

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of study

Environment, whether physical, biological or chemical, is composed of discrete components that are either biotic or abiotic, and are extremely related and dependent on each other for optimal functioning. Therefore, any interference with the natural state of these components, especially to an extreme condition, often leads to ecosystem disruption, ecological imbalance/stress and even global deterioration. Such interferences most often stem from the quest to satisfy one need or the other at the expense of another, without due caution on the potential consequences. However, such consequences when they occur have ways of attaining solutions, executing corrections and potentially itching for reclamations.

Most environmental consequences arise from the phenomenon known as “Pollution”, which is the occurrence of contaminants within a pre-existing natural environment with the aftermath effect of initiating adverse change. And considering the three basic domains of the earth; air, water and soil, pollution have been an age long concern. Regardless of the source of contaminants or point of contamination, pollution whether it is air, water or soil, can be borderless; hence the reason for the associated global impacts. In fact, air, water and soil pollutions in forms of particulate matter emissions, sea oil spills and polychlorinated biphenyls-impacted soil, respectively abound. These do occur to the magnitude that questions have been asked- ‘what/who is the cause?’

Definitely, the answer is humans- the embodiment of the biological part of the ecosystem that is saddled with the utmost responsibility of resource utilization and management. The wants of humans are insatiable and with rapid growth in population,

increase in demands and supply become inevitable. Changing lifestyle, globalization, infrastructural developments, renewed income earning trends and some other socio-cultural/economic inclinations have driven a different wave of anthropogenic activities against the backdrop of what is obtainable in the past, especially before the “March to civilization” era. Such wave of anthropogenic activities do not only help to attain economic empowerments and societal developments but have as well become the bane of most environmental pressure of which pollution is part of it. However, among the various aspects of anthropogenic activities that cause problems to our immediate environment, waste generated is of significant interest.

Environmental pressures from the generation and management of waste include but not limited to emissions to air (including greenhouse gases), water and soil, all with potential impacts on human health and nature (Fauziah et al., 2013; Emenike et al., 2012a). A number of waste management options are being used in the contemporary era to ensure disposal and treatment of waste, among which includes, composting, incineration, land/seafilling, and recycling. Whereas the developed economies of the world adopt more advanced, cleaner and sustainable principles towards waste minimization and handling, the developing societies are yet to embrace nascent technologies pivotal to managing waste in a manner that has less/or zero negative impact on the immediate environment. Landfilling of waste is one of such management options that involve less technology and energy dissipation in comparison to other systems that are more expensive, time consuming and high-tech oriented. Yet, the issue is whether there is any negative side to landfilling?

Therefore, landfilling remains the dominant waste disposal method in most Asian and developing countries. About 75% of the municipal waste generated in Malaysia is landfilled and this has significant pressure on the environment, as only 5% is recycled, while the rest are either burned or dumped into rivers or at illegal sites (Agamuthu et al., 2009). All landfills produce leachate which is liquid produced by the action of “leaching” when rain water percolates through any permeable material. As such, streams and other forms of water bodies are contaminated with leachate due to the vertical and lateral migration of leachate (Jaffar et al., 2009), if there are no geomembrane liners.

Groundwater and other forms of water course are precious part of the ecosystem and in order to prevent or minimize the possibility of water pollution, the degree of planning, engineering, waste stream control and management undertaken at municipal waste landfills has increased dramatically in recent years. The potential and degree of risk posed to groundwater, soil and even aquatic life by landfill leachate is extremely difficult to assess (Emenike et al., 2012a). Leachate composition varies based on the materials present in landfill, i.e. dissolved organic matters (alcohols, acids, aldehydes, and short chain sugars), inorganic macro components (common cations and anions including sulphate, chloride, and ammonium), heavy metals (Pb, Ni, Cu, Hg), xenobiotic organics and polychlorinated biphenyls (PCBs) (Ludwig et al., 2003; Christensen et al., 2001). Therefore, the need for a critical study of the composition of ‘modern’ municipal waste landfill leachate had been evaluated (Murray & Beck, 1990). The study opined that leachates may contain toxic and hazardous compounds, hence there is need to properly evaluate leachates from municipal waste landfills. Such investigation is absolutely necessary as landfilling is the predominant method of

municipal waste disposal in most industrialized countries (Carra & Cossu, 1990) and developing nations as well.

Due to the composition of wastes, during storage or disposal of municipal solid waste (MSW), wastewater is separated and is polluted by organic materials, heavy metals, and other toxic substances. The amount of leachate depends on the initial water content of the MSW, and the storage or disposal conditions such as temperature, humidity, and ventilation (Selic et al., 2007). Leachate is the potentially polluting liquor that accumulates beneath a landfill site resulting from the infiltration and percolation of rainfall, groundwater, runoff, or flood water into and through an existing or abandoned solid waste landfill site.

Landfill leachate is characterized by high levels of salts and  $\text{NH}_x\text{-N}$ , as well as, high organic concentration. Higher organic loading yields greater substrate availability for planktonic and epiphytic bacteria, and may induce inhibitory effect on sedimentary bacteria (Wendong et al., 2007). More than 200 organic compounds have been identified in municipal landfill leachate (Schwarzbauer et al, 2002), with about or more than 35 compounds having the potential to cause harm to the environment and human health (Paxeus, 2000). High level of ammonia is present in many older landfills, and is toxic to many living organisms in surface water and contributes to eutrophication, and dissolved oxygen depletion (Bae et al., 1997).

In terms of solid waste management, Malaysia is characterized with many uncontrolled landfills without appropriate bottom liners and leachate collection systems, and there are about 291 landfills of different sizes and ages recognized officially with an estimated

three times more illegal dumps (Emenike et al., 2012; Fauziah & Agamuthu, 2010). At the exception of a few, most of the landfills are devoid of sanitary status as they are characterized of none or inadequate leachate collection and/or treatment facilities and also lack infrastructure to exploit landfill gas (Fauziah & Agamuthu, 2010). Toxicological evaluations of the landfill leachate are in great demand, to ensure safe discharge of leachate from landfills. Now, it has gradually begun to be incorporated into the environmental legislations in some countries (Eun-ah et al., 2009).

Chemical oxidation has been developed as a method for the early-stabilization of landfills. However, by-products that are difficult to detect by chemical analysis can be compensated by toxicological evaluation. Therefore, toxicity tests have become useful tools for detecting the changes of leachate quality to complement the chemical oxidation method (Eun-ah et al., 2009). Both mortality and behavioural effect of landfill leachate on *Cyprinus caprio* had been evaluated (Jaffar et al., 2009). Toxicity of municipal dump leachate was tested on zebra fish (*Brachydanio rerio*) (Sisinno et al., 2000), while different concentrations of leachate were utilized to analyze the survival ability of tilapia (*Sarotherodon mossambicus*) (Wong, 1989). Another study had used larve and adult of Japanese Medaka (*Oryzias latipes*) to test the toxic potency of landfill leachate (Osaki et al, 2006). However, the absence of landfill toxicity data on a number of fresh water local fishes is a subject of concern due to their dominance and high economic value in the tropical and some temperate countries (Emenike et al., 2012a). More so, some of the toxicants associated with leachate have the potential of building up within the living systems in form of bioaccumulation.

In as much as, acute mortality and chronic conditions are vivid impacts of most effluents on the aquatic life, yet some other effects might be initiated without any observable changes. One of such effects is bioaccumulation. A number of toxicants are built up in living systems as a result of exposure. Therefore, bioaccumulation tests are used as a means of assessing and evaluating the potential build up of these toxicants within a given species with the view of extrapolating the tendency of absorption by another level of organism along the food chain/web hierarchy.

The determination of heavy metal accumulation in aquatic organisms especially fish is very important in order to assess the potential risk in the event of consumption of such organism by man. These metals in leachate may not necessarily exist as discrete elements or cations but might be in the form of metal complexes. Therefore, the fact that some metals like Zn, Mn and Fe play significant roles in biological systems (Tuzen, 2003) should not imply that they are non-toxic. Some studies had reported heavy metal uptake in fish where excessive intake of these metals can initiate toxic effects (Ubillus et al., 2000; Tuzen, 2003). While some landfills might have low concentrations of heavy metals (Kjeldsen et al., 2002), landfills in some places especially Asia have shown high heavy metal concentrations in leachate from closed and active landfills (Robinson, 2007; Emenike et al., 2012a; Agamuthu et al., 2011).

Therefore, considering the fact that soil is often a barrier between surface water and leachate from landfills/dump sites, it is necessary to control/or even prevent the eventual seepage of some contaminants into surface water. Among such leachate components is heavy metals, hence a suitable soil remediation approach is required. However, any suitable soil remediation approach should be sustainable.

This is to imply that any adopted technology to this respect of soil restoration, should preserve the soil quality and functions. Bioremediation is a nascent technology that meets such soil requirement. In as much as that this form of technology is based on the principle of microbes utilization, yet the build up of most highly toxic contaminants in the ecosystem proves that microorganisms, by themselves, are deficient to face the flux of anthropogenic pollution (Sprocati et al., 2011).

## **1.2 Problem Statement**

Toxicity testing, being a combination of techniques and principles ensures proper evaluation of resultant effects of some substances. Regardless of the fact that substances or compounds possess certain chemical and physical properties that helps to predict their perceived impact on our immediate environment, the effect of such substances when existing as mixture, makes impact evaluation or prediction difficult. Therefore, toxicity testing becomes paramount to understanding such perceived or unknown effects, and serves as a basic tool for identifying and assessing a better control mechanism. Priority is given to living organisms while analyzing the potential impact of a substance. This is to ensure conservation and sustainability. Toxic compounds can initiate varying effects on living system, hence evaluation of short term effect (often mortality impact) via acute toxicity test, and long term observation (chronic toxicity test) are employed to analyze the effect of such compounds (Buratini et al., 2004).

Toxic compounds may not exist in discrete forms but rather maybe a mixture in any form of the three kinetic states of matter. The most common is effluent form which is liquid/semi-fluid discharge emanating from various anthropogenic activities, especially

production and degradation (Emenike et al., 2013). Identifying the discrete compounds that will cause toxicity is an upheaval task considering the fact that effects may vary based on combination of many compounds. This is the reason for undertaking a whole effluent toxicity (WET) testing (Emenike et al., 2013).

In this research, raw leachate is the whole effluent of interest. Although the toxicity of landfill leachate was studied for numerous fish species, there is complete lack of landfill leachate toxicity data for most fish species which are widespread and presently cultured all over Asia in general and Malaysia in particular. Therefore, there is a need to identify fish species that can serve as bio-indicator of leachate pollution in Malaysia. In addition to that, the limitation of landfill leachate toxicity studies for Malaysian landfills in the open literature is the basis for this study.

As Asia is richly endowed with water bodies that serve as environment for aquatic organisms especially different fish species, assessing the impact of pollutants require critical evaluation because such environment have both ecological and economic relevance (Agamuthu et al., 2011). Solubility can enhance toxicity of compounds and it can be noted that a number of solids in leachate are soluble in water. Chian and Dewalle's (1976) study of 12 landfills proved that the reason for low concentration of total suspended solids (TSS) in leachate (averagely less than 2.5% of the total solids), is because most of the solids dissolve in the leachate. Such condition enhances the complexity of leachate characterization in relation to its potential environmental impacts.

However, regardless of the fact that leachate may contain many compounds; heavy metal content is a major concern due to the persistent nature. Even in the marine

environment, heavy metal pollution has been considered a serious environmental issue for a long time (Tuzen, 2003). Bioaccumulation of heavy metals in aquatic organism is a threat to human health. Giordano et al. (1991) reported that marine organisms and sediments easily accumulate pollutants which are eventually taken up by man via food chain/web. The movement of heavy metals in leachate tend to be enhanced by the complex nature of the contained organic and colloidal matter (Jensen et al., 1999).

Though most concerns about leachate only point towards groundwater and surface water pollutions, but considerations need to be accorded to soil contaminated with leachate. In most cases, leachate percolates through soil membrane before getting to surface waters like river, streams and lakes. Soil serves as the backbone of most terrestrial interactions. Excluding the physical and chemical characteristics of soil, the biological quality is very crucial. Therefore it is worthy of note that microorganisms play significant role in the soil. The microbial population and diversity has impaired consequent upon soil contamination by heavy metals and other chemical constituents. Leachate is often characterized with different concentrations of heavy metals, PCBs and other compounds (Ludwig et al., 2003); hence it creates much concern as it may impose stress on agricultural activities and even on some environmental bioremediation like composting, during its interaction with the soil microbial flora. Comparison of the diversity, activity and the composition of soil microbial community could be useful in evaluating the relationships of different environmental conditions on soil habitat (Lorena *et al.*, 2005) and thus lead to an effective bioremediation purpose which includes bioremediation of leachate contaminated soil.

Bioremediation applications are still being affected by severe bottlenecks, and as such, exploiting the intrinsic bioremediation potential of a contaminated soil for translating it

into improved bioremediation technologies is the daunting task which this research is trying to address. Availability of toxic metals inhibits/obstruct the activities of the degrader bacteria, and as such, co-contamination caused by both heavy metals and other organic compounds is a major complication (Sprocati et al., 2011).

### **1.3 Research Hypothesis**

The research therefore hypothesizes as follows;

1. The varied impact of leachate is a reflection of the variation in the heterogenous nature of leachate across different landfills rather than differences in exposed fish species/types.
2. Bioaugmentation is expected to be a sustainable approach to the reduction/removal of heavy metals from leachate polluted soil.

### **1.4 Objectives**

The objective of this doctoral dissertation includes the following;

- i. To characterize and compare the physico-chemical composition of both sanitary and non-sanitary landfills (active and non-active) leachate in Malaysia.
- ii. To assess the mortality, tissue impairment and bioaccumulation of heavy metals among fishes due to leachate pollution
- iii. To propose a leachate toxicity index in Malaysia.
- iv. To assess the distribution of microbes in leachate contaminated soil
- v. To bioremediate leachate contaminated soil (heavy metals) using leachate-site persistent microbes.
- vi. To use a kinetic model in generating the removal rate of heavy metals during bioremediation of leachate contaminated soil.

Therefore this work has been subdivided into chapters in order to enhance easy and proper concept of the study. Chapter one has formed the introductory stage with an overview of leachate toxicity and potential bioremediation option, while itemizing the objectives and hypothesis of this research. Literature review characterized chapter two, where the relationship that exists across waste generation, leachate toxicity and bioremediation interest is explained in details as obtained from most previous studies. Chapter three shows the methodology, result and discussion on the leachate toxicity studies as performed in this research, whereas chapter four explains the bioremediation aspect of this study. A general summary of the findings is explained in chapter five, while conclusions and recommendations on the study are in chapter six.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **SECTION A – LEACHATE STUDY**

##### **2.1 Introduction**

Malaysia has turned out to be among the centre of world's economic platform. In fact, economic empowerment and adaptation to the globalized lifestyle had made Asia in general a beehive of industries and sophisticated markets. This lead to the increased generation of both hazardous and non-hazardous wastes. Since waste generation is inevitable, managing it from the point source to the final disposal becomes a subject of concern due to perceived and identified impacts on our immediate environment. Waste is the accrued by-product of anthropogenic activities. Various forms of waste emanate as household waste, industrial/hazardous waste, and toxic waste, but the significant interest is based on the fact that they pose environmental threat. Hence the need for waste management and disposal arises. Unfortunately, the lack of useable and comparable data for many countries does not always allow reliable comprehensive assessment of waste-related issues. The generation of waste reflects a loss of materials or resources and energy, and imposed economic and environmental costs on society for its collection, treatment and disposal (Brunner & Fellner, 2006). Waste forms an increasing part of the total material flow through the economy (Brunner & Rechberger, 2004).

Waste is an issue in every Asian country, and waste quantities are generally growing. With over almost 60% of world's population situated in Asia, the region especially South East Asia is presently the most dynamic region in the world in reference to economic activity and industrialization. A closer look at the average annual percentage increase in real Gross Domestic Product (GDP) reflects the fastest economic growth by

nations like Vietnam, Malaysia, Indonesia, Singapore, Taiwan, Thailand and South Korea (Nguyen Ngoc & Schmitzer, 2008).

Today, it is undeniable that more than half of the world's growing population lives in urban area, which in turn cause enormous pressure on the immediate environment. Particularly in the large agglomerations of the developing countries, inadequate waste management is the cause of serious urban pollution and health hazards. Highly industrialized nations are facing an ever-increasing load of wastes and declining landfill space to dispose these materials. This is to imply that for nearly two decades now, the globe is challenged with the task of undertaking sustainable management of waste with the overall goal of minimizing its impact on the environment in an economically and socially acceptable way (Ludwig et al, 2003).

The impact of waste on the environment, resources and human health depends on its quantity and nature. Environmental pressures from the generation and management of waste include emissions to air (including greenhouse gases), water and soil, all with potential impacts on human health and nature.

Though a number of waste management options abound, such as incineration, composting, 3R (reduce, reuse and recycle), yet landfilling remains the dominant waste disposal method in most Asian and developing countries. All landfills produce leachate. The unscientific management of municipal solid waste will lead to contamination of soil. In turn, the contamination of soil results in the pollution of groundwater due to heavy metals and toxic organic compounds. When groundwater is contaminated, it may take some years to get purified. Groundwater may be susceptible to contamination with

the abundance of nearby dumping sites. This is mainly due to percolation of leachate. The quantity of leachate is often more in some countries due to heavy rainfall (Kortegast et al., 2007).

