator of cancer (Umanzor *et al.,* 2006). The target organs and clinical manifestations of chronic exposures to the metal are given in Table 1.3.

Table 1.3: The target organs and clinical manifestations of chronic exposures to the metal (Manju, 2015)

Metal	Target Organs	Primary Sources	Clinical Effects
Arsenic	Pulmonary Nervous System, Skin	Industrial Dusts, Medicinal Uses of Polluted Water	Perforation of Nasal Septum, Respiratory Cancer, Peripheral Neuropathy: Dermatomes, Skin, Cancer
Cadmium	Renal, Skeletal Pulmonary	Industrial Dust and Fumes and Polluted Water and Food	Proteinuria, Glucosuria, Osteomalacia, Aminoaciduria, Emphysemia
Chromium	Pulmonary	Industrial Dust and Fumes and Polluted Food	Ulcer, Perforation of Nasal Septum, Respiratory Cancer
Manganese	Nervous System	Industrial Dust and Fumes	Central and Peripheral Neuropathies
Lead	Nervous System, Hematopoietic System, Renal	Industrial Dust and Fumes and Polluted Food	Encephalopathy, Peripheral Neuropathy, Central Nervous Disorders, Anemia.
Nickel	Pulmonary, Skin	Industrial Dust, Aerosols	Cancer, Dramatis
Tin	Nervous, Pulmonary System	Medicinal Uses, Industrial Dusts	Central Nervous System Disorders, Visual Defects and EEG Changes, Pneumoconiosis
Mercury	Nervous System, Renal	Industrial Dust and Fumes And Polluted Water And Food	Proteinuria

Untreated effluents including landfill leachate, which consist of heavy metals elements may migrate through different pathways such as into water, soil sediments and air to the nearby agricultural fields and thus becoming the sources of heavy metal

pollution in agricultural soil (De Vries *et al.,* 2005). From the study conducted by Roy and McDonald (2013), it was reported that carrots which grown in soils contaminated with cadmium (Cd) have high tendency to cause toxicology problems in men, women and young children. Meanwhile, according to Morgan (2014), high level of Cd in soil was identified to be the cause of itai-itai disease in Toyama Prefecture, Japan. As metals can accumulate in the plant cells, this will lead to various adverse effects on plants including reduction of cell activities and inhibition of plant growth (Farooqi *et al.,* 2009).

Heavy metals also can be considered as the most serious pollution in the aquatic environment because metals can accumulate in the body of marine organisms (Malik *et al.,* 2014). According to Alina *et al.* (2012), diet and foods are the most predominant sources of heavy metals contaminant and various studies have been conducted on marine organisms especially fishes and shellfishes because these organisms contributed to a large percentage of dietary protein to human being globally. Transportation of heavy metal ions occur through the blood as metal ions bound to protein and then those ions will be transported to the organs and tissues of the marine organisms (Singh & Kalamdhad, 2011).

In order to control heavy metal pollution by means to minimize the impacts to the environment, the best method is to establish an innovative technology, which could economically remediate the metal toxicity, thereby reducing the adverse effects on living organisms and environment (Garbisu & Alkorta, 2003). There are different techniques used in remediating heavy metal especially in soil such as physical, chemical and thermal processes (Abioye, 2011). Moreover, according to McIntyre (2003), these

methods required a range of US$0.6 million to US$2.5 million in order to remove $1m^3$ soil from a 1 acre of contaminated site.

Bioremediation is an alternative to conventional treatments, which offers higher probability in destroying various contaminants by using natural biological activity (Shukla *et al.,* 2010). Bioremediation is the most cost-effective and eco-friendly treatment, and uses relatively low technology as compare to conventional methods. This is because the method tends to lead to a complete mineralization of the pollutants and yet leaving the ecosystem undamaged (Perelo, 2010). Bioremediation of contaminated soil actually has happened naturally since 3, 500 million years ago when the life first appeared on the Earth (Cortez *et al.,* 2010).

Different types of microorganisms have a different tendency to degrade contaminants. Most microorganisms use contaminants as source of carbon and energy needed for the growth and survival (Thapa *et al.,* 2012). Somehow, bioremediation does have some limitation but scientists have been able to figure out the special microbial population and a better reaction technique in order to reach the remediation purpose (Juwarkar *et al.,* 2010). Additionally, many studies on bioremediation have been reported and scientific literature has revealed various advance techniques in bioremediating waste compounds with the possibility of contaminants degradation (Juwarkar *et al.,* 2014). Most of the techniques applied in bioremediation are aerobic processes. However, anaerobic processes also have been developed in order to degrade pollutants in oxygen shortage areas (Franchi *et al.,* 2016).

1.2 Problem Statement

Landfilling of waste into non-sanitary landfill is still the main technique of waste disposal. The main concern of this situation is that non-sanitary landfill has poor leachate management system thus cannot prevent leachate from flowing to the surrounding area or seeping into underground water system. Leachate usually contains high concentration of heavy metals and high amount of heavy metals can be dangerous to human body (Roongtanakiat *et al.,* 2003). These heavy metals such as cadmium, chromium and mercury could cause kidney failure, skin lesions, fatigue lung and increased blood pressure (Castro-González & Méndez-Armenta, 2008).

According to Lenart-Boroń and Boroń (2014), adverse effects of metals on microorganisms has resulted in reduction of microbial growth, soil respiration, decreasing of decomposition of organic matter, decreased diversity, and declined activity of several soil enzymes. Hafeburg and Kothe (2007) stated that heavy metals had led to general changes in morphology, disruption of the life cycle and increase or decrease of pigmentation of microorganisms in the soil. Rajapaksha *et al.* (2004) had compared the reactions of bacteria and fungi to zinc and copper in soils, and they concluded that the bacterial community is more sensitive to increased concentrations of heavy metals in soils than the fungal community.

However, microorganisms also tend to evolve via several mechanisms to tolerate the uptake of heavy metal ions in order to survive under metal stressed conditions (Spain & Alm, 2003). Some mechanisms that might undergo evolution include reduction of the heavy metal ions to a less toxic state, accumulation and complexation of the metal ions inside the cell, and efflux of metal ions outside the cell (Zaidi *et al.,* 2009). According to Cortez *et al.* (2010), microorganisms also have the ability to immobilize or mobilize

these heavy metal contaminants in natural environments. Therefore, it is important to investigate the potential of microorganisms in converting heavy metal from landfill leachate contaminated soil, from the toxic phase to non-toxic phase.

1.3 Objectives

1) To isolate and identify bacterial species from contaminated landfill soil.
2) To generate a blend of microbial inoculum with high heavy metal resistance.
3) To evaluate the heavy metal removal performance in monometal and polymetal systems by microbes from contaminated landfill soil.
4) To investigate the behavioral changes of microorganisms based on the growth and pH due to metal pollution.

CHAPTER 2: LITERATURE REVIEW

2.1 Soil Pollution

Pollution can be defined as any substance that is present in an environment having the chemical properties and quantity that would restrain the function of natural processes and produces adverse environmental and health effects (Nathanson, 2018). Pollution is very costly because it may cause productivity losses, health problems, and damages to ecosystems (National Research Council, 2010). Household air and water pollution, the forms of pollution that were historically associated with profound poverty and traditional lifestyles, are slowly declining. However, ambient air pollution, chemical pollution, and soil pollution are all increasing (Smith & Ezzati, 2005; Omran, 2005). These types of pollution may result from the uncontrolled growth of cities; increasing mining, smelting, and deforestation; the global spread of toxic chemicals; heavier applications of insecticides and herbicides; and an increasing use of petroleum-powered vehicles (Wilkinson *et al.,* 2007).

Recently, scientific understanding of pollution and its effects on health have incredibly progressed (National Research Council, 2012; Brauer *et al.,* 2012). New technologies, including satellite imaging, have heightened the ability to map pollution, measure pollution levels, identify sources of pollution, and track temporal patterns (Brauer *et al.,* 2012; Sorek-Hamer *et al.,* 2016). Regardless of these advances in technology, there are still numerous gaps in information about pollution and its effects on health. These gaps include lack of information in numerous nations on pollution levels and the frequency of pollution-related disease and poor information on the lethal effects of chemicals used (Landrigan & Goldman, 2011; Grandjean & Landrigan, 2014).

Pollution prompts to endanger the stability of the Earth's support systems, threatens the continuing survival of human societies, endangers the health of people, and responsible for a massive global burden of disease, disability, and premature death (Rockström *et al.,* 2009; Landrigan *et al.,* 2017). The World Health Organization (WHO) estimates that 12.6 million persons die each year of polluted environment in 2012 (WHO, 2016). Pollution was also responsible for an estimated 9 million premature deaths in 2015 (Landrigan *et al.,* 2017). As shown in Figure 2.1, in 2015, pollution in the soil, air, and water (total pollution) killed three times more people than acquired immune deficiency syndrome (AIDS), tuberculosis, and malaria combined (Landrigan *et al.,* 2017).

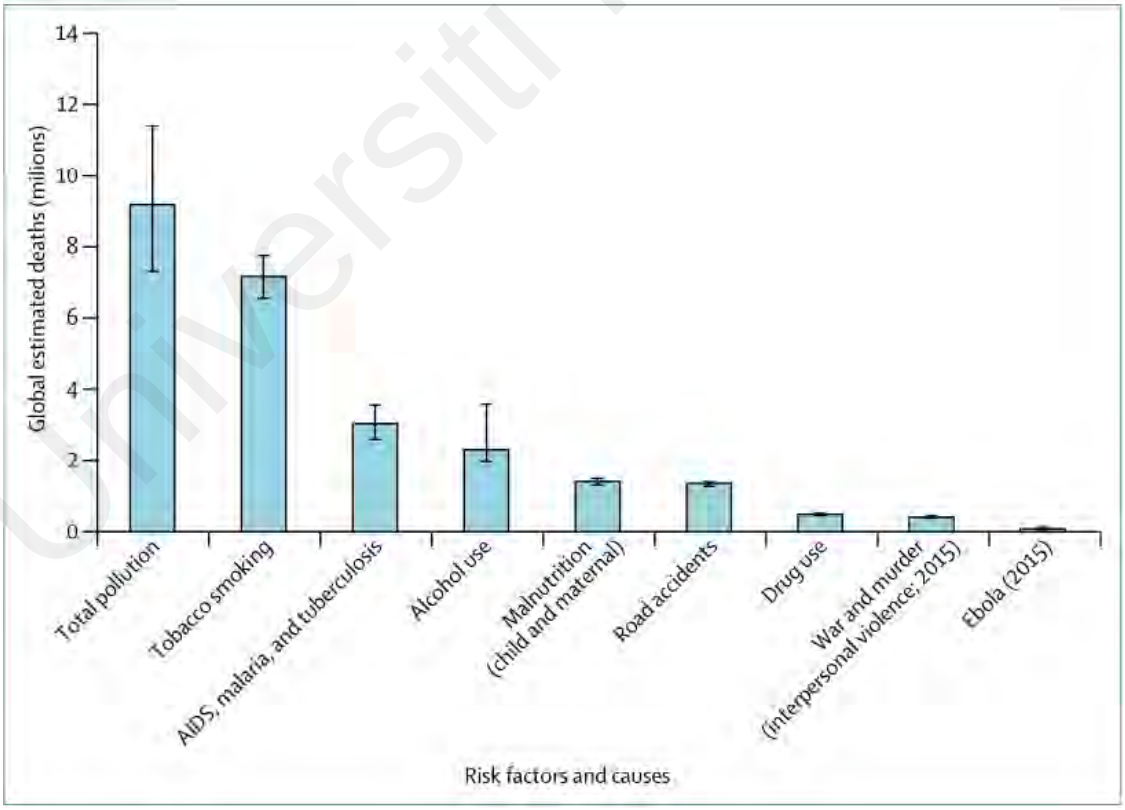


Figure 2.1: The Lancet Commission on causes of global mortality in 2015 (Landrigan *et al.*, 2017)

According to Mishra *et al.* (2015), soil is a crucial part of the natural environment because it influences the distribution of plant species and provides a habitat for a wide range of organisms. Soil tends to respond to any changes in the environment either temporary and reversible or permanent and irreversible (Mishra *et al.,* 2015). Soil pollution causes adverse effects on physical, chemical and biological properties of the soil, reduces soil productivity, affects plant growth, pollutes underground water, and affects human and other organisms (Rosen, 2002). There are many sources of soil pollution; either from anthropogenic activities or from natural phenomena, but the most typical sources are through chemicals from agricultural sectors, industrial activities, wastewater and improper waste disposal (Shayler *et al.,* 2009). Table 2.1 shows the estimation of waste generation by region in Malaysia with annul increment of 3%.

Table 2.1: Estimation of waste generation by region in Malaysia with annul increment of 3% (Sadeghi *et al.*, 2013)

Region	**Estimation of waste generation (tonnes/day)**					
	2015	**2016**	**2017**	**2018**	**2019**	**2020**
Central Peninsular Malaysia	8873.3	9096	9318.7	9541.4	9764.1	9986.8
Eastern Peninsular Malaysia	4903.8	5021.1	5138.4	5255.7	4317.3	4434.6
Southern Peninsular Malaysia	5456	5586.4	5716.8	5847.2	4804	4934.4
Northern	7070.9	7240	7436	7578.1	6225.7	6394.7
East Malaysia	6995.8	7163.2	7330.3	7497.7	6159.7	6326.8
Total	**34106.7**	**34940.2**	**35720.1**	**31270.8**	**32077.3**	**31686.4**

Agricultural activities can be classified as a major source of soil pollution. Many agricultural activities apply fertilizers, pesticides, and insecticides for a better crop yield. However, the excessive application of these chemicals may cause natural radionuclides and heavy metals pollution from mercury, lead, nickel, copper, and cadmium (Ilker *et al.,* 2007). The excessive usage of nitrogen fertilizers might contaminate the groundwater thus causing problems to human once consumed since these fertilizers consist of carcinogenic materials such as nitrosamines (Ilker *et al.,* 2007). According to Beseler *et al.* (2008), agricultural activities such as livestock farming, fish farming, and trees processing industries, dairy farming and animal slaughtering activities, have generated many wastes, which gives a serious impact to human-beings and other organisms.

Carbonell *et al.* (2011) reported that the usage of mineral fertilizers has resulted in the increases of the concentration of nickel and cadmium in the soil thus contribute to an adverse effect on human health and livestock once exposed. According to Zhong *et al.* (2007), the 13 years continuous application of inorganic fertilizers, which is nitrogen, and phosphorus fertilizers has resulted in greater nitrification and urease activity in the soil. Meanwhile, the use of nitrogen fertilizers will decrease the soil pH and without proper nitrogen management, it may result in declining of crop productions (Savci, 2012). The unbalance usage of nitrogen and phosphate fertilizers have increased the concentration of nitrate phosphate in soil causing the declining of soil health thus decreases the qualitative and quantitative production of crops (Yargholi & Azarneshan, 2014).

Industrial waste disposal into the land will lead to soil and groundwater pollution from the production of leachate (Pillai *et al.,* 2014). Leachate that flows from waste has

greater effects on the chemical and geotechnical properties of soil because leachate has a tendency to alter the soil properties as well as soil behavior (Ukpong & Agunwamba, 2011). According to De *et al.* (2016), leachate can be defined as high strength toxic effluent, which consists of complex organic and inorganic pollutants, produced by rainwater percolation at waste layers.

The increasing numbers of industrial activities have increased the disposal of effluent into the land area and water bodies (Kaur & Sharma, 2014). Effluent can be defined as treated or untreated wastewater that flows from the treatment plant, sewer or industrial activities (US-EPA, 1994). According to Kaur and Sharma (2014), the effluent that flows into soil might change the soil properties, pH, nutrient contents, soil infiltration rate, porosity, bulk density and hydraulic conductivity.

El- Arby and Elbordiny (2006) reported the effects of treated wastewater on soil where the total content of heavy metals such as lead, cadmium, chromium, and nickel in the surface layer of the soil is higher when compared to the lower layers. The heavy metal contamination does not only promote soil pollution but also reaches underground water and wells thus contaminate the water sources (Al- Musharafi *et al.,* 2012).

According to Karakas *et al.* (2006), irrigation of sewage along the discharge channel of Konya, Iran, increased the concentration of heavy metal such as lead, zinc, chromium, copper, cadmium, manganese and nickel in fertile soil. These metals tend to accumulate in plants thus affecting the plant growth (Pandey & Tripathi, 2011). The detail effects of contaminated soil will be further discussed in subchapter 2.2.

According to Noor *et al.* (2013), more than 200 organic compounds have been found in landfill leachate and out of 35 compounds are hazardous to environment and human health. The biggest issue with leachate is its tendency to migrate into soil, contaminate underlying soil and groundwater and affect surface water quality (Mohamed & Jahi, 2000). Heavy metal is one of the hazardous components found in leachate, which contaminated the soil through the disposal of metal wastes, fertilizers, pesticides, paints, sewage sludge, petrochemicals and coal combustion residues (Zhang *et al.,* 2010). Soil will trap those metals, which remain in the soil for a long period because most of the metals are nonbiodegradable (Wuana & Okieimen, 2011). Soil contaminated with heavy metal will result in food chain disturbance, reduction of food quality and problem in land occupancy (Ling *et al.,* 2008).

Heavy metals tend to lower the biomass, biodiversity, biodegradation, enzyme activity and respiration process of microorganisms (Wyszkowska *et al.,* 2008). High concentration of heavy metals will damage their nucleic acid, nutrient structure and form a complex protein molecule to induce cell membrane disruption or entire cell function failure (Bong *et al.,* 2010). According to Lenart-Boroń and Boroń (2014), different metal may affect different microbial species, for example, high copper concentration in soil will disturb the microorganisms that are important in nitrification and mineralization process of protein compound. Meanwhile, 100 ppm of zinc in soil will prohibit the nitrification activity and 1000 ppm of zinc will inhibit almost completely microbial activity in the soil.

Waste management concept adapted by Malaysia is the waste hierarchy concept including the "3R concept" which emphasizes on waste reduction, reuse and recycle in order to achieve waste minimization in the future (Fauziah & Agamuthu, 2013). Figure

2.2 illustrates the waste management hierarchy. The Solid Waste and Public Cleansing Act 2007 focuses on the waste hierarchy concept in order to reduce the current practice of the disposal of 95% of MSW to landfills (Aja *et al.,* 2014).

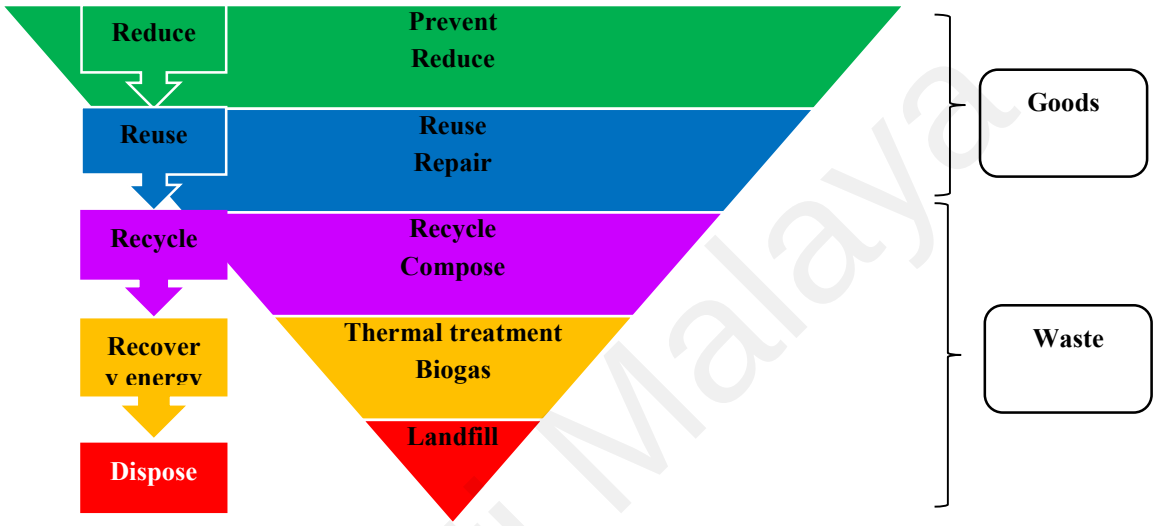


Figure 2.2: Waste hierarchy (Adapted from Wan Ahmad Nadzim, 2016)

2.2 Microorganisms and Heavy Metals in Soil

2.2.1 Heavy Metals Impacts on Microbial Community Structure and Microbial Process

Soil contamination is usually related to the types of activity in the area, for example, some areas in Upper Silesia, Poland has been contaminated with heavy metals because of the coal deposits and the number of coal processing plants (Rachwał *et al.,* 2015). The existence of heavy metal was usually initiated in industrialized and urbanized areas in the whole world (Máthé *et al.,* 2012). Heavy metals cannot be degraded thus they remain in the soil for a long time and affects microbial community structure, thus

interfere with the degradation and mineralization of organic matter, and nutrient cycles in the soil (Kozdrój & van Elsas, 2001; Simona *et al.,* 2004).

Heavy metals may affect the number, diversity and microbial activity in the soil, which resulted in the reduction of the growth rate and reproduction of microorganisms (Tayebi *et al.,* 2014). Prolong heavy metals contamination can trigger continuous changes in microbial composition, and microbial population that has continually exposed to contaminated environments can have a much higher biomass and activity than that measured in uncontaminated soils (Markowicz *et al.,* 2016; Joynt *et al.,* 2006). Heavy metals may accumulate in the tissues of organisms and appear in the food webs upon consumption and thus threatened human health and other organisms (Markowicz *et al.,* 2016).

Microbial communities are bioindicators of the soil quality and biomonitoring tools for assessing the recovery of soil quality throughout heavy metal remediation processes (Gómez-Sagasti *et al.,* 2012). Microbial communities in soil perform as the biological catalysts to promote diverse reactions and metabolic processes in the biogeochemical cycles of nutrients. They can also repair the soil structure, detoxify contaminants, and manufacture crucial compounds for other organisms and plants (Khan *et al.*, 2009).

Microbial communities in the soil are referred to as sensors towards any natural and anthropogenic activities occurring within the soil system (Wang *et al.,* 2007). Researchers have used the microbial enzymatic activities as bioindicators to control toxicological effects of assorted pollutants on soil microbial quality (Shen *et al.,* 2005). Microbial biomass carbon (MBC) is deliberated as one of the significant soil biological

activities normally influenced by heavy metal pollution. Within the past, MBC has been used as important indexes of soil quality assessment (Xu *et al.,* 2008). Even at moderate levels, heavy metal pollution could cause long term declines in microbial diversity (Gans *et al.,* 2005), which may affect the functional stability of microbial communities (Brandt *et al.,* 2010).

Prolonged exposure to heavy metal pollution apparently has damaging effects on the structure and the function of the microbial community, as dormancy might not be a helpful survival choice (Khan *et al.,* 2010). The amount of species loss apparently becomes a task of the mobility of resistance genes such as horizontal gene transfers and the behavior of the metal species within the environment (Cai *et al.,* 2009). Muhammad *et al.* (2005) established that this might be due to additional energy cost to soil microorganisms beneath heavy metals stress condition. The additional energy cost ought to cause a reducing amount of substrate that is available for microorganisms' growth (Zhang *et al.,* 2016).

Microbial communities in soil can immobilize heavy metals (Wyszkowska *et al.,* 2013). Alternatively, they contribute to a greater mobility of heavy metals, particularly because of the microbial metabolites (Kuffner *et al.,* 2008). Soil polluted with heavy metals in diverse quantities and forms resulted in modifications of microorganisms' counts and activity of microbial enzymes, which is the real replication of the actual microbiological condition in soil (Wyszkowska *et al.,* 2007).

As reported by Ancion *et al.* (2010), transformation in microorganism community structure was detected after only three days of exposure to metal. The short lifecycle of

bacteria is anticipated to be the main factor to the fast alterations in community structure resulting to environmental alterations (Paerl and Pinckney, 1996). Apparently, *Pseudomonas* species that have been used in this experiment gave the impression to thrive underneath high metal concentrations (Teitzel and Parsek, 2003).

2.2.2 Mechanisms of Heavy Metal Toxicity and Resistance of Microorganisms

Bioavailability is a crucial aspect when assessing metal toxicity. Bioavailability can be described as the ability of metals to be dissolved and released from the soil or other media, and the ability to desorbed toxic chemicals in target tissues (Kim *et al.,* 2015). The bioavailability of Cd (one of the most toxic heavy metals) relies upon on several factors, such as soil type, source of contamination and characteristic of the microorganisms (Vig *et al.,* 2003).

Vig *et al.,* (2003) also stated that the bioavailability of a heavy metal drops with the time it is in contact with the soil. Usually, heavy metal concentrations in the soil decline at neutral pH or alkaline (Munoz-Melendez *et al.,* 2000). Soluble forms of heavy metals are considered to be most available to microorganisms and their enzymes (Huang and Shindo 2000). Bhattacharyya *et al.* (2008a) stated that soluble and exchangeable forms of metals showed powerful inhibitory effects on soil enzyme activities. Karaca *et al.,* (2010) concluded that, high dissolved metal concentrations in agricultural soil have been very toxic to some enzymes.

Some metals are important to the life cycle of microorganisms. Calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium and zinc are micronutrients that help in redox processes, stabilize components in various enzymes and regulate the osmotic pressure (Olaniran *et al.,* 2013). However, there are some

metals such as aluminium, cadmium, lead and mercury that do not have any biological function and potentially toxic to microorganisms (Bruins *et al.,* 2000).

Heavy metals are difficult to be removed from contaminated environments because they are non-biodegradable, though the speciation and bioavailability of metals may change with different environmental factors (Kumar *et al.,* 2011). Higher concentration of metals has great effects on microbial communities because it may lead to a reduction of total microbial biomass, decrease in the numbers of microbial populations and change the microbial community structure (Azarbad *et al.,* 2013; Kumar *et al.,* 2011).

At higher concentrations, heavy metal ions will form complex compounds inside the microorganism's cell, which ends up to toxic condition for any physiological characteristic (Pernyeszi*,* 2011). Toxicity of metals happen through the displacement of important metals from their native binding sites or through ligand interactions in the microorganisms (Olaniran *et al.,* 2013; Bruins *et al.,* 2000). The toxic metal ions may substitute the essential ions within an enzyme causing the enzyme to be ineffective (Olaniran *et al.,* 2013). For example, mercury ion (Hg^{2+}), cadmium ion (Cd^{2+}) and silver ion (Ag^{2+}) tend to bind to sulfhydryl groups of enzymes that are crucial for microbial metabolism and thus inhibit the activity of the enzymes (Sinha *et al.,* 2009a). Toxic effect of most metal ions such as manganese ion (Mn^{2+}), iron ion (Fe^{2+}), cobalt ion (Co^{2+}), nickel ion (Ni^{2+}), copper ion (Cu^{2+}) and zinc ion (Zn^{2+}) happen only when those elements enter the microbial cell (Hobman & Crossman, 2015).

Most microorganisms encounter this problem through biosorption mechanisms for heavy metal ions (Prabhakaran *et al.,* 2016). One is fast, unspecific, constitutively expressed and determined by the chemiosmotic gradient throughout the cytoplasmic membrane of bacteria (Marais, 2012). The second one is inducible, has high substrate

specificity, slower, often uses adenosine triphosphate (ATP) hydrolysis as the energy source and is only produced by the cell in times of need, starvation or a special metabolic situation (Olaniran *et al.,* 2013). Metal biosorption is a complex process affected by some factors (Javanbakht *et al.,* 2014):

(i) the status of biomass (living or non-living),

(ii) types of biomaterials, chemistry or chemical properties of metal solution,

(iii) and ambient or environmental conditions such as pH and temperature influence the mechanism of metal uptake

Even though microorganisms have specific biosorbtion systems, excessive concentrations of nonessential metals may be transported into the cell by an unspecified system (Kowshik, 2013). In addition, at excessive ranges, both essential and nonessential metals can harm cell membranes, alter enzyme specificity, disrupt cellular functions, and damage the DNA structure (El-Meleigy *et al.,* 2013). Also, high concentration of heavy metals will bring an oxidative stress to microorganisms (Jan *et al.,* 2015). Therefore, some microorganisms have developed mechanisms to control the levels of toxic metals and eliminate those in excess (Hobman & Crossman, 2015).

Heavy metals may inhibit the enzymes that involve in biodegradation of pollutants or those that involved in general metabolism process (Joutey *et al.,* 2013). The ionic form of metal interferes with enzymes that are involved in heavy metal degradation processes, showing that metal toxicity is related to the concentration of bioavailable metal rather than total soluble metal concentration (Karigar & Rao, 2011; Parizanganeh *et al.,* 2012).

Enzyme activities are stimulated in diverse ways by different metals due to different chemical affinities of the enzymes in the soil system (Karaca *et al.,* 2010). Enzyme

reactions are inhibited by heavy metals in three distinctive approaches (Tejada *et al.,* 2008):

(i) complexation of the substrate;

(ii) combination with protein-active groups on the enzyme, and;

(iii) reaction with the enzyme–substrate complex.

According to D'Ascoli *et al.* (2006), heavy metals inhibit enzyme activity in numerous approaches as followed:

(i) by masking catalytically active groups;

(ii) denaturing the protein conformation, or;

(iii) competing with metal ions that are needed to form enzyme–substrate complexes.

A significant amount of evidence has been recorded on the reduction of enzyme activity in the soil as a result of long-term exposure to heavy metal pollution (Wang *et al.,* 2007). Soil urease activity was negatively associated with the available Cu. But soil protease and phosphatase activities were not drastically affected by heavy metal pollutants. The results propose that several enzymes are insensitive to Cu pollution in the soils for several years following a moderate pollution (Macdonald *et al.,* 2007).

Belyaeva *et al.* (2005) found that Pb reduced the activities of urease, catalase, invertase, and acid phosphatase significantly. Zeng *et al.* (2007) observed a stimulating impact of Pb on soil enzyme activities at low concentrations of Pb, but, when the level of Pb was increased to 500 mg/kg, soil enzyme activities reduced. Lorenz *et al.* (2006) stated that increasing the level of Cd reduced enzyme activities. Renella *et al.* (2005) determined that Cd inhibited alkaline phosphatase, arylsulfatase and protease, but did not affect acid phosphatase, b-glucosidase and urease.

Tejada *et al.* (2008) mentioned that soil enzyme activities reduced with increasing Ni concentration. Carine *et al.* (2009) found that phenoloxidase activity was inhibited by Al chloride salt at a higher rate and lower Al level than Al sulfate salt. Zeng *et al.* (2007) stated that it is widely recognized that any element beneath unique environmental situations would result in the unfavorable impact to plants and microorganisms if its concentration is higher than a certain range.

Sardar *et al.* (2007) examined soil enzyme activities (catalase, alkaline phosphatase, and dehydrogenase) when some levels of Cd and/or Pb were applied to the soil. Strong inhibition was detected at high heavy metal concentrations in both the single-metal and dual-metal systems; however, the inhibition was greater in the dual-metal system than the single-metal systems (Sardar *et al.,* 2007). However, according to Wyszkowska *et al.* (2006), Cu alone inhibited higher soil enzyme activity than Cu applied in conjunction with other heavy metals (Cu with Zn, Ni, Pb, Cd, and Cr).

Shen *et al.* (2005) observed a negative interplay between Zn and Cd as a result of the competition for sorption sites. This is because different metals have different effects on the enzymes of microorganisms (Karaca *et al.,* 2010). Lorenz *et al.* (2006) observed that As contaminations substantially affect arylsulfatase activity but xylanase, invertase, protease and alkaline phosphatase were unaffected; and Cd contamination had a negative impact on the activities of protease, urease, alkaline phosphatase and arylsulfatase but no significant impact on invertase. Wang *et al.* (2007) observed that soil phosphatase activity was substantially negatively correlated with Cu and Zn.

D'Ascoli *et al.* (2006) studied the consequences of heavy metal contamination on the enzyme properties (hydrolase, dehydrogenase, b-glucosidase, urease, arylsulfatase, and

acid phosphatase) of a soil onto a river contaminated with Cr (III) and Cu. The results showed negative correlations among the activities of dehydrogenase, arylsulfatase, and acid phosphatase and Cr fractions (soluble, exchangeable, and carbonate-bound). Despite the fact that Cu pollution negatively stimulated soil organic and biochemical properties, the soil organic matter was able to mask those negative effects of Cu on the microbial community.

Every enzyme displays a different sensitivity to heavy metals (Karaca *et al.,* 2010). Effron *et al.* (2004) concluded that heavy metals inhibited the activities of arylsulfatase, acid phosphatase, protease and urease. The relative toxicities of the metals towards enzyme activity were: Cd > Cu > Pb. Yang *et al.* (2006) examined the mutual effects of Cd, Zn, and Pb on catalase, urease, invertase, and alkaline phosphatase in soil. The results showed that Cd significantly inhibited the activities of all the enzymes studied, Zn only inhibited urease and catalase, while Pb was not significantly inhibiting the studied enzymes as compared to other heavy metals, and actually had a protective influence on catalase activity when all metals were present (Cd, Zn and Pb).

Acosta-Martinez and Tabatabai (2001) observed that Ag (I), Hg(II) and Cd(II) were the most efficient inhibitors than the alternative 18 trace elements tested. Shen *et al.* (2005) suggested that the order of inhibition of urease activity generally decreased in line with the series Cr > Cd > Zn > Mn > Pb. Wyszkowska *et al.* (2006) concluded that concentration of 50 mg/ kg of metals (Cu, Zn, Ni, Pb, Cd and Cr) inhibited soil enzyme activities (dehydrogenase, urease, acid phosphatase and alkaline phosphatase). Mikanova (2006) studied the effects of heavy metals on the enzyme activities (arylsulfatase, invertase, urease and dehydrogenase) of heavy metal contaminated alluvial soils. Increasing heavy metal concentration inhibited all the soil enzymes

studied, however arylsulfatase and dehydrogenase were highly sensitive to lower concentrations of metal than invertase and urease.

Hinojosa *et al.* (2004) conducted a study to determine enzyme sensitivity with a view to discover the scale of the heavy metal pollution (Cd, Pb, Cu and Zn) attributable to a mine spill. Further, increasing the degree of pollution induced the reduction of soil enzyme activities. The highest enzyme activity was discovered in unpolluted soil and the lowest was in the most polluted soil. Different types of heavy metals can affect soil enzymes in different ways. Wyszkowska *et al.* (2006) determined that the metal sensitivities of enzymes accompanied the order: dehydrogenase > urease > alkaline phosphatase > acid phosphatase.

Shen *et al.* (2005) examined the interactions of polycyclic aromatic hydrocarbons (PAHs) (e.g., phenanthrene, fluoranthene, benzo[a]pyrene) and heavy metals (Cd, Zn and Pb) with soil enzymes (urease and dehydrogenase). The outcomes confirmed that dehydrogenase was highly sensitive to the collective pollutants as compared to urease. Shen *et al.* (2005) suggested that urease and dehydrogenase may be appropriate indicators of collective pollutants (heavy metals and PAHs), particularly at the early stages of pollutants. In addition, Maliszewska-Kordybach and Smreczak (2003) revealed that dehydrogenase activity is highly sensitive to the collective outcomes of pollutants (heavy metals and PAHs).

Sardar *et al.* (2007) found that enzymes were inactivated by heavy metals, whereby heavy metals respond to sulfhydryl groups of enzymes and inhibit and/or inactivate the enzymatic activities. Lorenz *et al.* (2006) reported that enzyme activities declined because of the binding of Cd^{2+} to sulfhydryl groups of the enzyme. Bhattacharyya *et al.* (2008b) indicated that As ions inactivate enzymes by

reacting with sulfhydryl groups attributable to the formation of arsenic sulfide. They also suggested that As decreases enzyme activity in three methods:

(i) by way of interacting with the enzyme–substrate complicated;

(ii) via denaturing the enzyme protein, or;

(iii) interacting with the active protein groups.

Yang *et al.* (2006) stated that the degree of enzyme inhibition or activation depends on:

(i) the heavy metal ion;

(ii) the interaction among the heavy metals;

(iii) the reactions between the heavy metals in solution and the functional groups of the enzymes; and

(iv) the chemical and physical properties of the soil (pH, organic matter content, and type and amount of clay.

According to physicochemical approach, heavy metals biosorption by microorganism also affected through changes of pH (Congeevaram *et al.,* 2007). According to the study by Chen *et al.* (2000), biosorption for Cu (II) and Zn (II) is insignificant at pH ranges below 3.0 because of the high affinity of protons onto metallic binding sites on the cell wall of microorganisms.

Chen *et al.,* (2000) also mentioned that pH also affects the metal uptake because there are numerous functional groups on the bacterial cell walls. The functional groups (e.g., carboxyl, sulfate, phosphate and amino groups) might be deprotonated at high pH values (Javanbakht *et al.,* 2014). As pH increase, more functional groups are detached

and turn to be free for metal uptake because of the smaller rivalry from protons (Javanbakht *et al.,* 2014).

Chen *et al.* (2000) reported that pH modifications due to biotic sorption have been insignificant at pH 6.8. This indicates that the mechanism for biosorption is probably unrelated to ionic exchange. However, at pH 6.4, proton exchange has become significant, especially for metal biosorption at higher Zn (II) concentrations. Consequently, protons on the binding functional groups at pH 6.8 may be more stable than at pH 6.4 due to lower free energy of protein conformation at pH 6.8. Metal ions cannot be degraded or modified like toxic organic compounds. Therefore, six possible metal resistance mechanisms has been proposed as stated by Rampelotto (2010):

i. Exclusion by permeability barrier;
ii. Intracellular sequestration;
iii. Extracellular sequestration;
iv. Active transport efflux pumps;
v. Enzymatic detoxification;
vi. And reduction in the sensitivity of cellular targets to metal ions.

One or more of these resistance mechanisms allows microorganisms to function in metal contaminated environments (Fashola *et al.,* 2016). Thus, at high concentrations, either metal ions can completely inhibit the microbial population by inhibiting their various metabolic activities, as shows in Figure 2.3 or organisms can develop resistance or tolerance to the elevated levels of metals (Khan *et al.,* 2009).