Leachate migrates vertically and laterally into the environment by direct discharge into the adjacent streams or other forms of water body (Jaffar et al., 2009). Asia is richly endowed with water bodies and expanse of fertile land that serve as serene environment for aquatic organisms especially fish and terrestrial microorganisms, respectively. Due to these aquatic and terrestrial considerations that have both ecological and economic importance, the impact of leachate and its constituents need to be investigated in order to control pollution damages.

Leachate composition varies based on the materials present at landfill, i.e. dissolved organic matters (alcohols, acids, aldehydes, and short chain sugars), inorganic macro components (common cations and anions including sulphate, chloride, and ammonium), heavy metals (Pb, Ni, Cu, Hg, and others), xenobiotic organics and Polychlorinated Biphenyls (PCBs) (Ludwig et al., 2003). It is paramount to note that even the best liner and leachate collection system will ultimately fail due to natural deterioration. Recent improvements in landfill containment technologies suggest that releases may be delayed by many decades at some landfills. Though plastics are inert, the state-of-the-art plastic (HDPE) landfill liners (2.5mm thick) and plastic pipes which allow chemicals and gases to pass through their membranes can become brittle, swell and eventually break. Therefore, the risk of leachate emission to the adjacent area is inevitable.

## **2.2 Background of Solid Waste**

Solid waste is termed useless, unwanted and discarded non-liquid waste materials arising from domestic, trade, commercial, industrial, agriculture, as well as, public services. It is inextricably associated with resource consumption, urban lifestyle, attitude, education, regulation and economic activity (Taieba, 2008).

In the modern conceptual definition - Solid waste management encompasses all activities such as generation, storage, collection, transportation, processing, treatment, and disposal. It is in accordance with the best principle of public health, economics, engineering, conservation, aesthetic and other favorable environmental considerations in the framework of administrative, financial, legal, planning and engineering functions (Taieba, 2008).

The problems associated with municipal solid waste (MSW) management have acquired an alarming dimension in the developing countries during the last few decades. This is due to high population growth rate and increase of economic activities in the urban areas of developing countries combined with a lack of infrastructures, appropriate system and associated training, awareness and commitment in service. Compared to developed countries, the urban residents of developing countries produce less per-capita solid waste, but the capacity of the developing countries to collect, process, dispose or reuse it in a cost effective way is limited. The waste generated by human settlements and the associated problems are similar in the developing nations with variations between regions and locations based on geographic, socio-cultural, industrial, infrastructural, legal and environmental factors.

Management of vast quantities of solid waste generated by urban communities is a very complex process. Due to severe financial constraints, lack of motivation and the absence of effective legislation to protect the environment and to handle waste, the whole system is becoming a threat for city dwellers, planners and other concerned stakeholders. Inadequate or unavailable solid waste collection and disposal services result in the indiscriminate dumping of waste on streets and in public areas, clogging of urban drainage systems, contaminating water resources, and proliferating insects and rodent vectors. Industrial wastes are generally dumped and discharged on the nearest land, in ditches, canals, ponds, lakes and even in the rivers running within or besides major cities. Such conditions increase health risks by direct human contact with solid waste, and constitute major factors in the spread of gastrointestinal and parasitic diseases (Muhammed et al, 2005). In order to have a clear understanding of landfill use, an overview of solid waste generation is required.

### **2.3 Waste Sources**

The sources and types of MSW with the data composition and generation are the basic parameters in the design and operation of the functional elements associated with the management of solid waste (Tchobanoglous & Kreith, 2002). Sources of waste in a community are usually related to land use and zoning. In general, sources of MSW are categorized as residential, commercial, institutional, and street sweepings (Chan, 1993).

*Residential sources:* Residential wastes are the main sources of MSW in many developing nations. Major portion are generated due to household activities. The types of dwellings are single family, multi-family, low, medium and high-rise apartments. These wastes include food wastes, rubbish, ashes and others.

*Commercial sources:* Solid wastes in commercial sources are generated from stores, restaurants, markets, hotels, service station and others. These wastes include papers, plastics, packaging materials and others.

*Institutional sources:* the sources of these wastes are mainly universities, schools, hospitals, clinics, pathological laboratories, prisons, government and private centers/office/institutions. These wastes include mainly paper, plastics, office articles and medical waste.

*Street sweepings:* These wastes are mostly generated in open areas such as streets, alleys, parks, highways, vacant lots, playgrounds, beaches, terminals, and recreational areas. Street sweepings include dust, rubbish and others.

The term generator means any person, by site or location whose act or process produces solid waste or first causes it to become regulated (Alamgir et al., 2005). The locations of generators of MSW according to different sources are given in Table 2.1.

**Table 2.1 Typical location of solid waste generation associated with various sources**

Sources	Locations where wastes are being generated
<b>Residential</b>	Slum, town, houses, single family and multifamily detached dwellings, duplexes, low, medium, and high-rise apartments.
<b>Commercial</b>	Stores, restaurants, markets, shopping malls, city centers, hotels, motels, warehouses, print shops, service stations, airports and auto repair shops.
<b>Institutional</b>	Schools, hospitals, prisons, medical facilities, governmental and private offices/centers/institutions.
<b>Industrial</b>	Packaging of components, office wastes, lunchroom and restroom wastes (but not industrial process wastes)
<b>Open area</b>	Street cleaning, landscaping, catch basin cleaning, parks and beaches, and other recreational areas.

Source: Tchobanoglous et al., 1993

## 2.4 Solid Waste Generation in Asia

Population growth can increase the amount MSW significantly. The waste generation rate increased from 0.7kg/day in 1990s to 1.2kg/day in 2000 (Agamuthu, 2001) in the developing countries like Malaysia. Currently in Malaysia, the solid waste generation is expected to reach 1.5 kg/day in most cities, since it was already 1.3 kg/day in 2006 (Agamuthu et al., 2009). Nations like US, Sweden, Germany and UK generated about 1.96, 1.40, 1.58, and 1.54 kg/day of waste respectively (USEPA, 2012). Approximately, 1.3 billion tonnes per year of MSW is generated globally (considering data from 161 countries) and is expected to reach 2.2 billion tonnes per year by 2025 (World Bank, 2012). However, the generation can also vary with the level of income, urbanization trend, changing food habit, social and cultural habits, and lifestyle changes. Seasonal variations also influence the amount of waste generation (Taieba, 2008). Table 2.2 shows the generation of MSW in Malaysian States for the years 2003-2008. Also Figure 2.1 shows the waste generated in some countries in 2012, where Switzerland and the US generated 700 and 720 kg per capita, 2012.

Table 2.2 Malaysia MSW Generation

	<b>Solid Waste Generated (tonnes/day)</b>					
	<b>2003<sup>1</sup></b>	<b>2004<sup>1</sup></b>	<b>2005<sup>2</sup></b>	<b>2006<sup>2</sup></b>	<b>2007<sup>2</sup></b>	<b>2008<sup>2</sup></b>
Johor	2199	2310	2391	2475	2561	2651
Kedah	1520	1597	1653	1711	1771	1833
Kelantan	1188	1248	1292	1337	1384	1432
Melaka	590	620	642	664	687	711
Negeri Sembilan	869	913	945	978	1012	1047
Pahang	1099	1154	1194	1236	1279	1324
Perak	1753	1842	1906	1973	2042	2113
Perlis	224	235	243	252	261	270
Pulau Pinang	1248	1312	1358	1405	1455	1506
Selangor	3245	3410	3529	3653	3781	3913
Terengganu	1013	1064	1101	1140	1180	1221
Kuala Lumpur	2893	3040	3146	3257	3371	3489
WP Labuan	72	75	78	80	83	86
Sabah	2641	2802	2900	3002	3107	3216
Sarawak	1982	2063	2135	2210	2287	2367
<b>Total</b>	<b>22544</b>	<b>23691</b>	<b>24514</b>	<b>25372</b>	<b>26260</b>	<b>27179</b>

Note <sup>1</sup>Source: Ministry of Housing and Local Government, 2003.

<sup>2</sup>Figures calculated based on 3.5% annual increase from MHLG (2003).

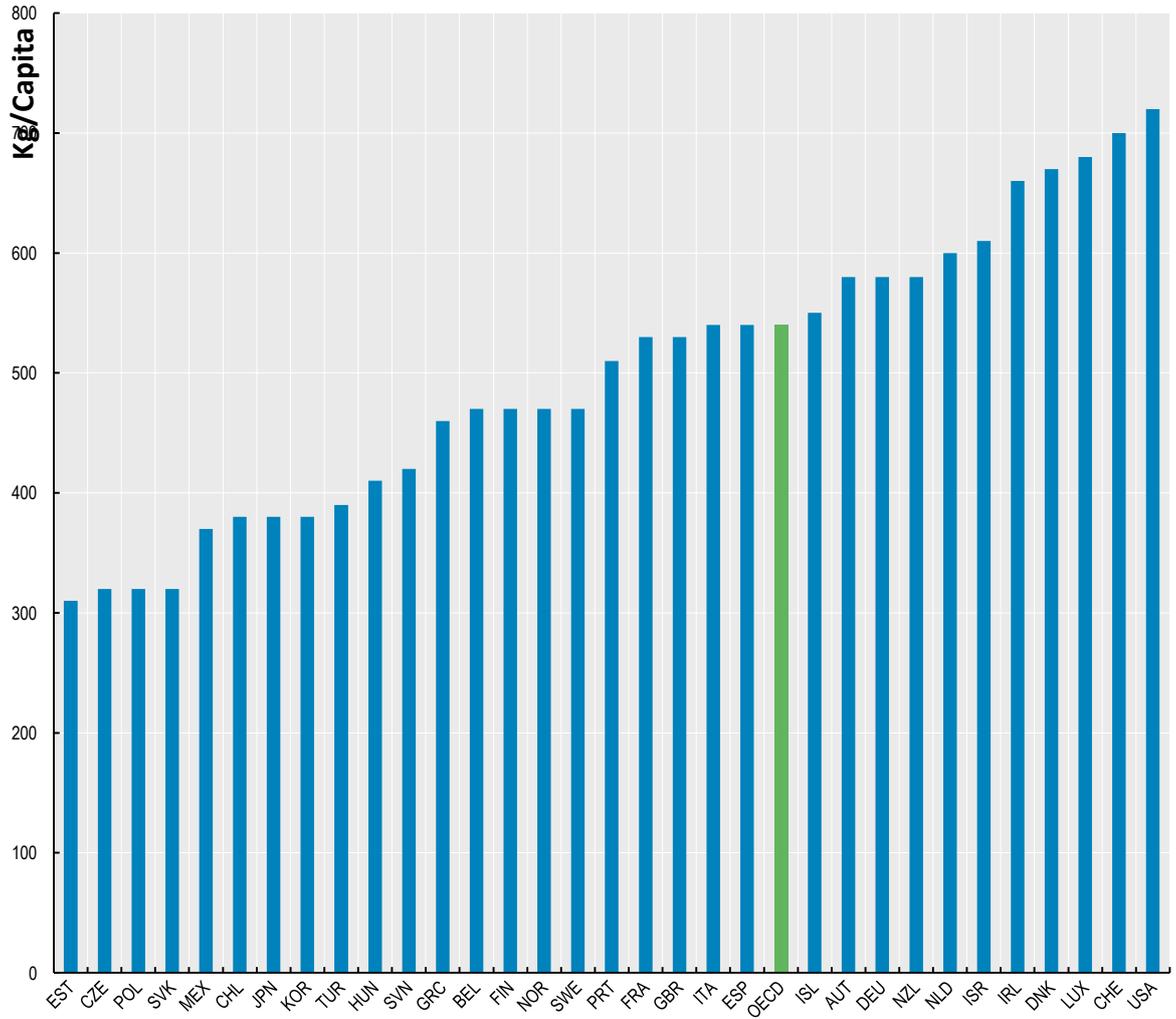


Figure 2.1. Municipal Waste Generation kg per capita, 2012 (OECD, 2013).

Residential wastes are the main sources of MSW in Malaysia. The other important sources are commercial wastes including markets, hotels and restaurants. Institutional wastes include the wastes from school, college and universities, as well as, the offices. Saeed et al., (2009) presented a forecasting study of MSW generation rate and potentials of its recyclables in Kuala Lumpur, Malaysia as shown in Tables 2.3 and 2.4.

MSW also include wastes from street sweeping and drain cleaning. The study analyzed the generation rates of various classes such as street cleansing, landscape and garden, industrial and construction, institutional, residential and commercial.

**Table 2.3 Estimation of Total MSWG of Kuala Lumpur**

Year	Population of KL city millions	MSWG Kg/Cap./day	MSWG tonnes/day	MSWG tonnes/year
<b>2008</b>	2.34	1.62	3798.88	1383642.0
<b>2010</b>	2.53	1.69	4274.86	1560323.9
<b>2012</b>	2.74	1.76	4810.49	1755828.9
<b>2014</b>	2.96	1.83	5413.23	1975828.9
<b>2016</b>	3.20	1.90	6091.49	2223393.9
<b>2018</b>	3.46	1.98	6854.73	2501976.5
<b>2020</b>	3.75	2.06	7713.61	2815467.7
<b>2022</b>	4.05	2.14	8680.09	3168232.9
<b>2024</b>	4.38	2.23	9767.68	3565203.2

(Adapted from Saeed et al., 2009)

**Table 2.4 Estimation of Sectoral MSWG of Kuala Lumpur (tonnes/day)**

Year	Residential (48%)	Street cleansing (11%)	Commercial (24%)	Institutional (6%)	Construction and industry (4%)	Landscape (7%)
<b>2008</b>	1823.46	417.88	911.73	227.93	151.96	265.92
<b>2010</b>	2051.93	470.24	1025.97	256.49	170.99	299.24
<b>2012</b>	2309.04	529.15	1154.52	288.63	192.42	336.73
<b>2014</b>	2598.35	595.46	1299.17	324.79	216.53	378.93
<b>2016</b>	2923.91	670.06	1461.96	365.49	243.66	426.40
<b>2018</b>	3290.27	754.02	1645.14	411.28	274.19	479.83
<b>2020</b>	3702.53	848.50	1851.27	462.82	308.54	540.00
<b>2022</b>	4166.45	954.81	2083.22	520.81	347.20	607.61
<b>2024</b>	4688.49	1074.44	2344.24	568.06	390.71	683.73

(Adapted from Saeed et al., 2009).

In 2009, Hong Kong generated about 6.45 million tonnes of solid waste which is more than double that of 1990 levels. Table 2.5 shows the waste generation rate in 2009 and its projection for 2025 in selected countries in Asia. The average per capita generation of MSW by a Malaysian is 1.3 kg daily; whereas 0.7kg is generated in Vietnam and Laos. While Bangladesh and Indonesia recorded an average daily per capita waste generation of 0.25 kg and 0.75 kg, respectively. India and Pakistan on the other hand generate 0.4 kg each. Variations and rise in per capita waste generation is highly

dependent on the country's socio-economic inclinations (Agamuthu et al., 2011). The amount of solid waste generated in the cities is much higher than in rural areas.

Table 2.5 Waste generation in 2009 and waste projection for 2025 in selected Asian countries

<b>Country</b>	<b>Waste Generation Rate (Kg/cap/day), 2009</b>	<b>Projected Waste Generation in 2025 (Kg/cap/day)</b>
Brunei	0.66	0.95
Cambodia	0.52	1.10
Indonesia	0.76	1.00
Laos	0.55	0.90
Malaysia	1.30	1.40
Myanmar	0.45	0.85
Philippines	0.52	0.80
Singapore	1.10	1.10
Thailand	0.64	1.50
Vietnam	0.67	1.00
Nepal	0.40	0.60
Bangladesh	0.25	0.60
Mongolia	-	0.60
China	0.80	0.90
Sri Lanka	0.2 – 0.9	1.00
Republic of Korea	1.00	1.40
Japan	1.10	1.30

(Agamuthu et al., 2011)

The generation rate in rural areas can be as low as 0.15 kg/cap/day, while in the urban areas the rate can reach up to 1.0 kg/cap/day (Environmental Management Centre, 2007). Table 2.6 shows the waste generation rate among some major cities in Asia.

Table 2.6 Solid waste generation rates of some major Asian cities

City	Country	Generation Rate (Kg/cap/day)
Delhi	India	0.47
Dhaka	Bangladesh	0.50
Urban	Islamic Republic of Iran	0.80
Penang	Malaysia	0.98
Katmandu	Nepal	0.30
Manila	Philippines	0.66
Singapore	Singapore	0.94
Colombo	Sri Lanka	0.62
Taipei	Republic of China	0.95
Bangkok	Thailand	0.88
Hanoi	Vietnam	0.63

Adopted from Environmental Management Centre (2007).

## 2.5 Municipal Solid Waste Composition among Asian Nations

MSW in a simple sense is a heterogeneous mixture of wastes; organic and inorganic, rapidly and slowly biodegradable, and hazardous and non-hazardous generated, from various sources. Within Malaysian cities, food and vegetable wastes are the dominant components in the waste stream. In Agamuthu et al., (2011), organic waste constitute 55% of the total waste stream in Malaysia (Table 2.7) as against 19.8% obtained in the neighbouring country, Singapore (Table 2.8).

Table 2.7 Waste Composition (% wet weight) in Malaysia from 1980 -2010

Waste Composition	1980	1985	1990	1995	2000	2005	2010
Organic	54.4	48.3	48.4	45.7	43.2	44.8	55.0
Paper	8.0	23.6	8.9	9.0	23.7	16.0	13.0
Plastics	0.4	9.4	3.0	3.9	11.2	15.0	19.0
Glass	0.4	4.0	3.0	3.9	3.2	3.0	2.0
Metal	2.2	5.9	4.6	5.1	4.2	3.3	3.0
Textiles	2.2	NA	NA	2.1	1.5	2.8	4.0
Wood	1.8	NA	NA	NA	0.7	6.7	1.0
Others	0.3	8.8	32.1	4.3	12.3	8.4	3.0

(Source: Agamuthu et al., 2009; Agamuthu, 2011)

The study showed that organic waste content was approximately 50% in the 1980s and 1990s and was mainly comprised of kitchen and food waste. Some other waste

components are polythene and plastics, paper and paper products, textile and woods, glass and ceramics, rubber and leathers, metal and tins. However, by 2007 about 46% of total waste stream was attributed to putrescible waste while paper waste and plastic-based waste contributed 14% and 15% respectively (Agamuthu et al., 2009).

Most Asian nations show high percentage of food waste (organic) in the solid waste stream (Table 2.8). However, this may vary based on population and level of development. Cambodia and Myanmar recorded approximately 70% of organic waste generation unlike the Republic of Korea and Japan which showed an average of 20% (Agamuthu et al., 2011; Borongan & Okumura, 2010).

**Table 2.8. Waste Composition of Asian Nations**

Country	Municipal Solid Waste Composition (%)					
	Food waste (organic)	Paper	Plastic	Metal	Glass	Others
Brunei	36	18	16	4	3	23
Cambodia	63.3	6.4	15.5	0.6	1.2	13
China	49	16	16	2	1	16
Indonesia	63	11	10	1	1.5	13.5
Japan	15	50	20	2	1	4
Republic of Korea	26.3	21.4	8.9	8	4.7	30.7
Lao PDR	30	15	30	25	25	-
Malaysia	55	15	14	4	3	17
Mongolia	16.8	25.2	12.1	2.5	4.4	39
Myanmar	73.7	2.24	17.75	0.20	0.45	6.09
Philippines	32.7	12.5	24.7	5	3.1	22
Singapore	19.8	22.8	22.8	3.36	2.32	28.92
Thailand	43	12.1	10.9	3.5	6.6	23.9
Vietnam	41.9	1.9	15.6	6	7.2	27.4

Adopted from Borongan and Okumura (2010)

These components may vary with the sources. Figure 2.2 shows municipal solid waste composition of seven OECD countries in comparison to seven Asian cities in 2002. The

bulk of the waste generated in Asia is landfilled. The detailed breakdown of all possible major types of MSW according to source is presented in Figure 2.3

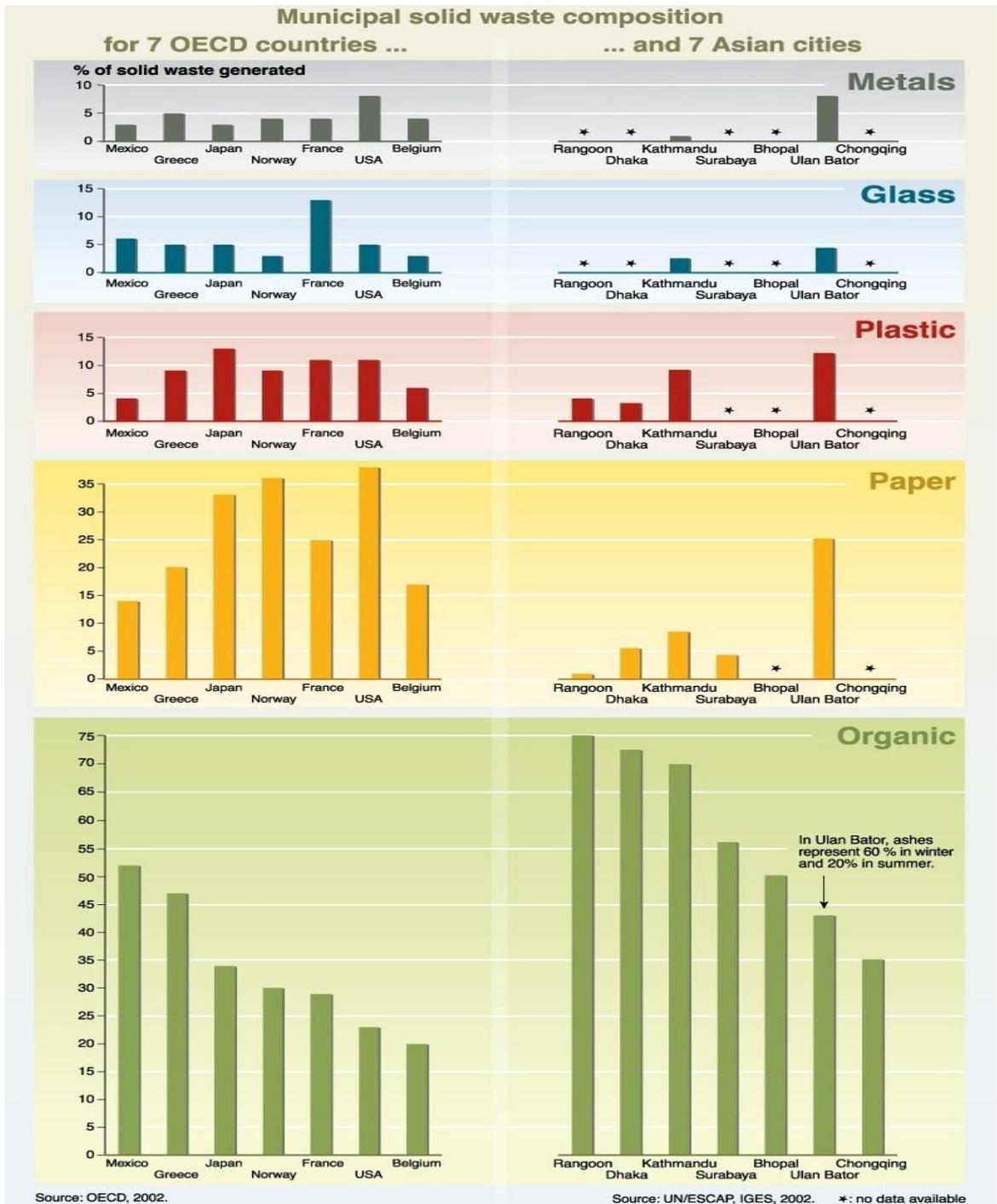
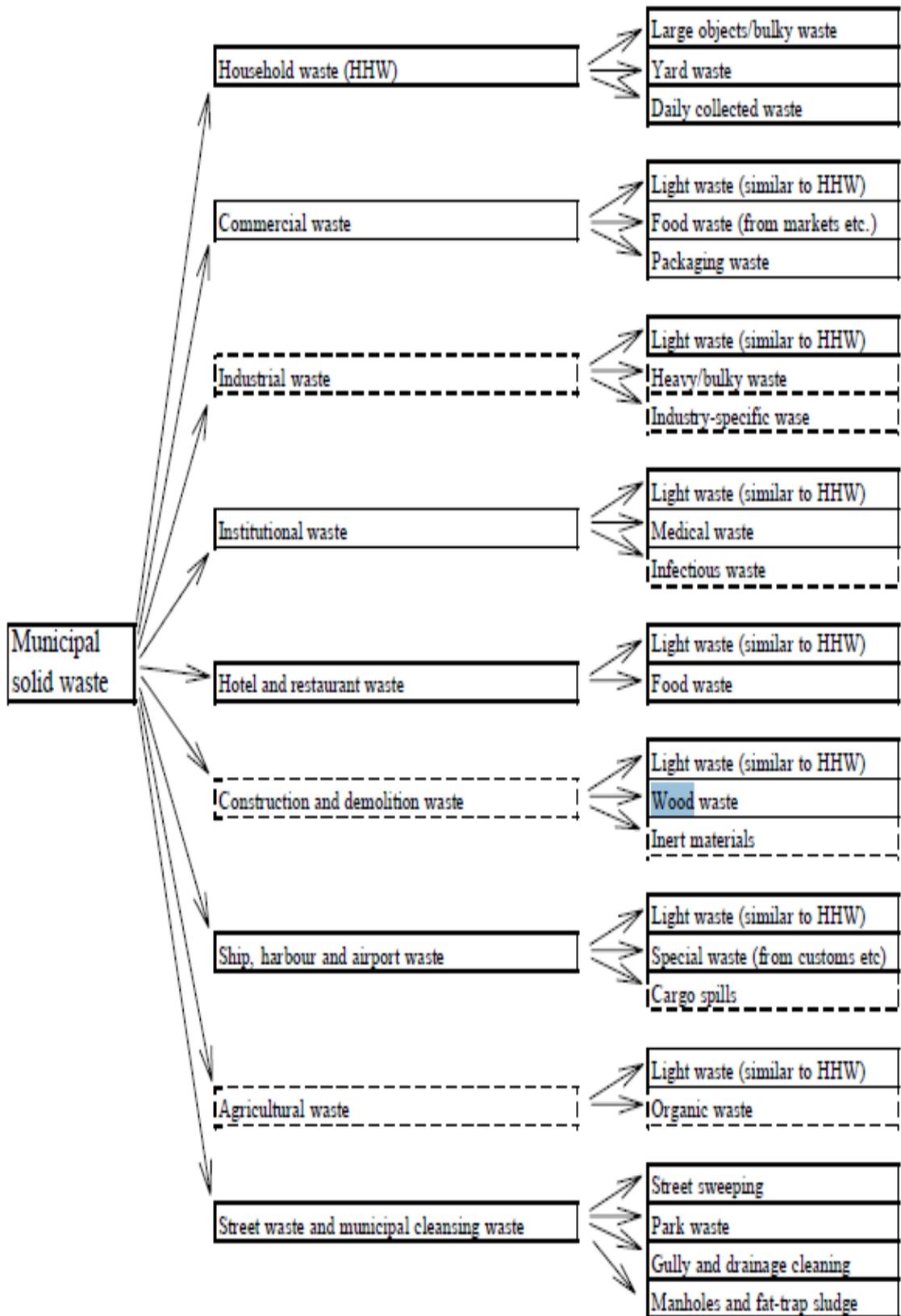


Figure 2.2 MSW compositions in OECD countries and Asian cities  
Source: <http://www.vitalgraphics.net/waste> (accessed 16th July, 2010)



Note: The waste types in the dotted frames only partly go into municipal solid waste stream.  
 Figure 2.3 Breakdown of major types in MSW (Jensen & Pipatti, 2004)

## 2.6 Characteristics of MSW

Generally, MSW is identified by the following four major characteristics;

1. Physical characteristics
2. Chemical characteristics
3. Biological characteristics
4. Mechanical characteristics

Determination of physical and chemical characteristics of solid wastes or its components would be necessary in order to ascertain the most appropriate type of treatment and management approach. The above categories are described subsequently.

### Physical characteristics of MSW

In addition to analyzing characteristics, it is strongly recommended that the sampling program include provisions for determining moisture content, bulk density and size distribution. The major physical characteristics of MSW are pH, moisture content, volatile solid content and ash residue, bulk density, and particle size distribution (Alamgir et al, 2005).

*pH*: It is the negative logarithm of hydrogen ion activity and if  $pH < 7$  represents acidic, while  $pH = 7$  is neutral and  $pH > 7$  is basic or alkaline. Organic materials with a wide range of pH values from 3 to 11 can be composted, but the more desirable pH range for composting is between 5.5 and 8.5 (Tchobanoglous et al., 1993).

*Moisture content*: One of the most important physical characteristics of solid waste is moisture content. Moisture content is a property of particular importance to incinerator design and operation because of its effect on the calorific value and the ignition

characteristics of the refuse. It is also very important in composting of MSW, as there is a close relationship between moisture and aeration (Diaz et al., 1996). The basis of the relationship is the fact that the principle source of the oxygen required by the microbial populations entraps the air in the voids; and there is an indispensable relation between moisture content and air (oxygen). Moisture content varies widely which is influenced by local climate conditions, refuse-storage practices, and method of refuse collection and refuse composition (Tchobanoglous et al., 1993).

*Volatile solid content and ash residue:* The portion of organic materials that can be released as a gas when organic materials is burned in a muffle furnace at the temperature of 550<sup>0</sup>C is known as volatile solid content (Tchobanoglous & Kreith, 2002). Volatile solids are a useful parameter particularly in gas production. Gas production is usually expressed in two terms: i) per unit of total solid, and ii) volatile solids introduced.

*Bulk density:* Bulk density is the important characteristics of solid waste for final sample size reduction operation. Density data is often required to obtain the mass and volume of waste that must be managed. Proper evaluation of this property is also important in designing the collection equipment and treatment plant capacity. It should be noted that the different value between the compacted and uncompacted densities (Diaz et al., 1996).

In general, the lower income level of country, higher the concentration of putrescible matters, moisture, ash and dirt in solid waste as well as less likely the possibility of

finding low density packaging materials (Diaz et al., 1996). Therefore, the lower level of income, higher the density of waste, this inherent trend is shown in Table 2.9.

Table 2.9 Variations of bulk density with income level of country

<b>Status of country</b>	<b>Loose bulk density (Kg/m<sup>3</sup>)</b>
Low income developing countries	300 – 600
Middle income developing countries	200 – 300
Industrialized countries	100 - 150

Source: Diaz et al., 1996

*Particle size distribution:* The size of the particles in the MSW is a nutrient-related factor, because the waste is the substrate in composting and the substrate is the source of nutrients. The relation to nutrition is the effect of size of the individual particles. Particle size also determines the ratio of mass-to-surface and hence the amount of a particle's mass that is exposed to microbial attack (Diaz et al., 1996).

#### Chemical characteristics of MSW

Information on the chemical characteristics of the components that constitute MSW is important in evaluating alternative processing and aid recovery options. For example, the feasibility of combustion depends on the chemical composition of the solid wastes. Typically, wastes can be thought of as a combination of semi-moist combustible and non-combustible materials. When there is need to degrade/compost the organic fraction of MSW or use it as feedstock for the production of other biological conversion products, information that is required is not limited to that of the major elements (ultimate analysis) that are contained in the waste, rather importance is also given to the

degree of trace elements present (Tchobanoglous et al., 1993). If solid wastes are to be used as fuel, some other components such as carbon and nitrogen.

### Biological Characteristics of MSW

Excluding plastic, rubber and leather components, the organic fraction of most MSW can be classified as follows:

1. Water-soluble constituents, such as sugars, starches, amino acids and various organic acids.
2. Hemicellulose, a condensation product of five- and six-carbon sugars;
3. Cellulose, a condensation product of the six-carbon sugar glucose;
4. Fats, oils and waxes, which are esters of alcohols and long-chain fatty acids;
5. Lignin, a polymeric material containing aromatic rings with methoxyl group ( $-\text{OCH}_3$ ), the exact chemical nature of which is still unknown (present in some paper products such as newsprint and fibreboard);
6. Lignocelluloses, a combination of lignin and cellulose; and
7. Proteins, which are composed of chains of amino acids.

Perhaps the most important biological characteristic of organic fraction of MSW is the fact that almost all the organic components can be converted biologically to gases and relatively inert organic and inorganic solids. The production of odours and the generation of flies are also related to the putrescible nature of the organic materials found in MSW (Tchobanoglous et al., 1993). Yet understanding the mechanical properties of waste is important for disposal.

### Mechanical characteristics of MSW

Proper design of processing plants as well as final disposal facilities should include a thorough understanding of the properties of refuse and its components, but this tend to be often ignored in some places. Perhaps this can be explained by the absence of reliable information readily available in the literature. This problem is particularly more pronounced in economically developing countries. Mechanical properties are especially important in the design of sanitary landfills and ancillary systems (Diaz et al., 1996).

Major mechanical characteristics of MSW are:

1. Stress-strain behaviour
2. Absorptive and field capacities
3. Permeability of compacted waste.

Triaxial compression tests on raw MSW and on mixtures of MSW with incinerator bottom ash has shown considerable impact of ash on behaviour of refuse (Diaz et al., 1996). Field capacity of MSW is the total amount of moisture that can be retained in a waste sample subject to downward pull of gravity. The field capacity of waste materials is of critical importance in determining the formation of leachate in landfills. it varies with the degree of applied pressure and the state of decomposition of waste. The field capacity of uncompacted commingled wastes from residential and commercial sources is in the range of 50 to 60% (Tchobanoglous et al., 1993). Similarly, the hydraulic conductivity of compacted wastes is an important physical property that, to a large extent, governs the movement of liquids and gases in a landfill. The coefficient of permeability is normally written as:

$$K = Cd^2y/\mu = ky/\mu$$

Where

$K$  = coefficient of permeability

$C$  = dimensionless constant or shape factor

$d$  = average size of the pores

$\gamma$  = specific weight of the water

$\mu$  = dynamic viscosity of water

$k$  = intrinsic permeability

Tests have been carried out using a large-scale compression cell to determine several hydrogeological and geotechnical properties of refuse. Results of these analyses are useful in the evaluation of leachate management systems (Diaz et al., 1996). Hence there is need to understand some conditions of waste characterization.

## **2.7 Conditions for Waste Characterization**

Improper waste management has become an acute problem in many countries. Management options require extensive waste characterization since many of them may contain compounds deleterious for the ecosystem, such as heavy metals and organic micropollutants. The concern and the necessity for assessing the environmental hazard from different waste management practices have been expressed in the Hazardous Waste Directive 91/689 of the EU (Mantis et al., 2005; EC, 1999). The toxicity of the influents from industries into urban wastewaters, of the effluents from treatment plants, as well as, of the water in the various compartments, must be continuously monitored. This is necessary to avoid damages to the activated sludge, to check, at any moment, the

effectiveness of the treatments and the quality of water released to the environment (Stefano et al., 2008).