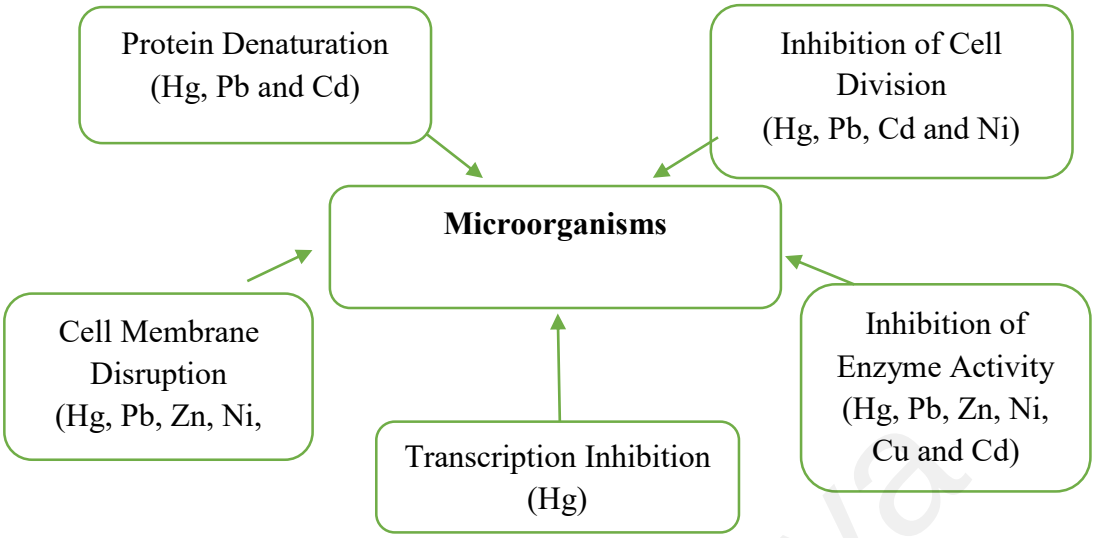


Figure 2.3: Mechanisms of heavy metal toxicity to microbes (Adapted from Khan *et al.*, 2009)

Resistance is the ability of microbes to survive in higher concentrations of toxic substances by detoxification mechanisms (Ahemad *et al.,* 2009). Therefore, toxic heavy metals need to be either completely removed from the contaminated soil, transformed or immobilized to produce much less or non-toxic species for bacteria to survive under the metal-stressed environment (Akhtar *et al.,* 2013).

Bacterial resistance mechanisms to heavy metals are encoded generally on plasmids and transposons through gene transfer or spontaneous mutation (Mindlin *et al.,* 2016; Ahemad, 2012). The regulation of the metal resistant gene expression is specific for each heavy metal and dependent upon metal species concentration, promoters, and regulatory genes, from the bacterial operons that can be used to create metal-specific biosensors (Große *et al.,* 2004).

2.2.3 Metal-microbe Interactions

The optimum rate of metal toxicity to soil microorganisms is not conclusive. Yet interactions between heavy metals and microbes do occur in nature because microorganisms can interact with metals via many mechanisms as shows in Figure 2.4. This has been used as the basis of potential bioremediation strategies (Marais, 2012). Meanwhile, Table 2.2 lists the mechanisms of metal-microbe interactions that influence the bioremediation of metals.

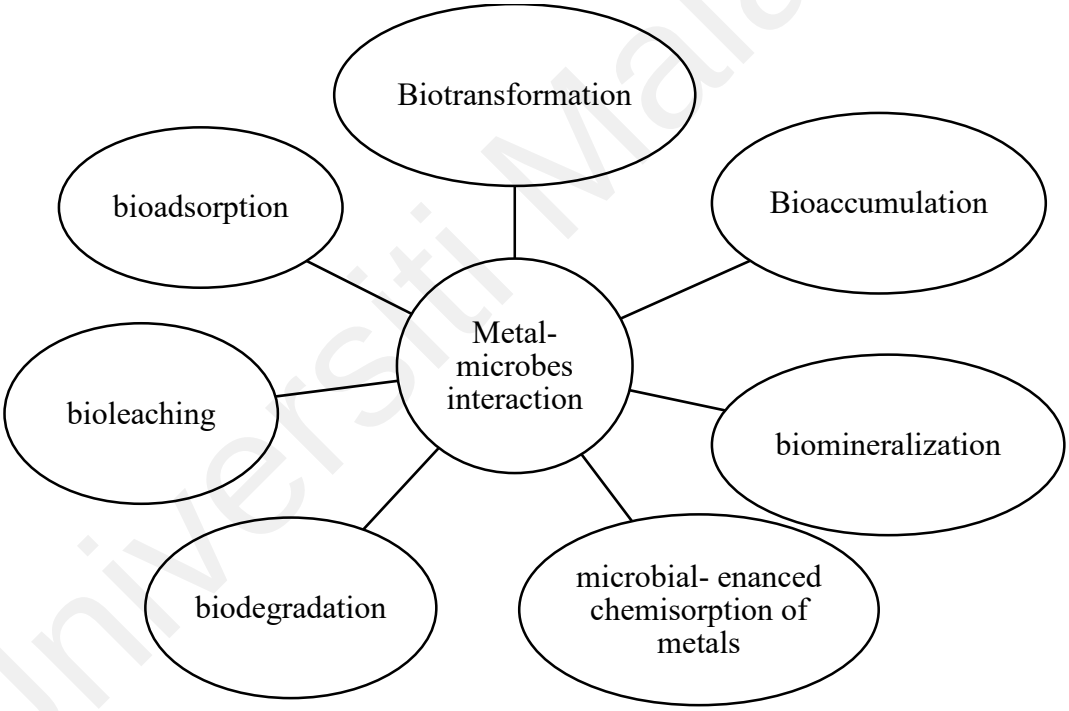


Figure 2.4: Metal-microbe interactions affecting bioremediation (Adapted from Marais, 2012)

Table 2.2: Description of major microbial processes that influence the bioremediation of metals.

Mechanisms	Descriptions
Biotransformation	A substance is changed from one chemical form to another chemical form by chemical reactions involving reduction, oxidation, methylation, demethylation and hydrogenation (Satyanarayana *et al.,* 2012; Diaz- Bone & Van de Wiele, 2010).
Biosorption	A process that uses biological materials such as algae, bacteria, fungal and yeast to bind to metal ions from aqueous solutions (Say *et al.,* 2001; Volesky, 2007).
Bioleaching	A process that uses microorganisms to transform the elements so that they can be extracted from a material when water is filtered through it (Mishra *et al.,* 2005).
Biodegradation of chelation agents	Chelating agents such as EDTA, NTA and DTPA has been used to extract the metal from soil phase to aqueous phase (Regmi *et al.,* 1996).
Bioaccumulation	An energy-dependent heavy metal transport system (Archana & Jaitly, 2015). Bioaccumulation mechanisms of heavy metal influx across the bacterial membranes include ion pumps, ion channels, carrier-mediated transport, endocytosis, complex permeation, and lipid permeation (Satyapal *et al.,* 2016).
Biomineralization	A process in which toxic metal ions combine with anions or ligands produced from the microbes to form precipitation (Patel & Kasture, 2014).
Microbially-enhanced chemisorption of metals	A series of chemical reactions where microbes first precipitate a bio-mineral of a non-target metal known as priming deposits, the priming deposits, then act as a nucleation focus on the subsequent deposition of the target metal (Tabak *et al.,* 2005).

2.3 Mechanism of Bioremediation through Biosorption

Biosorption can be defined as a heavy metal removal and recovery process by microorganisms from aqueous solutions (Abbas *et al.,* 2014)**.** The heavy metals adsorption occurs via physico-chemical interactions of metal ions with the cellular compounds of the bacteria (Dada *et al.,* 2015).

2.3.1 Adsorption by Microorganisms Cell Surface

Microorganisms cell surface consists of anionic functional groups, which resulted in the negative charge of the cell surface and allow the binding of metal cations. The negatively charged functional groups that involves are alcohols, amines, carboxyl, hydroxyl, ester, sulfhydryl, phosphoryl, sulfonate, thioester, thiol and many more. These functional groups play important role in metal biosorption (Kapahi and Sachdeva, 2019). In according to Shamim, (2018), bacteria have the highest surface-to-volume ratio as compared to other microorganisms, therefore bacteria have a higher biosorption capacity.

Gram-positive bacteria have a thick cell wall in outer shell, approximately 20 to 80 nm meanwhile Gram-negative bacteria have a relatively thin cell wall, therefore, Gram-positive bacteria has more rigid structure compared to Gram-negative bacteria (Mai-Prochnow *et al.,* 2016; Li and Tao, 2015). In accordance to Gupta et al., (2015), Gram-positive bacteria contain thick peptidoglycan layer in the outer cell which consists of large amounts of teichoic acids, polymers of glycerol joined by phosphate groups. Whereas the peptidoglycan in Gram-negative bacteria is thinner compare to Gram-positive bacteria and composed of phospholipids, lipopolysaccharides, enzymes, glycoproteins and lipoproteins. From previous research

study, the potential metal binding sites on the bacterial cell wall are found to be peptidoglycan, teichoic acids and lipoteichoic acids (Gupta *et al.,* 2015).

2.3.2 Biosorption by Extracellular Accumulation

According to Rehan and Alsohim (2019), microorganisms produce different types of metal-binding metabolites that made up of polysaccharides, proteins, uronic acids, humic substances, lipids, capsules, slimes, sheaths and biofilms. Igiri *et al.,* (2018) has reported that dead cells of *Pseudomonas putida*, *Brevibacterium sp.* and *Bacillus sp*. has the capability of extracellular accumulation of heavy metals.

2.3.3 Biosorption by Intracellular Accumulation

Microorganisms have high specific active transport mechanisms that can uptake heavy metals from the environment (Upadhyay *et al.,* 2021). Previous researchers have reported that the non-specificity of normal transport system and competitive nature of metals to bind with substrate has resulted in the intracellular accumulation of metals (Fang *et al.,* 2016).

2.3.4 Precipitate Formation

According to Jin *et al.* 2018, insoluble metal precipitates are forms when the functional groups present on the surface of the microbial cells bind with the metal ions. One crucial mechanism of precipitation is the metal complexation, which involve in metal–ligand interactions. These interactions have resulted in the formation of metal-complex with microbial metabolites such as sulphides and phosphates. Igiri *et al.*

(2018), reported that some iron reducing bacteria such as *Geobacter sp*. and sulphur reducing bacteria (SRB) like *Desulfuromonas sp.* has the ability to precipitate metals.

2.3.5 Transformation of Metals

Transformation of metals and metalloids by microorganisms are occur through different processes like oxidation, reduction, methylation and demethylation (Upadhyay *et al.,* 2021). Electron transport system in microorganisms help in partial metal reduction which resulted in immobilize the metals and metals becoming less toxic to the environment (Banerjee *et al.,* 2018).

According to Upadhyay *et al.* (2021), some microorganisms have plasmid-coded specific enzyme systems that help in methylation-demethylation reactions. Microorganisms are able to perform resistance towards particular metal due to these specific enzyme systems. However, as reported by Upadhyay *et al.* (2021), metals have the tendency of being volatilized during methylation-demethylation reactions and has high chances to escape from the treatment site which can pollute the atmosphere.

2.3.6 Potential Bacteria Used for Heavy Metal Removal

There are huge numbers of microorganism in the environment, the continuous and long-term exposure of heavy metals resulted in resistant behaviour of microorganisms through mutation (Anusha *et al.,* 2021). Microorganisms are globally used for the bioremediation of pollutant in soil and water (Kumar and Gunasundari, 2018). Microorganisms are able to grow at cold temperature, high temperature, in the water with oxygen, and in anaerobic conditions (Fingerman, 2016). Table 2.3 shows the

summary of heavy metal removal using bacteria by previous researchers in monometal system. Meanwhile, Table 2.4 shows the summary of heavy metal removal using bacteria by previous researchers in polymetal system.

Table 2.3: Summary of heavy metal removal using bacteria by previous researchers in monometal system

Bacteria	Heavy metal	Reference
Bacillus cereus *Bacillus pumilus* *Burkholderia sp.* *Delftia tsuruhatensis* *Pseudomonas alcaligenes*	Pb	Çolak *et al.,* 2011 Çolak *et al.,* 2011 Yang *et al.,* 2018 Dorian *et al.,* 2012 Liu *et al.,* 2011
Burkholderia sp.	Mn	Yang *et al.,* 2018
Cloacibacterium sp. *Pseudomonas mendocina* *Serratia marcescens*	Fe	Nouha *et al.,* 2016 DuBois, 2019 Nwagwu *et al.,* 2017
Brevundimonas diminuta *Burkholderia sp.* *Cloacibacterium sp.* *Delftia tsuruhatensis* *Serratia marcescens*	Zn	Ali *et al.,* 2021 Yang *et al.,* 2018 Nouha *et al.,* 2016 Dorian *et al.,* 2012 Nwagwu *et al.,* 2017
Burkholderia sp. *Cloacibacterium sp.* *Ochrobacterium sp.*	Cu	Yang *et al.,* 2018 Nouha *et al.,* 2016 Fan, 2013
Aeromonas caviae *Burkholderia sp.* *Ochrobacterium sp.* *Serratia marcescens* *Brevundimonas diminuta*	Cd	Loukidou *et al.,* 2004 Yang *et al.,* 2018 Fan, 2013 Nwagwu *et al.,* 2017 Ali *et al.,* 2021
Cloacibacterium sp. *Serratia marcescens*	Ni	Nouha *et al.,* 2016 Nwagwu *et al.,* 2017
Aeromonas caviae *Bacillus aryabhattai*	Cr	Loukidou *et al.,* 2004 Verma *et al.,* 2014
Cloacibacterium sp. *Rhodococcus sp.*	Al	Nouha *et al.,* 2016 Cayllahua *et al.,* 2010

Table 2.4: Summary of heavy metal removal using bacteria by previous researchers in polymetal system

Bacteria	Heavy metal	Reference
Bacillus cereus *Bacillus thuringiensis,* *Bacillus anthrocis* *Pseudomonas aeruginosa*	Zn, Cu and Pb	Anusha *et al.,* 2021
Aeromonas caviae	Cd and Cr	Loukidou *et al.,* 2004
Brevundimonas diminuta	Cd and Zn	Ali *et al.,* 2021
Burkholderia sp.	Zn, Pb, Mn, Cd and Cu	Yang *et al.,* 2018
Cloacibacterium sp.	Ni, Fe, Zn, Al and Cu	Nouha *et al.,* 2016
Delftia tsuruhatensis	Zn and Pb	Dorian *et al.,* 2012
Ochrobacterium sp.	Cd and Cu	Fan, 2013
Serratia marcescens	Ni, Cd, Zn and Fe	Nwagwu *et al.,* 2017

CHAPTER 3: METHODOLOGY

3.1 Description of Study Area

Soil samples were collected from Taman Beringin landfill and Bukit Beruntung disposal site in 2017. The sampling sites were selected based on the level of contamination of metals and organic compounds as published by Fauziah *et al.,* (2017) and Jayanthi *et al.,* (2017). Table 3.1 and 3.2 indicated the presence of heavy metal in the leachate contaminated soil (Bukit Beruntung and Taman Beringin) in comparison with local standards from Department of Environment, Malaysia (2009).

Table 3.1: Comparison of leachate contaminated soil in Taman Beringin landfill (Fauziah *et al.,* 2017) with local standards from Department of Environment, Malaysia (2009).

Test parameter	Range values (mg/L)	Standard value
As	0.01	0.05
Ca	242.1±42	N.A
Fe	134.6±16	5.0
Mn	3.1±0.32	0.2
Mg	52.2±8.7	N.A
Na	29.7±5.1	N.A
Cu	0.5±0.1	0.2
Zn	24.3±3	2.0
Pb	<0.01	0.10
Cd	0.4±0.1	0.01
Hg	0.03	0.005
Cr	6.2±1.4	0.20
Ni	0.85±0.1	0.20
Al	5.47±1.2	N.A

Table 3.2: Comparison of leachate contaminated soil in Bukit Beruntung disposable site (Jayanthi *et al.,* 2017) with local standards from Department of Environment, Malaysia (2009).

Test parameter	Range values (mg/L)	Standard value
As	0.21	0.05
Ca	91.2 ± 11.6	N.A
Fe	60 ± 18.2	5.0
Mn	5.1 ± 0.5	0.2
Mg	96.6 ± 16	N.A
Na	242.1 ± 22.8	N.A
Cu	2.62 ± 0.8	0.2
Zn	236 ± 11.8	2.0
Pb	1.12 ± 0.04	0.10
Cd	0.4 ± 0.1	0.01
Hg	0.04	0.005
Cr	17.3 ± 1.9	0.20
Ni	12 ± 4.4	0.20
Al	13.1 ± 3.2	N.A

Bukit Beruntung disposal site as shown in Figure 3.1 is situated in Hulu Selangor district and occupies an area of about 5 acres. Bukit Beruntung disposal site has been operating since 2001. Bukit Beruntung disposal site, which is visible from the North-South Highway (PLUS), receives approximately 1500 tonnes of waste daily (Jayanthi *et al.,* 2016).

Figure 3.1: Bukit Beruntung disposal site (https://goo.gl/maps/ahFccZrdet12)

Taman Beringin landfill as shown in Figure 3.2 is located in Jinjang Utara, Kuala Lumpur and owned by Kuala Lumpur City Hall (DBKL). This landfill occupied an area of more than 16 hectares, which operated from 1991 to 2005 (Jayanthi *et al.,* 2016). Along its northern flank is Sungai Jinjang.

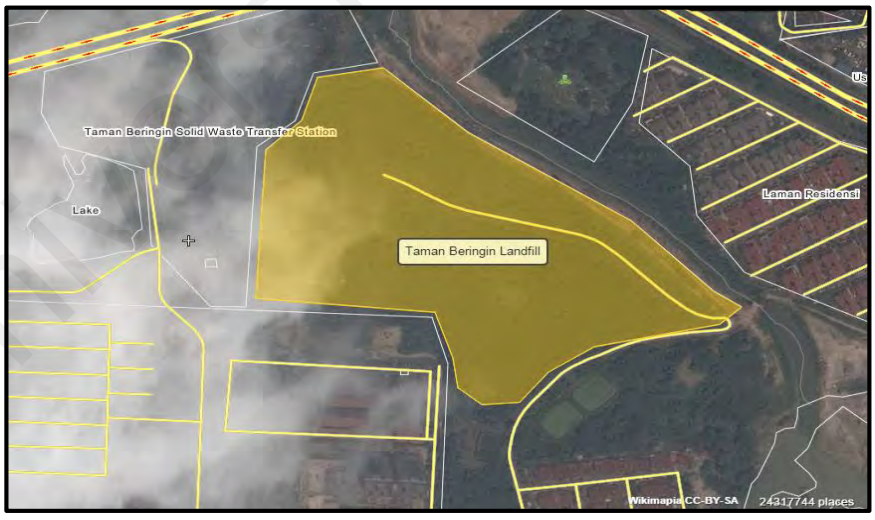


Figure 3.2: Taman Beringin Landfill (https://goo.gl/maps/4L1JjP7n97M2)

3.2 Soil Samples Collection

About 1 kg of soil (from surface to 30 cm deep) was scooped from four sampling points as shown in Plate 3.1. The GPS locations of the sampling points are depicted in Table 3.3. Collected soil samples were kept in clean airtight zipped plastic bags and immediately transported to the laboratory for further analysis.

Plate 3.1: One-foot deep of the top soil was removed before the desired soil sample was collected.

Table 3.3: GPS Coordinates for Sampling Points at Taman Beringin and Bukit Beruntung

Taman Beringin	Bukit Beruntung
1. Point A 3°13'40.17 N 101 ° 39'43.487 E	1. Point A 3°42'49.21 N 101 ° 54'55. 87 E
2. Point B 3°13'42.86 N 101 ° 39'37.16 E	2. Point B 3°42'49.81 N 101 ° 54'53.35 E
3. Point C 3°13'37.91 N 101 ° 39'51.74 E	3. Point C 3°25'31.88 N 101 ° 32'48.92 E
4. Point D 3°13'36.44 N 39'46.72 E	

3.3 Microbial Isolation

Twenty-three gram of Nutrient Agar (NA) was dissolved in 1000 ml distilled water and sterilized in autoclave for 15 minutes at 121ºC. 1 gram of soil (from each sampling points) was mixed with 10 ml saline water (0.9% NaCl) and the soil suspension was shaken using Lab-Line 3521 orbit shaker for 2 hours at 150 rpm (Auta, 2017). Serial dilutions were performed and then 0.1 ml of 10^{-3}, 10^{-5} and 10^{-7} dilution of soil suspension were dispensed and spread over the NA plates using sterile L rod (Gowsalya *et al.,* 2014).

To minimize error, each diluted suspension was plated onto three replicate plates. The inoculated plates were incubated at 37 °C for 24 hours (Sarita, 2015). Single colonies that developed on the plates were sub-cultured separately on freshly prepared NA through streaking plate method in order to generate pure culture for microbial identification (Gowsalya *et al.,* 2014).

3.4 Microbial Identification

Isolated bacteria were identified using the Biolog GEN III Microplate protocol (Bochner, 1989a; Bochner, 1989b). A cotton-tipped inoculator swab was used to pick up a 3 mm diameter area of a single colony from the surface of the agar into the cell suspension liquid. The cell suspension liquid was poured into the multichannel pipet reservoir. Eight sterile tips were fastened securely onto the channel repeating pipette and the tips were filled up by drawing up the cell suspension from the reservoir. All 96 wells were filled with 100 µl of cell suspension. The microplate was placed into the OmniLog incubator for 3 to 36 hours and incubated at 33 °C (Muthukrishnan *et al.,* 2015).

3.5 Microbial Inoculum Build Up

The identified bacterial strain was grown as pure culture in NA plates and incubated at 37 °C for 24 hours. Microbial inoculum builds up was set up by introducing each pure cultures into 1000ml Nutrient Broth (NB). They were set to grow to a stationary phase in rotating shaker at 29 °C at 150 rpm (Plate 3.2).

Plate 3.2: Microbial inoculum grown in rotating shaker.

The optical density (OD) of the innoculum were observed for every 12 hours using a Spectrophotometer at 600 nm until the OD obtained was approximately 2.0 ABS. After that, discrete suspensions at the same physiological phase (2.0 ABS at 600 nm) were then pooled in equal proportions to set up the innocula for the bioaugmentation (in order to determine the behavioral changes of those bacteria with the introduction of heavy metals). The experiment was designed according to the characteristics of the microbes namely Treatment A was for Gram-positive bacteria, Treatment B for Gram-negative bacteria and Treatment AB was a combination of all bacteria (Table 3.4).

Table 3.4: Identified bacterial species used as treatments in bioaugmentation set-ups.

Treatment A	Treatment B	Treatment AB	Control
Bacillus aryabhattai	-	*Bacillus aryabhattai*	-
Bacillus cereus	-	*Bacillus cereus*	-
Bacillus kochii	-	*Bacillus kochii*	-
Bacillus pumilus	-	*Bacillus pumilus*	-
Burkholderia vietnamiensis	-	*Burkholderia vietnamiensis*	-
Janibacter hoylei	-	*Janibacter hoylei*	-
Rhodococcus rubber	-	*Rhodococcus ruber*	-
-	*Acidovorax ebreus*	*Acidovorax ebreus*	-
-	*Aeromonas caviae*	*Aeromonas caviae*	-
-	*Brevundimonas diminuta*	*Brevundimonas diminuta*	-
-	*Chryseobacterium gleum*	*Chryseobacterium gleum*	-
-	*Cloacibacterium*	*Cloacibacterium*	-
-	*Delftia tsuruhatensis*	*Delftia tsuruhatensis*	-
-	*Ochrobacterium intermedium*	*Ochrobacterium intermedium*	-
-	*Pseudomonas alcaligenes*	*Pseudomonas alcaligenes*	-
-	*Pseudomonas mendocina*	*Pseudomonas mendocina*	-
-	*Serratia marcescens*	*Serratia marcescens*	-
-	*Stenotrophomonas acidaminiphilia*	*Stenotrophomonas acidaminiphilia*	-

3.6 Preparation of Heavy Metal Standard Solutions

Stock solutions of the heavy metals were prepared to achieve maximum solubility of the metal and the details of chemical elements used were shown in Table 3.5. The stock solutions were prepared with 1000 ppm concentration of respective metal in milli-Q grade deionized water by compensating for the salt or nonmetallic component and stored at 4^{o}C.

Table 3.5: Chemical elements used for heavy metal stock solutions.

Chemical Elements	Weight (g)
1. Pb from lead (II) chloride, ($PbCl_2$)	1.342
2. Mn from manganese (II) sulfate monohydrate, ($MnSO_4$. H_2O)	3.077
3. Fe from iron (II) sulfate heptahydrate, ($FeSO_4$. $7H_2O$)	4.978
4. Zn from zinc sulfate heptahydrate, ($ZnSO_4$. $7H_2O$)	4.396
5. Cu from copper (II) sulfate, ($CuSO_4$)	2.511
6. Cd from cadmium chloride hemipentahydrate, ($CdCl_2$. 2½ H_2O)	2.031
7. Ni from nickel (II) chloride hexahydrate, ($NiCl_2$. $6H_2O$)	4.049
8. Cr from potassium dichromate, ($K_2Cr_2O_7$)	2.828
9. Al from aluminium sulfate hexadecahydrate, (Al $(SO_4)_3$. $16H_2O$	11.68

Equation 3.1 was used to prepare the standard metal solutions at a concentration of 100 ppm in volumetric flask containing 100 ml distilled water:

$$M_1V_1 = M_2V_2$$
$$(1000\ ppm)\ (V_1) = (100\ ppm)\ (100\ ml)$$
$$V_1 = \frac{10ml}{} \quad (3.1)$$

3.7 Experimental Design

3.7.1 Monometal System

A total of 36 volumetric flasks with 180 ml NB were set up for bioaugmentation (the practice of adding cultured microbial consortia to perform a specific remediation task in a given contaminated habitat) as shown in Table 3.6.

Table 3.6: Bioaugmentation set up for monometal system

Number of flask	Set up for
9	Controls (no microbes)
9	Gram-positive bacteria as Treatment A (homogeneous)
9	Gram-negative bacteria as Treatment B (homogeneous)
9	all organisms as Treatment AB (heterogeneous)

Nine metal elements namely Zinc (Zn), Copper (Cu), Cadmium (Cd), Manganese (Mn), Iron (Fe), Nickel (Ni), Lead (Pb), Chromium (Cr) and Aluminium (Al) were prepared. 20 ml of each metal element was introduced into the volumetric flasks. Then, 10% (v/v) of microbes (20 ml) from stock solution was introduced into each flask.

3.7.2 Polymetal System

A total of 12 volumetric flasks with 180 ml NB were set up for bioaugmentation (the practice of adding cultured microbial consortia to perform a specific remediation task in a given contaminated habitat) as shown in Table 3.7.

Table 3.7: Bioaugmentation set up for polymetal system

Number of flask	Set up for
3	Controls (no microbes)
3	Gram-positive bacteria as Treatment A (homogeneous);
3	Gram-negative bacteria as Treatment B (homogeneous)
3	all organisms as Treatment AB (heterogeneous)

Nine metal elements namely Zinc (Zn), Copper (Cu), Cadmium (Cd), Manganese (Mn), Iron (Fe), Nickel (Ni), Lead (Pb), Chromium (Cr) and Aluminium (Al) were prepared. 20 ml of each metal element was mixed in a beaker. Then, 20 ml from the mixture was introduced into each volumetric flask. 10% (v/v) of microbes (20 ml) from stock solution was introduced into each flask.

All flasks were placed onto the Lab-line 3521 orbital shaker at 150 rpm in room temperature as shown in Plate 3.3. All samples were shaken for eight days. The samples were checked for their metal concentration, bacterial counts and pH readings. Metal concentration in each sample was determined using ICP-OES system analysis according to USEPA 3050B guidelines (Gadd, 2000) on Day 2 and Day 8. Day 2 was chosen as this was the optimum metal removal rate by bacteria based on two previous trial experiments. Meanwhile, Day 8 was chosen as the last day of removal rate because on

Day 8 the bacterial count was reduce as half as compared to initial bacterial counts. 40ml from each samples were taken for the ICP-OES analysis. Bacterial counts were determined using plate count method every 24 hours from Day 0 to Day 8. Lastly, pH readings for every sample were determined using multiprobe meter (YSI Professional Plus, USA) and examined for every 24 hours from Day 0 (first day) to Day 8.

Plate 3.3: All samples were put onto the orbital shaker at 150 rpm in room temperature for 8 days.

3.8 Heavy Metal Degradation by Bacteria

Heavy metal degradation from each treatment was calculated using;

$$\% \text{ of heavy metal removal} = \left(\frac{C_{0(x)} - C_{F(x)}}{C_{0(x)}} \right) X\ 100 \qquad (3.2)$$

Where,

$C_{0(x)}$ = initial concentration of metal x in the NB at the start of experiment

$C_{F(x)}$ = final concentration of metal x at the end of the experiment

The data was further processed to determine the rate constant of heavy metals removal via the use of First order kinetic model;

$$K = -\frac{1}{t}\left(ln\frac{c}{c_0}\right) \quad (3.3)$$

Where,

K= First order rate constant for metal uptake per day

t= time in days

C= concentration of residual metal in NB (mg/Kg)

C_0= initial concentration of metal in NB (mg/Kg)

3.9 Statistical Analysis

Data are expressed as means of the three (3) replicates. Comparison of metal removal rate among isolated microbes was analyzed using a one-way ANOVA. A *p* value below 0.05 was regarded as statistically significant. All statistical analyses were carried out using SPSS software (version 23) at 95% confidence limit. All graphical work was carried out using Excel (version 16.0).

CHAPTER 4: RESULT & DISCUSSION

4.1 Bacterial Isolation Study

A total of 18 strains of bacteria were isolated from the contaminated soil collected from Taman Beringin landfill and Bukit Beruntung disposal sites. These strains were of diverse genera that included both Gram-positive and Gram-negative bacteria (Table 4.1). Seven species were Gram-positive and the other 11 species were Gram-negative bacteria. The presence of these species in the leachate-contaminated soil implies their high resistance to heavy metals. These bacteria species are used in the consecutive bioaugmentation experiment.

Table 4.1: Bacterial isolated from Taman Beringin Landfill and Bukit Beruntung disposal site.

	Gram-positive Bacteria	Gram-negative Bacteria
1.	*Bacillus aryabhattai*	*Acidovorax ebreus*
2.	*Bacillus cereus*	*Aeromonas caviae*
3.	*Bacillus kochii*	*Brevundimonas diminuta*
4.	*Bacillus pumilus*	*Chryseobacterium gleum*
5.	*Burkholderia vietnamiensis*	*Cloacibacterium*
6.	*Janibacter hoylei*	*Delftia tsuruhatensis*
7.	*Rhodococcus rubber*	*Ochrobacterium intermedium*
8.		*Pseudomonas alcaligenes*
9.		*Pseudomonas mendocina*
10.		*Serratia marcescens*
11.		*Stenotrophomonas acidaminiphilia*

Observation on the reduction in the heavy metal concentration was done after two days and after eight days. Table 4.2 presents the percentage of metal removal by bacteria in monometal system for two and eight days of incubation period. Table 4.3 presents the percentage of metal removal by bacteria in polymetal system for two and eight days of incubation period.

Table 4.2: Percentage of metal removal by bacteria in monometal system

Monometal System								
Heavy Metal	**Initial Concentration (ppm)**	**Percentage of metal removal (%)**						
		Treatment A		**Treatment B**		**Treatment AB**		**Control**
		2 Days	**8 Days**	**2 Days**	**8 Days**	**2 Days**	**8 Days**	
Pb	100	52.3	53	32.1	33.5	29	62.5	0
Mn	100	14.3	75.9	12.9	7.9	8.3	23.8	0
Fe	100	29.9	43.6	49.1	53.3	53.1	73.8	0
Zn	100	18.8	7.5	10.1	0	9.6	15	0
Cu	100	30.2	15.7	21.2	28.5	29.1	35.1	0
Cd	100	34.3	16.4	18.6	18.2	16.1	8.6	0
Ni	100	15.1	24.8	18.6	17.8	16.6	6.4	0
Cr	100	8.6	12	0	0	0	0	0
Al	100	9.8	21.4	12.9	14.5	14.6	1	0

= increased, = decreased, = unchanged.

Table 4.3: Percentage of metal removal by bacteria in polymetal system

Polymetal System								
Heavy Metal	Initial Concentration (ppm)	Percentage of metal removal (%)						
		Treatment A		Treatment B		Treatment AB		Control
		2 Days	8 Days	2 Days	8 Days	2 Days	8 Days	
Pb	100	66.7	91.8	39.4	77.8	60.7	41.5	0
Mn	100	10.8	39.3	4.2	12.5	0	23.2	0
Fe	100	53.8	72.7	24.3	29.7	45.3	26.3	0
Zn	100	17.7	54.2	0.8	13.3	0	23.4	0
Cu	100	33.2	54.1	28.1	36.7	20.7	41	0
Cd	100	12.5	53.1	0	19.1	0	27.3	0
Ni	100	15.8	50.9	9.2	22.9	91.7	29.3	0
Cr	100	11.7	47.3	0	13.3	0	11.7	0
Al	100	5	54.25	0	13.3	0	13.3	0

= increased, = decreased.

Initial bacteria count for all treatments were 1.4 x 10^8 CFU/ml whereas the control contained no bacteria. The analysis was carried out on Day 2 where the bacteria were allowed to undergo adaptation phase. The exposure to the heavy metal ended on the Day 8 when bacterial counts reduced by half of the initial bacterial counts. In addition, maximum removal refers to maximum heavy metal capacity that bacteria can adsorb. The following sections discuss the adsorption in monometal and polymetal systems in details.

4.2 Metal Removal in Monometal System

4.2.1 Lead (Pb)

Figure 4.1 shows percentage removal of Pb across treatments in monometal system. For Control, there was no changes in heavy metal concentration (100 ppm) as there were no bacteria added to the control, and this applicable to all control in this research work. For Treatment A, 52% of Pb was removed in two days whereas 53% of Pb (an increase by 1%) was recorded on the eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.370 day^{-1} (2-Day) and 0.094 day^{-1} (8-Day). For Treatment B, 32% of Pb was removed in two days whereas 34% of Pb (an increase by 2%) was recorded in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.194 day^{-1} (2-Day) and 0.051 day^{-1} (8-Day). For Treatment AB, 29% of Pb was removed in two days whereas 63% of Pb (an increase by 34%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.171 day^{-1} (2-Day) and 0.123 day^{-1} (8-Day). Therefore, the order for Pb removal was A>B>AB (2-Day incubation period) and AB>B>A (8-Day incubation period).

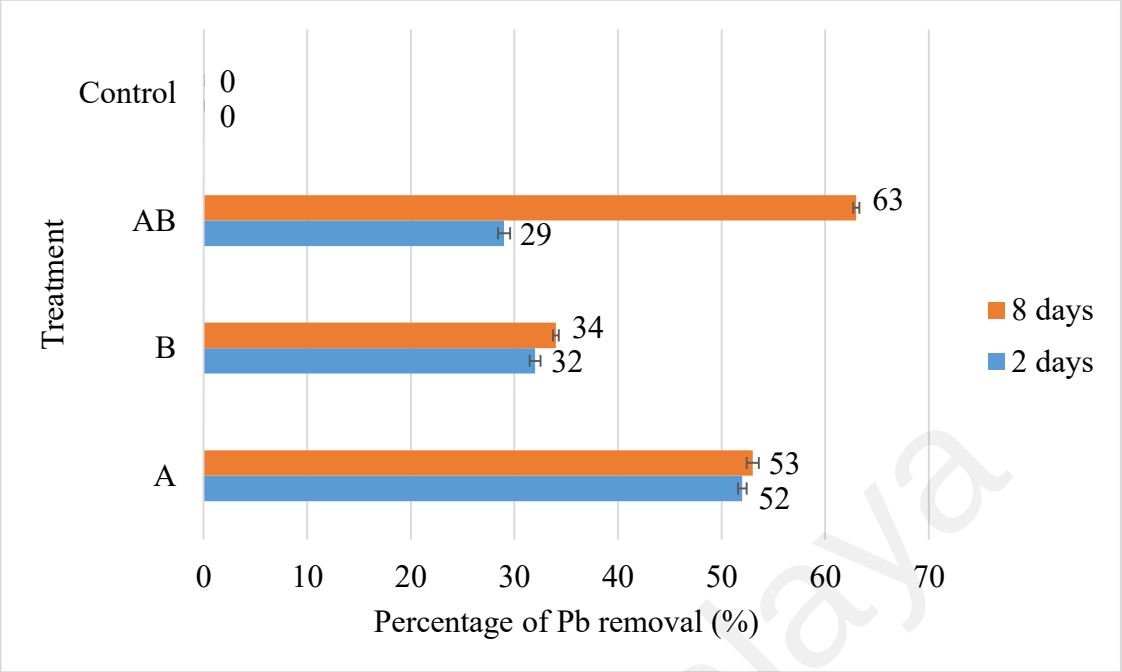


Figure 4.1: Percentage of Pb removal in monometal system

Among the homogeneous groups, Treatment A (Gram-positive) able to reduce 52% of Pb meanwhile Treatment B (Gram-negative) reduced 32% of Pb within two days. On 8-Day both treatments able to reduce 53% and 34% of Pb, respectively. Both Gram-positive and Gram-negative bacteria has optimum Pb removal on Day 2. This is evident from the slowdown in removal percentage of Pb on Day 8. The removal performance of Gram-positive and Gram-negative bacteria was in accordance to Ray *et al.* (2006) who reported that *Bacillus cereus* (Gram-positive) removed 85% of Pb. Meanwhile, Das *et al.* (2016) noted that *Bacillus pumilus* (Gram-positive) has a high ability in precipitating Pb. As for Gram-negative bacteria, Bautista-Hernández *et al.* (2012) reported that *Delftia tsuruhatensis* has the ability to adsorb 44.4 mg/g of Pb, which was almost half of the actual concentration. According to Leung *et al.* (2000), *Pseudomonas alcaligenes* (Gram-negative) was found to be capable of removing a significant amount of Pb. Gawali Ashruta *et al.,* (2014) reported that *Pseudomonas* species (Gram-negative) was able to remove 78.18% of Pb. Meanwhile, *Serratia marcescens* (Gram-negative) has

optimum Pb removal potential which was up to 0.213 µg/g compared to the other metals used in the research previously described by Cristani *et al.* (2012).