The composition, the leachability of potentially harmful/hazardous components, the long-term behavior, and the general properties of a waste must be known before any disposal practice can be formulated. The general characterization and testing of waste usually includes basic characterization, compliance testing, and on-site verification (1999/31/EC, EC, 1999). The wastes can be characterized as hazardous or not according to 14 properties and with the presence of 51 inorganic and organic compounds [(metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and others)]. Ecotoxicity of waste and resulting leachates also appears to be key criterion for waste landfilling (Mantis et al., 2005).

A wide variety of single leaching tests for waste characterization is employed in different countries. In recent years, standardization of leaching tests in Europe is under development. The Commission of European Nations (CEN) compliance test for leaching of granular waste materials and sludge (EN 12457/1-4) and the draft CEN up-flow percolation test for inorganic constituents have been incorporated in the Council Decision (EC, 2002). However, although much work on the tests employed for the evaluation of inorganic materials has been done, the leaching behavior of organic micropollutants is not well studied (Schultz et al., 2002). Until now, no recommendations or validated methods for wastes containing organic pollutants have been available (CEN, 1999). Recently, the European Commission (EC) established criteria for the characterization of wastes and their acceptance in landfills based on the chemical composition of the leachates derived from the application on the EN 12457- 2 (EC, 2002).

Chemical analysis of wastes provides only part of the knowledge necessary to evaluate and assess their toxic potential for wildlife and humans. The complex interactions between different environmental chemicals and their bioavailability are not completely understood and it is difficult to make hazard assessments and predictions of possible ecotoxicological effects based only on total concentrations. Toxicity evaluation is an important parameter in waste characterization as it provides the complete response of a test organism to all the compounds existing in the waste (Wang et al., 2003).

The utility of bioassay tests for environmental hazard assessment of wastes is well established. Many types of bioassays using representatives from microorganisms, plants, invertebrates, and fish are available. In recent years, use of bioluminescence bacterial tests has become particularly popular because they are rapid, reproducible, simple to use, unambiguous, cost effective, and they cause no ethical problems (Ribo and Kaiser, 1987; Kavka, 1989). Bioluminescence bioassays have been frequently employed to measure the toxicity of wastewaters, sludges, and solid wastes (Wang et al., 2002). However, only a few attempts to use waste leaching tests in conjunction with ecotoxicity tests have been made (Schultz et al., 2002). Therefore it became imperative to have an overview of landfill as a waste disposal option.

## **2.8 Landfilling: A Waste Disposal Option**

Landfilling is an important option in waste management hierarchy and is also one of the primary technologies used to dispose of solid waste. It is defined as a method of refuse disposal, where waste is systematically covered by layers of earth within a limited space. Buried waste degrades as a result of natural oxidation and microbial action (Agamuthu, 2001). Landfill according to International Solid Waste Association (ISWA) (1992) is “*the engineered deposit of waste onto and into land in such a way that*

*pollution or harm to the environment is prevented and, through restoration, land provided which may be used for another purpose”.*

Landfilling stands alone as the only waste disposal method that deal with all materials in the solid waste stream in many developing nations. It is also considered the simplest, and in many areas, the cheapest disposal method (Agamuthu, 2001). Landfills can be in various forms; hence even open dumps are considered as landfill. For a sustainable waste management, landfills can basically be in form of sanitary landfill or secure landfill. Slight variations in their designs help to make the function distinct.

Whereas secure landfill is predominately used for hazardous or toxic waste, sanitary landfill is more to MSW. This basically follows the principle of “controlled tipping”. It is a method of disposing refuse on land without creating nuisance or hazard to public health and safety by utilizing the principles of engineering to confine the refuse to the smallest practical area. Such reduces the waste to the smallest practical volume and is covered with a layer of earth at the conclusion of each day’s operation or at such more frequent intervals as deemed necessary (Agamuthu, 2001).

Daily operations at a landfill are supposed to be carried out under stringent regulations designed to protect the environment, public health, and safety. Environmental control systems are required by state laws and regulations to protect the environment and people. In US, landfill managers are required to adopt a stipulated solid waste disposal method and also adhere strictly to Resource Conservation and Recovery Act (RCRA) US EPA state regulations (USEPA, 2000). Also in UK, landfill managers are required to comply with the Landfill Directive where pollution impacts from landfill must be prevented or reduced to the maximum (Environmental Agency, 2006).

The primary aim of sanitary landfilling is for safe long-term disposal of solid waste with minimal health impact, or environmental degradation. Primarily in Asia, landfills form an integral part of waste management due to its low cost (Shekdar, 2009; Chong et al., 2005; Lucas and Shreeve, 2000).

About 230 officially recognized landfills and an estimated three times more illegal dumps exist in Malaysia (Jaffar et al., 2009; Agamuthu, 2001). Table 2.10 shows the distribution of landfills in Malaysia. Most landfills in Malaysia do not come under the sanitary landfill classification either because there are no facilities to collect and/or treat the leachate or there is no infrastructure to exploit the landfill gas. Globally there are more than 453 facilities for landfill gas usage and the estimated amount of gas from domestic solid waste is 730 billion m<sup>3</sup> (Gendebien et al., 1991; Agamuthu, 2001).

**Table 2.10 Locations of Landfills in Malaysia**

State	Number of landfills in operation	Number of inert landfill	Number of closed landfill	Number of Proposed landfill	Number of RDF in operation	Number of transfer stations in operation
<b>Perlis</b>	1	0	1	0	0	1
<b>Kedah</b>	10	0	5	0	0	0
<b>Penang</b>	1	0	2	0	0	2
<b>Perak</b>	20	0	9	2	0	0
<b>Pahang</b>	19	0	13	1	0	0
<b>Selangor</b>	6	1	12	1	1	0
<b>Wilayah Persekutuan Putrajaya</b>	0	0	0	0	0	0
<b>Wilayah Persekutuan KL</b>	1	0	7	0	0	1
<b>Negeri Sembilan</b>	8	0	10	1	0	0
<b>Melaka</b>	2	0	5	1	0	0
<b>Johor</b>	13	0	21	1	0	1
<b>Kelantan</b>	13	0	4	0	0	0
<b>Terengganu</b>	9	0	12	2	0	0
<b>Wilayah Persekutuan Labuan</b>	1	0	0	0	0	0
<b>Sabah</b>	21	0	1	0	0	0
<b>Sarawak</b>	51	0	12	0	0	0
<b>TOTAL</b>	176	1	114	9	1	5

*Source: Department of National Solid Waste, Malaysia (August, 2009)*

Common difficulty encountered in regulating and properly managing waste is attitude and behaviour of waste generators (Begum et al., 2009). Teo and Loosemore (2001) opined that attitudes regarding waste differ from one organization to another, depending on organization, culture and waste management policies. Like in the case of construction industry, some contractors do not practice source separation, source reduction, reuse or recycling nor do they dispose of their waste at a landfill (Begum et al., 2009). The reason for this may be due to cost, lack of knowledge regarding consequences of waste and the potential for waste reduction or minimization (Begum et al., 2009).

## **2.9 Sanitary Landfill**

Wastes is susceptible to causing contamination of air, groundwater and surface water, and as such need to be contained in an engineered safe containment system, known as engineered or sanitary landfills. In particular, landfill is the term used to describe the physical facilities used for the disposal of solid wastes and solid wastes residuals in the surface soils of the earth. Once containment has escaped into the ground, it flows from pore to pore through the soil, sometimes travelling several kilometres.

The manner and rate of transport depend on many factors, including:

- Whether the soil is saturated or unsaturated,
- The type of soil,
- The type of material flowing through the soil, especially its solubility in water and its specific gravity,
- The velocity and direction of natural groundwater flow,
- The rate of infiltration from the source.

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

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

### **2.9.1 Evolution of sanitary landfills**

Since the turn of the last century, the use of landfills, in one form or another, has been the most economical and environmentally acceptable method for the disposal of solid wastes throughout the world. Landfills, in various forms, have been used for many years (Table 2.11). The first recorded regulations to control municipal waste were implemented during the Minoan civilization, which flourished in Crete (Greece) from 3000 to 1000 B.C.E (Alagmir et al., 2005). Solid wastes from the capital, Knossos, were placed in large pits and covered with layers of earth at intervals (Tammemagi, 1999). This basic method of landfilling has remained relatively unchanged right up to the present day.

Table 2.11. The summary of the evolution of municipal landfills.

<b>Period</b>	<b>Development</b>	<b>Problems</b>	<b>Improvements</b>
<b>1970s</b>	Sanitary landfills	Health/nuisance i.e. odour, fires, litter	Daily cover, better compaction, engineered approach to containment
<b>Late 1970 – early 1980s</b>	Engineered landfills, recycling	Ground and ground water contamination	Engineered liners, cover, leachate and gas collection systems, increasing regulation, financial assurance
<b>Late 1980s – early 1990s</b>	Improved siting and containment, waste diversion and re-use	Stability, gas migration	Incorporation of technical, socio-potential factors into siting process, development of new lining materials, new cover concepts, increased post-closure use.
<b>2000s</b>	Improved waste treatment	?	Increasing emphasis on mechanical and biological waste pretreatment, leachate recirculation and bioreactors, “smart landfills”

Source: Bouzza et al., 2002

### 2.9.2 An overview of sanitary landfills types

Landfill may be categorized into three classes:

- (a) Class-I: Hazardous wastes,
- (b) Class-II: Designated and,
- (c) Class-III: MSW (Tchobanoglous & Kreith, 2002).

However, in class III landfills, limited amount of nonhazardous industrial wastes and sludge from water and wastewater treatment plants are also accepted. Designated wastes are nonhazardous wastes that may release constituents that are in excess of applicable

water quality objectives established by recognized authority. Again, based on the physical infrastructures and other associated facilities, landfill can also be designed as;

- a. Sanitary landfills; denote the facility in which waste placed in the landfill is covered at the end of each day's operation. Today, it refers to an engineering facility for the disposal of MSW designated and operated to minimize public health & environmental impacts (Figure 2.4).
- b. Monofills; contains individual wastes constituents such as combustion ash, asbestos, and other similar wastes.
- c. Secure landfills: denote the facility which is used for the disposal of hazardous wastes, and
- d. Uncontrolled land disposal sites: refer to those places where waste is dumped on or into the ground in no organized manner.

Concerns with the landfilling of solid wastes are related to the following (Tchobanoglous & Kreith, 2002):

- The impact of uncontrolled discharge of landfill gases on the greenhouse effect in the atmosphere,
- The uncontrolled release of leachate that might migrate to underlying groundwater or surface streams,
- The breeding and harbouring of disease vectors in improperly managed landfills, and
- The health and environmental impacts associated with the release of the trace gases found in landfills arising from the hazardous materials that were often placed in landfills in the past.

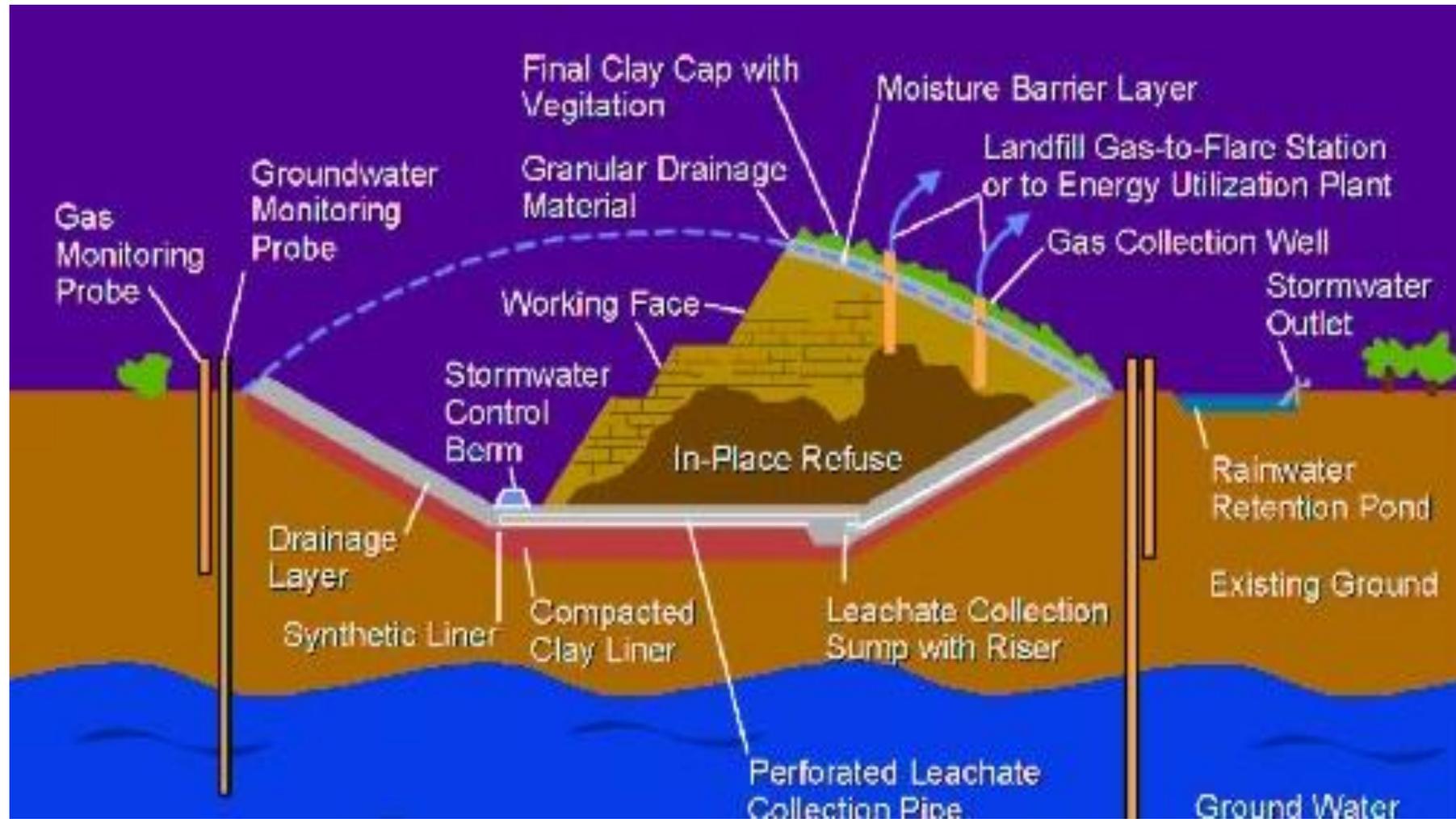


Figure 2.4. Typical Cross section of an engineered landfill

(Source: [blog.1800gotjunk.com](http://blog.1800gotjunk.com), 2012)

There are two major aspects associated with the installation and functioning of a landfill. These are:

*Management-* this consists of the following key elements of landfill; planning, design, operation, environmental monitoring, closure and post-closure control.

*Technical-* this consists of following key technical aspects associated mostly with the design: site selection, decomposition, liners, covers, leachate collection and treatment, gas collection and resource recovery or control, closure and post-closure (Tchobanoglous & Kreith, 2002).

In fact a landfill is a biochemical reactor, in which solid wastes and water are the major inputs, while leachate and landfill gas are the outputs. Landfill gas control systems are employed to prevent unwanted movement of landfill gas into the atmosphere. The covered landfill gas can be used to produce energy or flared under controlled conditions to eliminate the discharge of harmful constituents to the atmosphere.

Leachate, the contaminated by-product of water and solid wastes, is found under the bottom of the landfills at normal conditions and moves to the underlying strata. The effects of leachate depend on its chemical and biological constituents and the sub-soil conditions. It is recorded that a number of sanitary landfills contaminated groundwater through leakage. To save the groundwater from potential risk, double base liner, detection, collection and removal system are introduced in sanitary landfills.

## 2.10 Leachate Chemistry/Composition

In an attempt to reduce the potential for resulting groundwater pollution, the degree of planning, engineering, waste stream control and management undertaken at municipal waste landfills has increased dramatically in recent years. Hence, the some detailed chemical analysis of landfill leachate has been undertaken (Emenike et al., 2012a; Emenike et al., 2013). Without this information, the potential and degree of risk posed to groundwater, soil and even aquatic life by landfill leachate is extremely difficult to assess. Ehrig (1983) states that “without exact data (on the quantity and consumption of leachate) the discussion ‘to seal or not to seal’ (landfill with liner and cap) has no basis.

A number of studies of the chemical composition of municipal landfill leachate have been undertaken. Table 2.12 indicates the composition of leachate in two landfills in Malaysia.

Table 2.12 Leachate Composition of Two Sanitary Landfills in Malaysia

Parameter	Units	North Jinjang	Kelana Jaya
COD	mg/L	184.32	619.84
pH	-	8.18	8.15
Conductivity	mS	27.5	5.04
N-total	mg/L	19.36	20.25
P-total	mg/L	25.33	8.25
Mg	mg/L	327.49	32.99
Ca	mg/L	919.98	94.98
K	mg/L	5099.96	524.96
Na	mg/L	2319.88	389.88
Cl	mg/L	2979.8	439.42
F	mg/L	2.75	3.25
Fe	mg/L	115.5	5.4
Mn	mg/L	8.88	0.63
Zn	mg/L	11.65	0.65
Pb	mg/L	5.2	2.5
Cu	mg/L	0.79	0.79
Cd	mg/L	0.59	0.19

(Source: Agamuthu, 2001)

In the past, leachate analysis were predominantly restricted to major ions, metals and bulk organic parameters. Few investigations dealt with the analysis of individual organic compounds (Schultz and Kjeldsen, 1986). Even, Christensen et al., (1994) stated that “there is very little information on organic matter composition of leachate and we simply have to accept that differences in composition are not reflected in the employed bulk parameters”. Only a small proportion of total organic carbon (TOC) in leachate has been quantified or identified. Then, it was believed that about 90-95% of organic compounds in landfill leachates were unknown and therefore the potential for impacts on groundwater quality is unknown (Jones-Lee and Lee, 1993). Table 2.13 shows some landfill leachate composition from South Africa.

Table 2.13 Leachate Composition from Some Landfills in South Africa

Determinand	BISASAR ROAD (combined flow)		BISASAR ROAD (Randles Cell)		MARIANNHILL Whole site		VISSERSHOK		
	14.1.97	21.12.02	31.7.01	11.6.03	5.1.99	12.6.03	typical	maximum	
pH-value	-	7.5	7.7	7.6	7.8	7.6	7.9	8.1	8.3
COD	mg/l	2488	1382	2021	3949	1605	733	3382	5766
BOD <sub>5</sub>	mg/l	~300	~300	-	-	695	~300	-	627
ammoniacal-N	mg/l	1917	886	957	1060	675	387	1998	2894
chloride	mg/l	2133	2481	2437	3569	1197	1728	2977	3934
TOC	mg/l	-	-	-	-	-	-	-	-
fatty acids (as C)	mg/l	<0.1	<0.1	<0.1	<0.1	<0.1	-	-	-
alkalinity (CaCO <sub>3</sub> )	mg/l	4802	5665	5118	5823	5524	2654	9950	12421
EC	µS/cm	16240	17060	18050	21230	12650	9820	27000	29900
nitrate-N	mg/l	<0.1	<0.1	<0.05	<0.05	<0.1	<0.1	1.3	1.3
nitrite-N	mg/l	<0.1	<0.1	-	-	<0.1	<0.1	1.05	1.4
sulphate (SO <sub>4</sub> )	mg/l	<0.16	<0.16	15.2	35.6	96	26	94	409
phosphate (P)	mg/l	8.4	4.81	4.6	6.65	5.0	1.0	12.8	25.0
sodium	mg/l	1160	1291	1712	1905	914	826	2320	8235?
magnesium	mg/l	78	150	222	196	355	152	141	-
potassium	mg/l	721	658	1153	1060	940	541	1615	-
calcium	mg/l	47	75	112	93	208	161	136	-
chromium	µg/l	115	100	182	263	80	79	-	-
manganese	µg/l	12	20	-	-	860	-	-	-
iron	µg/l	2700	4560	2290	2840	18600	12000	7320	-
nickel	µg/l	90	80	-	-	120	120	-	-
copper	µg/l	<50	<50	<50	<50	<50	<10	-	-
zinc	µg/l	80	30	-	130	170	120	64	-
cadmium	µg/l	<10	<1	1.3	<1	<1	<1	-	-
lead	µg/l	56	<4	6.9	20	<4	7.3	-	-
arsenic	µg/l	-	8.2	7.6	24	62	-	-	-
mercury	µg/l	2.2	<0.5	<0.5	<0.5	-	<50	-	-

(Source: Robinson, 2007).

However a more detailed leachate composition analysis was carried out on Wysięka landfill near Bartosyce in Poland. It involved analysis for monocyclic aromatic hydrocarbons (benzene, xylene and others) as shown in Table 2.14.

Table 2.14 Leachate Composition from Wysięka in Poland

Leachate Constituent	Units	Maximum
pH	–	8.61
Total suspended solids	mg/L	740
BOD <sub>5</sub>	mg O <sub>2</sub> /L	701
COD	mg O <sub>2</sub> /L	1821
Organic nitrogen	mg N <sub>org</sub> /L	46
Ammonia nitrogen	mg N <sub>NH4</sub> /L	364
Total nitrogen	mg N/L	401
Total phosphorus	mg P/L	15.7
Chlorides	mg Cl <sup>-</sup> /L	1190
Magnesium	mg Mg <sup>2+</sup> /L	419
Calcium	mg Ca <sup>2+</sup> /L	430
Sulfate	mg SO <sub>4</sub> /L	374
Total dissolved solids	mg/L	6823
Zinc	mg Zn/L	0.435
Chromium	mg Cr/L	0.08
Cadmium	mg Cd/L	0.13
Copper	mg Cu/L	0.09
Nickel	mg Ni/L	0.07
Lead	mg Pb/L	1.84
Mercury	mg Hg/L	0.017
BTEX – sum	µg/L	496
Benzene	µg/L	1.3
Ethylbenzene	µg/L	89
Toluene	µg/L	182
Xylene	µg/L	225

(Source: Kulikowska & Klimiuk, 2008)

According to Kulikowska & Klimiuk (2008) “*investigations on leachate composition from municipal landfill in Wysieka were started up after 23 months of its exploitation and lasted for 4 years. It was shown that organics concentration (expressed as COD) decreased from over 1800 mg COD/L at the beginning to on average 610 mg COD/L after 4 years of landfill exploitation*”. It stated that the low COD concentration of 1000–2000 mg COD/L is typical of Polish landfill sites.

A number of important studies of the biological degradation of municipal waste in landfill settings (both field and laboratory scale) have described several phases of waste stabilization and resultant leachate composition (Farquahar and Rover, 1973; Chian et al 1976; Ehrig 1983; Harmsen, 1983). These authors have found that the dominant biochemical process occurring in a landfill affects the leachate composition, with some parameters such as pH, bicarbonate, sulfate, iron, manganese, bulk organics (TOC, BOD and COD) and volatile fatty acids (VFAs) being most affected.

In addition to the restricted nature of leachate analysis and the varying degree of waste stabilization, most of the landfill studied is either known or suspected to have received wastes other than municipal waste, such as sewage sludge, industrial wastes and other wastes such as road de-icing salts. As waste type is a major determinant of leachate composition (Christensen et al., 1994), it is reasonable to expect that there would be differences in the composition of leachate in these landfills compared with that of ‘modern’ municipal waste landfills that exclude these wastes. The phases of biological degradation are based on the dominant biochemical processes occurring (e.g. fermentation, methanogenesis) and are summarized in Table 2.15.

Table 2.15 Phases of Organic Waste Stabilizations in Landfills

PHASE	BIOCHEMICAL PROCESSES
1. AEROBIC	<p>Aerobic degradation of simple organic materials.</p> <ul style="list-style-type: none"> <li>• Simple carbohydrates <math>\rightarrow</math> <math>\text{CO}_2 + \text{H}_2\text{O}</math></li> </ul> <p>e.g. <math>\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{biomass} + \text{heat}</math></p> <ul style="list-style-type: none"> <li>• Simple lipids <math>\rightarrow</math> fatty acids and glycerol</li> <li>• Simple proteins <math>\rightarrow</math> amino acids <math>\rightarrow</math> <math>\text{CO}_2, \text{H}_2\text{O}, \text{NH}_4^+</math> and <math>\text{SO}_4^{2-}</math></li> </ul>
2. ANAEROBIC ACID FERMENTATION	<p>(i) Hydrolysis of complex dissolved and solid organic matter</p> <ul style="list-style-type: none"> <li>• Cellulose <math>\rightarrow</math> cellobiose by enzymes produced by fermentative bacteria</li> <li>• Lipids <math>\rightarrow</math> long chain fatty acids</li> <li>• Peptides <math>\rightarrow</math> amino acids</li> </ul> <p>(ii) Fermentation of hydrolysed organic compounds to simple organic compounds</p> <ul style="list-style-type: none"> <li>• Carbohydrates <math>\rightarrow</math> Volatile Fatty Acids (ethanoic, propanoic, butanoic, pentanoic and hexanoic), ethanol, <math>\text{CO}_2</math> and <math>\text{H}_2</math></li> </ul> <p>e.g. <math>\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{C}_2\text{H}_4\text{COOH} + 2\text{H}_2 + 2\text{CO}_2</math></p> <ul style="list-style-type: none"> <li>• Lipids <math>\rightarrow</math> long chain fatty acids to simple VFAs</li> <li>• Proteins <math>\rightarrow</math> deamination of amino acids VFAs, particularly isobutyric and iso-pentanoic, <math>\text{CO}_2</math> and <math>\text{NH}_3</math>, (minor <math>\text{SO}_4^{2-}</math>)</li> </ul> <p>(iii) Further fermentation of simple VFAs to ethanoic acid by acetogenic bacteria</p> <p>e.g. <math>\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2</math></p>
3. INTERMEDIATE ANAEROBIOSIS	<p>Slow conversion of ethanoic acid to <math>\text{CH}_4</math></p> <p>Slow growth of methanogenic bacteria</p> <p><math>\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2</math></p>
4. METHANOGENESIS (STEADY STATE)	<p>Production of methane by:</p> <p>(i) Acetophilic bacteria</p> <p>e.g. <math>\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2</math></p> <p>(ii) Hydrogenophilic bacteria</p> <p>e.g. <math>4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}</math></p>
5. METHANOGENESIS (DECLINING RATE)	<p>As for Phase 4 (methanogenesis-steady state)</p> <p>Characterised by presence of predominantly refractory organic matter. Low methane production, air may enter the upper layers producing a second aerobic phase.</p>

*Adapted from Rees (1980); Christen et al (1994); and Drever (1997).*

The need for a study of the composition of ‘modern’ municipal waste landfill leachate has been identified by Murray and Beck (1990). They noted that due to the potential for these leachates to contain toxic and hazardous compounds, particular emphasis should be placed on evaluating leachates from municipal waste landfills from which hazardous

waste has been excluded. This is particularly important as landfilling is the predominant method of municipal waste disposal in most industrialized countries (Carra & Cossu, 1990).

In as much as the principle of analysis involve the collection of representative samples of landfill leachate for chemical analysis, most studies on leachate chemistry simply mention where the samples were collected (e.g. leachate spring, leachate collection drain or sump). They rarely mention how the samples were collected and almost never discuss the potential of the sampling methodology to influence the results of leachate analysis. With respect to landfill leachate sampling, it is expected that the location of sample collection, as well as, the device used will be the most significant influences on resultant leachate composition.

According to Oman and Hynning (1991), an important factor in influencing analytical results (of organic compounds in landfill leachate) is, besides analytical method, the sampling point. A review of international landfill leachate composition studies reveals that leachate from a broad range of locations has shown some variations within the same landfill (Oman & Hynning, 1991). Such collection locations included leachate seeps/springs, leachate affected groundwater, artificial landfill cells, leachate collection systems (drains and sumps), and bores placed directly into a landfill.

A number of these sampling locations are expected to be barely indicative of 'in landfill' conditions. Artificial leachate are produced by placing wastes into a large container and adding deionised water while actual leachate samples collected from seeps and springs have been exposed to the atmosphere and light. Therefore, the

leachate affected groundwater has been diluted and may have been chemically changed (depending upon the aquifer and groundwater conditions) (Oman & Hynning, 1991).

Ettala et al. (1988) concluded that the concentrations of substances in leachate collected in external seepage drains around the landfill “did not generally correlate with those in leachate within the landfill”. Chian & DeWalle (1977) observed that exposure of landfill leachates to atmospheric oxygen (such as at leachate springs) resulted in an increase in chemical oxygen demand and total suspended solids. They also noted the potential for dissolved organic compounds to absorb suspended solids, thereby affecting the analytical results of dissolved constituents.

Some research has involved leachate sampled from within the landfill, either from the leachate collection system (drains and sumps) or from bores placed directly into the waste. In order to ‘minimize alterations of the leachate caused by, for example, sorption soil, oxidation from contact with air or dilution by surface water,’ Oman & Hynning (1991) collected samples from bores placed within the landfill. Although the atmospheric interface with leachate in a sump is much greater than in the bore, there has been no research determining whether this is significant enough to affect leachate composition prior to sampling.

Barcelona et al. (1984) found that, although no single type of (groundwater) sampling device could be expected to provide representative samples under all conditions for a wide range of chemical constituents, sampling for gas sensitive and volatile constituents can be significantly biased by degassing and loss of volatiles during sample collection. Chemical parameters that may be altered as a result of aeration and degassing during sampling include pH, dissolved oxygen (DO), inorganic carbon (e.g. bicarbonate),

alkalinity, TOC, volatile organic compounds, ammonium, nitrate, sulphide, mercury and dissolved cadmium, lead, iron and manganese (Parker, 1994).

## **2.11 Landfill in Asia and Associated Toxic Components**

In most European countries and some other places of the world, landfills now play a decreasing role in waste management. However, Asian countries still make use of many large landfill sites for waste disposal. Landfilling is an important option in waste management hierarchy and is also one of the primary technologies used to dispose of solid waste. It is defined as a method of refuse disposal, where waste is systematically covered by layers of earth within a limited space. Buried waste degrades as a result of natural oxidation and microbial action (Agamuthu, 2001). Landfill is assumed to be environmentally suitable if it is in sanitary form and even “secure” form as with the case of hazardous waste disposal.

The primary aim of sanitary landfilling is for safe long-term disposal of solid waste with minimal health impact, or environmental degradation. Primarily in Asia, landfills form an integral part of waste management due to its low cost (Shekdar, 2009; Chong et al., 2005; Lucas and Shreeve, 2000). Unfortunately, many landfills in Asia exist in non-sanitary form. Most landfills do not come under the sanitary landfill classification either because there are no facilities to collect and/or treat the leachate or there is no infrastructure to exploit the landfill gas.

It was observed that leachate from Pillar Point and Ma Yau Tong (Hong Kong), Lat Krabang (Thailand), Lewigadja (Indonesia) and Jeram (Malaysia) landfills were characterized of high BOD, COD and ammonia concentrations to the record of 27 000

mg/L, 51 200 mg/L and 3 032 mg/L, respectively (Table 2.16). Depending on the leachate state (acetogenic or methanogenic), it has the potential to contaminate both ground and surface waters, including surface and sub-surface soil. A leachate characteristic is a reflection of waste components that manifest after some biological and physico-chemical interactions in the landfill. Some of the components are contaminants which have toxic nature especially in the form of persistent organic pollutants (POP), monocyclic aromatic hydrocarbons, heavy metals and others.

Table 2.16 Leachate of some selected landfills in Asia

<b>Parameter</b>	<b>Pillar Point (Hong Kong)<sup>a</sup></b>	<b>Ma Yau Tong (Hong Kong)<sup>a</sup></b>	<b>Lat Krabang (Thailand)<sup>a</sup></b>	<b>Leuwigadja (Indonesia)<sup>a</sup></b>	<b>Jeram (Malaysia)<sup>b</sup></b>
pH	8.6	8.1	7.6	8.4	7.35
COD	2830	873	2700	-	51,200
BOD <sub>5</sub>	384	117	-	-	27,000
Ammoniacal-N	2700	1156	3032	2000	0.085
Chloride	2740	853	3802	2330	4150
TOC	-	-	392	968	380
Alkalinity	11,700	4940	23,910	7840	1980
EC	30.400	14,000	28,100	-	12.30
Nitrate-N	2.5	1.1	<1	<1	38.6
Nitrite-N	<0.1	<0.1	<0.7	<1	4.8
Sulphaete	-	-	15	159	54.89
Phosphate	125	29.7	1.7	12	113
Sodium	2100	600	2453	1130	58.7
Magnesium	32	21	121	56	11.4
Potassium	1130	375	1932	1600	530
Calcium	35	42	55	86	20.17
Chromium	-	-	780	250	25.27
Managese	-	-	240	473	540.76
Iron	6.6	8.5	2.77	6.23	97.76
Nickel	-	-	<1	0.38	19.50
Copper	-	-	<0.05	0.39	3.59
Zinc	2.2	1	0.15	0.46	827.7
Cadmium	-	-	<0.05	<0.02	<0.001
Lead	-	-	<1	<0.3	<0.001
Arsenic	-	-	-	-	-
Mercury	-	-	-	-	0.05

a- Adopted from Robinson (2007)

b- Adopted from Emenike et al (2011). All units in mg/L except EC ( $\mu$ S/cm) and pH

The level of pollution parameters such as BOD, COD and metal concentrations, differ from one landfill to the other (Fauziah & Agamuthu, 2005). This most often is dependent on the landfill status (whether it still receives MSW or not). Active landfills like Jeram, will record high BOD and COD due to the presence of biodegradable components of the waste stream, whereas inactive landfills (those that do not receive waste any more) showed a decline in some parameters due to reduction in organic matter in the waste cell. However, the increased concentration of ammoniacal -N in the leachate becomes an issue of concern (Emenike et al., 2011). Brought about by continued degradation of amino acids and nitrogenous organic matter, ammoniacal-N is a significant determinant for the pollution potential of every landfill or waste dump. The long term degradation of the organic components of the landfill waste into organic acids like humic acids have influenced the degree of alkalinity among Asian landfills (1980 – 11,700 mg/L). This is common with landfill leachate in the early phases of waste stabilization (Kjeldsen et al., 2002), which thereby signify that ammonia and alkalinity are known to be potential toxicants of landfill leachate.