In the heterogeneous group, Treatment AB, had the lowest removal percentage on Day 2 compared to Treatment B and A, i.e., 21%, 32% and 52%, respectively. On Day 8, its removal percentage increased drastically to be higher than both Treatment A and B, i.e., 63%, 53% and 34%, respectively. The slow start can be attributed to heterogeneous bacteria are adapting to the environment. It seems that heterogeneous bacteria has optimum Pb removal. A study by Singh & Vaishya (2017) reported that the combination of *Bacillus* and *Pesudomonas* species were able to reduce 84.33% of Pb in 72 hours of incubation. Migahed *et al.,* (2017) reported that the heterogeneous bacteria, which consist of *Bacillus*, *Serratia*, *Vibrio* and *Paenabacillus* species has removed 55% of Pb (II) ion. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 20.64-23.53% of Pb. Results obtained from the heterogenous bacterial application indicate its potential to be used for bioremediation of contaminated soil.

Figure 4.2 shows bacterial counts for all treatments in monometal system from Day 0 until Day 8. In the presence of Pb, the maximum of bacterial growth was on Day 2. On Day 2, bacterial count for Treatment A was 2.07 x 10^{11} CFU/ml, 1.22 x 10^{11} CFU/ml for Treatment B and 8.24 x 10^{10} CFU/ml for Treatment AB. On Day 2, Treatment A showed the highest bacterial count compared to other treatments. On Day 8, the bacterial count for Treatment A, B and AB were 1.8 x 10^5 CFU/ml, 2.16 x 10^5 CFU/ml and 6.56 x 10^5 CFU/ml, respectively.

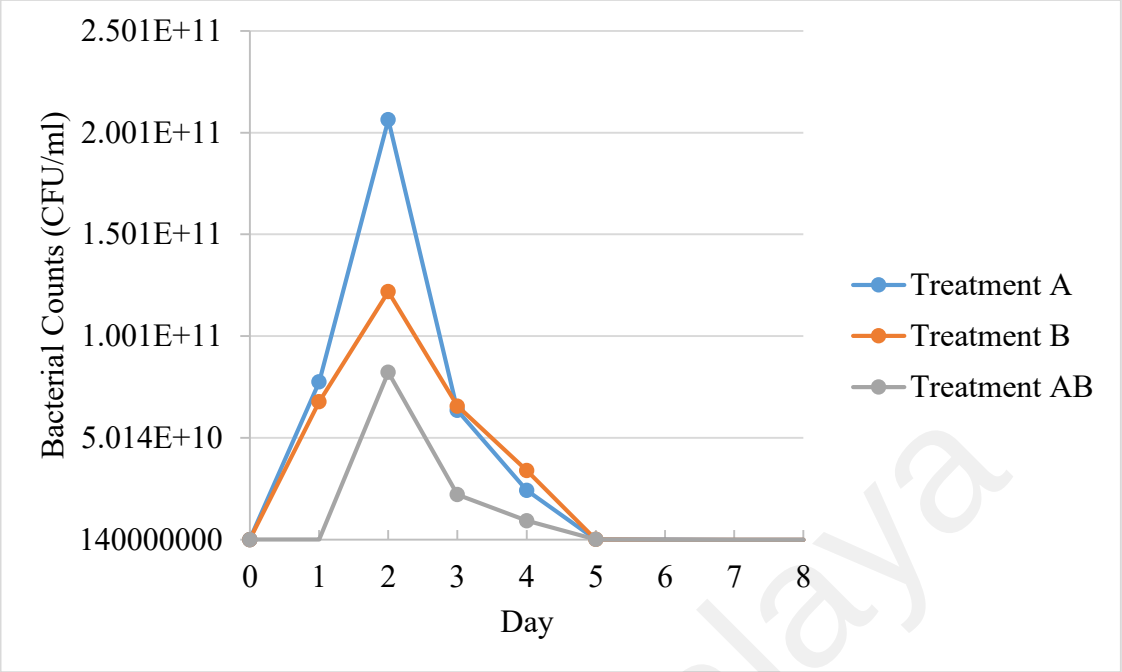


Figure 4.2: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A (Gram-positive) showed the highest Pb removal percentage along with the highest bacterial count, i.e., 2.07 x 10^{11} CFU/ml as compared to 1.22 x 10^{11} CFU/ml (B) and 8.24 x 10^{10} CFU/ml (AB). Gram-positive bacteria thrived in the presence of Pb but by Day 3, they had experienced a sharp decline in bacterial count. On Day 8, Gram-positive bacteria has the lowest count, i.e., 1.8 x 10^{5} CFU/ml as compared to 2.16 x 10^{5} CFU/ml (B) and 6.56 x 10^{5} CFU/ml (AB). Despite the stress, Gram-positive bacteria continued to remove Pb albeit at a low percentage. For Treatment B, Gram-negative bacteria thrived as well but not as much as Gram-positive bacteria. As for Treatment AB, the heterogeneous bacteria did not thrive as much as the homogeneous bacteria which explains Treatment AB having the lowest removal of Pb on Day 2. However, on Day 8, the heterogeneous bacteria has the highest count, as well as, the highest Pb removal percentage. Unlike homogeneous bacteria, Pb had not done any biological molecules disruption to heterogeneous bacteria as evident by the increase in 8-Day Pb removal percentage.

As shown in Figure 4.3, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH7.1-8.0, Treatment B ranged at pH7.2- 8.1, and Treatment AB ranged at pH6.7- 8.3. On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 7.4, 8.1 and 8.3, respectively, thus pH 7<A<B<AB. On Day 8, the pH for Treatment A, B and AB were 7.9, and 7.8 and 7.2, respectively, thus pH 7<AB<B<A. As described by Daboor (2014), neutral pH (pH 7) increases the negative charge of the bacterial cell, which favored electrochemical attraction and adsorption of Pb ions. This explains why Treatment AB had higher removal percentage on Day 8 than Day 2 (i.e., pH7.2 versus pH8.3). On Day 8, the pH of Treatment A and B increased to pH7.9 and pH7.8, respectively, resulted in the decreasing of Pb removal. This might be due to the formation of insoluble oxides, hydroxides and carbonates at pH above the neutrality, which reduced the free Pb ions as similarly reported by Daboor (2014). At the highest percentage of Pb removal, the pH for Treatment A, B and AB were pH7.4, pH8.1, and pH7.2, repectively. By Day 8, Treatment A (Gram-positive) and Treatment B (Gram-negative) became more alkaline while Treatment AB (heterogeneous) became less alkaline.

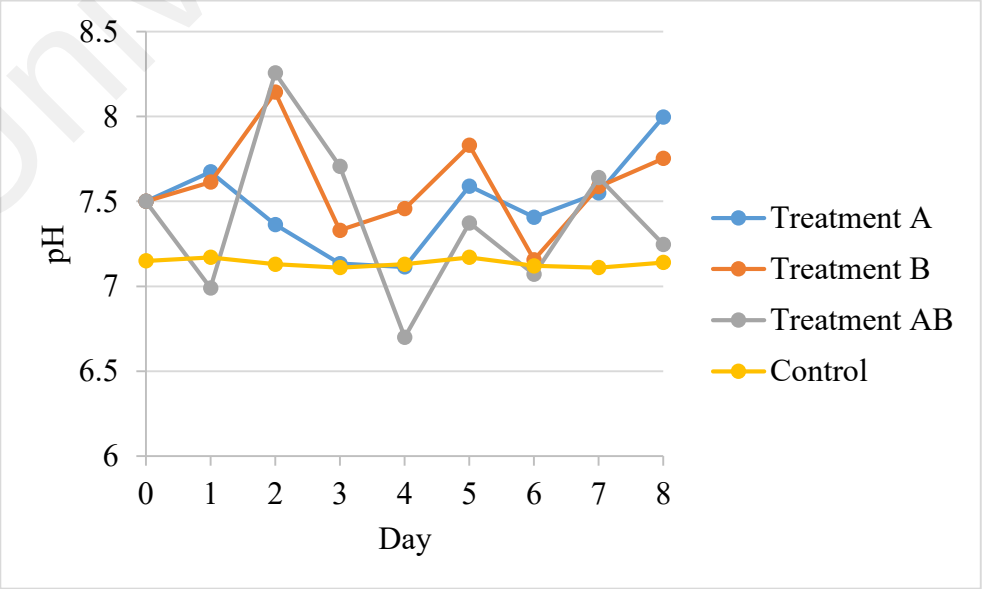


Figure 4.3: pH readings for all treatments in monometal system

4.2.2 Manganese (Mn)

Figure 4.4 shows the percentage of Mn removal across treatments in monometal system. For Treatment A, 14% of Mn was removed in two days whereas 76% of Mn (an increase by 62%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.077 day^{-1} (2-Day) and 0.178 day^{-1} (8-Day). For Treatment B, 13% of Mn was removed in two days whereas 8% of Mn (a decrease by 5%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.069 day^{-1} (2-Day) and 0.010 day^{-1} (8-Day). For Treatment AB, 8% of Mn was removed in two days whereas 24% of Mn (an increase by 16%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.043 day^{-1} (2-Day) and 0.034 day^{-1} (8-Day). Therefore, the order for Mn removal was A>B>AB (2-Day incubation period) and A>AB>B (8-Day incubation period).

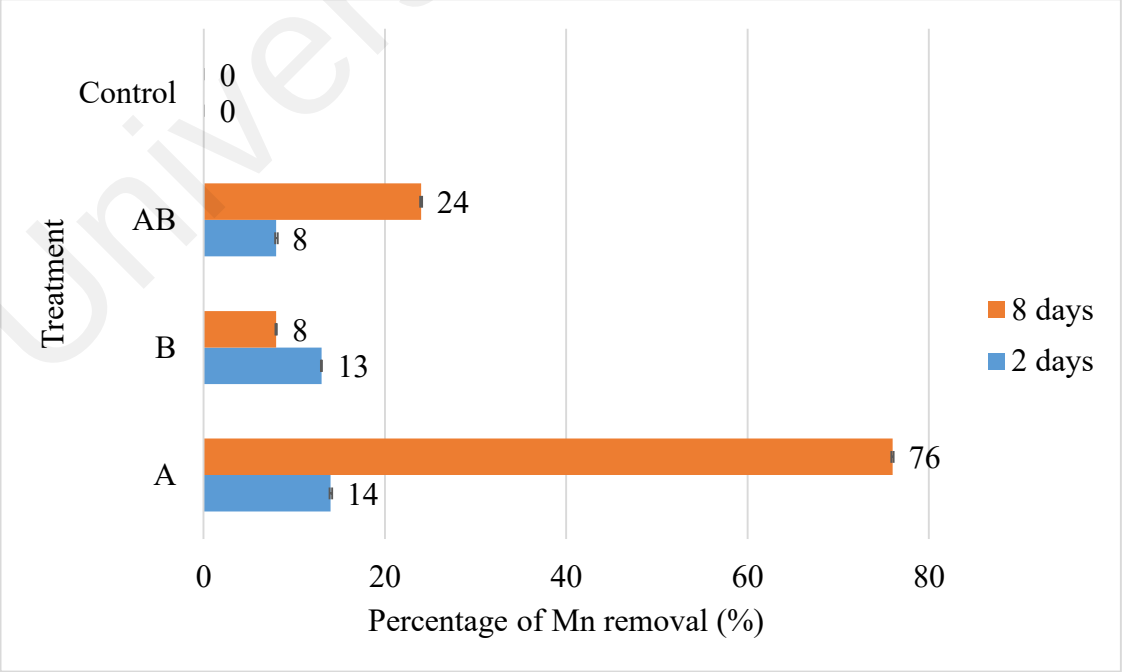


Figure 4.4: Percentage of Mn removal in monometal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Mn removal percentage, i.e., 14% as compared to 13% for Treatment B (Gram-negative) on Day 2. On Day 8, Treatment B also showed a higher Mn removal percentage, i.e., 76% as compared to 8% for Treatment B. Both have relatively similar removal percentages on Day 2 but contrasted with each other on Day 8. The removal percentage of Treatment A increased drastically while the removal percentage for Treatment B decreased. Gram-positive bacteria had not reached the highest Mn removal which explains the reason they continue to adsorb Mn on Day 8. Meanwhile, in Treatment B Mn seemed to be released back to the solution, hence the decrease in removal percentage on Day 8. Gram-negative bacteria had the highest Mn removal on Day 2 hence the decline in removal percentage and the release of Mn back into the solution on Day 8. Excessive Mn may have inhibited the respiratory chain of the bacteria and acted as potent disrupters of bacterial biological system (Basha & Rajaganesh, 2014). The removal performance of Gram-positive and Gram-negative bacteria was in accordance to previous findings. Mamba *et al.* (2009) reported that *Bacillus* species (Gram-positive bacteria) and *Pseudomonas* species (Gram-negative bacteria) could remove 96% and 72% of Mn, respectively.

Treatment AB (heterogeneous group) has the lowest removal percentage on Day 2, i.e., 8% as compared to 13% by Treatment B and 14% by Treatment A, and Day 8, i.e., 24% as compared to 8% by Treatment B and 76% by Treatment A. This may be caused by adsorption percentage of Gram-positive bacteria being higher than the release percentage of Gram-negative bacteria as seen in Treatment A and B. Thus, Mn removal by Treatment AB was higher than Treatment B but lower than Treatment A. Low removal percentage on Day 2 may be attributed to bacteria adjusting their interactions

and adopting the compatibilities among multispecies communities (Stubbendieck *et al.,* 2016). In addition, the highest Mn removal for heterogeneous bacteria may lie between that of Gram-positive and Gram-negative bacteria. Barboza *et al.* (2015) reported that the concentration of Mn (II) continuously decreased over time in the presence of the heterogeneous bacteria which consist of *Bacillus* and *Stenotrophomonas* species with a removal efficiency of 99.7%.

Figure 4.5 shows the bacterial counts for all treatments in monometal system from Day 0 until Day 8. In the presence of Mn, the maximum of bacterial growth was recorded on Day 2. On Day 2, bacterial count for Treatment A was 1.27 x 10^{11} CFU/ml CFU/ml, 9.26 x 10^{10} CFU/ml for Treatment B and 5.33 x 10^{10} CFU/ml for Treatment AB. On Day 8, final bacterial count was 9.2 x 10^{5} CFU/ml for Treatment A, 1.2 x 10^{5} CFU/ml for Treatment B, and 1.76 x 10^{5} CFU/ml Treatment AB.

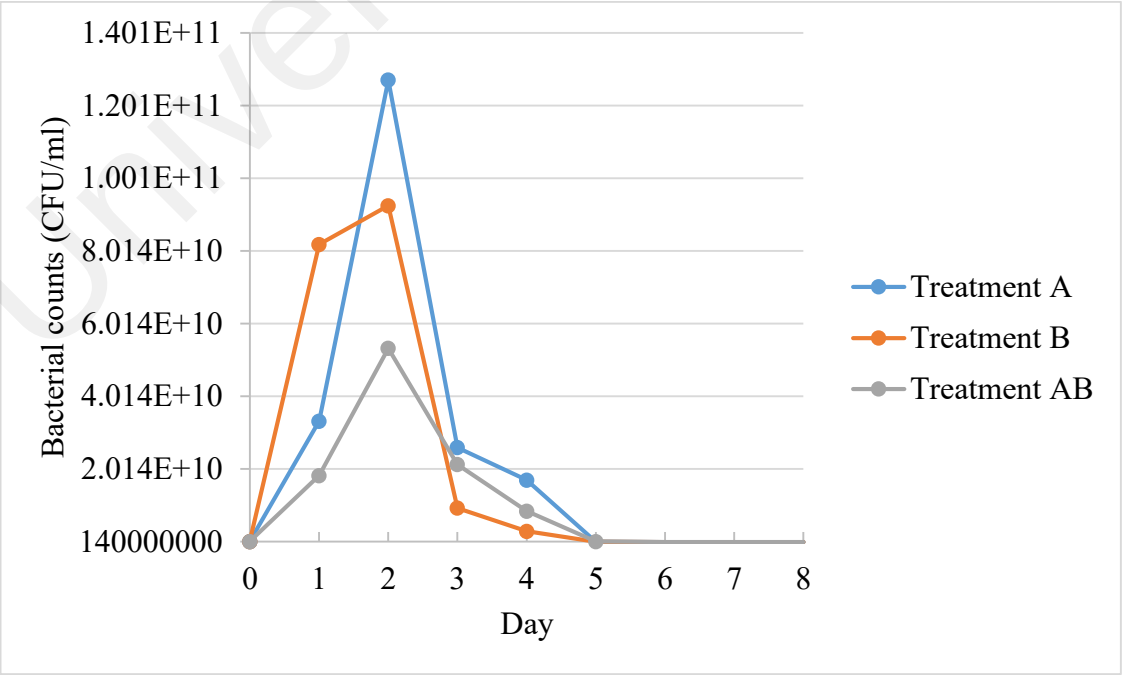


Figure 4.5: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A (Gram-positive) showed a drastic increase in Mn removal along with the increase in bacterial counts, i.e., 1.27 x 10^{11} CFU/ml as compared to 9.26 x 10^{10} CFU/ml (B) and 5.33 x 10^{10} CFU/ml (A). Gram-positive bacteria thrived in the presence of Mn but by Day 3, they had experienced a sharp decline in population. They were also resilient as evident by having the highest bacterial count on Day 8, i.e., 9.2 x 10^5 CFU/ml as compared to 1.76 x10^5 CFU/ml (AB) and 1.2 x 10^5 CFU/ml (B). For Treatment B, Gram-negative bacteria thrived as well but not as much as Gram-positive bacteria. Gram-negative bacteria experienced a sharp decline in bacterial count till Day 8. As for Treatment AB, the heterogeneous bacteria did not thrive as much as the homogeneous bacteria which explains why Treatment AB has the lowest removal percentage on Day 2. However, on Day 8, the heterogeneous bacteria had a higher bacterial count than Gram-negative bacteria.

As shown in Figure 4.6, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH6.7-8.1, Treatment B ranged at pH6.6-8.0 and Treatment AB ranged at pH6.9-8.1, On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 6.7, 7.2 and 7.9, respectively, thus pH A<7<B<AB. On Day 8, the pH for Treatment A, B and AB were 7.6, 7.1 and 7.2, respectively, thus pH 7<B<AB<A. On the day when Mn removal percentage was the highest, the pH observed for each treatment was 7.6 (Treatment A), 7.2 (Treatment B) and 7.2 (Treatment AB). It appears that by Day 8, Treatment A (Gram-positive) remained in alkaline condition while Treatment B (Gram-negative) and Treatment AB (heterogeneous) turned to near neutral.

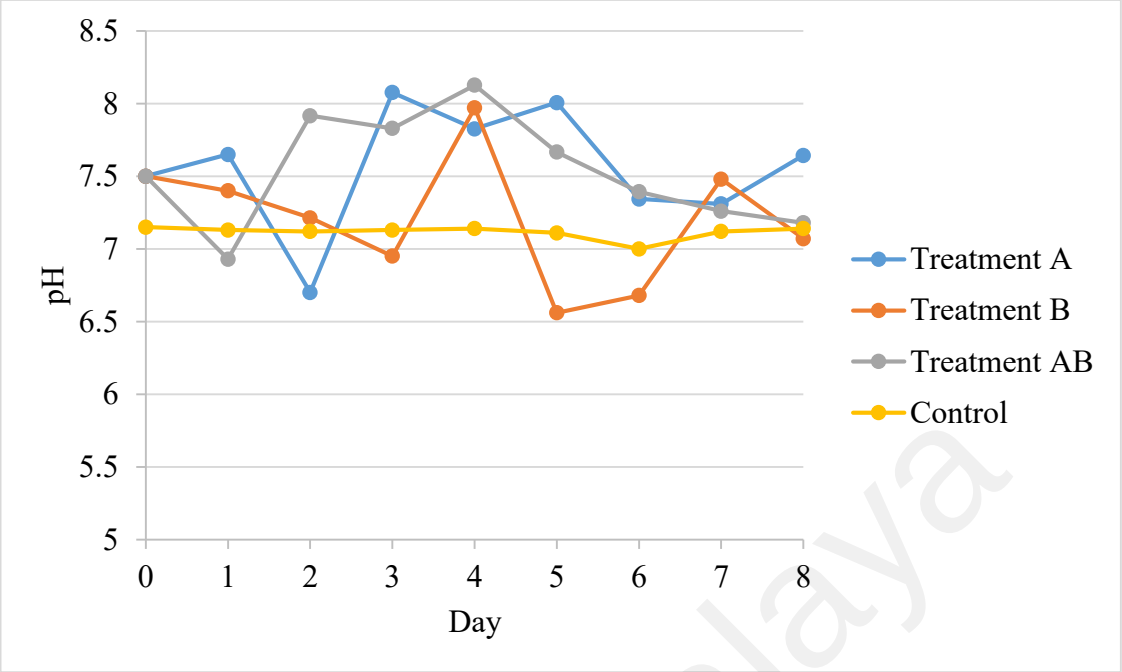


Figure 4.6: pH readings for all treatments in monometal system

4.2.3 Iron (Fe)

Figure 4.7 shows the percentage of removal for Fe across treatments in monometal system. For Treatment A, 30% of Fe was removed in two days whereas 44% of Fe (an increase by 14%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.178 day^{-1} (2-Day) and 0.072 day^{-1} (8-Day). For Treatment B, 49% of Fe was removed in two days whereas 53% of Fe (an increase by 4%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.338 day^{-1} (2-Day) and 0.095 day^{-1} (8-Day). For Treatment AB, 53% of Fe was removed in two Days whereas 74% of Fe (an increase by 21%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.376 day^{-1} (2-Day) and 0.167 day^{-1} (8-Day). Therefore, the order for Fe removal was AB>B>A (2-Day incubation period) and AB>A>B (8-Day incubation period).

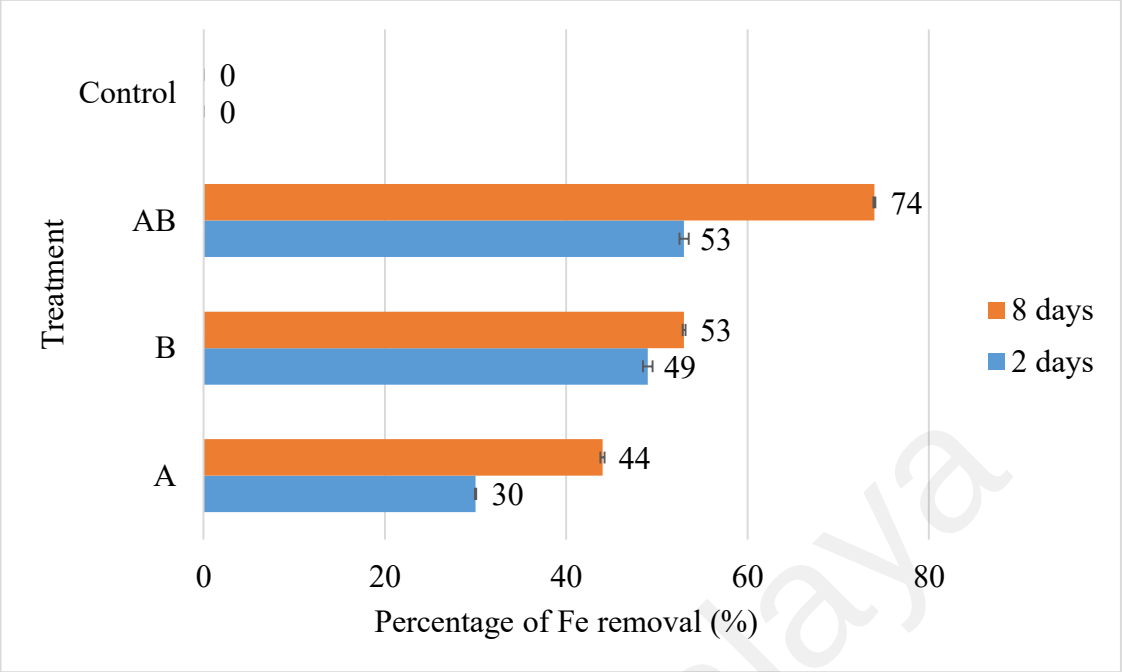


Figure 4.7: Percentage of Fe removal in monometal system

Among homogeneous groups, Treatment B (Gram-negative) has a higher Fe removal percentage than Treatment A (Gram-positive), i.e., 49% and 30% (2-Day) meanwhile 53% and 44% (8-Day), respectively. Gram-negative bacteria were to more active to interact with Fe than Gram-positive bacteria, allowing them to adsorb Fe better. On Day 8, the slowdown in Fe removal by Gram-positive bacteria is possibly due to them nearing the optimum Fe uptake. In comparison, Gram-negative bacteria is further from the optimum removal rate hence the higher Day-8 removal percentage. The removal performance of Gram-positive and Gram-negative bacteria was in accordance to previous findings by Štyriaková and Štyriak (2000) who reported that *Bacillus cereus* (Gram-positive bacteria) was able to remove 45% of Fe, while Zhu *et al.* (2013) noted that more than 87% of initial Fe has been precipitated by *Acidovorax ebreus* (Gram-negative bacteria). Chaudhari *et al.* (2013) previously described that Fe^{2+} and Fe^{3+} had a higher stimulatory effect on the enzyme activity of *Chryseobacterium gleum* (Gram-negative bacteria) as compared to other metals (141%). Meanwhile, Ams *et al.* (2004)

stated that *Pseudomonas mendocina* (Gram-negative bacteria) has the ability to increase the adsorption of Fe into their systems.

The heterogeneous group in Treatment AB has the highest removal (53% and 74%, respectively) as compared to other treatments on Day 2, i.e. 49% (B) and 30% (A), and Day 8, i.e. 53%(B) and 44% (A). The optimum Fe removal in Treatment AB surpassed both Treatment A and B. It seems that heterogeneous bacteria were mutualistic in reacting to Fe, hence led to the highest removal ability. According to Pan *et al.,* (2017), heterogeneous bacteria including *Bacillus, Pseudomonas, Clostridium, Anaeromyxobacter, Geothrix and Acinetobacter* species has promoted the activity of Fe reduction in Fe(II)-poor sediments. Together, *Bacillus* strains (Gram-positive bacteria) and *Pseudomonas* strains (Gram-negative bacteria) were able to remove up to 90% Fe in mine water (Mamba *et al.,* 2009).

Figure 4.8 shows the bacterial counts for all treatments in monometal system from Day 0 to Day 8. In the presence of Fe, the maximum of bacterial growth was recorded on Day 2. On Day 2, bacterial count for Treatment A was 1.963 x 10^{10} CFU/ml, while for Treatment B and Treatment AB, it was 7.72 x 10^{10} CFU/ml, and 3.17 x 10^{11} CFU/ml, respectively. Treatment AB showed the highest bacterial count on Day 2, as compared to other treatments. On Day 8, final bacterial count for Treatment A, B and AB were 3.64 x 10^{5} CFU/ml, 0.8 x 10^{5} CFU/ml, and 6 x 10^{5} CFU/ml, respectively.

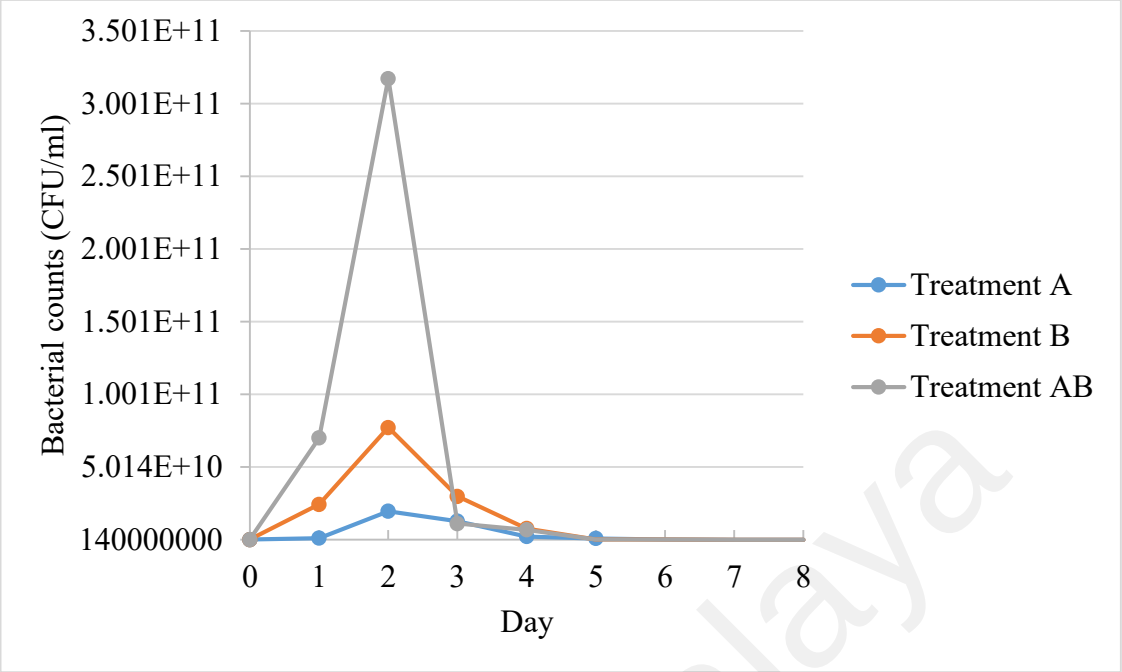


Figure 4.8: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment AB (heterogeneous) showed the highest increase in Fe removal percentage along with the highest increase in bacterial counts, i.e., 3.17 x 10^{11} CFU/ml as compared to 7.72.x 10^{10} CFU/ml (B) and 1.963 x 10^{10} CFU/ml (A). This explains the reason why Fe was reduced in the concentration. Heterogeneous bacteria thrived in the presence of Fe but by Day 3, they experienced a sharp decline in bacterial count. Nonetheless, Fe had not done any biological molecules disruption to heterogeneous bacteria as they continued to remove Fe quite substantially. They were also resilient as evidenced by the highest Day-8 bacterial count, i.e., 6 x 10^5 CFU/ml while for Treatment A and B, it was 3.64 x 10^5 CFU/ml and 0.8 x 10^5 CFU/ml, respectively. Both homogeneous bacterial set-ups (Treatment A & B) did not thrive in the presence of Fe. This means that Gram-negative bacteria were more tolerant and more adaptive to Fe than Gram-positive bacteria.

On Day 8, Gram-negative bacteria showed the lowest bacterial count, yet with an increase in the removal of Fe. This is possibly due to Fe adsorption by both living and dead cells. In Treatment A, Gram-positive bacteria were not tolerant to Fe that resulted with lowest bacterial count and lowest Fe removal on Day 2. On Day 8, Gram-positive bacteria were more tolerant to Fe than Gram-negative, as they did not decline as much. Gram-positive bacteria removed much more Fe than Gram-negative bacteria on Day 8 but collectively, the maximum Fe removed by Gram-negative bacteria was still higher than that of Gram-positive.

As shown in Figure 4.9, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH5.8-7.7, Treatment B ranged at pH6.5-7.8 and Treatment AB ranged at pH6.6-8.4. On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 6.7, 7.8 and 8.4, respectively, thus pH A<7<B<AB. On Day 8, the pH for Treatments A, B and AB were 7.7, 6.5 and 7.3, respectively, thus pH 7<AB<B<A. On the day when Fe removal percentage was the highest, the pH observed for each Treatment was 6.7 (Treatment A), 7.8 (Treatment B) and 8.4 (Treatment AB). On Day 8, Treatment A (Gram-positive) and Treatment AB (heterogeneous) remained to be in alkaline condition while Treatment B (Gram-negative) turned from alkaline to acidic.

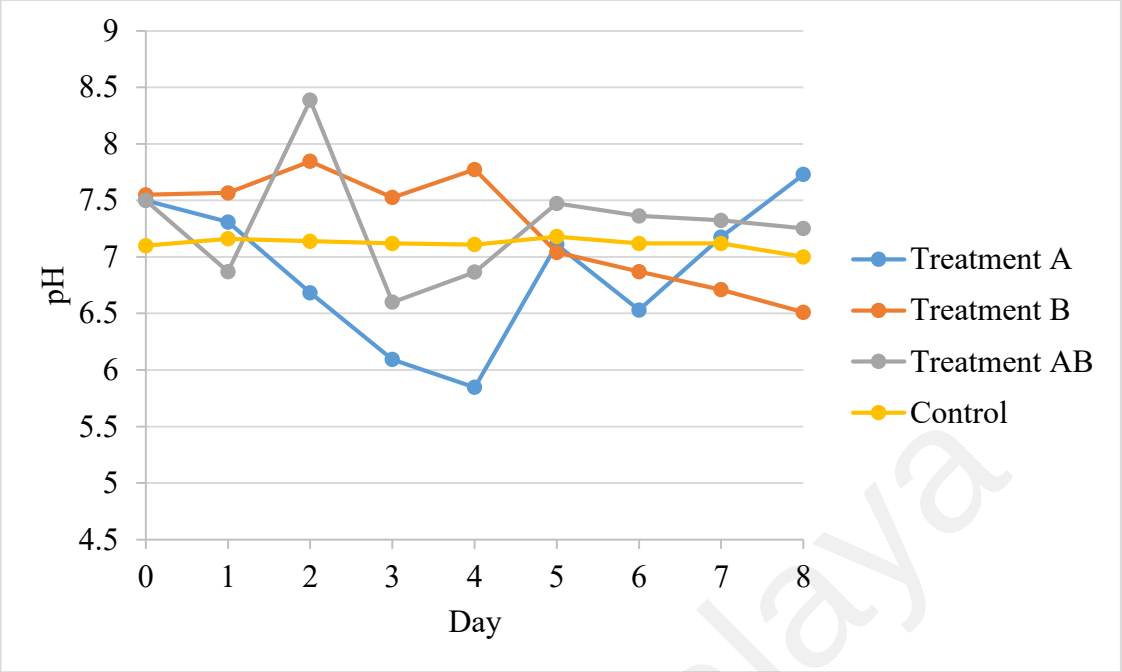


Figure 4.9: pH readings for all treatments in monometal system

4.2.4 Zinc (Zn)

Figure 4.10 shows the removal percentage of Zn across treatments in monometal system. For Treatment A, 19% of Zn was removed in two days whereas 8% of Zn (a decrease by 11%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.104 day^{-1} (2-Day) and 0.01 day^{-1} (8-Day). For Treatment B, 10% of Zn was removed in two days whereas 0% of Zn (a decrease by 10%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.053 day^{-1} (2-Day) and 0.0 day^{-1} (8-Day). For Treatment AB, 10% of Zn was removed in two days whereas 15% of Zn (an increase by 5%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.05 day^{-1} (2-Day) and 0.02 day^{-1} (8-Day). Therefore, the order for Zn removal was A>B>AB (2-Day incubation period) and AB>B>A (8-Day incubation period).

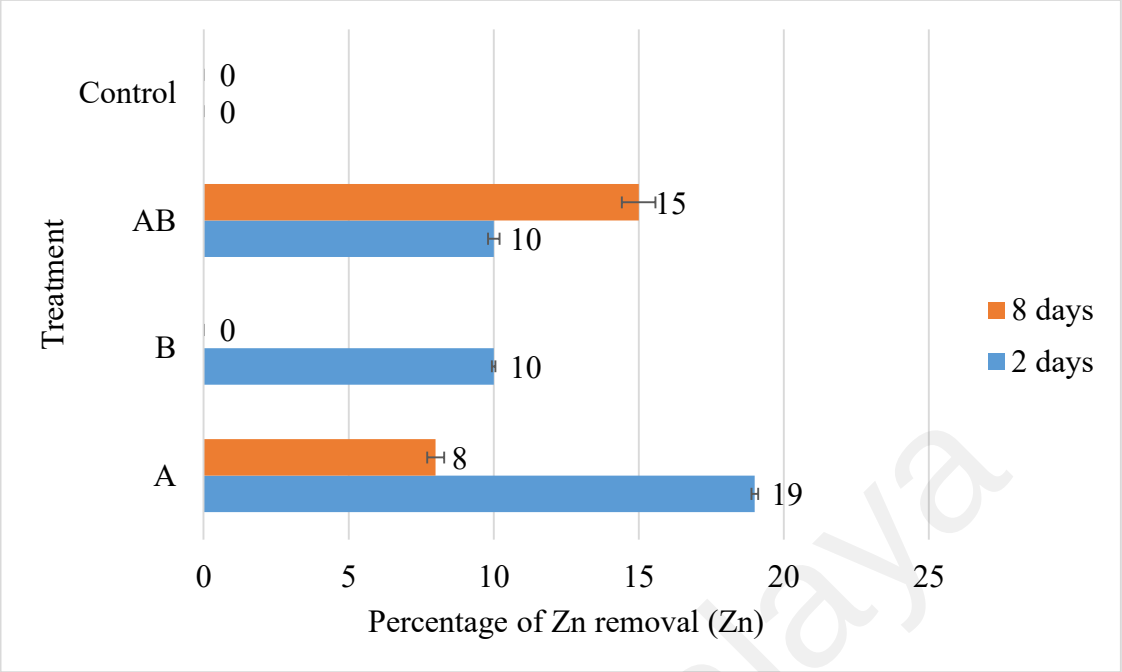


Figure 4.10: Percentage of Zn removal in monometal system

Among homogeneous groups, Treatment A (Gram-positive bacteria) showed a higher Zn removal percentage than Treatment B (Gram-negative bacteria). On Day 2, Treatment A showed 19% removal of Zn as compared Treatment B 10%, while on Day 8, the recorded removal were 8% and 0%, respectively. It is possible that both types of homogeneous bacteria had reached the maximum Zn removal by Day 2 and that insignificant removal occurred until Day 8. Gram-negative bacteria released 100% of adsorbed Zn into the solution whereas Gram-positive managed to retain 40% of adsorbed Zn. The reason for this is as bacterial cells age, the structural features of bacterial cell wall weaken, thus resulting in prevention of Zn adsorption (Daboor, 2014). The removal performance of Gram-positive and Gram-negative bacteria was in accordance to Ramesh *et al.* (2014) who reported that *Bacillus aryabhattai* (Gram-positive bacteria) was able to solubilize Zn. Meanwhile, Costa and Duta (2001) reported that the maximum Zn bioaccumulation by *Bacillus cereus* (Gram-positive bacteria) was 4.6 mol/g. According to a study by Vaid *et al.* (2014), *Burkholderia vietnamiensis*

(Gram-positive bacteria) has a total Zn uptake of 52.5%. Bautista-Hernández *et al.* (2012) reported that maximum adsorption of Zn by *Delftia tsuruhatensis* (Gram-negative bacteria) was 0.207 mmol/g, which can be considered as a median magnitude capacity when compared to other treatments used. Meanwhile, Gawali Ashruta *et al.* (2014) reported that *Pseudomonas* species (Gram-negative) was able to remove 77.15% of Zn.