Considering the fact that most landfills in Asia receive about 2000 metric tonnes of waste daily, it can then be estimated that about 300,000 L of leachate is produced daily from each landfill (average leachate production is estimated at 150 L/tonne). On an average, most Asian landfills have the daily potential to leach about 3 825 g (Fe), 23 400 g (Zn) and 4 095 g ( $\text{PO}_4^{3-}$ ) into nearby water courses (Agamuthu et al., 2011). Therefore, the release of these leachate components becomes of critical importance since aquatic life, especially fish might be affected; hence there is need for evaluate of leachate toxic effect on fish.

## **2.12 Fish and leachate toxicity**

The ecosystem has its base on three components namely; soil water and air, and as such any form of impairment on one of them may disrupt the flow of interaction in nature. Aquatic system is very crucial in maintaining ecological balance and harbors organisms that are of very significant importance to humans (Emenike et al., 2011). Fish is not just seen as the dominant organism resident in most waters but is vital for enhancing the energy flow in the ecosystem since it is edible food for humans in most cases. However, it may even serve as an indicator for degree of impacts of some anthropogenic activities that disrupt the ecosystem. The process by which fish consumes organisms is a salient aspect that regulates trophic structure. This therefore enhances the stability, resilience, and food web dynamics of aquatic systems (Holmulund & Hammer, 1999). Its ecological and economic importance cannot be over emphasized. In fact much effort has been devoted to establishing relationships (statistically) between reef fish communities and the various features of their habitat (Knudby et al., 2010). As such avails researchers the ecological details which enhance hypothetical postulates for future investigations. Such investigations become necessary since fish survival are negatively affected by multiple anthropogenic interruptions such as pumping, dredging, fishing and excessive pollution (Delpech et al., 2010). Being food for humans and source of income, its economic importance is very pronounced.

The survival and metabolic activities of aquatic organisms including fish is influenced by the presence of contaminants brought about by anthropogenic disturbances. This includes landfilling activities.

Toxicity testing, being a combination of techniques and principles ensures proper evaluation of resultant effects of some substances. Regardless of the fact that substances or compounds show some chemical and physical properties that helps to predict their perceived impact on our immediate environment, the effect of such substances when existing as mixture, makes impact evaluation or prediction difficult. Therefore, toxicity testing becomes paramount to understanding such perceived or unknown effects, and serves as a basic tool for identifying and assessing a better control mechanism. Priority is given to living organism while analyzing the potential impact of a substance. This is to ensure conservation and sustainability. Toxic compounds can initiate varying effects on living system; hence evaluation of short term effect (often mortality impact) via acute toxicity test, and long term observation (chronic toxicity test) are employed to analyze the effect of such compounds (Buratini, 2004).

The chemistry of natural surface waters is complex. It depends on the equilibrium reached with the normal physical, chemical and biological characteristics of the surrounding environment. Thus, there can never be a normal surface water quality; every natural water will have a different composition. Even rainwater varies in composition in different localities and regions. The precipitated water droplets will absorb acidic ions, volatile chemicals and fine particulate matter of natural and anthropogenic origin. A substantial proportion of the nitrogen input to soils comes with the winter rainfall. Recent data have shown that the level of atrazine in rain can be as high as  $1 \mu\text{g l}^{-1}$  in areas where there is widespread use of this herbicide (Svobodova et al., 1993).

Some indication has already been given of the added impact that can be caused by man's activities. Metal mining, by increasing the surface area of exposed rock to rainfall, can cause elevated concentrations of metals in drainage water. Commercial forestry can cause an increase in suspended mineral solids in the water after areas have been cleared by cutting and logging (Svobodova et al., 1993).

Fish have become genetically adapted to live in such diverse environments as cold, soft, arctic waters to warm muddy rivers in the tropics. Transfer of fish between these environments is not possible. In the same way, there is a limited potential for genetic adaptation to extreme conditions within a particular environment; for example, to extreme acidity in waters affected by acid rain, or to elevated levels of zinc in waters affected by historic mining activities (Svobodova & Vykusova, 1991).

In general, however, most of the adaptations that do occur are due to the limited ability of individual fish to detoxify the harmful chemicals entering the body, e.g. by enhancing the biochemical processes involved. High levels of ammonia in the water are toxic to fish. However, the end-product of protein catabolism in fish is ammonia which is excreted by the gills. A limited adaptation to ammonia can be obtained by enhancing the excretory mechanism.

Similarly, elevated levels of zinc and copper in the water can be harmful, although at lower levels they are essential elements for fish. The internal concentrations of these metals are maintained by translocating them as complexes with metallothioneins (proteins) and perhaps by depositing surplus metals in the form of inert granules. These mechanisms can be enhanced to a certain extent to cope with limited increased metal levels in the surrounding water.

Many organic compounds can be metabolized and detoxified in the liver; residues can be excreted in the urine or via the bile through the gut. Again, there is a limited capacity for these mechanisms to become enhanced to cope with increased uptake of potentially harmful chemicals from the water. The existence of such mechanism can be demonstrated by placing fish which have been exposed to sub-lethal concentrations of a toxicant into higher concentrations and comparing their survival times with those not previously exposed. In general, it is unusual to find that fish can achieve more than a four-fold increase in resistance to a toxic substance (Svobodova et al., 1993). It is very important to bear these adaptive potentials in mind when considering the effects of pollution on fish. In particular, the rate of change in the water quality may be important in determining whether the change is harmful; it may take some time for the adaption to be completed.

Toxicity tests have traditionally been performed with a variety of freshwater and saltwater species representing algae, fish and invertebrates (Castano, 1996). Although the initial aquatic toxicity tests were carried out using bacteria, invertebrates and other groups, they cannot replace the actual test performed on fish, which is the last chain in the aquatic food cycle (Castano, 1996).

There are a number of studies carried out to estimate the toxicity of landfill leachate using fish as test organism. Plotkin and Raham (1984) monitored the mortality of fathead minnows fish exposed to filtered and unfiltered leachates. Different concentrations of leachates were used to test the survival of tilapia (*Sarotherodon mossambicus*) (Wong, 1989). Sisunno *et al* (2000), evaluated toxicity of municipal dump leachate using zebrafish (*Brachydanio rerio*). Osaki *et al* (2006) determined the

toxic potency of the landfill leachates to the larvae and adult of Japanese Medaka (*Oryzias latipes*).

As Asia is richly endowed with water bodies that serve as environment for aquatic organisms, especially different fish species, assessing the impact of pollutants requires critical evaluation because such environment has both ecological and economic relevance (Agamuthu et al., 2011). Solubility can enhance toxicity of compounds and it can be noted that a number of solids in leachate are soluble in water. Chian and Dewalle's (1976) study of 12 landfills proved that the reason for low concentration of total suspended solids (TSS) in leachate (averagely less than 2.5% of the total solids), is because most of the solids dissolve in the leachate. Such condition enhances the complexity of leachate characterization in relation to its potential environmental impacts (Fauziah et al., 2013).

However, regardless of the fact that leachate may contain many compounds; heavy metal content is a major concern due to the persistent nature. Even in the marine environment, heavy metal pollution has been considered a serious environmental issue for a long time (Tuzen, 2003). Bioaccumulation of heavy metals in aquatic organisms is a threat to human health. Giordano et al. (1991) reported that marine organisms and sediments easily accumulate pollutants which are eventually taken up by man via food chain/web. The movement of heavy metals in leachate tend to be enhanced by the complex nature of the contained organic and colloidal matter (Jensen et al., 1999).

The determination of heavy metal accumulation in aquatic organisms especially fish is very important in order to assess the potential risk in the event of consumption of such

organism by man. These metals in leachate may not necessarily exist as discrete elements or cations but might be in the form of metal complexes. Therefore, the fact that some metals like Zn, Mn and Fe play significant roles in biological systems (Tuzen, 2003) should not imply that they are non-toxic. Some studies had reported heavy metals uptake in fish where excessive intake of these metals can initiate toxic effects (Ubillus et al., 2000; Tuzen, 2003). While some landfills might have low concentrations of heavy metals (Kjeldsen et al., 2002), landfills in some places especially in Asia have shown high heavy metal concentrations in leachate from closed and active landfills (Robinson, 2007; Emenike et al., 2011).

Chemical analysis alone is not enough for the assessment of risk potentials of leachate, rather, bioassay will allow a detailed characterization of the toxic potentials of landfill leachate as it integrates the biological effects of all its constituents (Kjeldsen et al., 2002). Therefore, there is a need to assess the impact of leachate and determine the level of heavy metals that can be accumulated in fish when exposed to characterized raw leachate from active and inactive landfills (Fauziah et al., 2013).

Considerable interest is shown over pollutants' impact on the aquatic system, hence assessing the leachate impact on fish species became the lead component of this research. However, it is necessary to note the fact that no landfill is situated directly over any surface water (except situations of seafilling as practiced in few places) or aquifer. This is to imply that a medium exists between the leachate source and the nearest aquatic system. The medium is soil.

While the rate of materials transport in water is faster than as obtainable in soil, soil serve as reservoir of both macro and micro components of the terrestrial ecosystem. Hence, the question arises on whether soil can be polluted by leachate discharge and seepage; will there be any negative effect on the soil biota; and what is the possibility of restoring the soil to original condition? Definitely it is no longer widely acceptable to use chemical methods to remedy environmental problems due to its residual impact. The same goes to the use of some physical methods since it attracts huge cost. Therefore, biological means especially the use of microbes then to be a greener and cheaper technology pivotal to more remediation situations. Yet, prior to review of bioremediation options in the next section, an overview of soil and microbial distribution is important in this section.

### **2.13 Soil and Microbial Diversity**

Soil is a complex micro habitat (Lorena *et al.*, 2005) with diversity of niches (Heribert, 2001) including microorganisms. Due to its complexity, microorganisms play an important role in maintaining the balance of soil ecosystem to ensure soil quality and plant productivity (Hull *et al.*, 2000). Previous study on soil microbes show that soil bacteria and fungi are observed and proven to play critical roles in various biogeochemical cycles (BGC) (Green *et al.*, 2004) and are responsible for the cycling of organic compounds. In addition, soil microbes also influence above-ground ecosystems. This is critical for nutrients supply to plant, ensuring healthy plant growth, and improving soil texture and soil fertility.

Some reviews had been done on developing bio-indicators for soil health (Nielson *et al.*, 2002; Van Bruggen and Semenov, 2000) and microorganisms that show the best results

of responding to environmental changes among other soil organisms are expected to be efficient bio-indicators. Doran and Safley (1997) defined soil health as “*the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote quality of air and water environments and maintain plant, animal and human health*”.

Basically, soil is used for producing crops and animals, housing, recreation, transport, industrial and other related activities. Microbial communities are integral parts of soil and their activity is very important to the functioning of soil. Therefore microorganism is included in the soil quality classification and assessment concepts (Winding et al., 2005). Although it appears inert, the topmost layer of soil teems with microorganisms mediating myriad chemical transformations vital to geochemical transformations and to soil fertility (Ingraham & Ingraham, 1995). Soil is a complex, heterogeneous medium consisting of minerals and other inorganic materials (oxides of iron, aluminium, and silicon) from the earth’s crust, living organisms, and the organic residues of dead ones. Some organic residues such as lignin, the stable component of woody plants, are long-lasting because they are resistant to microbial decomposition. They accumulate and form the organic fraction of soil called humus, which gives soil its brown or black colour.

The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities, given a specific set of environmental and cultural conditions, has made them likely candidates for solving particularly difficult problems in the life sciences and other fields as well. The various ways in which microorganisms have been used over the past 50 years to advance medical technology, human and animal health, food processing, food safety and quality, genetic engineering, environmental protection,

agricultural biotechnology, and more effective treatment of agricultural and municipal wastes provide a most impressive record of achievement (Higa & Parr, 1994). Many of these technological advances would not have been possible using straightforward chemical and physical engineering methods, or if they were, they would not have been practically or economically feasible (Higa & Parr, 1994).

Microorganisms respond sensitively to changes and environmental stress because they have intimate relations with their surroundings due to their high surface-to-volume ratio (Winding et al., 2005). In some instances, changes in microbial communities can precede detectable changes in soil properties or in plant and animal communities, thereby providing an early sign of soil improvement or an early warning of soil deterioration (Pankhurst et al., 1995).

In the biogeochemical transformation known as mineralization, microorganisms convert organic material in soil to an inorganic form. The rate and extent of mineralization depend on the availability of oxygen. Compared to anaerobic metabolism, aerobic metabolism is more versatile because more compounds are attacked. It is also more complete, producing carbon dioxide and water instead of organic acids and alcohols. Many organic materials are mineralized only if oxygen is available, but oxygen penetrates soil readily, down to a foot or so, when it is relatively dry and loose (Ingraham & Ingraham, 1995). Even the small regions within soil particles are anaerobic because oxygen-consuming microorganisms use oxygen faster than it diffuses in. When soils are flooded, they rapidly become completely anaerobic because water slows diffusion of oxygen to the rate lower than that needed by aerobic microorganism. As a result, mineralization proceeds slowly in waterlogged soils such as swamps and bogs. Such gradual mineralization was dramatically demonstrated in the 1960s when the

body of a Bronze Age man was found almost intact in a bog in Denmark (Ingraham & Ingraham, 2005). Waterlogged soils typically contain more than 90% organic material, while well-aerated agricultural soil usually contains less than 10%.

As reviewed by Nielsen and Windwing (2002), due to their numerous capabilities, microorganisms are used in several biomonitoring programs. Soil fertility describes a soil's ability to support plant growth. Fertility depends on the amount of inorganic nitrogen, phosphorus, and potassium in the soil. Forms of these nutrients that plants can use are produced by microorganisms as they mineralize organic material. Fertilizers are added to enrich a soil's complement of these elements. Potassium is added to fertilizer largely as an inorganic salt. Nitrogen and phosphorus can be added in either organic or inorganic form because the organic forms are readily mineralized by microorganisms. Thus, through mineralization, microorganisms improve soil fertility even when commercial fertilizers are used. Some bacteria improve soil fertility in different way, by fixing nitrogen (converting nitrogen gas from the atmosphere into solid form in the soil).

Environmental pollution, caused by excessive soil erosion and the associated transport of sediment, chemical fertilizers and pesticides to surface and groundwater, and improper treatment of human and animal wastes has caused serious environmental and social problems throughout the world (Reganold et al., 1990; Parr & Hornick, 1992). Often engineers have attempted to solve these problems using established chemical and physical methods. However, they have usually found that such problems cannot be solved without using microbial methods and technologies in coordination with agricultural production (Reganold et al., 1990; Parr & Hornick, 1992). Consequently,

changes in the microflora at specific site overtime indicate changes in environmental quality (Windwing et al., 2005).

The loss of biodiversity has become a major topic of concern in recent decades, and a significant amount of research has been devoted to understanding the consequences of ecosystem simplification. Although the extent of microbial diversity is not yet known, soil microorganisms probably represent the world's greatest reservoir of biological diversity (Torsvik et al., 1990; Dykhuizen 1998). However, little is known as to the forces that drive microbial community structure in soil (Tiedje 1995; Kowalchuk et al., 2002). Many organisms live in the soil. Some, including insects, worms, and small vertebrates, are visible to the naked eye. But the vast majority in terms of weight and metabolic capacity are microscopic, and most of these are bacteria.

### **2.13.1 Bacteria.**

Soil bacteria are extremely diverse. They include aerobes, anaerobes, and facultative anaerobes, which continue to proliferate as their habitat cycles between aerobic and anaerobic. Most soils contain thermophilic microorganisms, reflecting the fact that the soil surface can become extremely hot during the day. Soil temperature varies widely, and bacteria that inhabit the soil vary greatly in their optimum temperature for growth. Soil contains psychrophiles and mesophiles, as well as, thermophiles. Similarly, soil bacteria grow over a wide range of pH.

Actinomycetes, aerobic Gram-positive bacteria that form branching mycelia, are important contributors to the ecology of the soil. They break down plant and animal remains and keep the soil loose and friable. Over a million actinomycete colonies, representing more than 20 genera can be recovered from 1 g of soil (Ingraham &

Ingraham, 1995). The most numerous and widely distributed is the genus *Streptomyces*. The typical odour of soil is attributed to two volatile substances, geosmin and 2-methylisoborneol, produced by streptomycetes (Ingraham & Ingraham, 1995). *Streptomyces* colonies on laboratory media produce the same earthy odour. Availability of nutrients and oxygen determines the number and kinds of actinomycetes in soil. Actinomycetes are particularly significant degraders of complex polymers (including chitin) and hydrocarbons, which are relatively resistant to attack by other microorganisms.

A few bacteria that cause human disease are found in the soil. They include *Bacillus anthracis*, which causes anthrax, *Clostridium perfringens*, which causes food poisoning, *Clostridium tetani*, which causes tetanus, and *Clostridium botulinum*, which causes botulism. *Pseudomonas aeruginosa*, which causes opportunistic infections in burn patients and immunologically weakened individuals, occurs in almost all soils. Another significant organism outside bacteria that inhabit the terrestrial environment as well is fungi.

### **2.13.2 Fungi.**

Fungi are another group of active aerobes that degrade organic materials in the soil. They break down both simple compounds such as sugars and organic acids and complex polymers such as cellulose, starch, pectin, and lignin. Colony counts commonly underestimate the fungal population of soil because a mass of fungal hyphae may produce only a single colony when plated. Fungal biomass (total weight of organisms) is a more informative estimate of their impact on soil ecology. An acre of soil contains between 227 g and 2270 g of fungi in the complete soil layer (Ingraham & Ingraham, 1995).

Some fungi are predators. They produce special appendages or hyphal extensions that form rings to trap protozoa or nematodes. Then the fungal hyphae invade the captured prey and secrete enzymes that degrade it into smaller nutrients molecule to be absorbed. These predator fungi limit the populations of soil protozoa and nematodes.

Certain soil fungi, notably species of *Trichoderma* and *Laetisaria*, are mycoparasites. They attack other fungal species, including some that cause plant disease. Treating soil and seeds with mycoparasitic fungi can protect plants from disease (Ingraham & Ingraham, 1995). For example, *Trichoderma harzianum* controls damping-off (a disease that kills seedlings by blackening and shrinking their stems) beans, peas, while radishes diseases is controlled by *Rhizoctonia solani* or *Pythium spp.* *Trichoderma hamatum* improves survival of sugar beet seedlings, and *Laetisaria arvalis* protects seedlings of many species from fungal pathogens in the soil (Ingraham & Ingraham, 1995). For other reasons, however, commercial agriculture makes little use of mycoparasites.

Soil is also the major reservoir for some fungi that are pathogenic to humans. They include *Blastomyces dermatidis*, which causes blastomycosis; *Histoplasma capsulatum*, which causes histoplasmosis; and *Coccidioides immitis* which causes San Joaquin valley fever (Ingraham & Ingraham, 1995). However, the microflora of soil is not limited to bacteria and fungi, rather it also involves some other microorganisms which may have some roles in the ecological balance of the terrestrial environment in general.

### **2.13.3 Other Microorganisms.**

Algae are present on the surface of all soils, but usually in small numbers. A gram of soil contains 100 to 50,000 colony-forming units, amounting to between 3.18 g and 136 g of algal biomass per acre. Algae and phototrophic procaryotes do not contribute

significantly to soil fertility except in rice paddies, where cyanobacteria, free-living or in association with plants, fix considerable amounts of nitrogen.

The numbers of protozoa in the soil are small, but there is probably no soil that lacks them completely. Protozoan cell counts in soil vary between about 10,000 and 100,000 per gram. Their direct effect on biochemical transformations in the soil is minor. Indirectly, however, they play a critical role by preying on the bacterial population and thus regulating its size and composition (Ingraham & Ingraham, 1995).

Despite the realisation that microbial communities are key to the functioning of terrestrial ecosystems, their response to changes in macrophyte diversity has rarely been investigated directly (Broughton & Gross, 2000; Yin et al., 2000). The lack of information in this area is at least partly due to the difficulties associated with studying microbial diversity, especially in soil. The vast majority of soil microbes (>95%) are not culturable using currently available techniques. Morphological characters are insufficient to allow taxonomy, and the definition of a bacterial species is not at all clear. However, recent use of molecular biological techniques has opened up a new window of observation, which now allows us to view microbial diversity and community structure without the need for laboratory cultivation (Stephan & Kowalchuk, 2002). Thus, we are now in a position to address more directly this response of soil-borne microbial communities in response to changes in macrophyte diversity and species composition.

## 2.14 Microbes in Polluted Soil

Irrespective of the fact that normal soil is a serene habitat for numerous microorganisms, yet contaminated/polluted soils are characterized of harbouring some microbes. Soil is a habitat for diverse groups of microbes especially bacteria, fungi and algae (Megharaj et al., 2003). The soil environment contains a large variety of microbes, which reflect the habitat, and the relative ability of the individual microorganism to compete for the available nutrients (Parkes, 1982).

Some of the microbes in a polluted soil might be pre-existing in the soil before contamination or might be present from the source of contamination. Carlot et al., (2002), isolated several heavy metal resistant bacterial strains from soil contaminated with heavy metals. A study on soil contaminated with wastewater from Agege and Odo abattoirs both in Lagos state of Nigeria, showed a negative effect on the soil microbial population (Adesemoye et al., 2006). Among the organisms isolated from the contaminated soil were *Bacillus sp.*, *Pseudomonas aeruginosa*, *Vibrio sp.*, and *Lactobacillus plantarum* while the uncontaminated soil had *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus sp.*, and *Pseudomonas putida*.

Basically, microbial count can indicate their contribution to the natural environment, since they have important role in fundamental chemical and biological processes in soil fertility and plant growth (Parkes, 1982). Evaluation of microbial growth is a reasonable model for microbial activity investigations in a given system (Crittter et al., 2002). In microbial analysis of soil samples exposed to crude petroleum oil spills, the bacterial population ranged between  $9.5 \times 10^5$  and  $237.5 \times 10^5$  CFU/g soil (Saadoun, 2002).

In the study by Mailin & Firdausi (2006), residential wastewater and industrial oil-contaminated soils were chosen as sources of microorganisms for phenol degradation. The microbes isolated in their study were *Pseudomonas sp*, *Alcaligenes sp* and *Azotobacter sp*. The reason for the choice of such sites may be due to the high probability of presence of toxic pollutants (Nuhoglu & Yacin, 2004).

Metals tend to be of concern in the issue of associated soil toxicity. Most regulations that define a maximum concentration of metals in the receiving soil are based on total soil metal concentration. Still, the potential toxicity of a heavy metal in the soil depends on its specification and availability (Wang et al, 2007). Therefore Kizilkaya et al., (2004), used microbial characterization as indicators to evaluate the heavy metal contamination of agricultural soils (Table 2.17).

Table 2.17 Relationship between heavy metals and microbial characteristics

Heavy Metals	Microbiological characteristics				
	Dehydrogenase activity	Catalase activity	Urease activity	Basal soil respiration	Microbial biomass-C
Cd, $\mu\text{g g}^{-1}$	-0.407 *	-0.605 **	-0.216	-0.476 **	-0.367 *
Co, $\mu\text{g g}^{-1}$	-0.332 *	-0.386 *	-0.186	-0.379 *	-0.331 *
Cr, $\mu\text{g g}^{-1}$	0.076	-0.420 **	0.046	-0.334 *	-0.203
Cu, $\mu\text{g g}^{-1}$	-0.436 **	-0.497 **	0.045	-0.339 *	-0.477 **
Pb, $\mu\text{g g}^{-1}$	0.111	-0.489 **	-0.166	-0.351 *	-0.274
Ni, $\mu\text{g g}^{-1}$	-0.223	-0.423 **	0.040	-0.402 *	-0.545 **

\*P<0.05. \*\*P<0.001

Source: Kizilkaya et al., (2004)

Therefore, since surface water and groundwater pollution by leachate is critical, its ability to contaminate soil is of a major concern and bioremediation options ought to be adopted; reason why bioremediation options is reviewed in the next section of this study.

## **SECTION B – BIOREMEDIATION OPTIONS**

### **2.15 Introduction**

One of the contemporary issues the society must battle with is the dangerous accumulation of recalcitrant compounds in the soil due to prevalent chemical spills or inadequate strategies in disposal (Jansson et al., 2000). A number of the spills originated from associated activities which now make them site specific. For example, chlorinated phenolic compounds are found to accumulate in soil either as by-products from wood treatment processing plants or due to excessive utilization of chloroaromatic pesticides. In some fuel storage areas, military stations, airports, gasoline service stations and refineries, petroleum products are found to accumulate in soil due to petroleum fuel spills (Jansson et al., 2000)

Leaked leachate is a source of soil (Figure 2.5), groundwater, and occasionally surface water pollution that may persist for many decades (Belevi & Baccini, 1992; Kennedy & Everett, 2001). Therefore considering the unavoidable generation of leachate/other pollutants with their potential impacts on the environment, remedial and recovery options tend to be the significant task of most environmental studies. But having the economic balance and eco-protectionism in mind, every contemporary remedial research is weighed on the scale of cost and after-use impacts on the environment. The condition for microbial degradation tends to be jeopardized upon the introduction of high concentrations of such compounds into the environment.

Technological advances have being the backbone of the nascent industrialized society. The advent of many chemical compounds within the 20<sup>th</sup> century had led to modernization of lifestyle as it brought varieties of products. Yet, such chemical compounds had deteriorated the globe significantly in terms of environmental quality

(Iwamoto & Nasu; 2001). Chemicals of such importance include but not limited to heavy metals, organopesticides and xenobiotics. Some compounds like xenobiotic ones [trinitrotoluene (TNT), perchloroethylene (PCE), PCBs and trichloroethylene (TCE)], differ markedly in chemical structure from natural organic compounds due to their toxic impacts, resistance to biodegradation, and even biomagnifications through the food web.



Figure 2.5 Soil pollution due to leachate seepage (Source: Freudenrich, 2013)

Similarly, heavy metals give concern due to the persistent nature in the environment. With leachate being a heterogeneous compound, coupled with surface waters, it becomes imperative to have an overview of soil contamination with heavy metals.

Though much of leachate pollution concerns point towards groundwater and surface water contaminations, but soil is the major membrane through which leachate laterally

flows. Therefore considering the ecological importance of soil, its contamination with leachate is of significant interest. In cases of sanitary landfills, leachate collection systems and associated treatments avail the landfill operators the option of direct discharge of treated leachate into nearby watercourses; hence little or no contact with soil is observed. However, this is extremely different in the situation of non-sanitary landfills or open dump sites. Therefore, finding a corrective measure is necessary especially measures that is (are) green in nature such as bioremediation.

Bioremediation is a general concept that includes the use of biological processes to degrade, break down, transform, and remove contaminants which had altered the environment (Arvanitoyannis & Thassitou, 2001). Arvanitoyannis & Thassitou (2001) also stated that bioremediation is a natural process which relies on capable microorganisms mainly bacteria, fungi, and even plants to immobilize or bio-transform environmental contaminants to innocuous products. In most cases, bioremediation utilizes the metabolic versatility of microorganisms and plants which are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products. A major reservoir of microbes with such potential is soil.

Studies on the distribution and diversity of microbial populations provide knowledge for scientist to understand the interactions between microorganisms and pollutant which may lead to effective remediation planning (Ludvigsen *et al.*, 1999). This is because human activities had strongly influenced the soil conditions by altering it with sorts of pollutants such as pesticides, fertilizers and many more. One of the potential pollutants is landfill leachate generated from MSW disposal. Thus, detailed research need to be done to investigate the impacts of leachate contaminated soil on microbial ecology

while also trying to obtain persistent microbes in such contaminated such that will have a bioremediation potential for the heterogeneous components of leachate.

It is well documented that human industrial activities induced the negative impacts on biological systems and more in particular, the soil status (Lorena *et al.*, 2005). While many anthropogenic activities, such as urban development, agriculture, use of pesticides and pollution can potentially affect soil microbial diversity, it is known how changes in microbial diversity can influence below-ground and above-ground ecosystems (Jennifer *et al.*, 2004). Leachate, a liquid generated when percolation of excess rainwater to the ground is also one of the potential pollutants to contaminate the soil where the contamination level can affects soil texture. According to Nemes and Rawls (2006), soil texture is defined as “*a fundamental physical property of soils, correlated to just any other soil property*”.

Furthermore, a diversity of microbial communities has been identified in leachate plumes and is believed to be responsible for biogeochemical processes such as redox processes (Christensen *et al.*, 2001). Moreover, Ilyina *et al.* (2003) reported that microorganisms that are metabolically capable could occupy a suitable niche that enables them to survive under such contaminated habitat. Bacteria are capable of surviving in metal-contaminated environments.

## **2.16 Soil and Heavy Metals Contamination**

Contamination of soil may be due to accumulation of metalloids and heavy metals sequel to emissions from industrial areas, indiscriminate heavy metal wastes disposal, mine tailings, fertilizer applications, leaded gasoline and paints, incessant use of

pesticides, wastewater irrigation, petrochemicals spills, atmospheric deposition and even residues from coal combustion (Wuana & Okieimen, 2011; Khan et al., 2008; Zhang et al., 2010). Environmentally, heavy metals pose a great risk and amongst the commonly implicated within contaminated sites are arsenic (As), cadmium (Cd), Chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), zinc (Zn) (GWRTAC, 1997) and even iron (Fe).

Based on the anthropogenic activities mentioned *ab initio*, soils are known to serve as main heavy metals' sink. The irony of this fact is that while most organic contaminants under microbial interactions can be oxidized to CO<sub>2</sub>, metals tend not to be chemically and microbiologically degraded (Wuana & Okieimen, 2011; Kirpichtchikora et al., 2006). Consequent upon the introduction of heavy metals in the soil, the total concentration persists for a long time (Adriano, 2003), yet there are possibilities of having changes in their chemical forms and bioavailability.

Buttressing the risk effect of metals, in its toxic nature, biodegradation of organic contaminants can be impaired/ hindered. Yet toxicity of heavy metals can span across the entire ecosystem with humans inclusive via; direct ingestion or contact with polluted soil, drinking contaminated groundwater, food chain (soil- plant – human or soil- plant – animal – human), reduction in food quality (safe and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems (Wuana & Okieimen, 2011; McLaughlin et al., 2000a; McLaughlin et al., 200b; Ling et al, 2007).

Characterization and remediation are required for adequate protection and restoration of soil ecosystem that is contaminated with heavy metals. At both national and

international levels, the nascent legal framework and regulations that consider environmental protection and public health are all dependent on data that characterize chemical properties of environmental phenomenon (Kabata-Pendias & Pendias, 2001). Therefore to have an insight into heavy metal speciation and bioavailability, soil characterization becomes important, while an attempt to bioremediate heavy metal contaminated soils would involve knowledge of the contaminant, basic chemistry, environmental and associated health risks of these heavy metals.

For the remediation of heavy metal-contaminated soil, techniques like immobilization, soil washing and phytoremediation have been often listed among the best demonstrated available technologies (BDAT) (GWTAC, 1997). Regardless of their eco-friendliness and cost-effectiveness, the application in field is limited to developed countries. So far, such technologies are not commercially available in the developing countries because of inadequate awareness of their inherent advantages and principles of operation. Based on the increasing awareness by different sectors (government, public and private) on the dangers associated with contaminated soils on human and animal health, a simultaneous increase in solution-solving urge has been observed across scientists with view to develop technologies to remedy contaminated sites. High population density and scarcity of funds pose a threat for environmental restoration, however, cheaper and ecologically sustainable remedial options are required to recover polluted lands, with the intention of reducing the associated impacts, making the land resource available for agricultural production and enhance food security.

## 2.17 Sources of heavy metals in contaminated soils

At very minute levels which is often regarded as ‘trace’ ( $<1000 \text{ mg Kg}^{-1}$ ), heavy metals occur naturally within soil environment often due to weathering of parent materials at ready toxic conditions (Kabata-Pendias & Pendias, 2001; Pierzynski et al., 2000). The tendency of heavy metals in soils of rural and urban environments to accumulate to the degree capable of causing risks to animals, plants, human health, ecosystems, and or other media, is chiefly attributed to man’s interference and disruption of nature’s slowly occurring metals’ geochemical cycle (D’Amore et al., 2005). In soil environment, the contaminants status of heavy metal is because (i) they exhibit more rapid rates of generation via manmade cycles than natural ones, (ii) can be easily transferred from mines to diverse locations in the environment where occurrence due to direct exposure potential are higher, (iii) unlike receiving environment, the metal concentrations in discarded products are relatively high, and (iv) bioavailability may be enhanced in the receiving environmental system depending on the chemical form (species) of the metal (D’Amore et al., 2005). Below expression depicts a simple mass balance of heavy metals as found in the soil (Alloway, 1995; Lombi & Gerzabek, 1998):

$$M_{\text{total}} = (M_p + M_a + M_f + M_{\text{ag}} + M_{\text{ow}} + M_{\text{ip}}) - (M_{\text{cr}} + M_l), \quad (1)$$

where “ $M$ ” = heavy metal,

“ $p$ ” = parent material,

“ $a$ ” = atmospheric deposition,

“ $f$ ” = fertilizer sources,

“ $\text{ag}$ ” = agrochemical sources,

“ $\text{ow}$ ” = organic waste sources,

“ip” = inorganic pollutants,

“cr” = crop removal, and

“l” = losses by leaching, volatilization, and so forth.

The anthropogenic emission of heavy metals into the atmosphere was projected to be one-to-three of magnitude more than natural fluxes (Sposito & Page, 1984). Heavy metals in the soil of anthropogenic origin are characterized of high mobility which enhances bioavailability than found in the lithogenic or pedogenic ones (Kuo et al., 1983; Kaasalainen & Yli-Halla, 2003). Different anthropogenic sources of metal-bearing solids at contaminated sites can emanate from disposal of high metal wastes, unprotected landfills, animal manures, metal mine tailings, biosolids (sewage sludge), leaded gasoline and lead-based paints, fertilizer application on land, compost, petrochemicals, pesticides, coal combustion residues, and atmospheric deposition (Khan et al., 2008; Zhang et al., 2010; Basta et al., 2005) as discussed subsequently.

*Fertilizers.* Historically, the first significant influence of human on soil was agricultural practice (Scragg, 2006). Though macronutrients (N, P, K, S, Ca and Mg) are required for plant growth and completion of lifecycle, yet micronutrients also remain essential. Such micronutrients (Co, Cu, Fe, Mn, Ni and Zn) though also referred to as heavy metals are equally required for healthy plant growth (Lasat, 2000), hence it can be used as foliar spray or added to the soil for crops use. Soils deficient in Cu is often treated with Cu in order to be suitable for cereal crops, just as Mn can be similarly supplied to root crops and cereal (Wuana & Okieimen, 2011). During intensive farming, fertilizers are supplied in large quantities for the provision of sufficient N, P, K required for crop growth. Most times, trace amounts of heavy metals (Cd, Pb, and others) exist as impurities in the compounds used to supply these elements and persistent application of

fertilizer may significantly increase the concentrations of such heavy metals in the soil (Jones & Jarvis, 1981). While certain metals like Pb and Cd may have no known physiological activity, yet Cd and some other potentially toxic metals like F, Pb and Hg may be inadvertently added to the soil via the application of certain phosphatic fertilizers (Raven et al., 1984).