The heterogeneous group of Treatment AB had the highest Zn removal percentage. On Day 2, it had the lowest removal percentage which is 10% same as Treatment B and 19% by Treatment A. However, on Day 8, the removal percentage increased further to 15% as compared to 8% and 0% in Treatment A and B. This can be attributed to heterogeneous bacteria not yet reaching the optimum Zn removal. Heterogeneous bacteria seemed to retain Zn longer than homogeneous bacteria. Its slow start can be attributed to the bacteria adjusting their interactions between the different species and synergizing among the heterogeneous communities (Stubbendieck *et al.,* 2016). According to Fauziah *et al.* (2017), the mixed of *Cloacibacterium* sp., *Chryseobacterium gleum, Bacillus aryabhattai, Rhodococcus rubber, Bacillus Pumilus, Bacillus kochii, Janibacter hoylei and Bacillus cereus* were able to remove 50.3% of Zn. According to Carpio *et al.,* 2016, Bacillus species and Pseudomonas species able to remove more than 50% of Zn. According to Singh and Vaishya (2017), *Bacillus* and *Stenotrophomonas* species were able to degrade 80.26% of Zn. This highlighted that bacterial combinations worked better as compared to single-species bacteria.

Figure 4.11 shows the bacterial counts for all treatments in monometal system at Day 0 until Day 8. In the presence of Zn, the maximum of bacterial growth was recorded at

Day 2. On Day 2, bacterial count for Treatment A was 1.045 x 10^{11} CFU/ml (1.091 ABS). The bacterial count for Treatment B was 8.88 x 10^{10} CFU/ml (0.87 ABS) and Treatment AB was 4.05 x 10^{10} CFU/ml. On Day 2, Treatment A showed the highest bacterial count compared to other treatments. On Day 8, final bacterial count for Treatment A was 9.6 x 10^{4} CFU/ml, for Treatment B was 7.2 x 10^{4} CFU/ml and Treatment AB was 1.96 x 10^{5} CFU/ml.

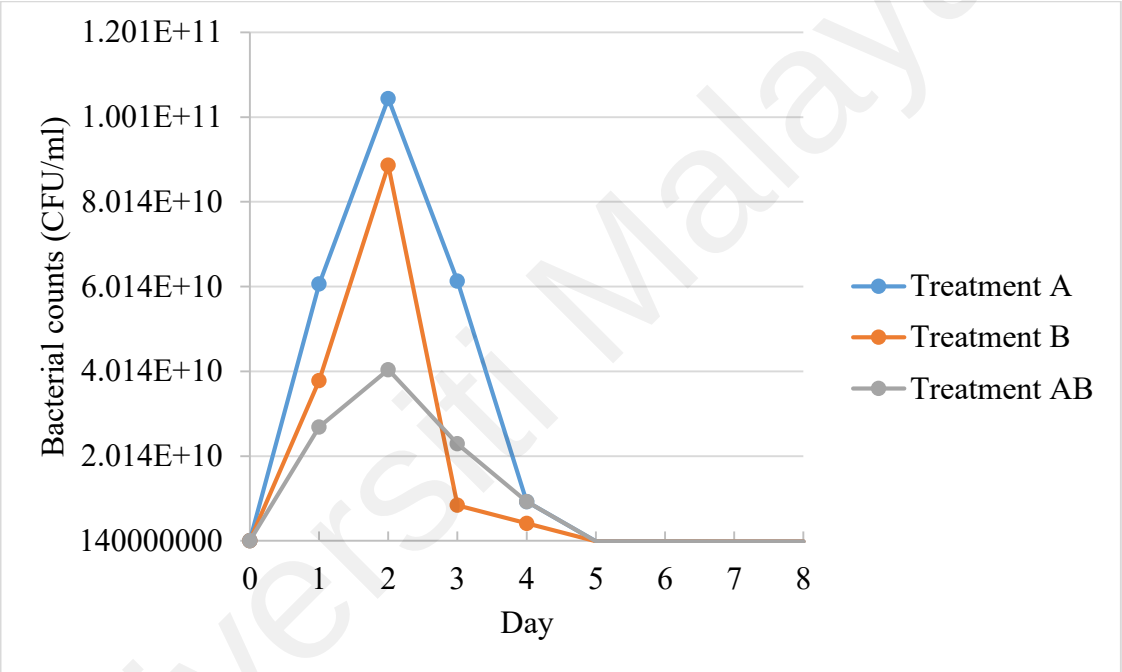


Figure 4.11: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A (Gram-positive) showed a drastic increase in Zn removal percentage along with the increase in bacterial counts, i.e., 1.045 x 10^{11} CFU/ml as compared to 8.88 x 10^{10} CFU/ml (B) and 4.05 x 10^{10} CFU/ml (AB). Gram-positive bacteria thrived in the presence of Zn but by Day 8, Zn begun to create toxicity that led to a decline in bacterial count, i.e., 0.72 x 10^{5} CFU/ml (B), 0.96 x 10^{5} CFU/ml (A), and 1.96 x 10^{5} CFU/ml (AB). The stress had caused them to release Zn back to the solution. For Treatment B, Gram-negative bacteria thrived as well but not as much as Gram-positive bacteria. Gram-negative bacteria experienced a sharp decline in bacterial count

until Day 8 as they were more stress of Zn as compared to Gram-positive bacteria. As for Treatment AB, the heterogeneous bacteria did not thrive as much as the homogeneous bacteria which explains Treatment AB having the lowest removal percentage on Day 2. Towards Day 8, the heterogeneous bacteria were more resilient than the homogeneous bacteria as evident from their steady increase in bacterial count. Their resilience can be associated by the high tolerance of heterogeneous bacteria to Zn, which increased the efficiency of Zn removal.

As shown in Figure 4.12, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH6.6-8.0, Treatment B ranged at pH6.0- 7.7 and Treatment AB ranged at pH6.4-7.8. On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 7.5, 6.8 and 6.7, respectively, thus pH AB<B<7<A. On Day 8, the pH for Treatment A, B and AB were 7.2, 5.8 and 6.8, respectively, thus pH B<AB<7<A. On the Day when Zn removal percentage was the highest, the pH observed for each treatment was 7.5 (Treatment A), 6.8 (Treatment B) and 6.7 (Treatment AB). It appears that by Day 8, Treatment A (Gram-positive) became less basic, Treatment B (Gram-negative) transitioned from basic to acidic and Treatment AB (heterogeneous) became slightly acidic.

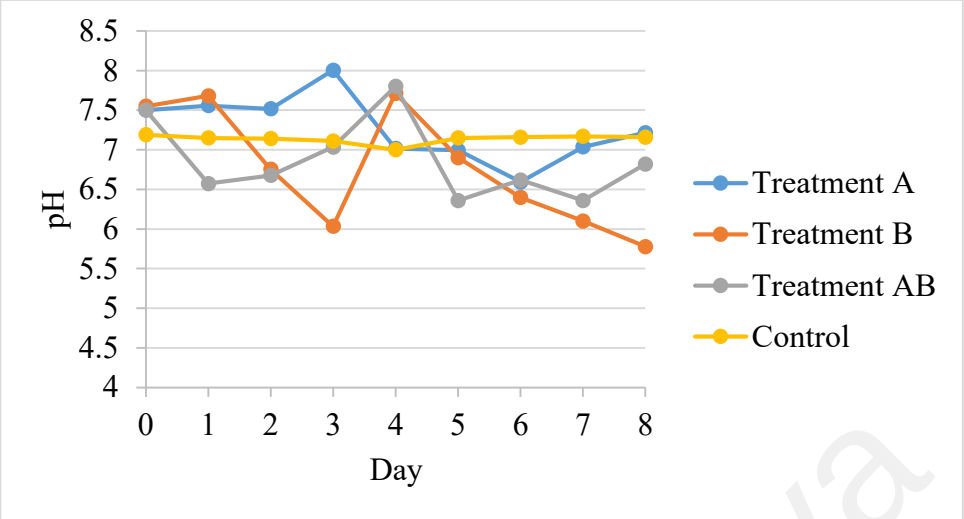


Figure 4.12: pH reading for all treatments in monometal system

4.2.5 Copper (Cu)

Figure 4.13 shows the removal percentage of Cu across treatments in monometal system. For Treatment A, 30% of Cu was removed in two Days whereas 16% of Cu (a decrease by 14%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.18 day^{-1} (2-Day) and 0.072 day^{-1} (8-Day). For Treatment B, 21% of Cu was removed in two days whereas 29% of Cu (an increase by 8%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.119 day^{-1} (2-Day) and 0.042 day^{-1} (8-Day). For Treatment AB, 29% of Cu was removed in two days whereas 35% of Cu (an increase by 6%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.172 day^{-1} (2-Day) and 0.054 day^{-1} (8-Day). Therefore, the order for Cu removal was A>AB>B (2-Day incubation period) and AB>B>A (8-Day incubation period).

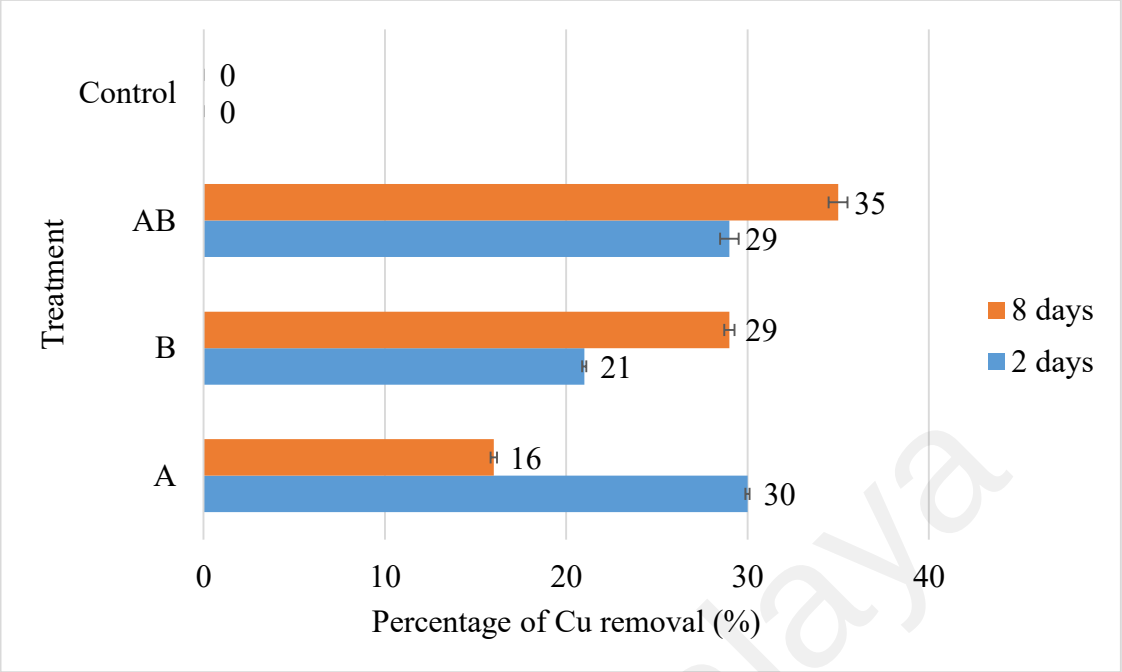


Figure 4.13: Percentage of Cu removal in monometal system

Among homogeneous groups, Treatment B (Gram-negative) showed higher Cu removal than Treatment A (Gram-positive). On Day 2, Treatment A has a higher removal percentage than Treatment B, i.e., 30% and 21%, respectively. In the beginning, it appears Gram-positive bacteria (Treatment A) was more adaptive to Cu than Gram-negative bacteria (Treatment B) which allowed them to adsorb a higher amount of Cu. However, on Day 8, Gram-positive bacteria had a large decline in removal percentage and released almost half of adsorbed Cu, i.e., 16%. This is because Gram-positive bacteria had earlier passed the optimum Cu removal, possibly on Day 2. On the other hand, Gram-negative bacteria continued to remove Cu on Day 8—the removal percentage for Day 8 was smaller than that of Day 2, suggesting that Gram-negative bacteria were nearing their maximum Cu removal. Based on maximum removal, Gram-positive bacteria removed slightly more Cu than Gram-negative bacteria, i.e., 30% and 29%, respectively. Furthermore, Gram-positive bacteria were faster in adsorbing Cu than Gram-negative bacteria. The removal performance of Gram-

positive and Gram-negative bacteria was in accordance to previous findings. By Ghosh and Saha (2013) who reported that *Bacillus pumilus* (Gram-positive bacteria) was able to remove Cu by 60% while a study by Shen *et al.* (2017) reported that *Stenotrophomonas* strain (Gram-negative bacteria) was also able to remove Cu. A finding by Leung *et al.* (2000) reported that over 90% of Cu was adsorbed on the cells of *Pseudomonas alcaligenes* (Gram-negative bacteria). According to Gawali Ashruta *et al.* (2014), *Pseudomonas* species (Gram-negative) was able to remove 71% of Cu, while, Narasimhulu and Rao (2009) reported that *Pseudomonas* species was able to remove 95% of Cu.

As the heterogeneous group, Treatment AB showed an almost similar removal performance to Treatment A on Day 2, i.e., 29% and 30%, respectively. On Day 8, it showed similar performance to Treatment B, i.e., 35% and 29%, respectively (an increase in removal percentage). The maximum removal for Treatment AB surpassed both Treatment A and B. It seems that heterogeneous bacteria were mutualistic in reacting to Cu, hence led to the highest removal percentage. According to Ilamathi *et al.* (2014), mixed consortium of yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* was able to remove 84.62% of Cu. Sannasi *et al.* (2009), reported that a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 18.85-18.99% of Cu.

Figure 4.14 shows the bacterial counts for all treatments in monometal system from Day 0 until Day 8. In the presence of Cu, the maximum bacterial growth was recorded

on Day 2. On Day 2, bacterial count for Treatment A was 7.55 x 10^{10} CFU/ml CFU/ml, Treatment B was 4.78 x 10^{10} CFU/ml (0.617 ABS) and Treatment AB was 7.0 x 10^{10} CFU/ml. Treatment A showed the highest Day-2 bacterial count as compared to other treatments. On Day 8, final bacterial count for Treatment A was 0.96 x 10^{5} CFU/ml (0.173 ABS). For Day 8 Treatment B bacterial count was 1.24 x 10^{5} CFU/ml (0.184 ABS) and Treatment AB was 5.265 x 10^{5} CFU/ml (0.196 ABS).

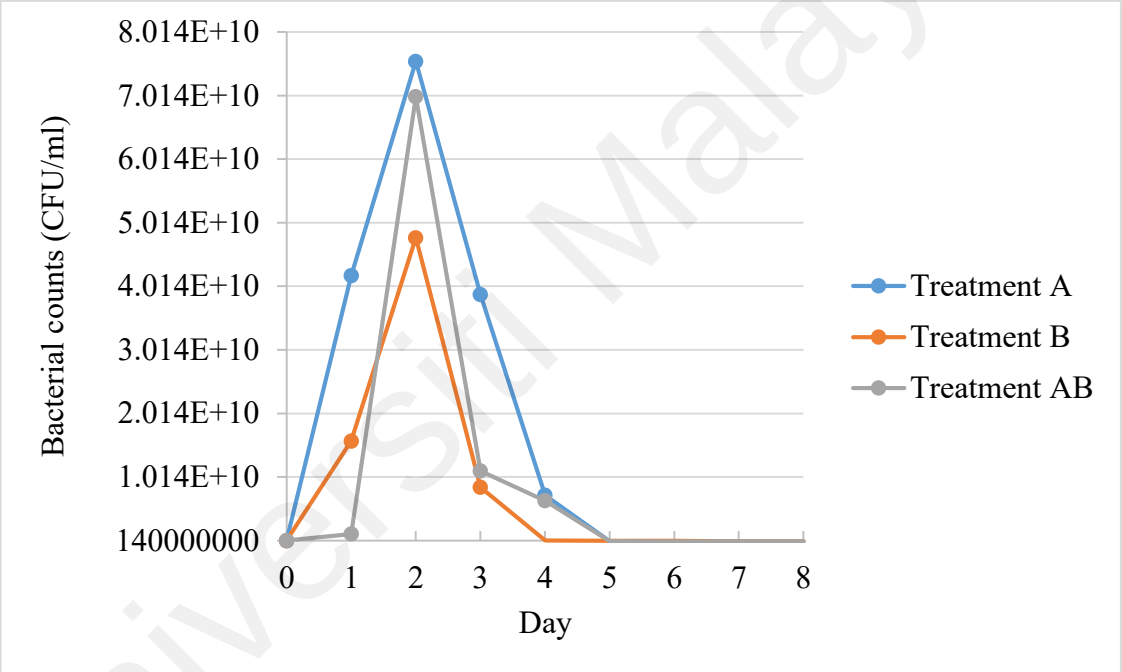


Figure 4.14: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A (Gram-positive bacteria) showed a rapid increase in Cu removal along with the increase in bacterial counts, i.e., 7.55 x 10^{10} CFU/ml. The bacterial growth enhances the Cu uptake which resulted in higher removal capacity. Gram-positive bacteria thrived in the presence of Cu but by Day 8, Cu had done some biological molecules disruption to them and led to a sharp decline in bacterial count, i.e., 0.96 x 10^{5} CFU/ml as compared to 1.24 x 10^{5} CFU/ml (B) and 5.265 x 10^{5} CFU/ml

(AB). The stress had caused them to release Cu back to the solution. Gram-negative bacteria, on the other hand, did not thrive. Gram-negative bacteria continued to remove Cu until Day 8 possibly because they have not reached the maximum Cu removal. As for Treatment AB, the heterogeneous bacteria showed high bacterial count on Day 2 but later declined drastically by Day 3, similar to that observed in Gram-negative bacteria. However, the heterogeneous bacteria were resilient as they formed the highest count and also showed the highest removal percentage by the end of incubation period.

As shown in Figure 4.15, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A (6.0-8.1), Treatment B ranged at pH5.5-8.1 and Treatment AB ranged at pH6.0-7.5. On Day 2 (the maximum bacterial growth), the pH for Treatments A, B and AB were 8.1, 7.9 and 6.0, respectively, thus pH AB<7<B<A. On Day 8, the pH for Treatment A, B and AB were 6.0, 5.5 and 6.8, respectively, thus pH B<A<AB<7. On the day when Cu removal percentage was the highest, the pH observed for each treatment was 8.1 (Treatment A), 7.9 (Treatment B) and 6.0 (Treatment AB). It appears that by Day 8, both Treatments B and C (homogeneous) transitioned from basic to acidic whereas Treatment AB (heterogeneous) transitioned from basic to almost neutral.

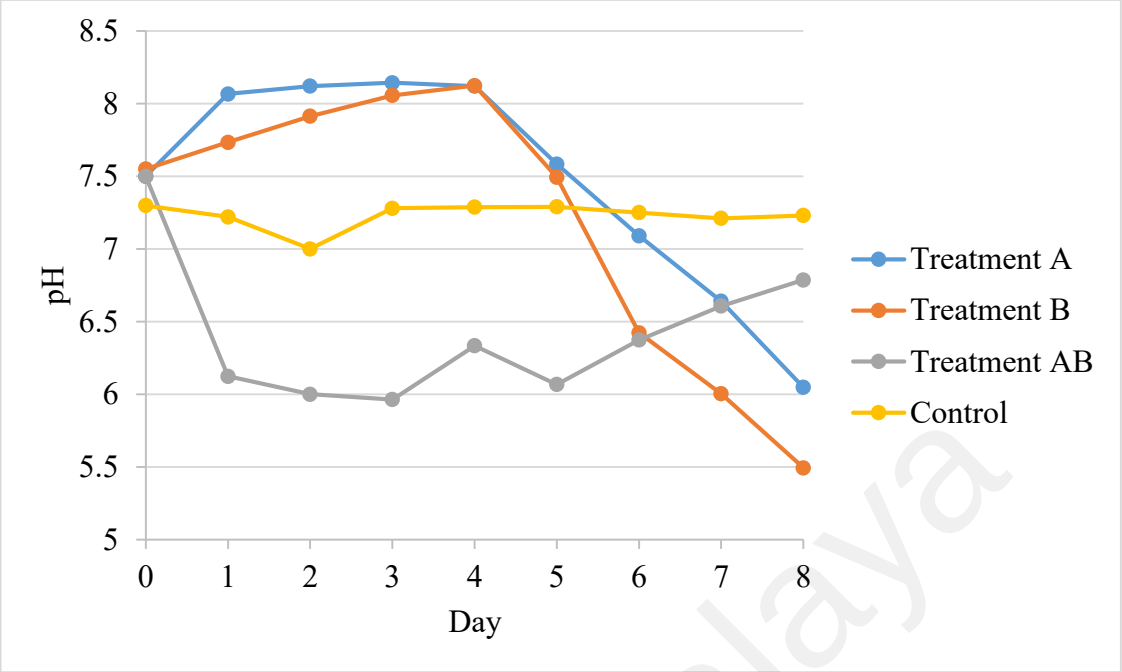


Figure 4.15: pH reading for all treatments in monometal system

4.2.6 Cadmium (Cd)

Figure 4.16 shows the removal of Cd across treatments in monometal system. For Treatment A, 34% of Cd was removed in two days whereas 16% of Cd (a decrease by 18%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.21 day^{-1} (2-Day) and 0.022 day^{-1} (8-Day). For Treatment B, 19% of Cd was removed in two Days whereas 18% of Cd (a decrease by 1%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.103 day^{-1} (2-Day) and 0.025 day^{-1} (8-Day). For Treatment AB, 16% of Cd was removed in two days whereas 9% of Cd (a decrease by 7%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.088 day^{-1} (2-Day) and 0.011 day^{-1} (8-Day). Therefore, the order for Cd removal was A>B>AB (2-Day incubation period) and B>AB>A (8-Day incubation period).

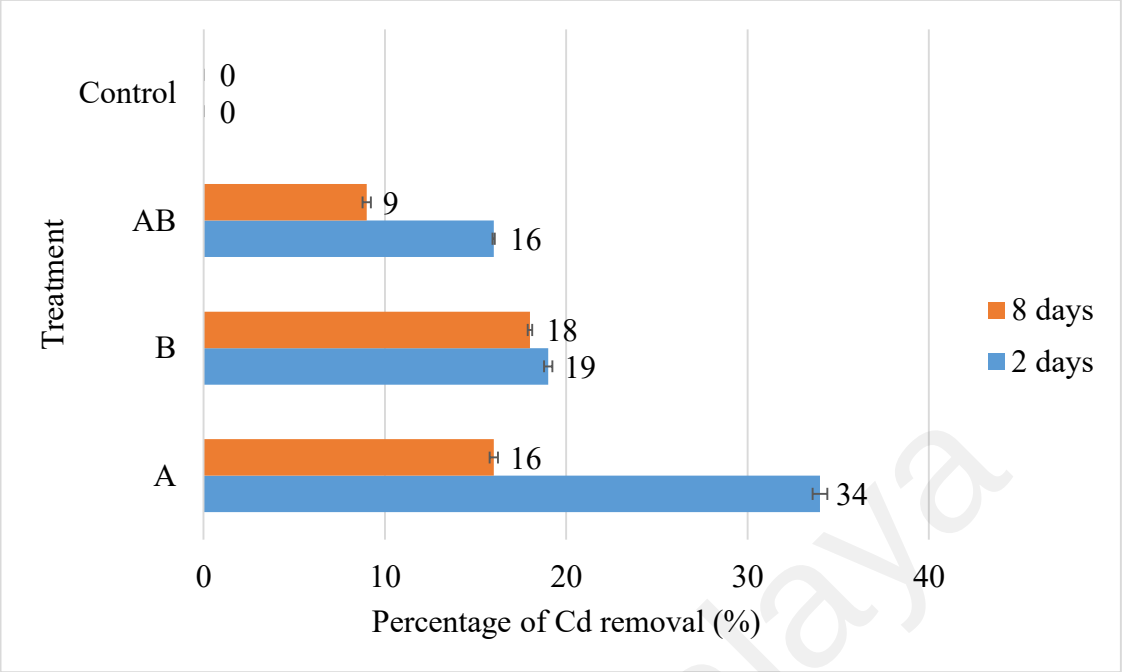


Figure 4.16: Percentage of Cd removal in monometal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Cd removal than Treatment B (Gram-negative). On Day 2, the removal percentage in Treatment A was much higher than in Treatment B, i.e., 34% and 19%, respectively. However, on Day 8, it became slightly lower than Treatment B, i.e., 16% and 18%, respectively. In the beginning, it appears Gram-positive bacteria was more adaptive to Cd than Gram-negative bacteria which allow them to adsorb a greater amount of Cd. However, both released Cd back into the solution by Day 8, hence the decrease in removal percentage. Apparently, Gram-negative bacteria have a higher Cd retention percentage than Gram-positive bacteria (i.e., decreased by 0.4% and 17.9%, respectively). Excessive Cd may have inhibited the respiratory chain of the bacteria and acted as potent disrupters of bacterial biological system (Basha & Rajaganesh, 2014). The removal performance of Gram-positive and Gram-negative bacteria was in accordance to previous findings of Arivalagan *et al.* (2014) who reported that the

maximum biosorption capacity of Cd by *Bacillus cereus* (Gram-positive bacteria) was 82%. Meanwhile, in a study by Jayanthi *et al.* (2016), *Burkholderia vietnamiensis* (Gram-positive bacteria) expressed a higher metal resistance compared to other microbes tested. Pandey *et al.* (2010) reported that *Ochrobactrum intermedium* (Gram-negative bacteria) capable of accumulating Cd from the external environment and can be implied in bioremediation of Cd. According to Gawali Ashruta *et al.,* (2014), *Pseudomonas* species (Gram-negative) was able to remove 72.71% of Cd. Narasimhulu and Rao (2009) reported that *Pseudomonas* species was able to remove 90% of Cd. Meanwhile, Cristani *et al.* (2012) noted that *Serratia marcescens* (Gram-negative bacteria) showed a biosorption potential of Cd with a range of 0.097 µg/g to 0.1853 µg/g.

Treatment AB (heterogeneous group) had the lowest removal percentage on both Day 2, i.e., 16% and Day 8, i.e., 9%. This may be caused by the weak interactions between different species and compatibilities among multispecies communities (Stubbendieck *et al.,* 2016). The heterogeneous bacteria seemed to have a low Cd retention like Gram-positive bacteria. According to Wong *et al.,* (2015), heterogeneous bacteria which consist of *Bacillus, Pseudomonas, Serratia, Agrobacterium, Chryseomonas, Flavobacterium, , Xanthomonas, Arthobacter,* and *Micrococcus* species is shown to degrade 23.6% of Cd. Ilamathi *et al.* (2014) reported that the mixed consortium of yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* were able to remove 67.17% of Cd. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive

(*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 9.42-10.62% of Cd.

Figure 4.17 shows the bacterial counts for all treatments from initial day (Day 0) until Day 8. In the presence of Cd, the maximum bacterial growth was on Day 2. On Day 2, bacterial count for Treatment A was 7.125 x 10^{10} CFU/ml CFU/ml. However, in Treatment B and AB, the bacterial count was 5.205 x 10^{10} CFU/ml and 2.5 x 10^{10} CFU/ml, respectively. Treatment A showed the highest Day-2 bacterial count as compared to other treatments. The final bacterial count for Treatment A, B and AB was 0.56 x 10^{5} CFU/ml, 2.96 x 10^{5} CFU/ml, and 1.28 x 10^{5} CFU/ml, respectively.

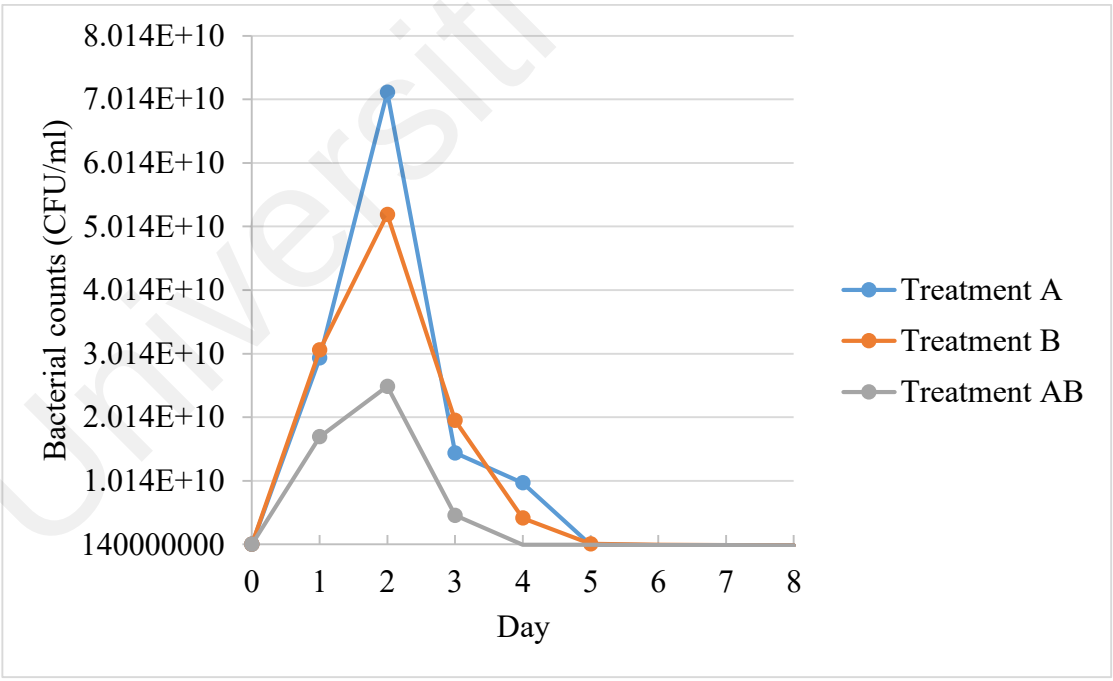


Figure 4.17: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A (Gram-positive) showed a drastic increase in Cd removal (34 %) along with the increase in bacterial counts, i.e., 7.125 x 10^{10} CFU/ml . On Day 8, Cd have done some biological molecules disruption to Gram-positive bacteria hence, resulting in the lowest bacterial count and the release of Cd back to the solution, i.e., 0.56 x 10^5 CFU/ml as compared to 1.28 x 10^5 CFU/ml (AB) and 2.96 x 10^5 CFU/ml (B). As for Treatment B, Gram-negative bacteria was less adaptive to Cd. Its maximum Cd removal (Day 2) was almost half of that of Gram-positive bacteria but by Day 8, it still retained more than 90% Cd removal. Gram-negative bacteria did not decline as much as Gram-positive bacteria. As for Treatment AB, heterogeneous bacteria did not thrive in the presence of Cd (i.e., the highest count was the lowest on Day 2), similar to that of Gram-negative bacteria. Although it did not thrive, the final bacterial count was higher than that of Gram-negative but lower than that of Gram-positive bacteria.

As shown in Figure 4.18, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH6.9-8.3, Treatment B ranged at pH6.8-8.1 and Treatment AB ranged at pH6.3-7.2. On Day 2 (the maximum bacterial growth), the pH for treatment A, B and AB were 8.1, 7.8, 7.1, respectively, thus pH 7<A<B<AB. On Day 8, the pH for Treatment A, B and AB were 6.9, 6.8 and 6.7, respectively, thus pH AB<B<A<7. On the day when Cd removal percentage was the highest, the pH observed for each treatment was 8.1 (Treatment A), 7.8 (Treatment B) and 7.1 (Treatment AB). It appears that by Day 8, Treatment A and B (homogeneous) transitioned from basic to almost neutral whereas Treatment AB (heterogeneous) remained almost neutral.

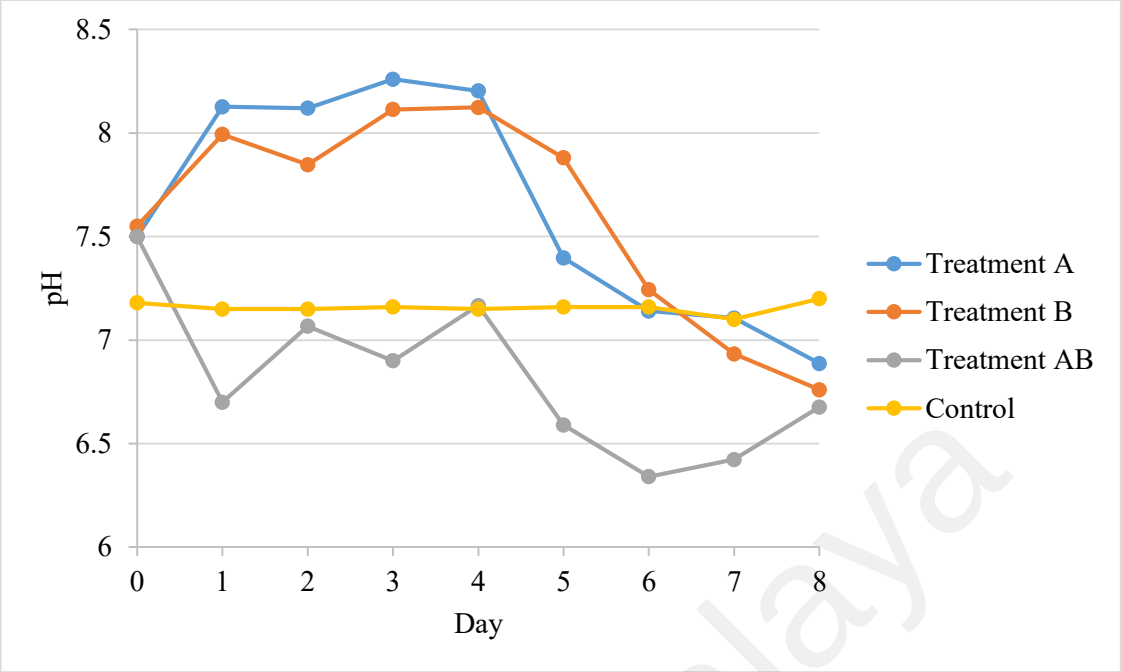


Figure 4.18: pH reading for all treatments in monometal system

4.2.7 Nickel (Ni)

Figure 4.19 shows the percentage of Ni removal across treatments in monometal system. For Treatment A, 15% of Ni was removed in two days whereas 25% of Ni (an increase by 10%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.082 day^{-1} (2-Day) and 0.036 day^{-1} (8-Day). For Treatment B, 19% of Ni was removed in two days whereas 18% of Ni (a decrease by 1%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.103 day^{-1} (2-Day) and 0.025 day^{-1} (8-Day). For Treatment AB, 17% of Ni was removed in two days whereas 6% of Ni (a decrease by 11%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.091 day^{-1} (2-Day) and 0.008 day^{-1} (8-Day). Therefore, the order for Ni removal was B>AB>A (2-Day incubation period) and A>B>AB (8-Day incubation period).

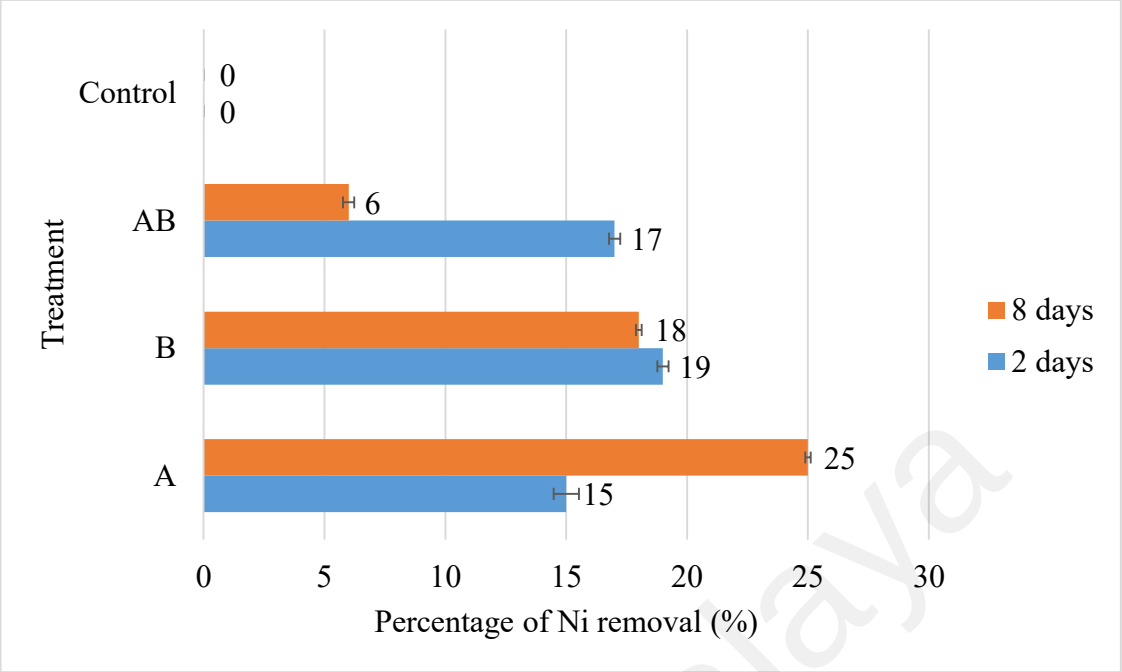


Figure 4.19: Percentage of Ni removal in monometal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Ni removal percentage than Treatment B (Gram-negative). On Day 2, the removal by Treatment B was higher 19% than Treatment A, 15%. On Day 8, the removal percentage of Treatment B was slightly decreased to 18% when the removal of Ni in Treatment A increased drastically to 25%. In the beginning, Gram-negative bacteria was more adaptive to Ni than Gram-positive bacteria, which allowed them to adsorb a higher amount of Ni. However, Gram-negative bacteria released Ni back into the solution by Day 8 albeit in a small amount. It appears Gram-positive bacteria have a higher ability to retain Ni in their cell as compared to Gram-negative bacteria. This allowed them to continue adsorbing Ni on Day 8. Gram-positive bacteria continued to remove Ni until Day 8 as they have not reached the maximum removal of Ni. The removal performance of Gram-positive and Gram-negative bacteria was in accordance to previous findings by Parameswari *et al.* (2009) who reported that the maximum Ni removal by *Bacillus* sp. (Gram-positive bacteria) was 84.32%. Jia *et al.* (2014) reported that the removal

percentage of Ni by *Stenotrophomonas* sp. (Gram-negative bacteria) was above 90% while Gawali Ashruta *et al.* (2014), concluded that *Pseudomonas* species (Gram-negative) was able to remove 66.63% of Ni. Narasimhulu and Rao (2009) also reported that *Pseudomonas* species was able to remove 90% of Ni. Meanwhile, Nouha *et al.* (2016) previously reported that *Cloacibacterium* sp. (Gram-negative bacteria) was able to remove 85% of Ni. In addition, *Serratia marcescens* (Gram-negative bacteria) has a maximum removal of Ni in a range of 25.51 to 28.08 mol/g as noted by Kannan and Ramteke (2002).

On Day 2, Treatment AB showed a slightly lower removal of Ni than that of Treatment B. Nevertheless, the removal by treatment AB was slightly higher than Treatment A (15%). On Day 8, the heterogeneous bacteria in Treatment AB released Ni back to the solution, hence reduced the removal capacity to 6%. In addition, the heterogeneous bacteria seemed to retain Ni the shortest which can be attributed to the non-mutualistic interaction among heterogeneous bacteria. Similar findings were reported by Fauziah *et al.* (2017) on the mixture of *Ochrobacterium intermedium, Burkholderia vietnamiensis, Stenotrophomonas acidaminiphilia, Acidovorax ebreus, Brevundimonas diminuta, Delftia tsuruhatensis, Aeromonas caviae, Pseudomonas alcaligenes, Pseudomonas mendocina, Serratia marcescens marcescens,* with the ability to remove 50.8% of Ni. According to Ilamathi *et al.* (2014) reported that the mixed consortium of yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* were able to remove 61.02% of Ni. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 13.34-15.43% of Ni.

Figure 4.20 shows the bacterial counts for all treatments in monometal system from Day 0 until Day 8. The highest bacterial growth was recorded on Day 2 where the bacterial count for Treatment B, AB and A was 3.797 x 10^{11} CFU/ml CFU/ml, 8.618 x 10^{11} CFU/ml, and 8.235 x 10^{11} CFU/ml, respectively. Treatment B showed the highest bacterial counts compared to other treatments. On Day 8, final bacterial counts for Treatment A was 2.44 x 10^{5} CFU/ml, followed by Treatment B at 1.84 x 10^{5} CFU/ml and Treatment AB at 1.56 x 10^{5} CFU/ml.

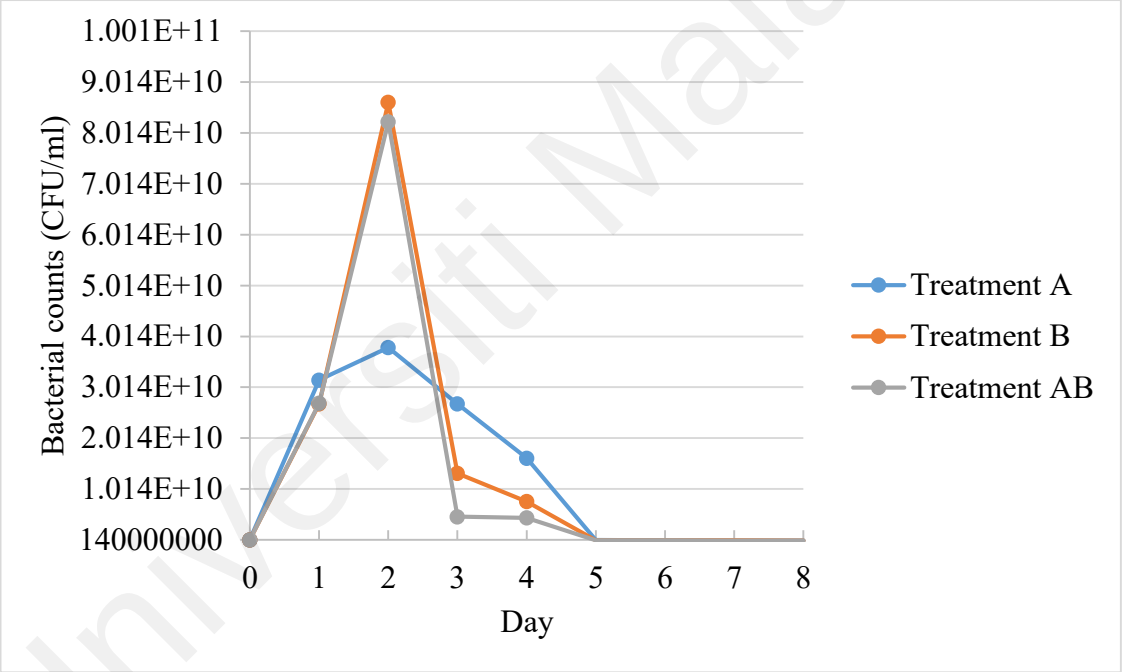


Figure 4.20: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment B (Gram-negative) showed a drastic increase in Ni removal along with the increase in bacterial counts at 8.618 x 10^{10} CFU/ml. Gram-negative bacteria thrived in the presence of Ni but by Day 8, Ni had done some biological molecules disruption to them and led to sharp decline in bacterial count to 1.84 x 10^{5} CFU/ml. The stress induced by toxicity probably caused the release of Ni back to the solution. For

Treatment A, Gram-positive bacteria did not thrive in the presence of Ni and Ni removal was the least on Day 2. However, they were resilient and did not decline drastically which explains the reason they continued to uptake Ni until Day 8 resulting with the highest removal capacity. As for Treatment AB, the heterogeneous bacteria showed high bacterial count on Day 2 like Gram-negative bacteria but later declined to the lowest by Day 8. Initially, the heterogeneous bacteria thrived in the presence of Ni but by Day 8, the bacterial count began to decline, most likely due to toxicity stress. As a result, more Ni was detected in the solution. Compared to Gram-negative bacteria, the heterogeneous bacteria were more affected by the stress as evident in Treatment AB having the lowest count and the lowest net Ni removal percentage on Day 8.

As shown in Figure 4.21, the pH for all treatments fluctuated during the 8-Day incubation period. Treatment A ranged at pH7.1-8.2, Treatment B ranged at pH7.3-8.2 and Treatment AB ranged at pH6.9-8.1. On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 7.1, 8.2 and 8.1, respectively, thus pH 7<A<AB<B. On Day 8, the pH for Treatment A, B and AB were 7.6, 7.1 and 7.2, respectively, thus pH 7<B<AB<A. On the Day when Ni removal percentage was the highest, the pH observed for each treatment was 7.1 (Treatment A), 8.2 (Treatment B) and 8.1 (Treatment AB). It appears that by Day 8, Treatment A (Gram-positive) became more basic, Treatment B (Gram-negative) became less basic and Treatment AB (heterogeneous) remained basic.

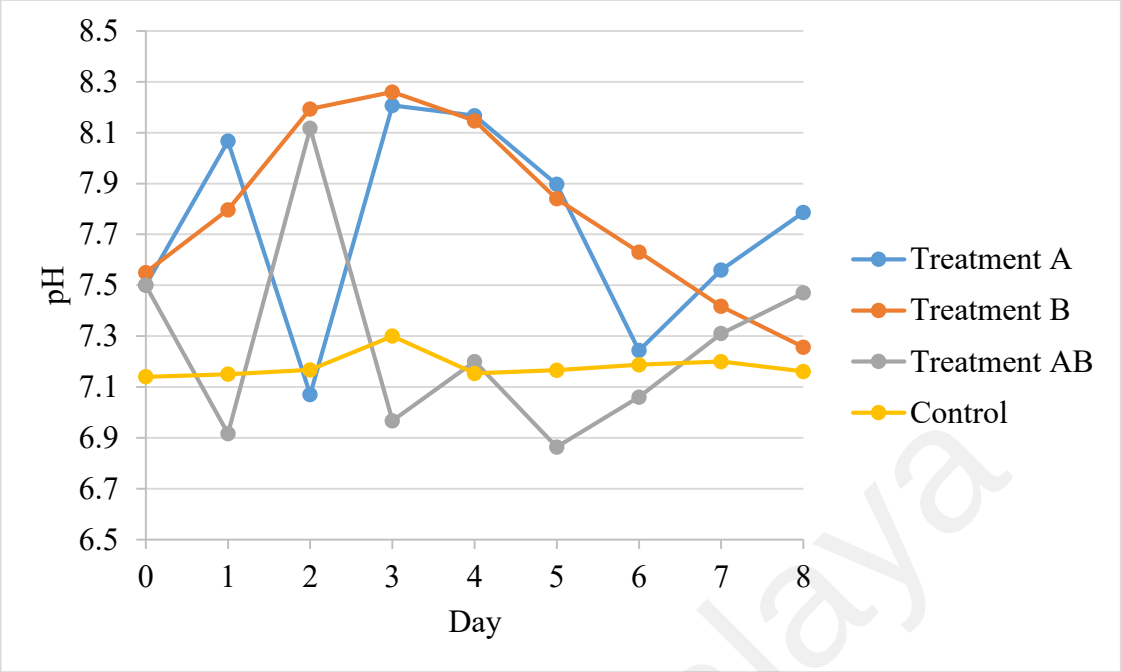


Figure 4.21: pH reading for all treatments in monometal system

4.2.8 Chromium (Cr)

Figure 4.22 shows the removal of Cr across treatment in monometal system. For Treatment A, 9% of Cr was removed within two days and 12% within Day 8. Based on these values, the first order rate constants for Treatment A were determined to be 0.045 day^{-1} (2-Day) and 0.016 day^{-1} (8-Day). Treatment AB and Treatment B showed no Cr removal, thus the first order rate constants of these two Treatments determined to be 0 day^{-1} for both 2-Day and 8-Day. Overall, only Treatment A showed Cr removal where A> (B & AB).

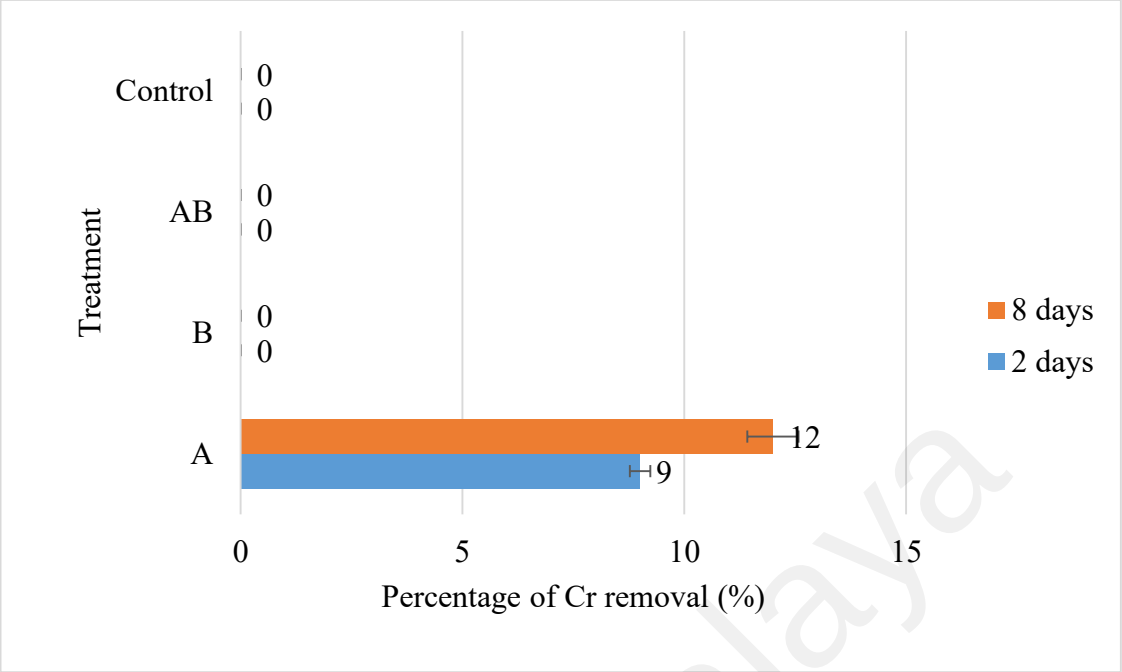


Figure 4.22: Percentage of Cr removal in monometal system

Among the homogeneous groups, only Treatment A was able to remove Cr. Its removal percentage was observed to increase until Day 8, albeit the low percentage from 9 % to 12%. Gram-positive bacteria probably have higher tolerance to Cr than Gram-negative bacteria which allow them to continue adsorbing Cr on Day 8. Excessive Cr may have inhibited the respiratory chain of the bacteria and acted as potent disrupters in bacterial biological system (Basha & Rajaganesh, 2014). The removal performance of Gram-positive was in accordance to findings of Parameswari *et al.* (2009) who reported that *Bacillus* sp. (Gram-positive) was able to remove 89.50% Cr. In addition, as reported by Naik *et al.* (2012), *Bacillus cereus* (Gram-positive) was able to remove Cr more than 75%. In a study by Basu *et al.* (2015), *Bacillus pumilus* (Gram-positive) was reported to remove 1610 µg/ml of Cr. Meanwhile, according to Heipieper (2017), *Rhodococcus ruber* (Gram-positive) has the ability to remove Cr for 71.43%. Gawali Ashruta *et al.* (2014) reported that *Pseudomonas* species (Gram-negative) was able to

remove 74.48% of Cr. Narasimhulu and Rao (2009) also reported that *Pseudomonas* species was able to remove 40% of Cr.

As for the heterogeneous group, Treatment AB was not able to remove Cr. Apparently, there was no mutualism between multispecies communities due to lack of compatibilities in Cr interaction. However, according to Singh & Vaishya (2017), *Bacillus* sp*., Pseudomonas* sp., and *Paenibacillus* sp. were able to reduce 84.13% of Cr. Migahed *et al.,* (2017) reported that *Bacillus* sp., *Serratia* sp., *Vibrio* sp. and *Paenabacillus* sp. were able to remove 100% of Cr. Meanwhile, Ilamathi *et al.* (2014) reported that yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* were able to remove 49.25% of Cr. Kader *et al.* (20017) reported that the bacterial consortium which consists of *Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., (Gram-negative bacteria), *Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp. (Gram-positive bacteria) were able to remove 50-90% of Cr. Also, as reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 5.98-6.68% of Cr.

Figure 4.23 shows the bacterial counts for all treatments in monometal system from Day 0 until Day 8. The maximum bacterial growth was recorded on Day 2 where the bacterial count for Treatment A, B and AB was 2.07 x 10^{11} CFU/ml, 4.91 x 10^{10} CFU/ml, and 4.01 x 10^{10} CFU/ml, respectively. On Day 8, bacterial counts for Treatment A was 2.36 x 10^{5} CFU/ml, followed by Treatment B at 5.68 x 10^{5} CFU/ml, and Treatment AB at 6.48 x 10^{5} CFU/ml.

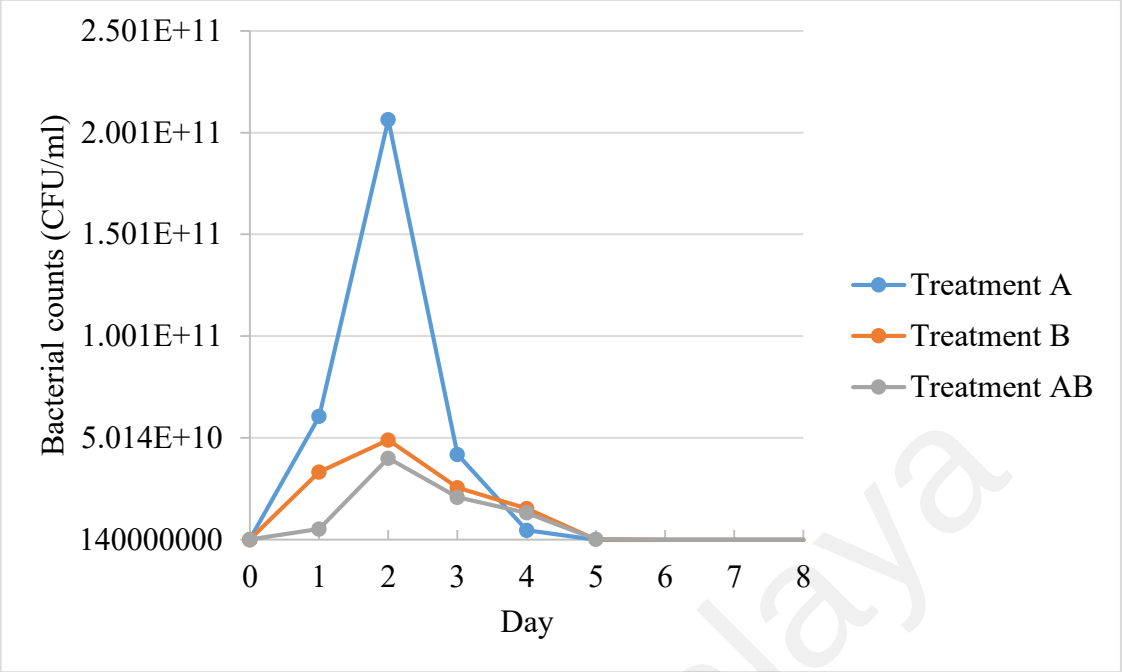


Figure 4.23: Bacterial counts (CFU/ml) for all treatments in monometal system

On Day 2, Treatment A showed a much increase in bacterial population at 2.07 x 10^{11} CFU/ml as compared to Treatment B and AB, at 4.91 x 10^{10} CFU/ml, and 4.01 x 10^{10} CFU/ml, respectively. Treatment B and AB were not able to remove Cr, but they still manage to grow in the presence of Cr, albeit at much lower count than Treatment A. As for the bacterial count, Treatment AB was relatively more resilient than Treatment B. The sharp decline in Treatment A can be attributed to toxicity effect of Cr on Gram-positive bacteria earlier on Day 2.

As shown in Figure 4.24, the pH for all treatments fluctuated during the 8-Day incubation period. The pH ranges are: Treatment A ranged at pH7.1-8.0, Treatment B ranged at pH6.9-8.0 and Treatment AB ranged at pH6.8-8.2. On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 7.3, 7.8 and 7.3, respectively, thus pH 7<AB<A<B. On Day 8, the pH for Treatment A, B and AB were 7.9, 7.7 and 7.8, respectively, thus pH 7<B<A<AB. On the day when Cr removal percentage was the highest, the pH observed for Treatment A was 7.3. It appears that by

Day 8, all treatments became more basic. For Treatment B, the pH readings followed a similar pattern to that of control. As for Treatment AB, its pattern was only similar half way from Day 4 to 8. The difference between the pH pattern among Treatment B and AB may have resulted from the diversity of the homogeneous group and heterogeneous group bacteria. The similarity in pH pattern in reference to control was due to no reaction to Cr.

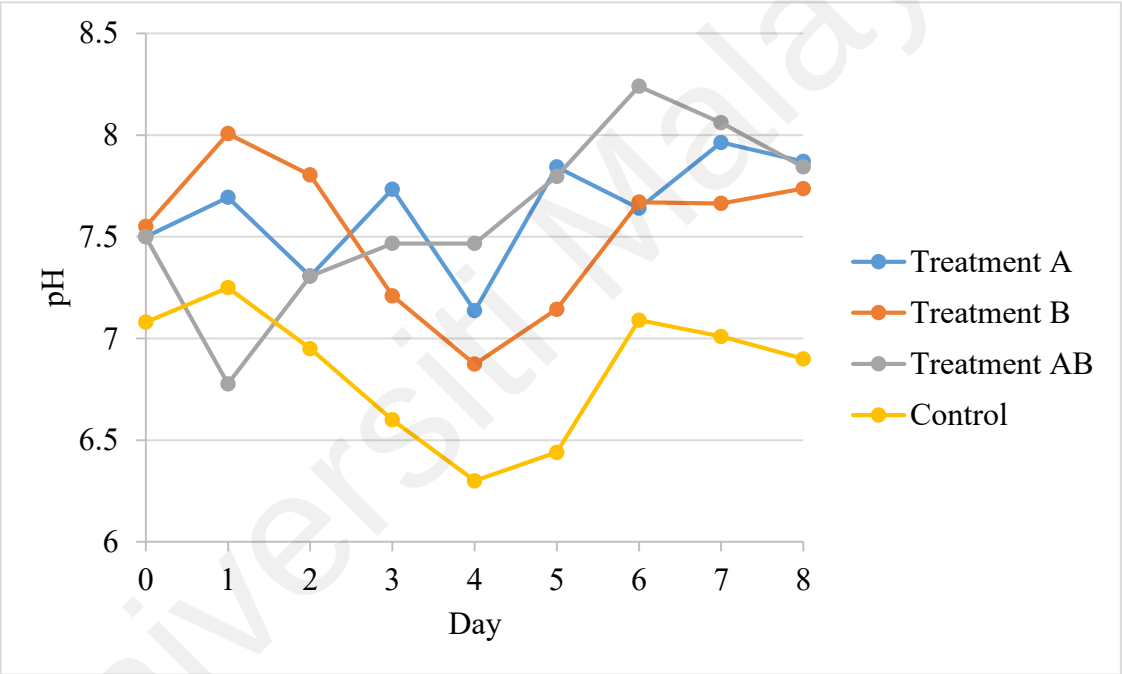


Figure 4.24: pH reading for all treatments in monometal system

4.2.9 Aluminium (Al)

Figure 4.25 shows the removal of Al across treatments in monometal system. For Treatment A, 10% of Al was removed in two days whereas 21% of Al (an increase by 11%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.052 day^{-1} (2-Day) and 0.030 day^{-1} (8-Day). For Treatment B, 13% of Al was removed in two days whereas 15% of Al (an increase by

2%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.069 day^{-1} (2-Day) and 0.020 day^{-1} (8-Day). For Treatment AB, 15% of Al was removed in two days whereas 1% of Al (a decrease by 14%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.316 day^{-1} (2-Day) and 0.001 day^{-1} (8-day). Therefore, the order for Al removal was AB>B>A (2-Day incubation period) and A>B>AB (8-Day incubation period).

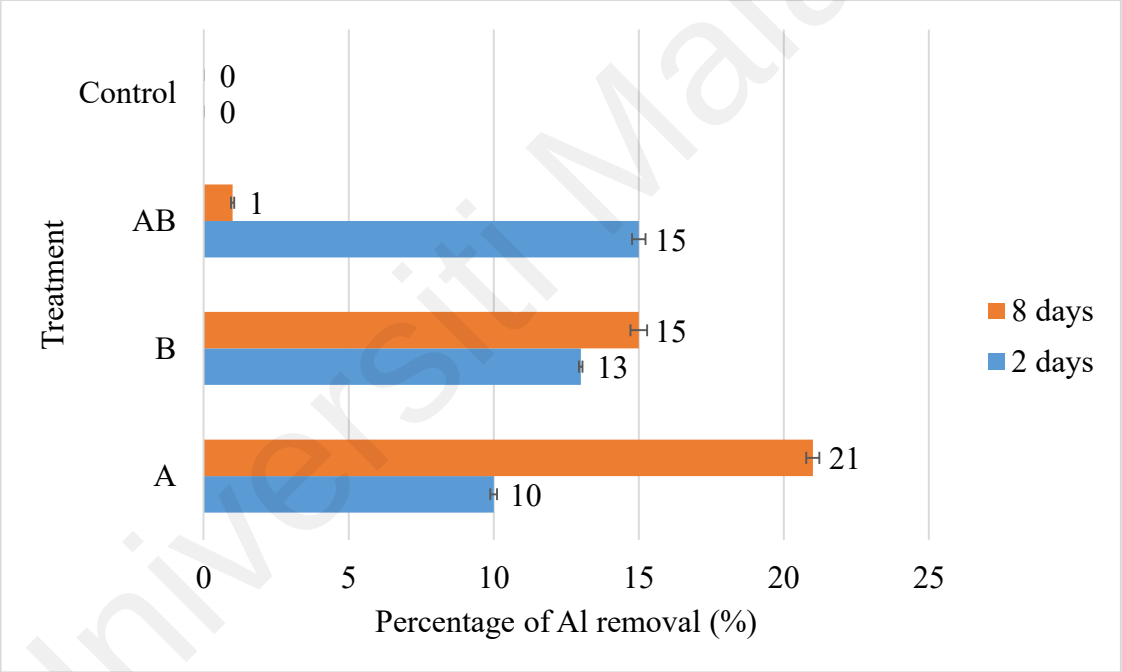


Figure 4.25: Percentage of Al removal in monometal system

Among the homogeneous groups, Treatment B showed a higher Al removal percentage than Treatment A. On Day 2, Treatment B has higher percentage than Treatment A. But, on Day 8, the removal of Al in Treatment B was 13% as compared to 10% removal in Treatment A. In the beginning, it appears that Gram-negative bacteria (Treatment B) has taken up more Al than that of Gram-positive bacteria (Treatment A). However, on Day 8, Gram-negative bacteria has slowed down in the Al uptake, possibly

because optimum Al uptake has been reached. On the other hand, Gram-positive bacteria experienced a spike in Al removal reaching the highest in Day 8. Gram-positive bacteria continued to remove Al until Day 8 as they have not yet reached the optimum adsorption of heavy metal. The removal performance of Gram-positive and Gram-negative bacteria was in accordance to the findings of Rajasekar and Ting (2010) who reported that *Bacillus cereus* (Gram-positive bacteria) was able to degrade 85% of Al while *Serratia marcescens* (Gram-negative bacteria) was able to degrade 60% of Al.

The heterogeneous group in Treatment AB uptake the most Al. On Day 2, Treatment AB (heterogeneous group) had the highest removal percentage compared to other treatments, i.e., for Treatment A and B, at 10% and 15%, respectively. However, on Day 8, there was a sharp decline in Al removal percentage, i.e., 1%, 15% (B) and 21% (A), respectively. The heterogeneous bacteria seemed to have the lowest Al retention as more than 90% was released back to the solution by Day 8. This is probably because heterogeneous bacteria were the earliest to reach optimum Al removal. The relationship among heterogeneous bacteria had resulted in the optimal removal percentage only for Day 2 but not until Day 8. According to Fauziah *et al.* (2017), the mixture of *Ochrobacterium intermedium, Burkholderia vietnamiensis, Stenotrophomonas acidaminiphilia, Acidovorax ebreus, Brevundimonas diminuta, Delftia tsuruhatensis, Aeromonas caviae, Pseudomonas alcaligenes, Pseudomonas mendocina, Serratia marcescens marcescens* were able to remove 89.15% of Al.

Figure 4.26 shows the bacterial counts for all treatments in monometal system from Day 0 until Day 8. The highest bacterial growth was recorded on Day 2 where the bacterial count for Treatment A, B and AB was 5.077 x 10^{10} CFU/ml CFU/ml, 6.059 x

10^{10} CFU/ml, and 2.287 x 10^{11} CFU/ml, respectively. Treatment A showed the highest bacterial counts compared to other treatments. On Day 8, final bacterial count for Treatment A was 0.44 x 10^5 CFU/ml, followed by Treatment B was 7.92 x 10^5 CFU/ml and Treatment AB was 0.4 x 10^5 CFU/ml.

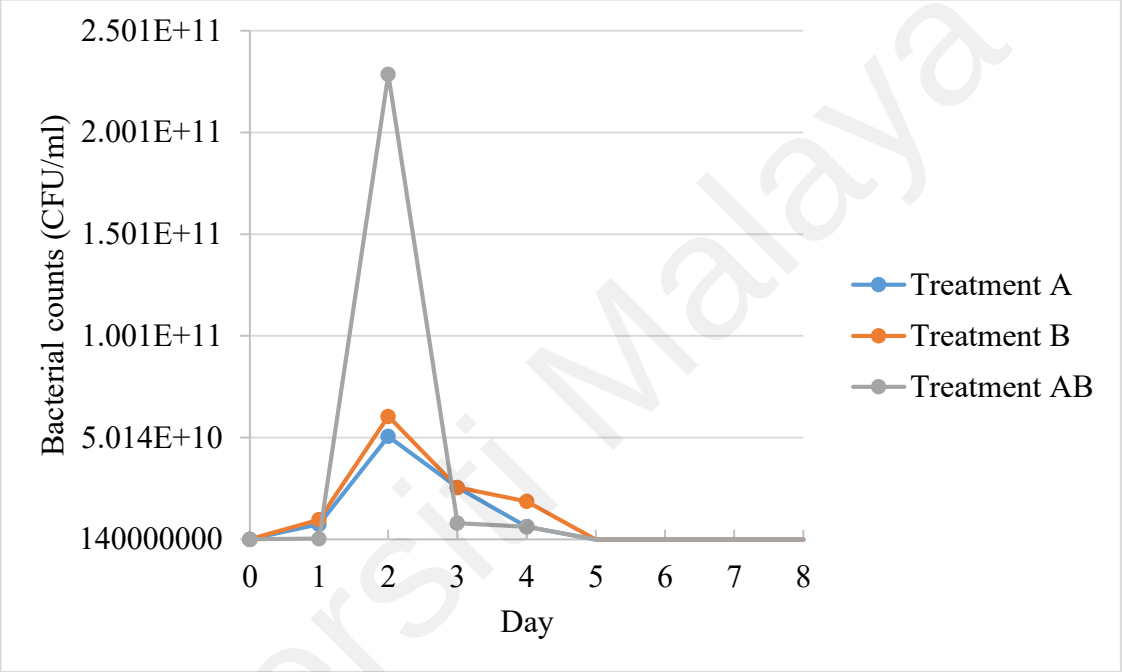


Figure 4.26: Bacterial counts (CFU/ml) for all treatments in monometal system

Gram-negative (Treatment B) bacteria had slightly higher bacterial count and were more resilient (i.e., did not decline drastically) than Gram-positive bacteria (Treatment A). Both Gram-negative and Gram-positive bacteria continued to remove Al until Day 8 but Gram-positive bacteria removed more Al despite having the least count. On Day 2, Treatment AB (heterogeneous) showed a drastic increase in Al removal percentage along with the increase in bacterial counts. Heterogeneous bacteria thrived in the presence of Al but by Day 8, Al had exhausted them and led to a sharp decline in bacterial count. The stress had caused them to release Al back to the solution.

According to Daboor (2014), as bacterial cells age, the structural features of bacterial cell wall weaken, thus resulting in prevention of Al adsorption.

As shown in Figure 4.27, the pH for all treatments fluctuated during the 8-Day incubation period. The pH ranges for Treatment A (4.9-7.5), Treatment B (5.1-7.5) and Treatment AB (4.3-7.5). On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 5.3, 5.1 and 4.5, respectively, thus pH A<B<AB<7. On Day 8, the pH for Treatment A, B and AB were 5.4, 5.3 and 5.0, respectively, thus pH A<B<AB<7. On the Day when Al removal percentage was the highest, the pH observed for each treatment was 5.4 (Treatment A), 5.1 (Treatment B) and 4.5 (Treatment AB). It appears that by Day 8, all treatments transitioned from basic to acidic.

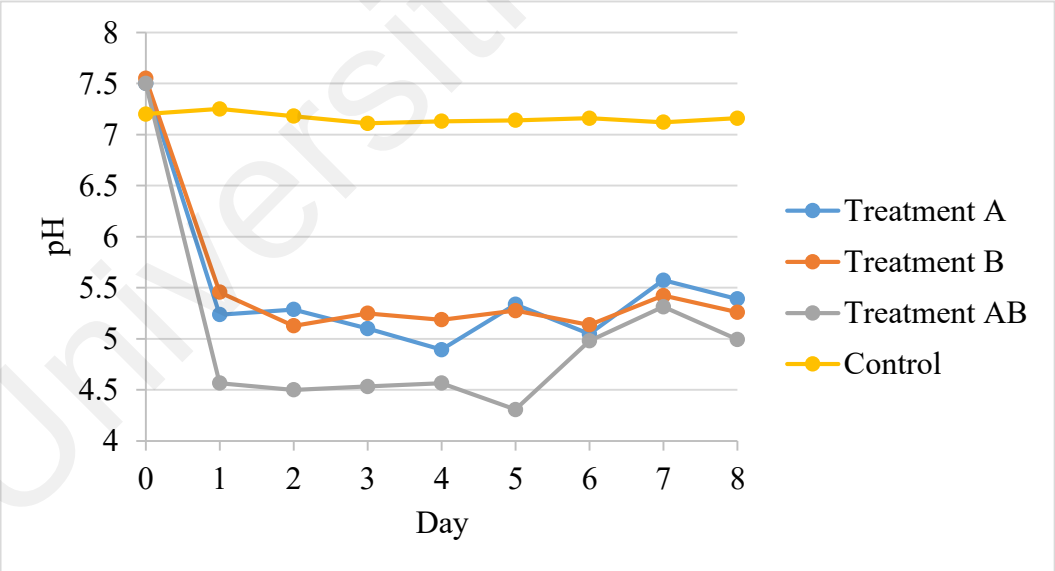
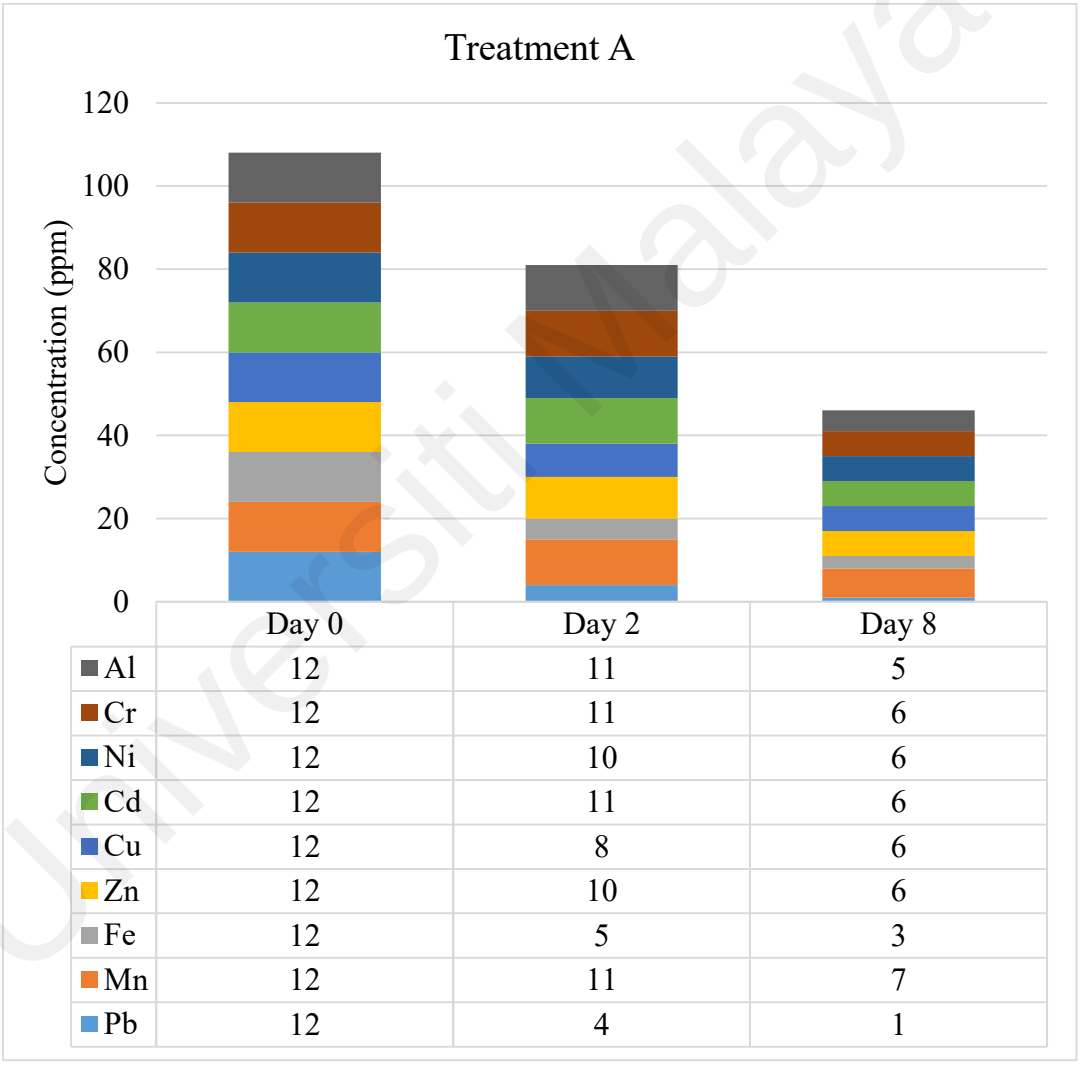


Figure 4.27: pH reading for all treatments in monometal system

4.3 Metal Removal in Polymetal System

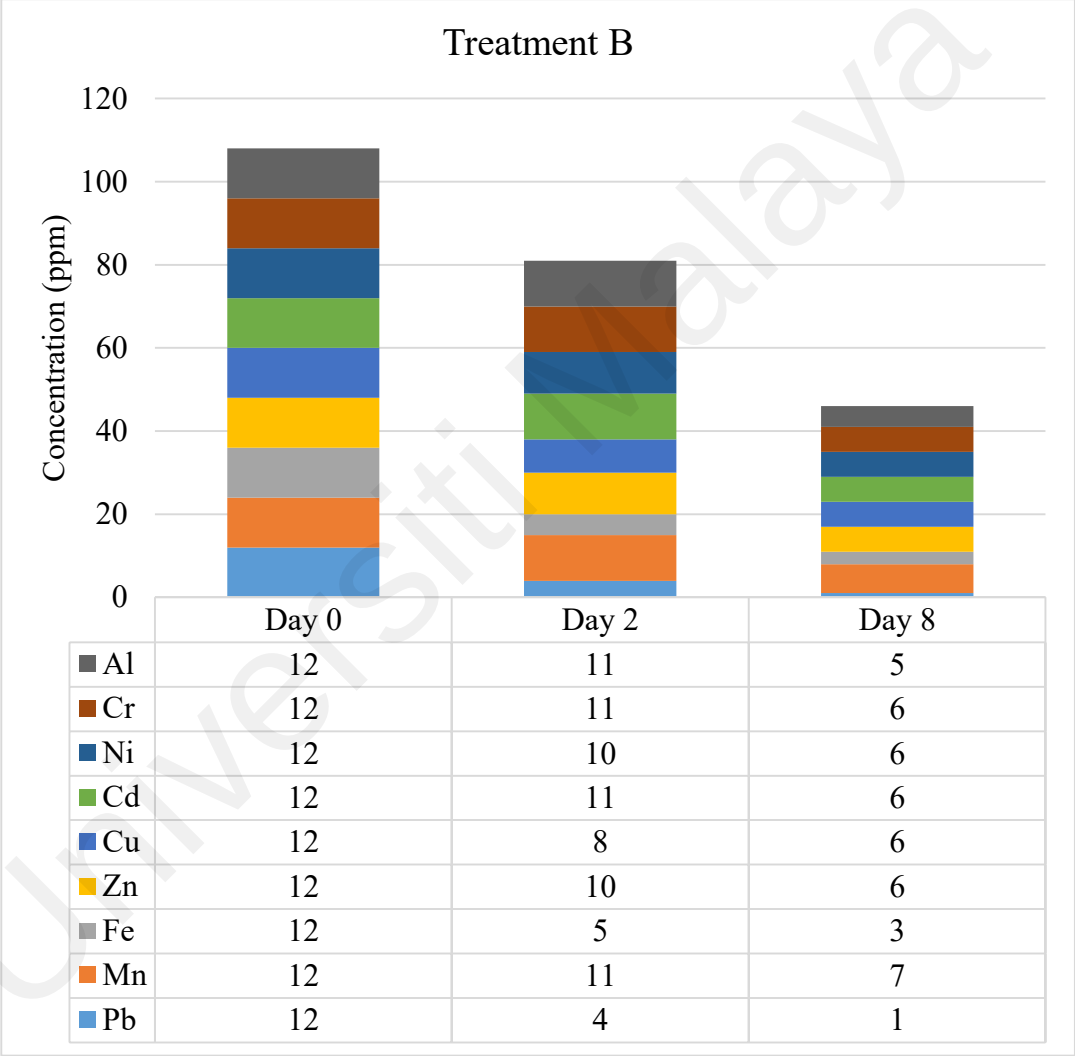
Figure 4.28 shows the residual concentration of Treatment A in polymetal system. After two Days, the collective concentration of heavy metals dropped from 108 ppm to 81 ppm which indicates 25% removal. After eight days, the collective concentration of heavy metals further dropped to 46 ppm (57% removal, i.e., an increase by 32%).



	Day 0	Day 2	Day 8
Al	12	11	5
Cr	12	11	6
Ni	12	10	6
Cd	12	11	6
Cu	12	8	6
Zn	12	10	6
Fe	12	5	3
Mn	12	11	7
Pb	12	4	1

Figure 4.28: Residual concentration (ppm) of Treatment A in polymetal system

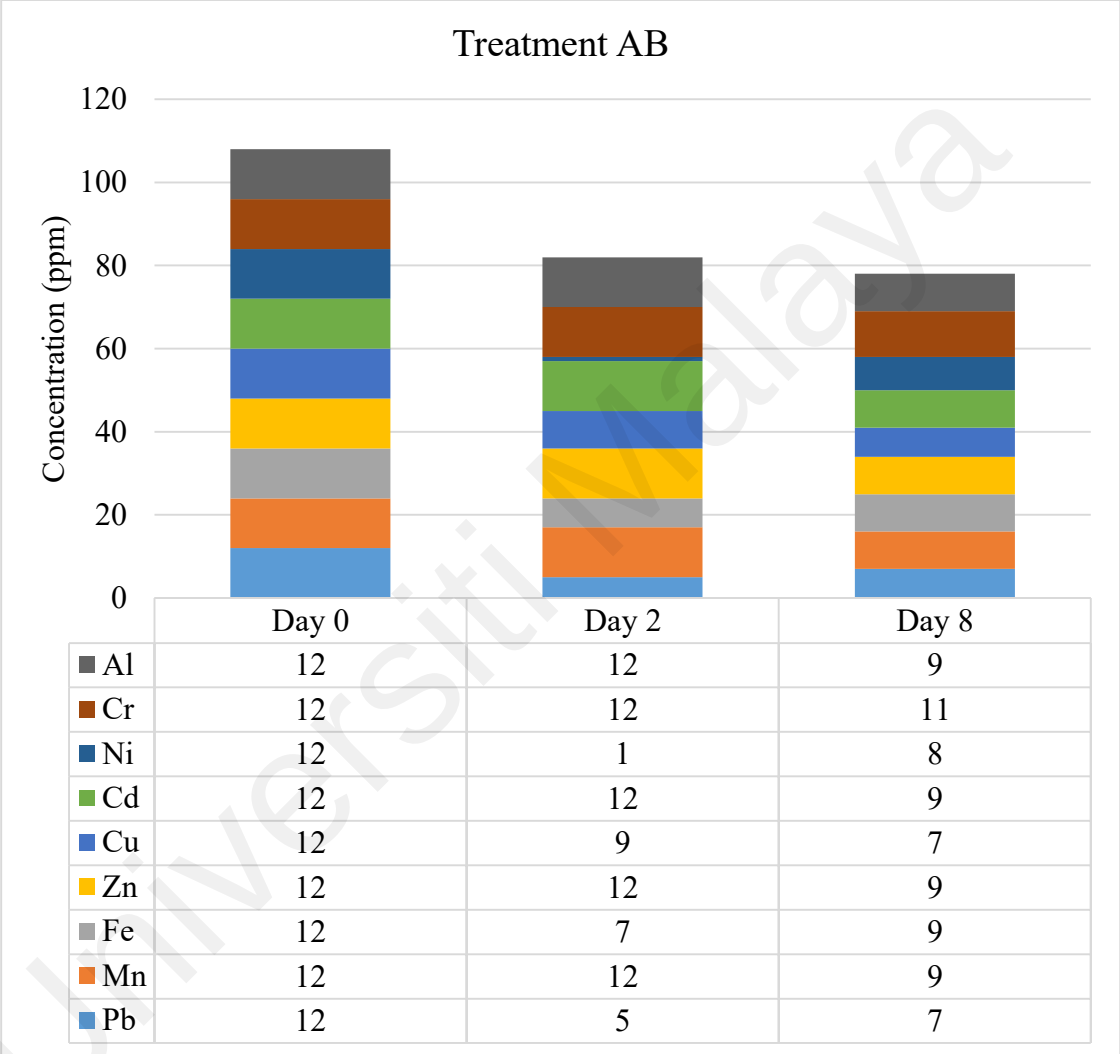
Figure 4.29 shows the residual concentration of Treatment B in polymetal system. After two days, the collective concentration of heavy metals dropped from 108 ppm to 95 ppm which indicates 12% removal. After eight days, the collective concentration of heavy metals further dropped to 80 ppm (26% removal, i.e., an increase by 14%).



	Day 0	Day 2	Day 8
Al	12	11	5
Cr	12	11	6
Ni	12	10	6
Cd	12	11	6
Cu	12	8	6
Zn	12	10	6
Fe	12	5	3
Mn	12	11	7
Pb	12	4	1

Figure 4.29: Residual concentration (ppm) of Treatment B in polymetal system

Figure 4.30 shows the residual concentration of Treatment AB in polymetal system. After two days, the collective concentration of heavy metals dropped from 108 ppm to 82 ppm which indicates 24% removal. After eight days, the collective concentration of heavy metals further dropped to 78 ppm (28% removal, i.e., an increase by 4%).



	Day 0	Day 2	Day 8
Al	12	12	9
Cr	12	12	11
Ni	12	1	8
Cd	12	12	9
Cu	12	9	7
Zn	12	12	9
Fe	12	7	9
Mn	12	12	9
Pb	12	5	7

Figure 4.30: Residual concentration (ppm) of Treatment AB in polymetal system

Therefore, the order of adsorption effectiveness in terms of 2-Day collective removal is A>AB>B where 25% > 24% > 12%. In terms of 8-Day collective removal, the order is A>AB>B where 57% > 28% > 26%. The breakdown of collective removal is discussed as follows:

4.3.1 Lead (Pb)

Figure 4.31 shows Pb removal percentage across treatments in polymetal system. For Treatment A, 67% of Pb was removed in two days whereas 92% of Pb (an increase by 25%.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.550 day^{-1} (2-Day) and 0.313 day^{-1} (8-Day). For Treatment B, 39% of Pb was removed in two days whereas 78% of Pb (an increase by 39%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.251 day^{-1} (2-Day) and 0.188 day^{-1} (8-Day). For Treatment AB, 61% of Pb was removed in two days whereas 42% of Pb (a decrease by 19%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.467 day^{-1} (2-Day) and 0.067 day^{-1} (8-Day). Therefore, the order for Pb removal was A>AB>B (2-Day incubation period) and A>B>AB (8-Day incubation period).

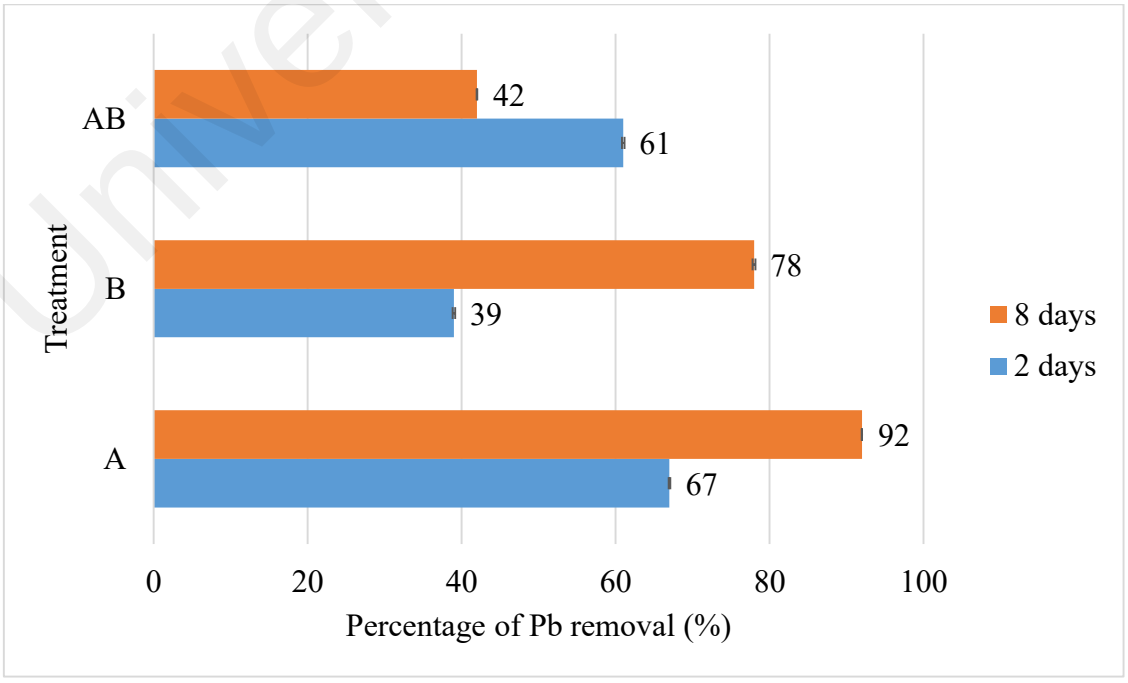


Figure 4.31: Percentage of Pb removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Pb removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 67% and 39% (2-Day) meanwhile 92% and 78% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached optimum Pb (polymetal) removal on Day 2. This is evident from continuous albeit slower Pb removal performance till Day 8. No study has reported on Pb removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Pb removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Pb such as Leung *et al.* (2000), Ray *et al.* (2006), Bautista-Hernández *et al.* (2012), Cristani *et al.* (2012), Gawali Ashruta *et al.,* (2014) and Das *et al.* (2016).

On Day 2, the heterogeneous Treatment AB had a slightly lower removal percentage (61%) compared to Treatment A (67%) but a higher removal percentage than Treatment B (39%). On Day 8, the heterogeneous bacteria released Pb back to the system, hence the decrease in removal percentage. They may have reached the optimum Pb (polymetal) removal on Day 2. In addition, the heterogeneous bacteria seemed to have a low Pb retention that can be attributed to non-mutualistic interaction among heterogeneous bacteria. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 14.58-15.10% of Pb in the mixture of five metals (Cd, Cr, Cu, Ni and Pb). Meanwhile, Singh *et al.,* (2012) reported that bacterial consortium was efficient in removing 44.74% of Pb from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.2 Manganese (Mn)

Figure 4.32 shows Mn removal percentage across treatments in polymetal system. For Treatment A, 11% of Mn was removed in two days whereas 39% of Mn (an increase by 28%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.057 day^{-1} (2-day) and 0.062 day^{-1} (8-Day). For Treatment B, 4% of Mn was removed in two days whereas 13% of Mn (an increase by 9%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.021 day^{-1} (2-Day) and 0.017 day^{-1} (8-Day). For Treatment AB, 0% of Mn was removed in two days whereas 23% of Pb (an increase by 23%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0 day^{-1} (2-Day) and 0.033 day^{-1} (8-Day). Therefore, the order for Mn removal was A>B>AB (2-Day incubation period) and A>AB>B (8-Day incubation period).

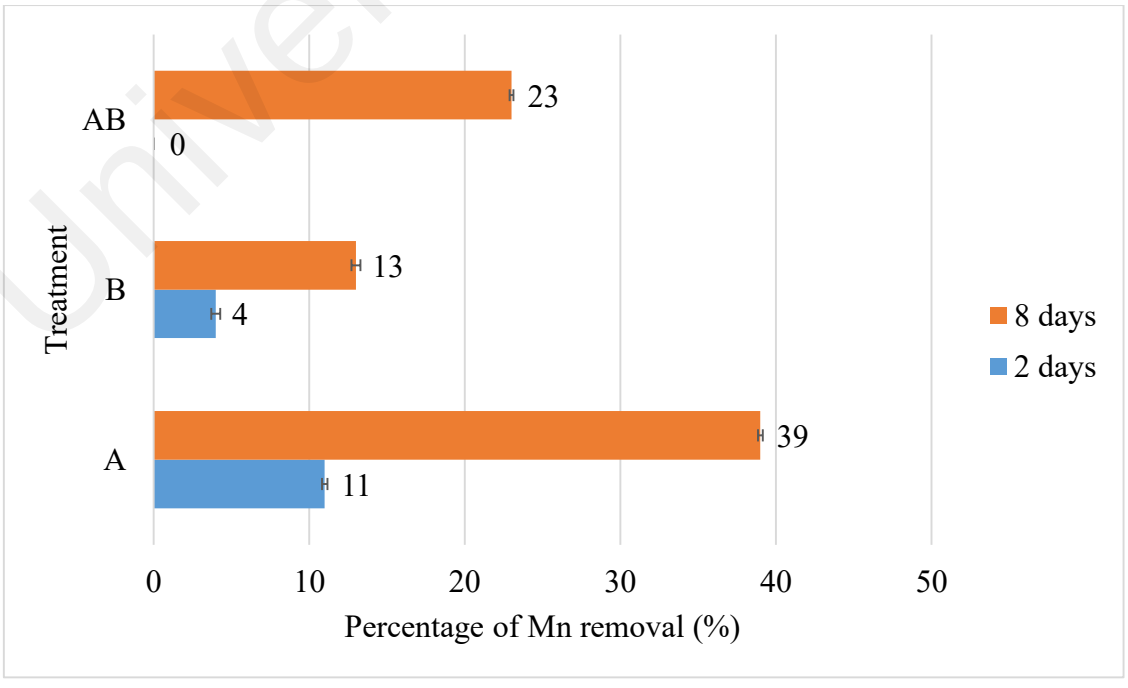


Figure 4.32: Percentage of Mn removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Mn removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 11% and 4% (2-Day) meanwhile 39% and 13% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Mn (polymetal) removal on Day 2. This is evident from continuous Mn removal performance of both treatments until Day 8. No study has reported on Mn removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, there was a study on Mn removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Mn, namely, Mamba *et al.* (2009).

On Day 2, the heterogeneous Treatment AB had zero removal percentage compared to Treatment A and B. However, on Day 8, the heterogeneous bacteria has increased the removal percentage (23%) to be lower than Treatment A (39%) but higher than Treatment B (13%). Heterogeneous bacteria have not reached the optimum Mn (polymetal) removal on Day 2. This is evident from continuous Mn removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Mn retention, which can be attributed to mutualistic interaction among heterogeneous bacteria. Apparently, no study has reported on heterogeneous bacteria's performance in removing Mn. However, there was a study on Mn removal performance of heterogeneous bacteria in monometal system as mentioned in the monometal subchapter on Mn, namely, Barboza *et al.* (2015).

4.3.3 Iron (Fe)

Figure 4.33 shows Fe removal percentage across treatments in polymetal system. For Treatment A, 54% of Fe was removed in two days whereas 73% of Fe (an increase by 19%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.386 day^{-1} (2-Day) and 0.162 day^{-1} (8-Day). For Treatment B, 24% of Fe was removed in two days whereas 30% of Fe (an increase by 6%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.139 day^{-1} (2-Day) and 0.044 day^{-1} (8-Day). For Treatment AB, 45% of Fe was removed in two days whereas 26% of Pb (a decrease by 19%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.301 day^{-1} (2-Day) and 0.038 day^{-1} (8-Day). Therefore, the order for Fe removal was A>AB>B (2-Day incubation period) and A>B>AB (8-Day incubation period).

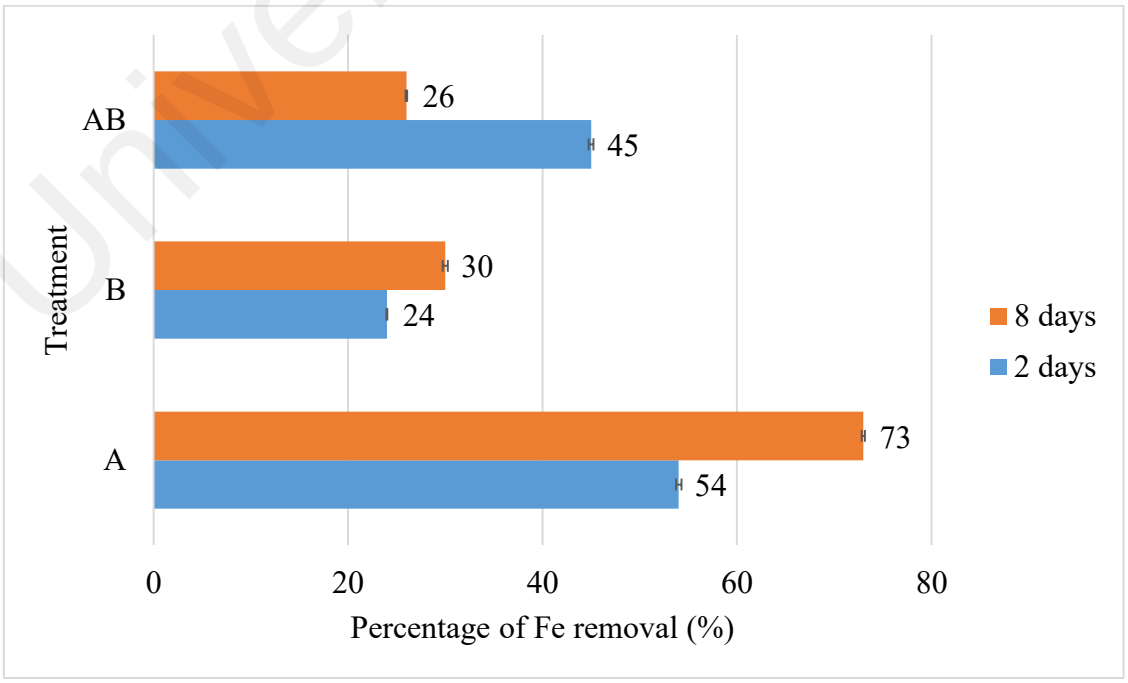


Figure 4.33: Percentage of Fe removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Fe removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 54% and 24% (2-Day) meanwhile 73% and 30% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Fe (polymetal) removal on Day 2. This is evident from continuous albeit slower Fe removal performance till Day 8. No study has reported on Fe removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Fe removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Fe such as Štyriaková and Štyriak (2000), Ams *et al.* (2004), Zhu *et al.* (2013) and Chaudhari *et al.* (2013).

On Day 2, the heterogeneous Treatment AB had a slightly lower removal percentage (45%) compared to Treatment A (54%) but higher than Treatment B (24%). On Day 8, the heterogeneous bacteria released Fe back to the system, hence the decrease in removal percentage. They may have reached the optimum Fe (polymetal) removal on Day 2. In addition, the heterogeneous bacteria seemed to have a low Fe retention, which can be attributed to non-mutualistic interaction among heterogeneous bacteria. Apparently, no study has reported on heterogeneous bacteria's performance in removing Fe. However, many studies have reported on Fe removal performance of heterogeneous bacteria in monometal system as mentioned in the monometal subchapter on Fe such as Pan *et al.* (2017) and Mamba *et al.* (2009).

4.3.4 Zinc (Zn)

Figure 4.34 shows Zn removal percentage across treatment in polymetal system. For Treatment A, 18% of Zn was removed in two days whereas 54% of Fe (an increase by 36%) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.097 day^{-1} (2-Day) and 0.098 day^{-1} (8-Day). For Treatment B, 1% of Zn was removed in two days whereas 13% of Zn (an increase by 12%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.004 day^{-1} (2-Day) and 0.018 day^{-1} (8-Day). For Treatment AB, 0% of Zn was removed in two days whereas 23% of Zn (an increase by 23%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0 day^{-1} (2-Day) and 0.033 day^{-1} (8-Day). Therefore, the order for Zn removal was A>B>AB (2-Day incubation period) and A>AB>B (8-Day incubation period).

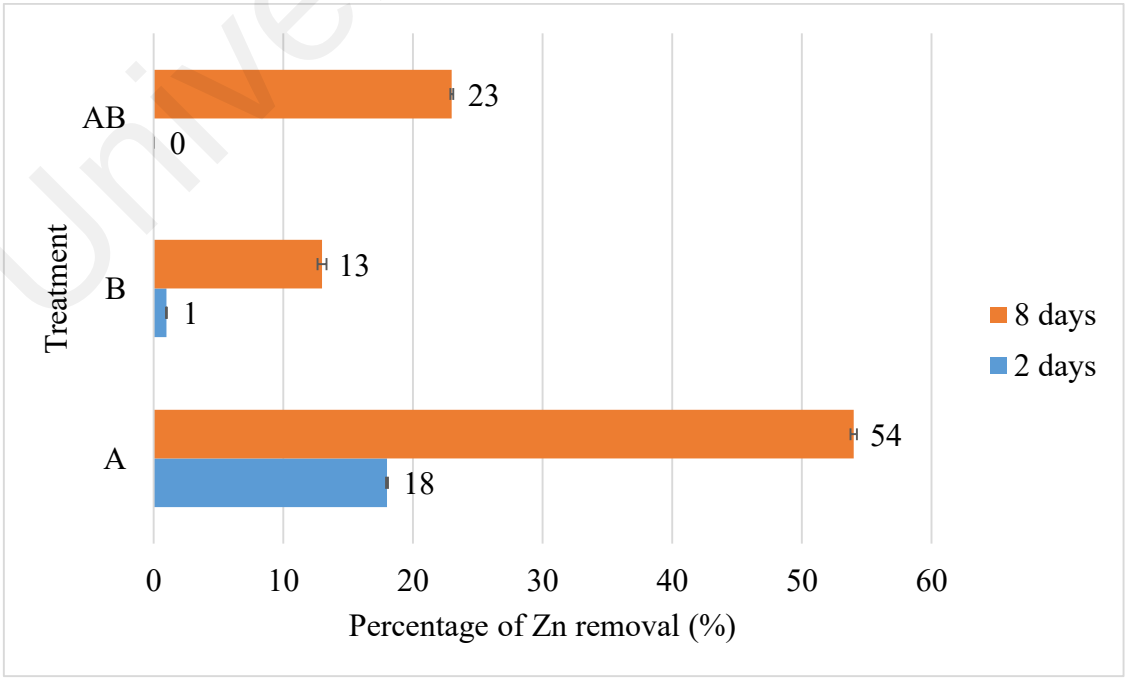


Figure 4.34: Percentage of Zn removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Zn removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 18% and 1% (2-Day) meanwhile 54% and 13% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Zn (polymetal) removal on Day 2. This is evident from high Zn removal performance until Day 8. No study has reported on Zn removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Zn removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Zn such as Costa and Duta (2001), Bautista-Hernández *et al.* (2012), Ramesh *et al.* (2014), Vaid *et al.* (2014) and Gawali Ashruta *et al.* (2014).

On Day 2, the heterogeneous Treatment AB had zero removal percentage. However, on Day 8, the heterogeneous bacteria have increased the removal percentage (23%) to be lower than Treatment A (54%) but higher than Treatment B (13%). Heterogeneous bacteria have not reached the optimum Zn (polymetal) removal on Day 2. This is evident from continuous Zn removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Zn retention, which can be attributed to mutualistic interaction among heterogeneous bacteria. According to Singh *et al.* (2012), bacterial consortium was efficient in removing 87.656% of Zn from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.5 Copper (Cu)

Figure 4.35 shows Cu removal percentage across treatments in polymetal system. For Treatment A, 33% of Cu was removed in two days whereas 54% of Cu (an increase by 21 %.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.201 day^{-1} (2-Day) and 0.097 day^{-1} (8-Day). For Treatment B, 28% of Cu was removed in two days whereas 37% of Cu (an increase by 9%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.165 day^{-1} (2-Day) and 0.057 day^{-1} (8-Day). For Treatment AB, 21% of Cu was removed in two days whereas 41% of Cu (an increase by 20%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.116 day^{-1} (2-Day) and 0.066 day^{-1} (8-Day). Therefore, the order for Cu removal was A>B>AB (2-Day incubation period) and A>AB>B (8-Day incubation period).

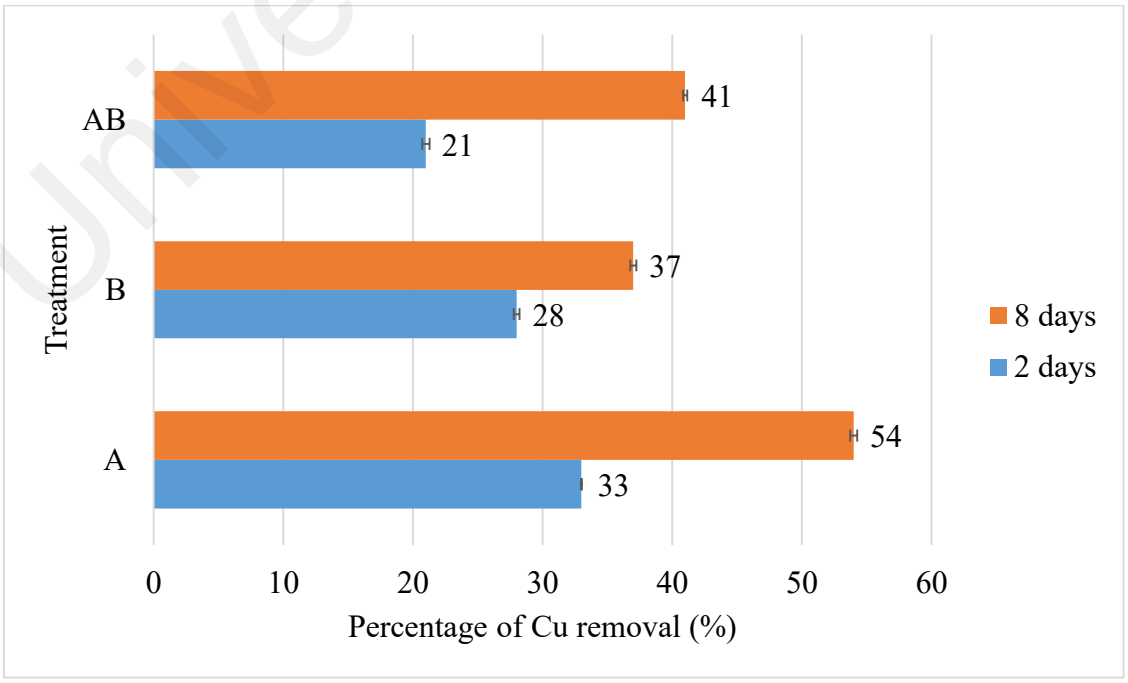


Figure 4.35: Percentage of Cu removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Cu removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 33% and 28% (2-Day) meanwhile 54% and 37% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Cu (polymetal) removal on Day 2. This is evident from continuous Cu removal performance until Day 8. No study has reported on Cu removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Cu removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Cu such as Leung *et al.* (2000), Narasimhulu and Rao (2009), Ghosh and Saha (2013), Gawali Ashruta *et al.* (2014) and Shen *et al.* (2017).

On Day 2, the heterogeneous Treatment AB had the lowest removal percentage (21%) compared to Treatment A (33%) and Treatment B (28%). On Day 8, the heterogeneous bacteria increased the removal percentage to 41% to be higher than Treatment B (37%) but lower than Treatment A (54%). Heterogeneous bacteria have not reached the optimum Cu (polymetal) removal on Day 2. This is evident from continuous Cu removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Cu retention that can be attributed to mutualistic interaction among heterogeneous bacteria. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 8.82-13.88% of Cu in the mixture of five metals (Cd, Cr, Cu, Ni and Pb). Meanwhile, Singh *et al.,* (2012) reported that bacterial consortium was efficient in removing 83.176% of Cu from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.6 Cadmium (Cd)

Figure 4.36 shows Cd removal percentage across treatments in polymetal system. For Treatment A, 13% of Cd was removed in two days whereas 53% of Cd (an increase by 40 %.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.067 day^{-1} (2-Day) and 0.095 day^{-1} (8-Day). For Treatment B, 0% of Cd was removed in two days whereas 19% of Cd (an increase by 19%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0 day^{-1} (2-Day) and 0.026 day^{-1} (8-Day). For Treatment AB, 0% of Cd was removed in two days whereas 27% of Cd (an increase by 27%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0 day^{-1} (2-Day) and 0.04 day^{-1} (8-Day). Therefore, the order for Cd removal was A> (B & AB) (2-Day incubation period) and A>AB>B (8-Day incubation period).

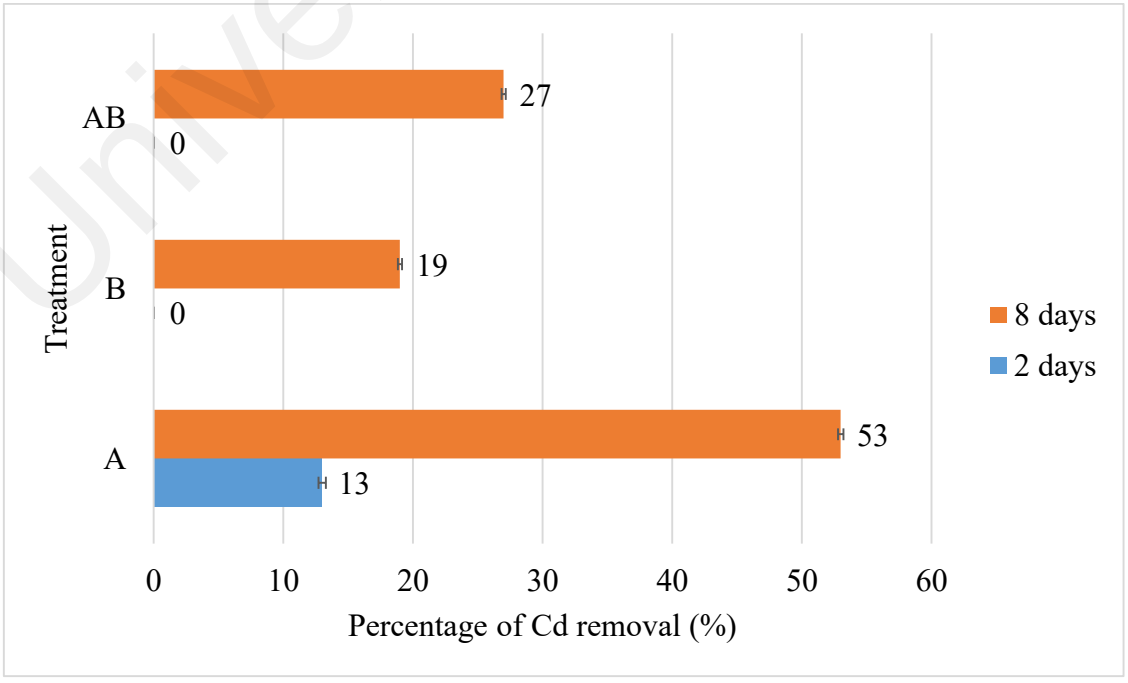


Figure 4.36: Percentage of Cd removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Cd removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 13% and 0% (2-Day) meanwhile 53% and 19% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Cd (polymetal) removal on Day 2. This is evident from continuous Cd removal performance till Day 8. No study has reported on Cd removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Cd removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Cd such as Narasimhulu and Rao (2009), Pandey *et al.* (2010), Cristani *et al.* (2012), Arivalagan *et al.* (2014), Gawali Ashruta *et al.,* (2014) and Jayanthi *et al.* (2016).

On Day 2, the heterogeneous Treatment AB had 0% of Cd removal percentage same with Treatment B. However, on Day 8, the heterogeneous bacteria have increased the removal percentage to 27% to be higher than Treatment B (19%) but lower than Treatment A (53%). Heterogeneous bacteria have not reached the optimum Cd (polymetal) removal on Day 2. This is evident from continuous Cd removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Cd retention that can be attributed to mutualistic interaction among heterogeneous bacteria. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 5.04-5.92% of Cd in the mixture of five metals (Cd, Cr, Cu, Ni and Pb). Meanwhile, Singh *et al.,* (2012) reported that bacterial consortium was efficient in removing 85.226% of Cd from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.7 Nickel (Ni)

Figure 4.37 shows Ni removal percentage across treatments in polymetal system. For Treatment A, 16% of Ni was removed in two days whereas 51% of Ni (an increase by 35%.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.086 day^{-1} (2-Day) and 0.089 day^{-1} (8-Day). For Treatment B, 9% of Ni was removed in two days whereas 23% of Ni (an increase by 14%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0.048 day^{-1} (2-Day) and 0.033 day^{-1} (8-Day). For Treatment AB, 92% of Ni was removed in two days whereas 29% of Ni (a decrease by 63%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 1.242 day^{-1} (2-Day) and 0.043 day^{-1} (8-Day). Therefore, the order for Ni removal was AB>A>B (2-Day incubation period) and A>B>AB (8-Day incubation period).

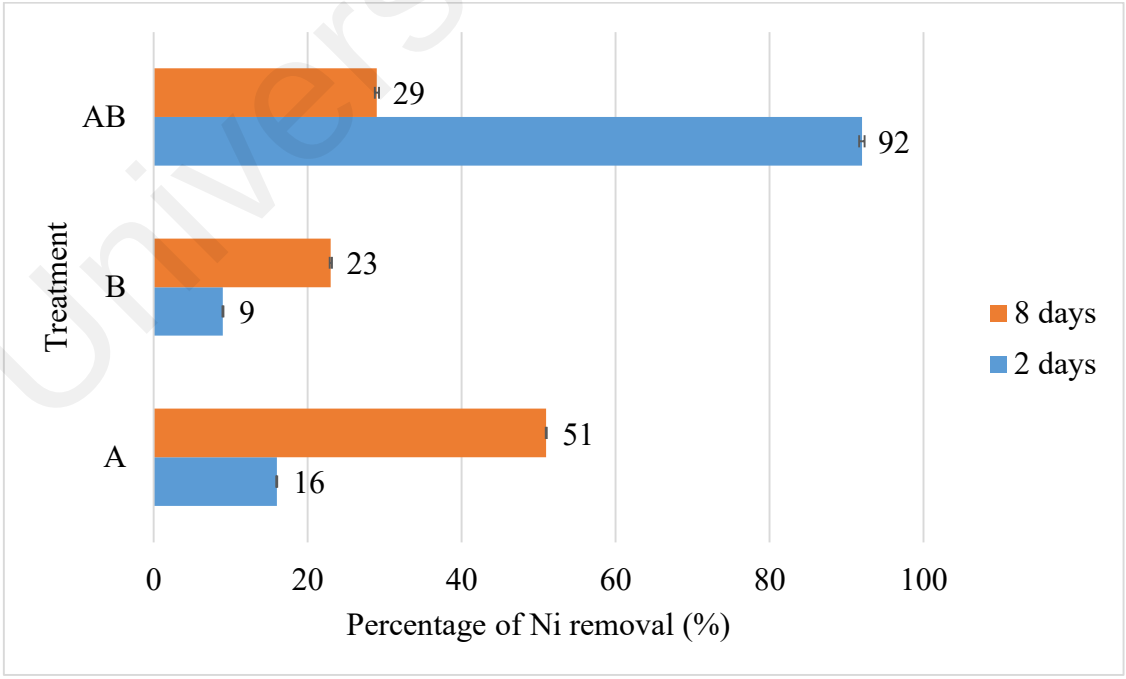


Figure 4.37: Percentage of Ni removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Ni removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 16% and 9% (2-Day) meanwhile 51% and 23% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Ni (polymetal) removal on Day 2. This is evident from continuous Ni removal performance till Day 8. No study has reported on Ni removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Ni removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Ni such as Kannan and Ramteke (2002), Narasimhulu and Rao (2009), Parameswari *et al.* (2009), Jia *et al.* (2014), Gawali Ashruta *et al.* (2014) and Nouha *et al.* (2016).

On Day 2, the heterogeneous Treatment AB had the highest removal percentage (92%) compared to Treatment A (16%) and Treatment B (9%). On Day 8, the heterogeneous bacteria released Ni back to the system, hence the decrease in removal percentage. They may have reached the optimum Ni (polymetal) removal on Day 2. In addition, the heterogeneous bacteria seemed to have a low Ni retention that can be attributed to non-mutualistic interaction among heterogeneous bacteria. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 6.34-6.41% of Ni in the mixture of five metals (Cd, Cr, Cu, Ni and Pb). Meanwhile, Singh *et al.,* (2012) reported that bacterial consortium was efficient in removing 85.744% of Ni from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.8 Chromium (Cr)

Figure 4.38 shows Cr removal percentage across treatments in polymetal system. For Treatment A, 12% of Cr was removed in two days whereas 47% of Cr (an increase by 35%.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.062 day^{-1} (2-Day) and 0.320 day^{-1} (8-Day). For Treatment B, 0% of Cr was removed in two days whereas 13% of Cr (an increase by 13%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0 day^{-1} (2-Day) and 0.018 day^{-1} (8-Day). For Treatment AB, 0% of Cr was removed in two days whereas 12% of Cr (an increase by 12%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0 day^{-1} (2-Day) and 0.016 day^{-1} (8-Day). Therefore, the order for Cr removal was A> (B & AB) (2-Day incubation period) and A>B>AB (8-Day incubation period).

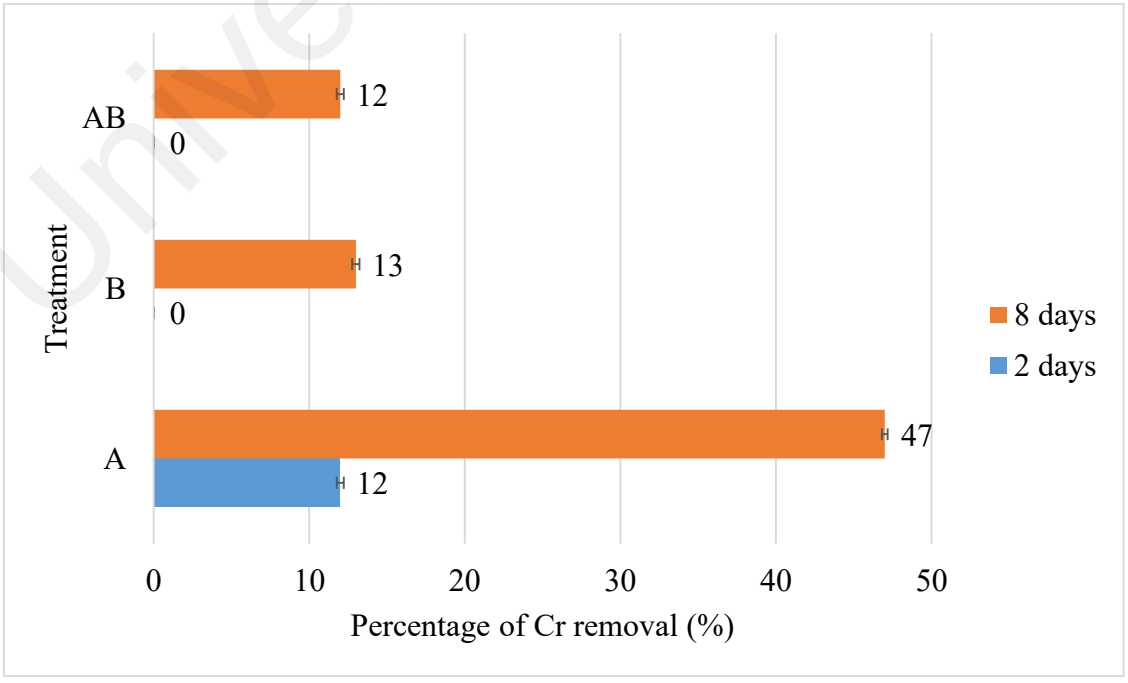


Figure 4.38: Percentage of Cr removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Cr removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 12% and 0% (2-Day) meanwhile 47% and 13% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Cr (polymetal) removal on Day 2. This is evident from continuous albeit slower Cr removal performance till Day 8. No study has reported on Cr removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Cr removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Cr such as Narasimhulu and Rao (2009), Parameswari *et al.* (2009), Naik *et al.* (2012), Gawali Ashruta *et al.* (2014), Basu *et al.* (2015) and Heipieper (2017).

On Day 2, the heterogeneous Treatment AB had 0% of Cr removal percentage like Treatment B. However, on Day 8, the heterogeneous bacteria have increased the removal percentage to 12% but not as high as Treatment A (47%) and Treatment B (13%). Heterogeneous bacteria have not reached the optimum Cr (polymetal) removal on Day 2. This is evident from continuous Cr removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Cr retention that can be attributed to mutualistic interaction among heterogeneous bacteria. As reported by Sannasi *et al.* (2009), a combination of six Gram-negative (*Pseudomonas* sp., *Serratia* sp., *Flavobacterium* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive (*Bacillus* sp., *Arthrobacter* sp., and *Micrococcus* sp.) bacteria were able to remove 4.01-5.16% of Cr in the mixture of five metals (Cd, Cr, Cu, Ni and Pb). Meanwhile, Singh *et al.,* (2012) reported that bacterial consortium was efficient in removing 71.618% of Cr from coal with six metals tested (Ni, Zn, Cd, Cu and Cr).

4.3.9 Aluminium (Al)

Figure 4.39 shows Al removal percentage across treatments in polymetal system. For Treatment A, 5% of Al was removed in two days whereas 54% of Al (an increase by 49%.) was removed in eight days. Based on these values, the first order rate constants for Treatment A were determined to be 0.026 day^{-1} (2-Day) and 0.098 day^{-1} (8-Day). For Treatment B, 0% of Al was removed in two days whereas 13% of Al (an increase by 13%) was removed in eight days. Based on these values, the first order rate constants for Treatment B were determined to be 0 day^{-1} (2-Day) and 0.018 day^{-1} (8-Day). For Treatment AB, 0% of Al was removed in two days whereas 27% of Al (an increase by 27%) was removed in eight days. Based on these values, the first order rate constants for Treatment AB were determined to be 0.004 day^{-1} (2-Day) and 0.04 day^{-1} (8-Day). Therefore, the order for Al removal was A>AB>B (2-Day incubation period) and A>AB>B (8-Day incubation period).

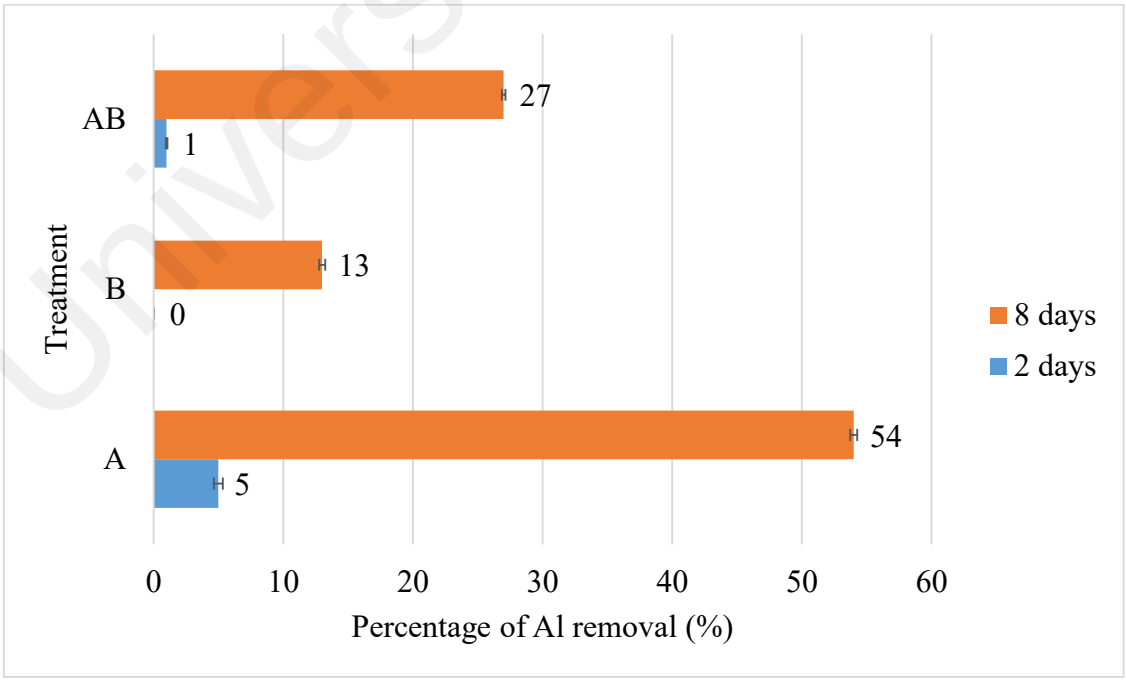


Figure 4.39: Percentage of Al removal in polymetal system

Among homogeneous groups, Treatment A (Gram-positive) showed a higher Al removal percentage than Treatment B (Gram-negative) in polymetal system, i.e., 5% and 0% (2-Day) meanwhile 54% and 13% (8-Day), respectively. Both Gram-positive and Gram-negative bacteria have not reached the optimum Al (polymetal) removal on Day 2. This is evident from continuous Al removal performance till Day 8. No study has reported on Al removal performance of Gram-positive and Gram-negative bacteria in polymetal system. However, many studies have reported on Al removal performance of Gram-positive and Gram-negative bacteria in monometal system as mentioned in the monometal subchapter on Al such as Rajasekar and Ting (2010).

On Day 2, the heterogeneous Treatment AB had 1% removal which was higher than Treatment B (0%) but lower than Treatment A (5%). On Day 8, a similar trend was shown where the heterogeneous bacteria had a higher removal percentage (27%) than Treatment B (13%) but lower than Treatment A (54%). Heterogeneous bacteria have not reached the optimum Al (polymetal) removal on Day 2. This is evident from continuous Al removal performance till Day 8. In addition, the heterogeneous bacteria seemed to have a high Al retention that can be attributed to mutualistic interaction among heterogeneous bacteria. Apparently, no study has reported on heterogeneous bacteria's performance in removing Al. However, there was a study on Al removal performance of heterogeneous bacteria in monometal system as mentioned in the monometal subchapter on Al, namely, Fauziah *et al.* (2017).

4.3.10 Bacterial count and pH value for all treatment in polymetal system

Figure 4.40 showed a comparison of the initial bacterial count from Day 0 to Day 8. The maximum bacterial growth was on Day 2, i.e., Treatment A was 1.894 x 10^{11} CFU/ml, Treatment B was 1.536 x 10^{11} CFU/ml and Treatment AB was 2.014 x 10^{11} CFU/ml. On Day 8, the final bacterial count for Treatment A was 5.28 x 10^5 CFU/ml, for Treatment B was 5.76 x 10^5 CFU/ml and Treatment AB was 5.52 x 10^5 CFU/ml.

On Day 2, Treatment AB (heterogeneous) showed the highest bacterial count, i.e., 22.014 x 10^{11} CFU/ml as compared to 1.894 x 10^{11} CFU/ml (A) and 1.536 x 10^{11} CFU/ml (B). The increase may be due to the optimized interaction among Gram-positive and Gram-negative bacteria. Also, it may be a reflection of higher resistance by the heterogeneous bacteria which might have added to the inocula ability to bioremediate greater mixture of the heavy metals. However, by Day 3, they had experienced a sharp decline in bacterial count and continued to decrease until Day 8. For Treatment A, Gram-positive bacteria showed high bacterial count (1.894 x 10^{11} CFU/ml) on Day 2 like heterogeneous bacteria but later decline by Day 3 until Day 8 to be the lowest count (5.28 x 10^5 CFU/ml). As for Treatment B, bacterial count increased (1.536 x 10^{11} CFU/ml) on Day 2 but lower than Treatments A and AB. However, like other Treatments, the bacterial count also decreased by Day 3 until Day 8. This may imply depletion in available nutrient required for bacterial survival as confirmed by Lin *et al.,* (2010). Also, the bacterial species may be stressed due to metabolic processes required for the heavy metals removal, causing mortality or inhibition of cell duplication to take place.

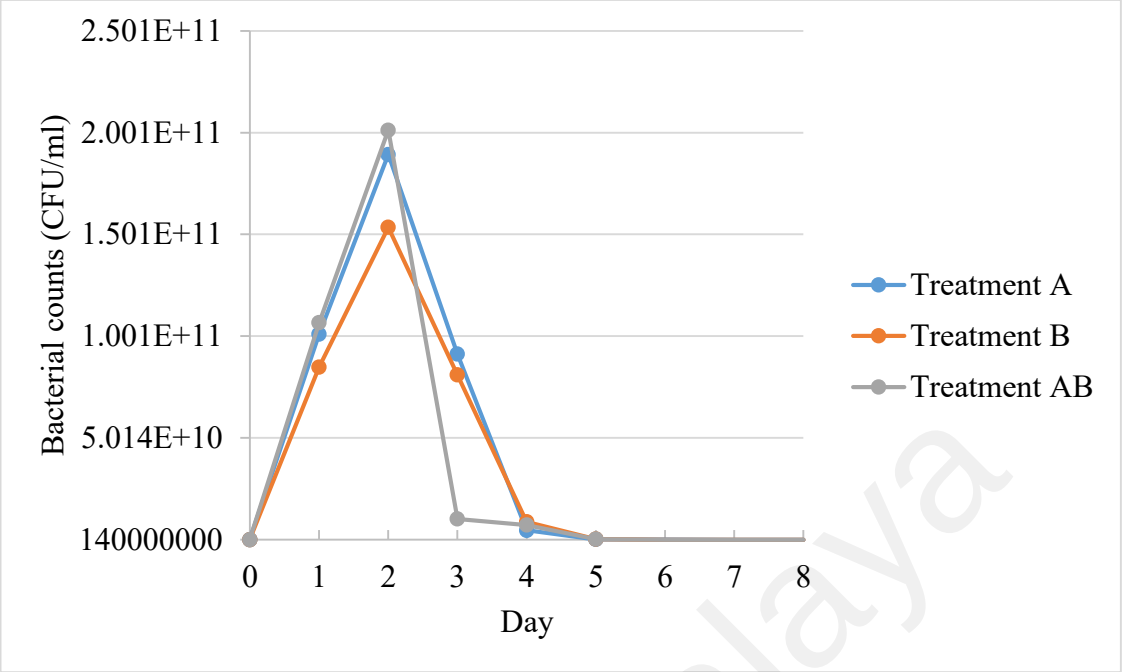


Figure 4.40: Bacterial counts (CFU/ml) for all treatments in polymetal system

As shown in Figure 4.41, the pH for all treatments fluctuated during the 8-Day incubation period. The pH ranges are: Treatment A (5.4-7.8), Treatment B (5.7- 8.1) and Treatment AB (5.1- 6.2). On Day 2 (the maximum bacterial growth), the pH for Treatment A, B and AB were 7.8, 6.6 and 6, respectively, thus pH AB<7<B<AB. On Day 8, the pH for Treatment A, B and AB were 5.4, 6.3 and 5.1, respectively, thus pH 7<AB<A<B. Neutral pH (pH 7) increases the bacterial cell wall negative charge, which favored electrochemical attraction and adsorption of metal ions. This explains why Treatment AB had a higher removal percentage on Day 2 (i.e., pH 7.8). It appears that by Day 8, Treatment A and AB became more acidic while Treatment B (heterogeneous) returned to original acidity, relatively.

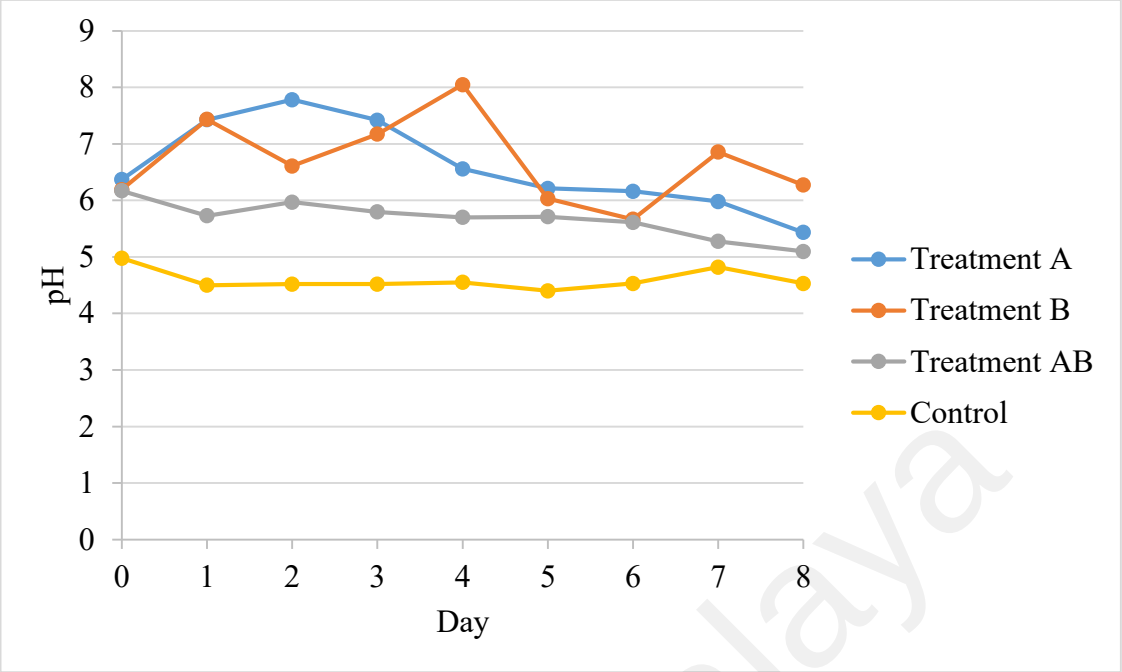


Figure 4.41: pH readings for all treatments during incubation period in polymetal system

4.4 Heavy Metal Removal Rate Constant

4.4.1 Monometal System

Table 4.4 shows the first order rate constant for heavy metal removal in monometal system for 2- and 8-Day incubation period. In two days, the highest rate constant value for Treatment A is Pb (K= 0.370 day^{-1}) while Treatment B is Fe (K= 0.338 day^{-1}) and Treatment AB is Fe (K= 0.376 day^{-1}). In eight days, the highest rate constant value for Treatment A is Mn (K= 0.178 day^{-1}) while Treatment B is Fe (K= 0.095 day^{-1}) and Treatment AB is Fe (K= 0.167 day^{-1}). Therefore, this suggest that complex interactions exist within the microcosms. Based on the sum of first order rate constants for the two days removal, Treatment AB was the most effective (1.307 day^{-1}), followed closely by Treatment A (1.298 day^{-1}) and Treatment B (1.090 day^{-1}). Thus, the increase in bacterial diversity has probably increase the metal removal capacity (Emenike *et al.,* 2017). For the eight days removal, Treatment A was the most effective (0.479 day^{-1}), followed closely by Treatment AB (0.418 day^{-1}) and Treatment B (0.268 day^{-1}).

Table 4.4: First order rate constant (K) for heavy metal removal across treatments in monometal system

Heavy metal	Removal per Day (Day^{-1})						
	Treatment A		Treatment B		Treatment AB		Control
	2-Day	8-Day	2-Day	8-Day	2-Day	8-Day	
Pb	0.370	0.094	0.194	0.051	0.171	0.123	0
Mn	0.077	0.178	0.069	0.010	0.043	0.034	0
Fe	0.178	0.072	0.338	0.095	0.376	0.167	0
Zn	0.104	0.01	0.053	0	0.050	0.02	0

Table 4.4, continued.

Heavy metal	Removal per Day (Day^{-1})						
	Treatment A		Treatment B		Treatment AB		Control
	2-Day	8-Day	2-Day	8-Day	2-Day	8-Day	
Cu	0.18	0.021	0.119	0.042	0.172	0.054	0
Cd	0.21	0.022	0.103	0.025	0.088	0.011	0
Ni	0.082	0.036	0.103	0.025	0.091	0.008	0
Cr	0.045	0.016	0	0	0	0	0
Al	0.052	0.030	0.069	0.02	0.316	0.001	0
Sum, ∑	**1.298**	**0.479**	**1.090**	**0.268**	**1.307**	**0.418**	**0**

4.4.2 Polymetal System

Table 4.5 shows the first order rate constant for removal percentage in polymetal system for 2- and 8-Day incubation period. In two days, the highest rate constant value for Treatment A is Pb (K= 0.550 day^{-1}) and Treatment B also is Pb (K= 0.251 day^{-1}) while Treatment AB is Ni (K= 1.242 day^{-1}). In eight days, the highest rate constant value for Treatment A is Cr (K= 0.320 day^{-1}) while Treatment B is Pb (K= 0.188 day^{-1}) and Treatment AB is Pb (K= 0.067 day^{-1}). This suggests that complex interactions may have existed within the microcosms. Based on the sum of first order rate constants for the two days removal, Treatment AB was the most effective polymetal treatment (2.13 day^{-1}), followed by Treatment A (1.532 day^{-1}) and Treatment B (0.628 day^{-1}). This result was the same as monometal system where the highest rate constant was

Treatment AB. Therefore, as mention by Emenike *et al.,* (2017), the increase in bacterial diversity has probably increase the metal removal capacity. For the 8-Day exposure, Treatment A was the most effective treatment (1.334 day^{-1}), followed by Treatment B (0.419 day^{-1}) and Treatment AB (0.376 day^{-1}).

Table 4.5: First order rate constant (K) for heavy metal removal across treatments in polymetal system

Heavy metal	Removal per Day (Day^{-1})						
	Treatment A		Treatment B		Treatment AB		Control
	Day 2	Day 8	Day 2	Day 8	Day 2	Day 8	
Pb	0.550	0.313	0.251	0.188	0.467	0.067	0
Mn	0.057	0.062	0.021	0.017	0	0.033	0
Fe	0.386	0.162	0.139	0.044	0.301	0.038	0
Zn	0.097	0.098	0.004	0.018	0	0.033	0
Cu	0.201	0.097	0.165	0.057	0.116	0.066	0
Cd	0.067	0.095	0	0.026	0	0.04	0
Ni	0.086	0.089	0.048	0.033	1.242	0.043	0
Cr	0.062	0.079	0	0.018	0	0.016	0
Al	0.026	0.098	0	0.018	0.004	0.04	0
Sum, ∑	**1.532**	**1.334**	**0.628**	**0.419**	**2.130**	**0.376**	**0**

CHAPTER 5: CONCLUSION

This study demonstrated heavy metal adsorption by selected bacteria isolated from landfills. Eighteen species of bacteria were isolated and identified from soil polluted with leachate. Seven species were Gram-positive and 11 species were Gram-negative. From these isolates, three combinations of microbial blend were produced to test the optimal removal of heavy metals. The three combinations are microbial blend with Gram-positive bacteria only, microbial blend with Gram-negative only, and one a combined blend of Gram-positive and Gram-negative bacteria.

Different treatments displayed varying capacity in heavy metal removal. In the monometal system, the highest rate constant value for Treatment A is Pb (K= 0.370 day^{-1}), Treatment B is Fe (K= 0.338 day^{-1}) and Treatment AB is Fe (K= 0.376 day^{-1}) while in the polymetal systems the highest rate constant value for Treatment A is Pb (K= 0.550 day^{-1}), Treatment B also is Pb (K= 0.251 day^{-1}) and Treatment AB is Ni (K= 1.242 day^{-1}). The removal of heavy metal was also found to be dependent with exposure duration and metal complexity. The efficiency of the bioremediation can be optimized by taking these factors into consideration.

In general, Gram-positive bacteria displayed a better heavy metal removal performance than Gram-negative bacteria. In the presence of heavy metals, Gram-positive and Gram-negative bacteria have different optimum of tolerance as reflected by their bacterial count and the final pH condition. Therefore, it can be concluded that different microbial blends have different optimal conditions to achieve the best heavy metal removal performance.

REFERENCES

Abbas, S. H., Ismail, I. M., Mostafa, T. M., & Sulaymon, A. H. (2014). Biosorption of heavy metals: a review. *Journal of Chemical Science and Technology*, *3*(4), 74-102.

Abioye, O. P. (2011). Biological remediation of hydrocarbon and heavy metals contaminated soil. *Soil contamination*, *7*, 127-142.

Acosta-Martínez, V., & Tabatabai, M. A. (2001). Arylamidase activity in soils: effect of trace elements and relationships to soil properties and activities of amidohydrolases. *Soil Biology and Biochemistry*, *33*(1), 17-23.

Aderemi, A. O., Oriaku, A. V., Adewumi, G. A., & Otitoloju, A. A. (2011). Assessment of groundwater contamination by leachate near a municipal solid waste landfill. *African Journal of Environmental Science and Technology*, *5*(11), 933-940.

Ahemad, M., Khan, M. S., Zaidi, A., & Wani, P. A. (2009). Remediation of herbicides contaminated soil using microbes. *Microbes in Sustainable Agriculture*, 261-284.

Ahkowshikemad, M. (2012). Implications of bacterial resistance against heavy metals in bioremediation: a review. *Journal of Institute of Integrative Omics and Applied Biotechnology (IIOAB)*, *3*(3).

Aja, O. C., Oseghale, S. D., & Al-Kayiem, H. (2014). Review and Evaluation of Municipal Solid Waste Management Practices in Malaysia. *The Journal of Solid Waste Technology and Management*, *40*(3), 215-232.

Akhtar, M. S., Chali, B., & Azam, T. (2013). Bioremediation of arsenic and lead by plants and microbes from contaminated soil. *Research in Plant Sciences*, *1*(3), 68-73.

Al Raisi, S. A. H., Sulaiman, H., Suliman, F. E., & Abdallah, O. (2014). Assessment of heavy metals in leachate of an unlined landfill in the Sultanate of Oman. *International Journal of Environmental Science and Development*, *5*(1), 60.

Ali, A., Li, M., Su, J., Li, Y., Wang, Z., Bai, Y., ... & Shaheen, S. M. (2021). Brevundimonas diminuta isolated from mines polluted soil immobilized cadmium (Cd2+) and zinc (Zn2+) through calcium carbonate precipitation: Microscopic and spectroscopic investigations. *Science of The Total Environment*, *813*, 152668.

Alina, M., Azrina, A., Mohd Yunus, A. S., Mohd Zakiuddin, S., Mohd Izuan Effendi, H., & Muhammad Rizal, R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *International Food Research Journal*, *19*(1), 135-140.

Al-Musharafi, S. K., Mahmoud, I. Y., & Al-Bahry, S. N. (2014). Environmental contamination by industrial effluents and sludge relative to heavy metals. *Journal of Geoscience and Environment Protection*, *2*, 14-18.

Ams, D. A., Fein, J. B., Dong, H., & Maurice, P. A. (2004). Experimental measurements of the adsorption of Bacillus subtilis and Pseudomonas mendocina onto Fe-oxyhydroxide-coated and uncoated quartz grains. *Geomicrobiology Journal*, *21*(8), 511-519.

Ancion, P.-Y., Lear, G., Dopheide, A. & Lewis, G.D. (2013). Metal concentrations in stream biofilm and sediments and their potential to explain biofilm microbial community structure. *Environmental Pollution,173*, 117–124.

Anusha, P., Natarajan, D., & Narayanan, M. (2021). Heavy metal removal competence of individual and bacterial consortium, evolved from metal contaminated soil. *Materials Today: Proceedings*.

Archana, A., & Jaitly, A. K. (2015). Mycoremediation: utilization of fungi for reclamation of heavy metals at their optimum remediation conditions. *Biolife*, *3*(1), 77-106.

Arivalagan, P., Singaraj, D., Haridass, V., & Kaliannan, T. (2014). Removal of cadmium from aqueous solution by batch studies using Bacillus cereus. *Ecologica lEengineering*, *71*, 728-735.

Auta, S. H., Emenike, C. U., & Fauziah, S. H. (2017). Screening for Polypropylene Degradation Potential of Bacteria Isolated from Mangrove Ecosystems in Peninsular Malaysia. *International Journal of Bioscience, Biochemistry and Bioinformatics, 7 (4),* 245-251.

Azarbad, H., Niklińska, M., van Gestel, C. A., van Straalen, N. M., Röling, W. F., & Laskowski, R. (2013). Microbial community structure and functioning along metal pollution gradients. *Environmental Toxicology and Chemistry*, *32*(9), 1992-2002.

Banerjee, A., Jhariya, M. K., Yadav, D. K., & Raj, A. (2018). Micro-remediation of metals: a new frontier in bioremediation. Handbook of environmental materials management. Springer.

Barboza, N. R., Amorim, S. S., Santos, P. A., Reis, F. D., Cordeiro, M. M., Guerra-Sá, R., & Leão, V. A. (2015). Indirect manganese removal by Stenotrophomonas sp. and Lysinibacillus sp. isolated from Brazilian mine water. *BioMed Research International*, *2015*.

Basha, S. A., & Rajaganesh, K. (2014). Microbial bioremediation of heavy metals from textile industry dye effluents using isolated bacterial strains. *International Journal of Current Microbiology and Applied Science*, *3*, 785-794.

Basu, A., Panda, S. S., & Dhal, N. K. (2015). Potential Microbial Diversity in Chromium Mining Areas: A Review. *Bulletin of Environment, Pharmacology and Life Sciences, 4*(8), 158-169.

Bautista-Hernández, D. A., Ramírez-Burgos, L. I., Duran-Páramo, E., & Fernández-Linares, L. (2012). Zinc and lead biosorption by Delftia tsuruhatensis: a bacterial strain resistant to metals isolated from mine tailings. *Journal of Water Resource and Protection*, *4*, 207-216.

Belyaeva, O. N., Haynes, R. J., & Birukova, O. A. (2005). Barley yield and soil microbial and enzyme activities as affected by contamination of two soils with lead, zinc or copper. *Biology and Fertility of Soils*, *41*(2), 85-94.

Beseler, C. L., Stallones, L., Hoppin, J. A., Alavanja, M. C., Blair, A., Keefe, T., & Kamel, F. (2008). Depression and pesticide exposures among private pesticide applicators enrolled in the Agricultural Health Study. *Environmental Health Perspectives*, *116*(12), 1713-1719.

Bhattacharyya, P., Tripathy, S., Kim, K., & Kim, S. H. (2008). Arsenic fractions and enzyme activities in arsenic-contaminated soils by groundwater irrigation in West Bengal. *Ecotoxicology and environmental safety*, *71*(1), 149-156.

Bijaksana, S., & Huliselan, E. K. (2010). Magnetic properties and heavy metal content of sanitary leachate sludge in two landfill sites near Bandung, Indonesia. *Environmental Earth Sciences*, *60*(2), 409-419.

Bochner, B. (1989). "Breathprints" at the microbial level. *ASM news*, *55*, 536-539.

Bochner, B. (1989). Sleuthing out bacterial identities. *Nature*, *339*(6220), 157-158.

Bong, C. W., Malfatti, F., Azam, F., Obayashi, Y., & Suzuki, S. (2010). The effect of zinc exposure on the bacteria abundance and proteolytic activity in seawater. *Interdisciplinary studies on environmental chemistry—biological responses to contaminants. Terrapub, Tokyo*, 57-63.

Brandt, K. K., Frandsen, R. J., Holm, P. E., & Nybroe, O. (2010). Development of pollution-induced community tolerance is linked to structural and functional resilience of a soil bacterial community following a five-year field exposure to copper. *Soil Biology and Biochemistry*, *42*(5), 748-757.

Brauer, M., Amann, M., Burnett, R. T., Cohen, A., Dentener, F., Ezzati, M., ... & Van Donkelaar, A. (2012). Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. *Environmental science & technology*, *46*(2), 652-660.

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and environmental safety*, *45*(3), 198-207.

Cai, L., Liu, G., Rensing, C., & Wang, G. (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiology*, *9*(1), 1-11.

Carbonell, G., de Imperial, R. M., Torrijos, M., Delgado, M., & Rodriguez, J. A. (2011). Effects of municipal solid waste compost and mineral fertilizer amendments on soil properties and heavy metals distribution in maize plants (Zea mays L.). *Chemosphere*, *85*(10), 1614-1623.

Carine, F., Enrique, A. G., & Stéven, C. (2009). Metal effects on phenol oxidase activities of soils. *Ecotoxicology and Environmental Safety*, *72*(1), 108-114.

Carpio, I. E. M., Franco, D. C., Sato, M. I. Z., Sakata, S., Pellizari, V. H., Ferreira Filho, S. S., & Rodrigues, D. F. (2016). Biostimulation of metal-resistant microbial consortium to remove zinc from contaminated environments. *Science of the Total Environment*, *550*, 670-675.

Castro-González, M. I., & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental toxicology and pharmacology*, *26*(3), 263-271.

Cayllahua, J. E. B., & Torem, M. L. (2010). Biosorption of aluminum ions onto Rhodococcus opacus from wastewaters. *Chemical Engineering Journal*, *161*(1-2), 1-8.

Chaudhari, P. N., Chaudhari, B. L., & Chincholkar, S. B. (2013). Iron containing keratinolytic metallo-protease produced by Chryseobacterium gleum. *Process Biochemistry*, *48*(1), 144-151.

Chen, B. Y., Utgikar, V. P., Harmon, S. M., Tabak, H. H., Bishop, D. F., & Govind, R. (2000). Studies on biosorption of zinc (II) and copper (II) on Desulfovibrio desulfuricans. *International Biodeterioration & Biodegradation*, *46*(1), 11-18.

Çolak, F., Atar, N., Yazıcıoğlu, D., & Olgun, A. (2011). Biosorption of lead from aqueous solutions by Bacillus strains possessing heavy-metal resistance. Chemical Engineering Journal, 173(2), 422-428.

Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., & Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of hazardous materials*, *146*(1-2), 270-277.

Cortez, H., Pingarrón, J., Muñoz, J. A., Ballester, A., Blázquez, M. L., González, F. & Coto, O. (2010). Bioremediation of soils contaminated with metalliferous mining wastes. *Trends bioremediation phytoremediation. Research Signpost, Trivandrum*, 283-299.

Costa, A. C. A. D., & Duta, F. P. (2001). Bioaccumulation of copper, zinc, cadmium and lead by Bacillus sp., Bacillus cereus, Bacillus sphaericus and Bacillus subtilis. *Brazilian Journal of Microbiology*, *32*(1), 1-5.

Cristani, M., Naccari, C., Nostro, A., Pizzimenti, A., Trombetta, D., & Pizzimenti, F. (2012). Possible use of Serratia marcescens in toxic metal biosorption (removal). *Environmental Science and Pollution Research*, *19*(1), 161-168.

Cristani, M., Naccari, C., Nostro, A., Pizzimenti, A., Trombetta, D., & Pizzimenti, F. (2012). Possible use of Serratia marcescens in toxic metal biosorption (removal). *Environmental Science and Pollution Research*, *19*(1), 161-168.

Daboor, S. M. (2014). Application of bacterial biomass as a potential heavy metal bio-removal agent. *African Journal of Microbiology Research*, *8*(22), 2229-2237.

Dada, E. O., Njoku, K. I., Osuntoki, A. A., & Akinola, M. O. (2015). A review of current techniques of Physico-chemical and biological remediation of heavy metals polluted soil. Ethiopian Journal of Environmental Studies and Management, 8(5), 606-615.

Das, S., Dash, H. R., & Chakraborty, J. (2016). Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. *Applied microbiology and biotechnology*, *100*(7), 2967-2984.

D'Ascoli, R., Rao, M. A., Adamo, P., Renella, G., Landi, L., Rutigliano, F. A., ... & Gianfreda, L. (2006). Impact of river overflowing on trace element contamination of volcanic soils in south Italy: Part II. Soil biological and biochemical properties in relation to trace element speciation. *Environmental Pollution*, *144*(1), 317-326.

De Vries, W., Schütze, G., Lofts, S., Tipping, E., Meili, M., Römkens, P. F. A. M., & Groenenberg, J. E. (2005). *Calculation of critical loads for cadmium, lead and mercury; background document to a mapping manual on critical loads of cadmium, lead and mercury* (No. 1104). Alterra.

De, S., Maiti, S. K., Hazra, T., Debsarkar, A., & Dutta, A. (2016). Leachate characterization and identification of dominant pollutants using leachate pollution index for an uncontrolled landfill site. *Global Journal of Environmental Science and Management*, *2*(2), 177-186.

Department of Environment. (2009). Contaminated land management and control guidelines no. 1: Malaysian recommended site screening levels for contaminated land. Ministry of Natural Resource and Environment, Putrajaya, Malaysia.

Diaz-Bone, R. A., & Van de Wiele, T. (2010). Biotransformation of metal (loid) s by intestinal microorganisms. *Pure and Applied Chemistry*, *82*(2), 409-427.

Dorian A, B. H., Landy I, R. B., Enrique, D. P., & Luis, F. L. (2012). Zinc and lead biosorption by Delftia tsuruhatensis: a bacterial strain resistant to metals isolated from mine tailings. *Journal of Water Resource and Protection*, *2012*.

DuBois, J. L. (2019). Pseudomonas mendocina: Versatile Remediator from a Versatile Genus. *Encyclopedia of Water: Science, Technology, and Society*, 1-7.

Effron, D., De la Horra, A. M., Defrieri, R. L., Fontanive, V., & Palma, R. M. (2004). Effect of cadmium, copper, and lead on different enzyme activities in a native forest soil. *Communications in Soil Science and Plant Analysis*, *35*(9-10), 1309-1321.

El-Arby, A. M., & Elbordiny, M. M. (2006). Impact of reused wastewater for irrigation on availability of heavy metals in sandy soils and their uptake by plants. *Journal of Applied Sciences Research*, *2*(2), 106-111.

El-Meleigy, M. A., Abed, N. N., & Sari, I. P. (2013). Fate of cobalt and nickel in B. firmus and B. subtilis. *Natural Science*, *9*(8), 175-189.

Emenike, C. U., Liew, W., Fahmi, M. G., Jalil, K. N., Pariathamby, A., & Hamid, F. S. (2017). Optimal removal of heavy metals from leachate contaminated soil using bioaugmentation process. CLEAN–Soil, Air, Water, 45(2), 1500802.

Fan, J. (2013). Application of *Cupriavidus metallidurans* and *Ochrobactrum intermedium* for copper and chromium biosorption (Doctoral dissertation).

Fang, L., Li, X., Li, L., Li, S., Liao, X., Sun, J., & Liu, Y. (2016). Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids from E. coli isolates of food-producing animals. *Scientific Reports, 6*(1), 1-8.

Farooqi, Z. R., Iqbal, M. Z., Kabir, M., & Shafiq, M. (2009). Toxic effects of lead and cadmium on germination and seedling growth of Albizia lebbeck (L.) Benth. *Pakistan Journal of Botany*, *41*(1), 27-33.

Fashola, M. O., Ngole-Jeme, V. M., & Babalola, O. O. (2016). Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. *International Journal of Environmental Research and Public Health*, *13*(11), 1047.

Fauziah, S. H, Jayanthi, B., Emenike, C. U. and 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.

Fauziah, S. H., & Agamuthu, P. (2013). Challenges in 3R Implementation and Public Participation. *Malaysian Journal of Science*, *32*(2), 49-58.

Fauziah, S. H., & Agamuthu, P. (2012). Trends in sustainable landfilling in Malaysia, a developing country. *Waste Management & Research*, *30*(7), 656-663.

Fauziah, S. H., Khairunnisa, A. K., Siti Zubaidah, B., & Agamuthu, P. (2009). Public perception on Solid Waste and Public Cleansing Management Bill 2007 towards sustainable waste management in Malaysia.

Fingerman, M. (Ed.). (2016). Bioremediation of aquatic and terrestrial ecosystems. CRC Press.

Fomina, M., & Gadd, G. M. (2014). Biosorption: current perspectives on concept, definition and application. Bioresource technology, 160, 3-14.

Franchi, E., Agazzi, G., Rolli, E., Borin, S., Marasco, R., Chiaberge, S. & Barbafieri, M. (2016). Exploiting Hydrocarbon-Degrading Indigenous Bacteria for Bioremediation and Phytoremediation of a Multicontaminated Soil. *Chemical Engineering & Technology*, *39*(9), 1676-1684.

Gadd, G. M. (2000). Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology, 11*(3), 271-279.

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science*, *309*(5739), 1387-1390.

Garbisu, C., & Alkorta, I. (2003). Basic concepts on heavy metal soil bioremediation. *European Journal of Mineral Processing and Environmental Protection*, *3*(1), 58-66.

Gawali Ashruta, A., Nanoty, V., & Bhalekar, U. (2014). Biosorption of heavy metals from aqueous solution using bacterial EPS. *International Journal of Life-Sciences, 2*, 373-377.

Ghosh, A., & Saha, P. D. (2013). Optimization of copper reduction from solution using Bacillus pumilus PD3 isolated from marine water. *Elixir Pollution*, *55*, 12910-12914.

Gómez-Sagasti, M. T., Alkorta, I., Becerril, J. M., Epelde, L., Anza, M., & Garbisu, C. (2012). Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. *Water, Air, & Soil Pollution*, *223*(6), 3249-3262.

Gowsalya, A., Ponnusami, V., & Sugumaran, K. R. (2014). Isolation of Bacteria from Soil sample for Exo-Polysaccharide production. *International Journal of ChemTech Research*, *6*(5), 2925-2928.

Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *The Lancet Neurology*, *13*(3), 330-338.

Große, C., Anton, A., Hoffmann, T., Franke, S., Schleuder, G., & Nies, D. H. (2004). Identification of a regulatory pathway that controls the heavy-metal resistance system Czc via promoter czcNp in Ralstonia metallidurans. *Archives of Microbiology*, *182*(2-3), 109-118.

Gupta, V. K., Nayak, A., & Agarwal, S. (2015). Bioadsorbents for remediation of heavy metals: current status and their future prospects. *Environmental Engineering Research, 20*(1), 1-18.

Haferburg, G., & Kothe, E. (2007). Microbes and metals: interactions in the environment. *Journal of Basic Microbiology*, *47*(6), 453-467.

Han, J. X., Shang, Q., & Du, Y. (2009). Effect of environmental cadmium pollution on human health. *Health*, *1*(03), 159.

He, P. J., Xiao, Z., Shao, L. M., Yu, J. Y., & Lee, D. J. (2006). In situ distributions and characteristics of heavy metals in full-scale landfill layers. *Journal of Hazardous Materials*, *137*(3), 1385-1394.

Heipieper, H. J. (Ed.). (2007). *Bioremediation of soils contaminated with aromatic compounds* (Vol. 76). Springer Science & Business Media.

Hinojosa, M. B., Carreira, J. A., García-Ruíz, R., & Dick, R. P. (2004). Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. *Soil Biology and Biochemistry*, *36*(10), 1559-1568.

Hobman, J. L., & Crossman, L. C. (2015). Bacterial antimicrobial metal ion resistance. *Journal of Medical Microbiology*, *64*(5), 471-497.

Hoornweg, D., & Bhada-Tata, P. (2012). What a waste: a global review of solid waste management. Retrieved on October 7, 2014 from https://openknowledge.worldbank.org/handle/10986/17388

Huang, Q., & Shindo, H. (2000). Effects of copper on the activity and kinetics of free and immobilized acid phosphatase. *Soil Biology and Biochemistry*, *32*(13), 1885-1892.

Igiri, B. E., Okoduwa, S. I., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: A Review. *Journal of Toxicology, 2018*.

Ilamathi, R., Nirmala, G. S., & Muruganandam, L. (2014). Heavy metals biosorption in liquid solid fluidized bed by immobilized consortia in alginate beads. *International Journal of ChemTech Research*, *6*, 652-662.

Ilker, S., Kaplan, M., & Sahriye, S. (2007). Investigation of seasonal changes in nitrate contents of soils and irrigation waters in greenhouses located in Antalya-Demre region. *Asian Journal of Chemistry*, *19*(7), 5639.

Ismail, S. N. S., & Manaf, L. A. (2013). The challenge of future landfill: A case study of Malaysia. *Journal of Toxicology and Environmental Health Sciences*, *5*(6), 86-96.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60-72.

Jan, A. T., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, Q. M. R. (2015). Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. *International Journal of Molecular Sciences*, *16*(12), 29592-29630.

Javanbakht, V., Alavi, S. A., & Zilouei, H. (2014). Mechanisms of heavy metal removal using microorganisms as biosorbent. *Water Science and Technology*, *69*(9), 1775-1787.

Jayanthi, B., Emenike, C. U., Auta, S. H., Agamuthu, P., & Fauziah, S. H. (2017). Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia. *International Biodeterioration & Biodegradation*, *119*, 467-475.

Jayanthi, B., Emenike, C. U., Agamuthu, P., Simarani, K., Mohamad, S., & 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.

Jayanthi, B., Emenike, C. U., Auta, S. H., Agamuthu, P., & Fauziah, S. H. (2016). Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia. *International Biodeterioration & Biodegradation*, *30*, 1-9.

Jia, C., Zhang, Y., Zhu, Y., Wang, H., & Wang, J. (2014). Isolation, identification and adsorption characteristics of an highly nickel-resistant Stenotrophomonas sp. strain. Environmental Science & Technology (China), 37(10), 55-61.

Jin, Y., Luan, Y., Ning, Y., & Wang, L. (2018). Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. *Applied Sciences, 8*(8), 1-17.

Joutey, N. T., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013). Biodegradation: involved microorganisms and genetically engineered microorganisms. *Biodegradation-Life of Science*, *1*, 289-320.

Joynt, J., Bischoff, M., Turco, R., Konopka, A., & Nakatsu, C. H. (2006). Microbial community analysis of soils contaminated with lead, chromium and petroleum hydrocarbons. *Microbial Ecology*, *51*(2), 209-219.

Juwarkar, A. A., Misra, R. R., & Sharma, J. K. (2014). Recent trends in bioremediation. In *Geomicrobiology and Biogeochemistry* (pp. 81-100). Springer, Berlin, Heidelberg.

Juwarkar, A. A., Singh, S. K., & Mudhoo, A. (2010). A comprehensive overview of elements in bioremediation. *Reviews in Environmental Science and Bio/Technology*, *9*(3), 215-288.

Kader, J., Sannasi, P., Othman, O., Ismail, B. S., & Salmijah, S. (2007). Removal of Cr (VI) from aqueous solutions by growing and non-growing populations of environmental bacterial consortia. *Global Journal of Environmental Research*, *1*(1), 12-17.

Kamarudzaman, A. N., Aziz, R. A., & Jalil, M. F. (2011). Removal of heavy metals from landfill leachate using horizontal and vertical subsurface flow constructed wetland planted with Limnocharis flava. *International Journal of Civil and Environmental Engineering*, *11*(5), 85-91.

Kannan, A., & Ramteke, P. W. (2002). Uptake of nickel (II) by Serratia marcescens. *Journal of Environmental Biology*, *23*(1), 57-59.

Kapahi, M., & Sachdeva, S. (2019). Bioremediation options for heavy metal pollution. *Journal of Health and Pollution, 9*(24).

Karaca, A., Cetin, S. C., Turgay, O. C., & Kizilkaya, R. (2010). Effects of heavy metals on soil enzyme activities. In *Soil heavy metals* (pp. 237-262). Springer, Berlin, Heidelberg.

Karakas, M., Dursun, Ş., Özdemir, C., & Argun, M. E. (2006). Heavy metal accumulation in irrigated soil with wastewater. *Selçuk Tarım Bilimleri Dergisi*, *20*(38), 64-67.

Karigar, C. S., & Rao, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. *Enzyme research*, *2011*, 1-11.

Kaur, V., & Sharma, G. (2014). Effects of industrial effluent on soil characteristics: A review. *International Journal of Advanced Engineering Science and Technological Research*, *3*, 201-207.

Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental chemistry letters*, *7*(1), 1-19.

Khan, S., Hesham, A. E. L., Qiao, M., Rehman, S., & He, J. Z. (2010). Effects of Cd and Pb on soil microbial community structure and activities. *Environmental Science and Pollution Research, 17*(2), 288-296.

Kim, R. Y., Yoon, J. K., Kim, T. S., Yang, J. E., Owens, G., & Kim, K. R. (2015). Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review. *Environmental Geochemistry and Health*, *37*(6), 1041-1061.

Kowshik, M. (2013). Mechanisms of metal resistance and homeostasis in haloarchaea. *Archaea*, *2013*.

Kozdrój, J., & van Elsas, J. D. (2001). Structural diversity of microbial communities in arable soils of a heavily industrialised area determined by PCR-DGGE fingerprinting and FAME profiling. *Applied Soil Ecology*, *17*(1), 31-42.

Kuffner, M., De Maria, S., Puschenreiter, M., Fallmann, K., Wieshammer, G., Gorfer, M., ... & Sessitsch, A. (2010). Culturable bacteria from Zn-and Cd-accumulating Salix caprea with differential effects on plant growth and heavy metal availability. *Journal of Applied Microbiology*, *108*(4), 1471-1484.

Kumar, A., Bisht, B. S., Joshi, V. D., & Dhewa, T. (2011). Review on bioremediation of polluted environment: A management tool. *International Journal of Environmental Sciences*, *1*(6), 1079-1093.

Kumar, P. S., & Gunasundari, E. (2018). Bioremediation of heavy metals. In Bioremediation: applications for environmental protection and management (pp. 165-195). Springer, Singapore.

Landrigan, P. J., & Goldman, L. R. (2011). Children's vulnerability to toxic chemicals: a challenge and opportunity to strengthen health and environmental policy. *Health Affairs*, *30*(5), 842-850.

Landrigan, P. J., Fuller, R., Acosta, N. J., Adeyi, O., Arnold, R., Baldé, A. B. & Chiles, T. (2017). The Lancet Commission on pollution and health. *The Lancet*.

Lenart-Boroń, A., & Boroń, P. (2014). The effect of industrial heavy metal pollution on microbial abundance and diversity in soils—a review. *Environmental Risk Assessment of Soil Contamination*.

Leung, W. C., Wong, M. F., Chua, H., Lo, W., Yu, P. H. F., & Leung, C. K. (2000). Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water science and technology*, *41*(12), 233-240.

Li, P. S., & Tao, H. C. (2015). Cell surface engineering of microorganisms towards adsorption of heavy metals. *Critical Reviews in Microbiology, 41*(2), 140-149.

Lin, T. C., Pan, P. T., & Cheng, S. S. (2010). Ex situ bioremediation of oil-contaminated soil. *Journal of Hazardous Materials*, *176*(1-3), 27-34.

Ling, W., Shen, Q., Gao, Y., Gu, X., & Yang, Z. (2008). Use of bentonite to control the release of copper from contaminated soils. *Soil Research*, *45*(8), 618-623.

Liu, H. H., & Sang, S. X. (2010). Study on the law of heavy metal leaching in municipal solid waste landfill. *Environmental Monitoring and Assessment*, *165*(1-4), 349-363.

Liu, R. Q., Luo, L. X., Xia, F., & Zhu, L. H. (2011, January). Biosorption characteristics of Pseudomonas alcaligenes biomass and its use for lead removal. In *2011 Third International Conference on Measuring Technology and Mechatronics Automation* (Vol. 1, pp. 645-648). IEEE.

Lorenz, N., Hintemann, T., Kramarewa, T., Katayama, A., Yasuta, T., Marschner, P., & Kandeler, E. (2006). Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. *Soil Biology and Biochemistry*, *38*(6), 1430-1437.

Macdonald, C. A., Singh, B. K., Peck, J. A., Van Schaik, A. P., Hunter, L. C., Horswell, J., ... & Speir, T. W. (2007). Long-term exposure to Zn-spiked sewage sludge

alters soil community structure. *Soil Biology and Biochemistry*, *39*(10), 2576-2586.

Mai-Prochnow, A., Clauson, M., Hong, J., & Murphy, A. B. (2016). Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Scientific reports, 6(1), 1-11.

Malaysia, Ministry of Housing and Local Government (MHLG). (2015). *Solid Waste Management Lab 2015.* Retrieved on January 11, 2016 from http://www.kpkt.gov.my/resources/index/user_1/Attachments/hebahan_slider/slaid_dapatan_makmal.pdf

Malik, D., Singha, S., Thakura, J., Singhb, R. K., Kapura, A., & Nijhawana, S. (2014). Microbial Fuel Cell: Harnessing Bioenergy from Yamuna Water. *International Journal of Current Microbiology and Applied Sciences*, *3*(7), 44-54.

Maliszewska-Kordybach, B., & Smreczak, B. (2003). Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination. *Environment International*, *28*(8), 719-728.

Mamba, B. B., Dlamini, N. P., Nyembe, D. W., & Mulaba-Bafubiandi, A. F. (2009). Metal adsorption capabilities of clinoptilolite and selected strains of bacteria from mine water. *Physics and Chemistry of the Earth, Parts A/B/C*, *34*(13-16), 830-840.

Mamba, B. B., Krause, R. W., Matsebula, B., & Haarhoff, J. (2009). Monitoring natural organic matter and disinfection by-products at different stages in two South African water treatment plants. *Water SA*, *35*(1).

Manaf, L. A., Samah, M. A. A., & Zukki, N. I. M. (2009). Municipal solid waste management in Malaysia: Practices and challenges. *Waste Management*, *29*(11), 2902-2906.

Manju, M. (2015). Effects of heavy metals on human health. *International Journal of Research–GRANTHAALAYAH*, *530*, 2394-3629.

Marais, L. M. (2012). *Characterization of bacteria isolated from a platinum mine tailings dam* (Doctoral dissertation, North-West University).

Markowicz, A., Cycoń, M., & Piotrowska-Seget, Z. (2016). Microbial community structure and diversity in long-term hydrocarbon and heavy metal contaminated soils. *International Journal of Environmental Research*, *10*(2), 321-332.

Máthé, I., Benedek, T., Táncsics, A., Palatinszky, M., Lányi, S., & Márialigeti, K. (2012). Diversity, activity, antibiotic and heavy metal resistance of bacteria from petroleum hydrocarbon contaminated soils located in Harghita County (Romania). *International biodeterioration & biodegradation*, *73*, 41-49.

McIntyre, T. (2003). Phytoremediation of heavy metals from soils. In *Phytoremediation* (pp. 97-123). Springer Berlin Heidelberg.

Meena, V., Kaur, H., & Mohini, M. (2005). Toxic metals and environmental pollution. *Journal of Industrial Pollution*, 21-101.

Migahed, F., Abdelrazak, A., & Fawzy, G. (2017). Batch and continuous removal of heavy metals from industrial effluents using microbial consortia. *International Journal of Environmental Science and Technology*, *14*(6), 1169-1180.

Mikanova, O. (2006). Effects of heavy metals on some soil biological parameters. *Journal of Geochemical Exploration*, *88*(1-3), 220-223.

Mindlin, S., Petrenko, A., Kurakov, A., Beletsky, A., Mardanov, A., & Petrova, M. (2016). Resistance of permafrost and modern Acinetobacter lwoffii strains to heavy metals and arsenic revealed by genome analysis. *BioMed Research International*, *2016*.

Mishra, D., Kim, D. J., Ahn, J. G., & Rhee, Y. H. (2005). Bioleaching: a microbial process of metal recovery; a review. *Metals and Materials International*, *11*(3), 249-256.

Mishra, R. K., Mohammad, N., & Roychoudhury, N. (2015). Soil pollution: Causes, effects and control. *Tropical Forest Research Institute*, *3*(1), 20-30.

Mohamed, A. F., & Jahi, J. (2000). Implication of manufacturing industries within langat drainage basin: issues and challenges. *Malaysian Journal of Environmental Management*, *1*, 143-161.

Morgan, R., Brevik, E. C., & Burgess, L. C. (2013). Soil, heavy metals, and human health. *Soils and Human Health*, 59-82.

Muhammad, A., Xu, J., Li, Z., Wang, H., & Yao, H. (2005). Effects of lead and cadmium nitrate on biomass and substrate utilization pattern of soil microbial communities. *Chemosphere*, *60*(4), 508-514.

Munoz-Melendez, G., Korre, A., & Parry, S. J. (2000). Influence of soil pH on the fractionation of Cr, Cu and Zn in solid phases from a landfill site. *Environmental Pollution*, *110*(3), 497-504.

Muthukrishnan, S., Sabaratnam, V., Tan, G. Y. A., & Chong, V. C. (2015). Identification of Indigenous Bacteria Isolated from Shrimp Aquaculture Wastewater with Bioremediation Application: Total Ammoniacal Nitrogen (TAN) and Nitrite Removal. *Sains Malaysiana*, *44*(8), 1103-1110.

Naik, U. C., Srivastava, S., & Thakur, I. S. (2012). Isolation and characterization of Bacillus cereus IST105 from electroplating effluent for detoxification of hexavalent chromium. *Environmental Science and Pollution Research*, *19*(7), 3005-3014.

Narasimhulu, K., & Rao, P. S. (2009). Studies on removal of toxic metals from wastewater using Pseudomonas species.

Nathanson, J. A. (2018). Pollution. *Encyclopædia Britannica*. Retrieved from https://www.britannica.com/science/pollution-environment

National Research Council. (2010). *Hidden costs of energy: unpriced consequences of energy production and use*. National Academies Press.

National Research Council. (2012). *Exposure Science in the 21st Century: a Vision and a Strategy*. National Academies Press.

Noor, Z. Z., Yusuf, R. O., Abba, A. H., Hassan, M. A. A., & Din, M. F. M. (2013). An overview for energy recovery from municipal solid wastes (MSW) in Malaysia scenario. *Renewable and Sustainable Energy Reviews*, *20*, 378-384.

Nouha, K., Kumar, R. S., & Tyagi, R. D. (2016). Heavy metals removal from wastewater using extracellular polymeric substances produced by *Cloacibacterium normanense* in wastewater sludge supplemented with crude glycerol and study of extracellular polymeric substances extraction by different methods. *Bioresource Technology*, *212*, 120-129.

Nwagwu, E. C., Yilwa, V. M., Egbe, N. E., & Onwumere, G. B. (2017). Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation. *African Journal of Biotechnology*, *16*(1), 32-40.

Odum, H. T., & Odum, E. C. (2006). The prosperous way down. *Energy*, *31*(1), 21-32.

Olaniran, A. O., Balgobind, A., & Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal of Molecular Sciences*, *14*(5), 10197-10228.

Olaniran, A. O., Balgobind, A., & Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal of Molecular Sciences*, *14*(5), 10197-10228.

Öman, C. B., & Junestedt, C. (2008). Chemical characterization of landfill leachates–400 parameters and compounds. *Waste Management*, *28*(10), 1876-1891.

Omran, A. R. (2005). The epidemiologic transition: a theory of the epidemiology of population change. *The Milbank Quarterly*, *83*(4), 731-757.

OpenLearn Works. (2016). *Study session 8 solid waste reduction, reuse and recycle*. Retrieved on August 28, 2018 from https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=80574&printable=1

Paerl, H. W., & Pinckney, J. L. (1996). A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microbial Ecology*, *31*(3), 225-247.

Pan, Y., Yang, X., Xu, M., & Sun, G. (2017). The Role of Enriched Microbial Consortium on Iron-Reducing Bioaugmentation in Sediments. *Frontiers in Microbiology*, *8*, 462.

Pandey, P., & Tripathi, A. K. (2011). Effect of heavy metals on morphological and biochemical characteristics of Albizia procera (Roxb.) Benth. seedlings. *International Journal of Environmental Sciences*, *1*(5), 1009-1018.

Pandey, S., Saha, P., Barai, P. K., & Maiti, T. K. (2010). Characterization of a Cd2+-resistant strain of Ochrobactrum sp. isolated from slag disposal site of an iron and steel factory. *Current Microbiology*, *61*(2), 106-111.

Parameswari, E., Lakshmanan, A., & Thilagavathi, T. (2009). Biosorption of chromium (VI) and nickel (II) by bacterial isolates from an aqueous solution. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(3), 150-156.

Parizanganeh, A. H., Bijnavand, V., Zamani, A. A., & Hajabolfath, A. (2012). Concentration, distribution and comparison of total and bioavailable heavy metals in top soils of Bonab District in Zanjan province. *Open Journal of Soil Science*, *2*(02), 123.

Patel, S., & Kasture, A. (2014). E (electronic) waste management using biological systems-overview. *International Journal of Current Microbiology and Applied Sciences, 3*(7), 495-504.

Patlolla, A. K., Barnes, C., Yedjou, C., Velma, V. R., & Tchounwou, P. B. (2009). Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental Toxicology*, *24*(1), 66-73.

Pawan, K. B. (2012). *Heavy Metals in Environment*. Lap Lambert Academic Publ.

Perelo, L. W. (2010). In situ and bioremediation of organic pollutants in aquatic sediments. *Journal of Hazardous Materials*, *177*(1-3), 81-89.

Pernyeszi, T. (2011). Bioadsorption of lead (II) and cadmium (II) ions onto the lyophilized cell surface of Pseudomonas aeruginosa in aqueous suspension. *Agriculture and Environment*, *3*, 5-17.

Pillai, S., Peter, A. E., Sunil, B. M., & Shrihari, S. (2014, March). Soil pollution near a municipal solid waste disposal site in India. In *International Conference on Biological, Civil and Environmental Engineering (BCEE-2014) March* (Vol. 1718).

Prabhakaran, P., Ashraf, M. A., & Aqma, W. S. (2016). Microbial stress response to heavy metals in the environment. *Rsc Advances*, *6*(111), 109862-109877.

Rachwał, M., Magiera, T., & Wawer, M. (2015). Coke industry and steel metallurgy as the source of soil contamination by technogenic magnetic particles, heavy metals and polycyclic aromatic hydrocarbons. *Chemosphere*, *138*, 863-873.

Rajapaksha, R. M. C. P., Tobor-Kapłon, M. A., & Bååth, E. (2004). Metal toxicity affects fungal and bacterial activities in soil differently. *Applied and Environmental Microbiology*, *70*(5), 2966-2973.

Rajasekar, A., & Ting, Y. P. (2010). Microbial corrosion of aluminum 2024 aeronautical alloy by hydrocarbon degrading bacteria Bacillus cereus ACE4 and Serratia marcescens ACE2. *Industrial & Engineering Chemistry Research*, *49*(13), 6054-6061.

Ramesh, A., Sharma, S. K., Sharma, M. P., Yadav, N., & Joshi, O. P. (2014). Inoculation of zinc solubilizing Bacillus aryabhattai strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology*, *73*, 87-96.

Rampelotto, P. H. (2010). Resistance of microorganisms to extreme environmental conditions and its contribution to astrobiology. *Sustainability*, *2*(6), 1602-1623.

Ray, L., Paul, S., Bera, D., & Chattopadhyay, P. (2006). Bioaccumulation of Pb (II) from aqueous solutions by Bacillus cereus M^{1}_{16}. *Journal of Hazardous Substance Research*, *5*(1), 1-22.

Regmi, T., Banerji, S. K., & Hong, A. (1996, May). Biodegradation of chelating agents used for metal removal from contaminated soils. In *HSRC/WERC Joint Conference on the Environment* (pp. 21-23).

Rehan, M., & Alsohim, A. S. (2019). Bioremediation of heavy metals. In Environmental Chemistry and Recent Pollution Control Approaches (p. 145). IntechOpen.

Renella, G., Mench, M., Gelsomino, A., Landi, L., & Nannipieri, P. (2005). Functional activity and microbial community structure in soils amended with bimetallic sludges. *Soil Biology and Biochemistry*, *37*(8), 1498-1506.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin III, F. S., Lambin, E. F., ... & Nykvist, B. (2009). A safe operating space for humanity. *Nature*, *461*(7263), 472-475.

Roongtanakiat, N., Nirunrach, T., Chanyotha, S., & Hengchaovanich, D. (2003). Uptake of heavy metals in landfill leachate by vetiver grass. *Kasetsart Journal - Natural Science, 37*(2), 168-175.

Rosen, C. J. (2002). Contaminants in the Home garden and urban soil environment. *Extension Guide FO-02453. Grand Rapids, MN: University of Minnesota*

Extension Services, Department of Soil, Water and Climate. Retrieved August, *15*, 2009.

Roy, M., & McDonald, L. M. (2015). Metal uptake in plants and health risk assessments in metal-contaminated smelter soils. *Land degradation & development*, *26*(8), 785-792.

Sadeghi, O., Fazeli, A., & Bakhtiarinejad, M. (2013). Waste to energy in Malaysia.

Saheri, S., Mir, M. A., Basri, N. E. A., Begum, R. A., & Mahmood, N. Z. B. (2017). Solid waste management by considering composting potential in Malaysia toward a green country. *e-BANGI*, *4*(1).

Sannasi, P., Kader, J., Othman, O., & Salmijah, S. (2006). Single and Multi-Metal Removal by an Environmental Mixed Bacterial Isolate. *Modern Multidisciplinary Applied Microbiology: Exploiting Microbes and Their Interactions*, *1*, 136-141.

Sannasi, P., Kader, J., Othman, O., & Salmijah, S. (2009). Physical growth and biomass characterization of bacterial cells exposed to Cd (II), Cr (VI), Cu (II), Ni (II), and Pb (II). *Journal of Environmental Research and Development*, *4*(1).

Sardar, K. H. A. N., Qing, C. A. O., Hesham, A. E. L., Yue, X., & He, J. Z. (2007). Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. *Journal of Environmental Sciences*, *19*(7), 834-840.

Saritha, P. 2015. Article of Bacteria Present in a Sample by Serial Dilution Agar Plating Method or Total Plate Count (TPC) Method. Retrieved on May 1, 2018 from http://www.yourarticlelibrary.com/experiments/bacteria-present-in-a-sample-by-serial-dilution-agar-plating-method-or-total-plate-count-tpc-method/26577

Satyanarayana, T., Johri, B. N., & Prakash, A. (Eds.). (2012). *Microorganisms in environmental management: microbes and environment*. Springer Science & Business Media.

Satyapal, G. K., Rani, S., Kumar, M., & Kumar, N. (2016). Potential role of arsenic resistant bacteria in bioremediation: current status and future prospects. *Journal of Microbial & Biochemical Technology, 8*(3), 256-258.

Savci, S. (2012). An agricultural pollutant: chemical fertilizer. *International Journal of Environmental Science and Development*, *3*(1), 73.

Say, R., Denizli, A., & Arıca, M. Y. (2001). Biosorption of cadmium (II), lead (II) and copper (II) with the filamentous fungus Phanerochaete chrysosporium. *Bioresource Technology*, *76*(1), 67-70.

Shamim, S. (2018). Biosorption of heavy metals. Biosorption, 2, 21-49.

Shayler, H., McBride, M., & Harrison, E. (2009). Sources and impacts of contaminants in soils.

Sheik, C. S., Mitchell, T. W., Rizvi, F. Z., Rehman, Y., Faisal, M., Hasnain, S., ... & Krumholz, L. R. (2012). Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PloS one*, *7*(6), 1-13.

Shen, G., Cao, L., Lu, Y., & Hong, J. (2005). Influence of phenanthrene on cadmium toxicity to soil enzymes and microbial growth (5 pp). *Environmental Science and Pollution Research*, *12*(5), 259-263.

Shen, J., Huang, L., Zhou, P., Quan, X., & Puma, G. L. (2017). Correlation between circuital current, Cu (II) reduction and cellular electron transfer in EAB isolated from Cu (II)-reduced biocathodes of microbial fuel cells. *Bioelectrochemistry*, *114*, 1-7.

Shukla, K. P., Singh, N. K., & Sharma, S. (2010). Bioremediation: developments, current practices and perspectives. *Genetic Engineering and Biotechnology Journal*, *3*(8), 1-20.

Simona, C., Angela, R. F., & de Santo Amalia, V. (2004). Suitability of soil microbial parameters as indicators of heavy metal pollution. *Water, Air, and Soil Pollution*, *158*(1), 21-35.

Singh, J., & Kalamdhad, A. S. (2011). Effects of heavy metals on soil, plants, human health and aquatic life. *International Journal of Research in Chemistry and Environment*, *1*(2), 15-21.

Singh, K. K., & Vaishya, R. C. (2017). Bioremediation of Heavy Metal Using Consortia Developed from Municipal Wastewater Isolates. *SAMRIDDHI: A Journal of Physical Sciences, Engineering and Technology*, *9*(01), 57-66.

Singh, P. K., Singh, A. L., Kumar, A., & Singh, M. P. (2012). Mixed bacterial consortium as an emerging tool to remove hazardous trace metals from coal. *Fuel*, *102*, 227-230.

Sinha, R. K., Valani, D., Sinha, S., Singh, S., & Herat, S. (2009a). Bioremediation of contaminated sites: a low-cost nature's biotechnology for environmental clean-up by versatile microbes, plants & earthworms. *Solid Waste Management and Environmental Remediation*, 978-1.

Smith, K. R., & Ezzati, M. (2005). How environmental health risks change with development: the epidemiologic and environmental risk transitions revisited. *Annu. Rev. Environ. Resour.*, *30*, 291-333.

Sorek-Hamer, M., Just, A. C., & Kloog, I. (2016). The Use of Satellite Remote Sensing in Epidemiological Studies. *Current opinion in pediatrics*, *28*(2), 228.

Spain, A., & Alm, E. (2003). Implications of microbial heavy metal tolerance in the environment.

Stubbendieck, R. M., Vargas-Bautista, C., & Straight, P. D. (2016). Bacterial communities: interactions to scale. *Frontiers in microbiology*, *7*, 1234.

Štyriaková, I. V. E. T. A., & Štyriak, I. G. O. R. (2000). Iron removal from kaolins by bacterial leaching. *Ceramics-silikaty*, *44*(4), 135-141.

Tabak, H. H., Lens, P., van Hullebusch, E. D., & Dejonghe, W. (2005). Developments in bioremediation of soils and sediments polluted with metals and radionuclides–1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport. *Reviews in Environmental Science and Bio/Technology*, *4*(3), 115-156.

Tayebi, B., & Ahangar, A. G. (2014). The influence of heavy metals on the development and activity of soil microorganisms. *International Journal of Plant, Animal and Environmental Sciences*, *4*, 74-85.

Teitzel, G. M., & Parsek, M. R. (2003). Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. *Applied and Environmental Microbiology*, *69*(4), 2313-2320.

Tejada, M., Moreno, J. L., Hernández, M. T., & García, C. (2008). Soil amendments with organic wastes reduce the toxicity of nickel to soil enzyme activities. *European Journal of Soil Biology*, *44*(1), 129-140.

Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu University Journal of Science, Engineering and Technology*, *8*(1), 164-170.

Theng, L. C., Hassan, M. N., Awang, M., & Saman, M. Y. (2004). Evaluation of the pollution levels of landfill sites–the landfill pollution index (LPI). *Malaysian Journal of Environmental Management*, *5*, 31-44.

Ukpong, E. C., & Agunwamba, J. C. (2011). Effect of Open Dumps on Some Engineering and Chemical Properties of Soil. *Continental Journal of Engineering Sciences, 6*(2), 45-55.

Umanzor, J., Aguiluz, M., Pineda, C., Andrade, S., Erazo, M., Flores, C., & Santillana, S. (2006). Concurrent cisplatin/gemcitabine chemotherapy along with radiotherapy in locally advanced cervical carcinoma: a phase II trial. *Gynecologic oncology*, *100*(1), 70-75.

United Nations (undated) Urban and rural areas 2009. Retrieved on June 22, 2014 from http://www.un.org/en/development/desa/population/publications/urbanization/urban-rural.shtml.

United Nations Development Programme (UNDP). (2008). Malaysia Developing a Solid Waste Management Model for Penang.

United States Environmental Protection Agency (US-EPA), 1994.

Upadhyay, K. H., Vaishnav, A. M., Tipre, D. R., & Dave, S. R. (2021). Metal Bioremediation, Mechanisms, Kinetics and Role of Marine Bacteria in the Bioremediation Technology. In Biotechnology for Sustainable Environment (pp. 173-199). Springer, Singapore.

Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K., & Srivastava, P. C. (2014). Effect of Zn solubilizing bacteria on growth promotion and Zn nutrition of rice. *Journal of Soil Science and Plant Nutrition*, *14*(4), 889-910.

Verma, D. K., Hasan, S. H., Singh, D. K., Singh, S., & Singh, Y. (2014). Enhanced biosorptive remediation of hexavalent chromium using chemotailored biomass of a novel soil isolate Bacillus aryabhattai ITBHU02: process variables optimization through artificial neural network linked genetic algorithm. *Industrial & Engineering Chemistry Research*, *53*(9), 3669-3681.

Verma, R., & Dwivedi, P. (2013). Heavy metal water pollution-A case study. *Recent research in science and technology*, *5*(5).

Vig, K., Megharaj, M., Sethunathan, N., & Naidu, R. (2003). Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Advances in Environmental Research*, *8*(1), 121-135.

Volesky, B. (2007). Biosorption and me. *Water research*, *41*(18), 4017-4029.

Vonck, K. J. (2009). *Towards more sustainable urban surface drainage: A comparative case study of impervious cover policies in Portland, Oregon, and Seattle, Washington*. University of Delaware.

Wan Ahmad Nadzim. (2016). Boosting the culture of Reduce and Reuse in Kuala Lumpur with the help of Location Intelligence. Keynote at Asian Researcher Symposium 2016 at Universitas Indonesia, Depok, Indonesia. 26th April 2016.

Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X., & Chen, Y. (2007). The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. *Ecotoxicology and Environmental Safety*, *67*(1), 75-81.

Wilkinson, P., Smith, K. R., Beevers, S., Tonne, C., & Oreszczyn, T. (2007). Energy, energy efficiency, and the built environment. *The lancet*, *370*(9593), 1175-1187.

Williams, J. (2006). Bioremediation of contaminated soils: a comparison of in situ and ex situ techniques.

Wong, K. K., Quilty, B., Hamzah, A., & Surif, S. (2015). Phenol biodegradation and metal removal by a mixed bacterial consortium. *Bioremediation Journal*, *19*(2), 104-112.

World Health Organization (WHO) (2016). An estimated 12.6 million deaths each year are attributable to unhealthy environments. Retrieved Mac 2, 2018 from http://www.who.int/mediacentre/news/releases/2014/air-pollution/en/

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Isrn Ecology*, *2011*.

Wyszkowska, J., Boros, E., & Kucharski, J. (2007). Effect of interactions between nickel and other heavy metals on the soil microbiological properties. *Plant Soil and Environment*, *53*(12), 544-552.

Wyszkowska, J., Borowik, A., Kucharski, M., & Kucharski, J. (2013). Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. *Journal of Elementology*, *18*(4), 769-796.

Wyszkowska, J., Kucharski, J., & Lajszner, W. (2006). The Effects of Copper on Soil Biochemical Properties and Its Interaction with Other Heavy Metals. *Polish Journal of Environmental Studies*, *15*(6), 927-934.

Wyszkowska, J., Kucharski, J., Borowik, A., & Boros, E. (2008). Response of bacteria to soil contamination with heavy metals. *Journal of Elementology*, *13*(3), 443-453.

Xu, Q., Jiang, P., & Xu, Z. (2008). Soil microbial functional diversity under intensively managed bamboo plantations in southern China. *Journal of Soils and Sediments*, *8*(3), 177.

Yang, R., Tang, J., Chen, X., & Hu, S. (2007). Effects of coexisting plant species on soil microbes and soil enzymes in metal lead contaminated soils. *Applied Soil Ecology*, *37*(3), 240-246.

Yang, Z., Shi, W., Yang, W., Liang, L., Yao, W., Chai, L., ... & Liao, Q. (2018). Combination of bioleaching by gross bacterial biosurfactants and flocculation: A potential remediation for the heavy metal contaminated soils. *Chemosphere*, *206*, 83-91.

Yang, Z. X., Liu, S. Q., Zheng, D. W., & Feng, S. D. (2006). Effects of cadium, zinc and lead on soil enzyme activities. *Journal of Environmental Sciences*, *18*(6), 1135-1141.

Yargholi, B., & Azarneshan, S. (2014). Long-term effects of pesticides and chemical fertilizers usage on some soil properties and accumulation of heavy metals in the soil (case study of Moghan plain's (Iran) irrigation and drainage network). *International Journal of Agriculture and Crop Sciences*, *7*(8), 518.

Yong, Z. J., Bashir, M. J., & Hassan, M. S. (2020). Assessment of environmental, energy and economic prospective of anaerobic digestion of organic municipal solid waste in Malaysia. In IOP Conference Series: Earth and Environmental Science (Vol. 463, No. 1, p. 012054). IOP Publishing.

Zaidi, A., Khan, M. S., Ahemad, M., Oves, M., & Wani, P. A. (2009). Recent advances in plant growth promotion by phosphate-solubilizing microbes. In *Microbial strategies for crop improvement* (pp. 23-50). Springer Berlin Heidelberg.

Zeng, L. S., Liao, M., Chen, C. L., & Huang, C. Y. (2007). Effects of lead contamination on soil enzymatic activities, microbial biomass, and rice physiological indices in soil–lead–rice (Oryza sativa L.) system. *Ecotoxicology and Environmental Safety*, *67*(1), 67-74.

Zhang, M. K., Liu, Z. Y., & Wang, H. (2010). Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Communications in Soil Science and Plant Analysis*, *41*(7), 820-831.

Zhang, Y., Yuan, S., Feng, X., Li, H., Zhou, J., & Wang, B. (2016). Preparation of nanofibrous metal–organic framework filters for efficient air pollution control. *Journal of the American Chemical Society*, *138*(18), 5785-5788.

Zhong, W. H., Zu-Cong, C. A. I., & Zhang, H. (2007). Effects of long-term application of inorganic fertilizers on biochemical properties of a rice-planting red soil. *Pedosphere*, *17*(4), 419-428.

Zhu, H., Carlson, H. K., & Coates, J. D. (2013). Applicability of anaerobic nitrate-dependent Fe (II) oxidation to microbial enhanced oil recovery (MEOR). *Environmental Science & Technology*, *47*(15), 8970-8977.

Zhu, X. F., Lei, G. J., Wang, Z. W., Shi, Y. Z., Braam, J., Li, G. X., & Zheng, S. J. (2013). Coordination between apoplastic and symplastic detoxification confers plant aluminum resistance. *Plant Physiology*, *162*(4), 1947-1955.

Zin, M., Shaylinda, N., Aziz, H. A., Adlan, M., & Ariffin, A. (2012). Characterization of leachate at Matang Landfill site, Perak, Malaysia. *Academic Journal of Science*, *1*(2), 317-322.