*Pesticides.* In the past, most of the extensively used pesticides in agriculture and horticulture contained substantial amounts of metals. For example, previously in UK, about 10% of chemicals that contain Cu, Hg, Mn, Pb or Zn were approved for use as fungicides and insecticides. *Bordeaux* mixture (copper sulphate) and copper oxychloride which are copper-containing fungicidal sprays were examples of such pesticides (Jones & Jarvis, 1981). In fruit orchards, lead arsenate was used for many years as control for some parasitic insects (Wuana & Okieimen, 2011). Similarly, in New Zealand, compounds containing arsenic were extensively used to control cattle ticks and pests in banana, just as timbers were preserved with formulations of Cu, Cr and As (CCA) in Australia; hence many derelict sites exist where soil concentrations of these elements are now above the background concentrations (Wuana & Okieimen, 2011). Problems can emanate from such contamination, especially in situations where such sites become reutilized for either agricultural or other non-agro related activities. Therefore, the utilization of some of such materials has been more localized when compared with fertilizers and are being restricted to specific sites or crops (McLaughlin et al., 2000).

*Biosolids and Manures.* The accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb in soil also occur from the numerous soil application of livestock manures, composts and municipal sewage sludge, which are known to be

biosolids (Basta et al., 2005). Poultry, pig and cattle manures which are considered animal wastes as produced in agriculture are usually being applied in form of solid or slurries to crops and pasture (Sumner, 2000). In as much as manure is viewed as valuable fertilizer, the addition of Zn and Cu to pig and poultry diets to serve as growth promoters tend to be another source of heavy metal contamination of soil (Sumner, 2000; Chaney & Oliver, 1996). Use of such dietary options cause high concentrations of As, Cu, and Zn in the subsequently generated manure and the continued application of the manure to restricted land enhance high bioaccumulation of these metals in the soil after a long time.

Primarily, biosolids (sewage sludge) are solid organic products resulting from treatment of wastewater which can be beneficially recycled. For countries that permit the reuse of biosolids produced by urban population, application of biosolids to land is a common practice (Wegler et al., 2004). Due to wide recognition and the regulatory definition of the term “sewage sludge”, it is used in many references, but replacing it with another term “biosolids” is gaining more grounds because it is viewed to specifically represent the beneficial characteristics associated to sewage sludge (Silveira et al., 2003). About half of the 5.6 million dry tonnes of sewage sludge generated in United States are applied to land and biosolids are agriculturally utilized in every region of the country (Wuana & Okieimen, 2011). More than 30% of generated sewage sludge in Europe serves as agricultural fertilizer (Silveira et al., 2003). Similarly, Australia recorded the utilization of more than 175, 000 tonnes of dry solids generated yearly across the metropolitan authorities on agricultural lands by incorporating them into the soil for arable cropping (McLaughlin et al., 2000).

Also the use of some other materials like straw, sawdust or garden waste to compost biosolids has been in practice, and the implication of continued application of these to soil is metal contamination of soils. Hence, the perceived potential to contaminate soil with heavy metals has always been a subject of concern over its agricultural use (Canet et al., 1998). Pb, Ni, Cd, Cr, Cu, and Zn are the major heavy metals often found in biosolids and the nature and intensity of industrial activity, in conjunction with the adopted biosolids' treatment, determine the concentrations of such heavy metals (Mattigod & Page, 1983). At certain situations, soil profile and groundwater may be contaminated as a result of the leaching of the heavy metals added to soils from applied biosolids. Study on New Zealand soils treated with biosolids had indicated high levels of Cd, Ni, and Zn in leachates from drainages (Keller et al., 2002).

*Wastewater.* The use of industrial, and municipal wastewater, and some other effluents on land has been in existence hundreds of years back and is currently a common practice in different parts of the world (Reed et al., 1995). Globally, wastewater is used to irrigate over 20 million hectares of agricultural lands (Wuana & Okieimen, 2011). In fact, agro-based wastewater irrigation accounts for 50% of vegetable supply in most urban areas of African and Asian cities (Bjuhr, 2007). While having much interest in increasing crop yields and profit maximization, most farmers show no regards for environmental benefits or hazards. In as much as the concentrations of metals tend to be relatively low in wastewater effluents, yet a long-term use of such wastewater on land can ultimately lead to accumulation of heavy metals in the soil (Wuana & Okieimen, 2011).

*Metal Mining and Milling Processes and Industrial Wastes.* The trend in wide distribution of heavy metal contamination of soil had been passed on to many countries

due to the excessive mining and milling of metal ores in combination with other associated industrial activities. During mining activity, particles (tailings) which are larger and heavier settle at the bottom of the flotation cell, and in most cases, are discharged directly into natural depressions and outside wetlands; hence generating increased metal concentrations (DeVolder et al., 2003). Risk on humans and ecological health had often occurred as in situations of extensive Zn and Pb ore mining and smelting that results into soil contamination (Wuana & Okieimen, 2011). Restoration of soil productivity is difficult because of the lengthy and expensive nature of most reclamation methods adopted for such contaminated sites. Environmental risk of soil heavy metal to humans is highly related to bioavailability. Pathways through which the accumulation can take place include the direct ingestion (oral bioavailability) of, or ingestion of plant material grown in (food chain), contaminated soil (Basta & Gradwohl, 1998).

Some other industrial materials generated, like tanning, textile, petrochemicals from accidental spills or utilized petroleum-based products, pesticides and pharmaceutical components, often differ in composition. In as much as many of such products are disposed of on land but beneficial importance of most of them to agriculture or forestry are very minimal. In fact, most are potential risk because of heavy metals contents (Cr, Pb, and Zn), or toxic organic components which are not desirable for land application. Some lack any importance in terms of soil conditioning properties and are relatively low in supply of plant nutrients (Sumner, 2000).

*Air-Borne Sources.* These include but not limited to duct or stack emissions of air, vapour streams, or gas. It can also be dust from storage areas or piles of waste, which are known to be fugitive emissions. Hence, metals which are airborne are usually

emitted from gas stream in form of particulates. During high temperature processing, volatilization of some metals like As, Cd and Pb can occur (Wuana & Okieimen, 2011). Such metals will undergo conversion to metal oxides before condensing as fine particulates expect in situations where a reducing atmosphere is maintained (Smith et al., 1995). Stack emissions can be dispersed across a wide area due to effect of natural air currents until it is removed from the gas stream by dry and/or wet precipitation mechanisms.

Fugitive emissions are often made near the ground and as such the distribution covers only a smaller area unlike stack emissions. This implies that stack emissions release higher contaminant concentrations than in fugitive emissions. However, metal concentrations and types released from both sources (fugitive and stack) depends on site-specific conditions. Since the dawn of industrial revolution, large scale contamination has been prevalent due to utilization of fossil fuels which often contain some heavy metals. The ensuing activities allow solid particles in smokes from fires and other emissions like those from factory chimneys to be eventually deposited on land and sea (Wuana & Okieimen, 2011). For example, soil and plants that were located adjacent to smelting works have shown high concentrations of accumulated Cd, Pb and Zn. Also, the combustion of petrol that contains tetraethyl lead give rise to aerial emission of Pb and is another source of soil contamination; it significantly accounts for Pb concentrations in soils within urban areas and adjacent major roads (Wuana & Okieimen, 2011). Tyres and lubricant oils can as well add Cd and Zn to soils adjacent roads (USEPA, 1996).

## 2.18 Basic Soil Chemistry and Potential Risk of Heavy Metals

Pb, Cr, As, Zn, Cd, Cu, and Hg is the order at which these common heavy metals are abundant in contaminated sites (USEPA, 1996). The relevance of the aforementioned heavy metals is embedded in the fact that they possess the ability of reducing crop production because of their bioaccumulation and biomagnification potentials in food chain. Similarly, they pose risk of contamination of both surfacewater and groundwater. Therefore, the understanding of the fundamental chemistry, environmental and health related impacts of the heavy metals are very important in explaining their speciation, bioavailability, and remediation options. The pathway of heavy metals in soil in terms of the fate and transport is determined by the forms in which the metal exists chemically and the speciation. Immediately heavy metals are in the soil, an initial fast reaction absorbs them within minutes or hours, and is subsequently trailed by gradual adsorption reactions that take some days or even years to occur, and as such, they become reallocated into diverse chemical types accompanied by variations in toxicity, mobility and bioavailability potentials (Shiowatana et al., 2001; Buekers, 2007). It is assumed that such reallocation is directed by certain heavy metal reactions which take place in soil and includes (i) precipitation of minerals and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake (Levy et al., 1992).

*Lead.* In the periodic table, Pb belongs to group IV and period VI, and the values of its atomic number, atomic mass, density, melting and boiling points are 82, 207.2, 11.4 g cm<sup>-3</sup>, 327.4<sup>0</sup>C and 1725<sup>0</sup>C, respectively. Pb occurs naturally as a bluish gray metal in a mineral state often in combination with other elements like sulphur (PbS, PbSO<sub>4</sub>), or oxygen (PbCO<sub>3</sub>), and its concentrations within the earth's crust is 10 to 30 mg kg<sup>-1</sup> (USDHHS, 1999). However, global surface soils contain about 32 mg kg<sup>-1</sup> of Pb but can

vary from 10 to 67 mg kg<sup>-1</sup> (Kabata-Pendias & Pendias, 2001). Based on industrial scaling of metals production, Pb is next in rank after Fe, Cu, Al, and Zn (Wuana & Okieimen, 2011). In US, about 50% of Pb utilized is actually used in the production of Pb storage batteries. It is also used in soldering, cable coverings, production of bearings, arms, caulking, pigments and plumbing (Wuana & Okieimen, 2011). A number of Pb alloys exist and can be found in the production of storage batteries (Sb), maintenance-free type of storage batteries (Ca & Sn), solder and anodes (Ag), electro-winning process (Sr & Sn), sheet and pipes used in chemical installations and nuclear shielding (Te), printing, sleeve bearings and advanced castings (Sb & Sn) (Manahan, 2003).

Oxides of Pb, Pb (II), and Pb-metal oxoanion complexes represent the common forms of Pb that easily find their way into surfacewaters, soil profile and eventually, groundwater. Pb (II) and lead-hydroxy complexes are the most stable Pb forms, but with Pb (I) as the commonest and most reactive, and have the ability to form monocuclear and polynuclear oxides and hydroxides (GWARTC, 1997). Insoluble Pb predominantly exists as Pb carbonates and (hydr) oxides (Raskin & Ensley, 2000). In soil matrix, PbS is characterized of more stable solid form and increasing the sulphide concentrations enhances its formation under reducing conditions. Also, tetramethyl Pb which is volatile organo-Pb is formed under anaerobic conditions due to microbial alkylation (GWARTC, 1997).

Compounds of Pb (II) are mostly ionic (Pb<sup>2+</sup> SO<sub>4</sub><sup>2-</sup>), whereas Pb (IV) compounds tend to be covalent (e.g., tetraethyl lead, Pb(C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>). Some Pb(IV) compounds, such as PbO<sub>2</sub>, are strong oxidants. Many basic salts can be formed from Pb, like Pb(OH)<sub>2</sub> · 2PbCO<sub>3</sub>, which was sometime the most generally utilized white paint pigment and the source of considerable chronic lead poisoning to children that had the habit of eating peeled white

paint. Many compounds of Pb (II) and some Pb(IV) compounds are useful. Lead dioxide and lead sulphate were the most common forms of the aforementioned and take part in the reversible reaction that occurs during the charge and discharge of lead storage battery.

While inorganic Pb compounds exist, some other organo-Pb compounds also abound, such as tetraethyl-Pb. It is worthy to note the toxic and environmental effects of organo-Pb compounds due to the previous excessive utilization of tetraethyl-Pb as a gasoline additive (Wuana & Okieimen, 2011). In as much as there have been synthesis of above 1000 organo-Pb compounds, yet those of alkyl Pb (methyl & ethyl) compounds with their salts are the ones that possess commercial and toxicological relevance.

Major routes of Pb exposure are via inhalation and ingestion, with both having same effect. Plumbum which is Pb poisoning can occur in humans due to bioaccumulation of Pb in any organ(s) of the body (brain) and may eventually cause death. Hence, central nervous system, kidneys and gastrointestinal tract are highly susceptible to Pb exposure (Wuana & Okieimen, 2011). When children are exposed to Pb, growth impairment, reduced intelligence quotient, limited attention span and hyperactivity are imminent. In some cases, mental retardation occurs in children less than six years old. However, when the exposure occurs in adults, then reduced reaction time, memory loss, anorexia, insomnia, nausea and joint's weakness are prevalent. Hence, Pb is not an essential element. Its toxicity and associated effects are well known and unlike other trace metals, its review is extensive; it has high injury potential on red blood cells (Baldwin & Marshall, 1999). Diverse range of biological effects can take place as a result of Pb exposure and such depends on the degree and duration of exposure. As effects vary across different levels of exposure, vulnerability is enhanced in young and infants than

in adults (Wuana & Okieimen, 2011). However, with the exception of some developing areas, it is now rare to witness Pb poisoning to the tune of visible illness.

Directly ingesting contaminated soil or dust is the most pronounced source of exposure to soil Pb. While in the normal sense, accumulation of Pb in plant is not possible because plants do not absorb it, but existence of the Pb in very high concentrations cause eventual uptake by plants. Research have demonstrated near impossible accumulation of Pb in vegetable fruiting parts and some other fruit crops like beans, tomatoes, corn, apples, squash and strawberries, but it may be found in leafy vegetables such as lettuce and on the surface of root crops like carrots (Wuana & Okieimen, 2011). Considering plants' inability to absorb high concentrations of soil Pb, the levels of Pb in soil that is considered safe for plants will be much higher than soil Pb levels in situations where eating of soil is a concern (pica). Generally, at less than 300 ppm of Pb in garden soil, produce grown in such soil is considered safe (Rosen, 2002). Increase in Pb concentration above the aforementioned level is considered to cause Pb poisoning risk in food chain. However, regardless of Pb levels in soil ( $> 300$  ppm), much risk can be attributed to Pb contaminated soil or dust deposited on the plants as against suspected biouptake of Pb by the plant (Rosen, 2002).

*Chromium.* In the periodic table, this element is a transition metal that is d-block from group VI B with 24, 52,  $7.19 \text{ g cm}^{-3}$ ,  $1875^{\circ}\text{C}$ , and  $2665^{\circ}\text{C}$  as the values of its atomic number, atomic mass, density, melting and boiling points, respectively. It is a rare element and does not naturally exist in elemental form rather as compounds. As a primary ore product, the mining of Cr occur in mineral chromite form,  $\text{FeCr}_2\text{O}_4$ . Cr contamination can emanate from discharges associated to electroplating processes and the disposal of wastes that contain Cr (Smith et al., 1995). In most contaminated site, Cr

is usually found in form of Cr (VI). This metal exist in the +III oxidation state, consequent upon the existing pH and redox conditions. Even, in shallow aquifiers, Cr (VI) is common since the existing environment there is aerobic (Smith et al., 1995). However, under anaerobic situation, organic matters in the soil, Fe and  $S^{2-}$  can enhance reduction of Cr (VI) to Cr (III) especially within deeper groundwater. Chromate ( $CrO_4^{2-}$ ) and dichromate ( $Cr_2O_7^{2-}$ ) are the major species of Cr (VI) that can easily be precipitated in the presence of  $Ba^{2+}$ ,  $Pb^{2+}$ , and  $Ag^+$  (metal cations). Both Cr (VI) species can be adsorbed on soil surfaces such as Fe and Al oxides. Below pH 4, Cr (III) becomes the dominant form of Cr. In combination with  $Cl^-$ ,  $F^-$ ,  $CN^-$ ,  $NH_3$ ,  $SO_4^{2-}$  and  $OH^-$ ,  $Cr^{3+}$  give rise to the formation of solutions and organic ligands that are soluble. Mobility of Cr (VI) is higher and as such exhibit higher toxicity than other forms of Cr. With any pH less than 5, the mobility of Cr (III) is reduced by clays and oxide adsorption just as its solubility is also low under such ph as a result of the  $Cr(OH)_3(s)$  formulation (Chrostowski et al., 1991). The mobility of Cr depends on the soil's sorption characteristics, clay content concentrations of iron oxide, and organic matter present (Wuana & Okieimen, 2011). When in precipitated or soluble forms, transport of Cr from surface run-off to surface waters can occur, where as both Cr complexes (soluble and un-absorbed) can potentially get to groundwater as a result of soil leaching. Higher pH values enhance the leaching potential of Cr (VI) (Smith et al., 1995). In most cases Cr is found in sediments and its release into natural water is often associated with release of particles (Smith et al., 1995) and allergic dermatitis is the common health risks of Cr in humans (Scragg, 2006).

*Arsenic.* In the periodic table, the group and period of arsenic (Ar) is VA and IV, respectively, and its occurrence is observable in different types of minerals especially  $AS_2O_3$ . Its optimal recovery can be achieved from the ores processing especially when

the ores contain Ag, Au, Cu, Pb and Zn. Similarly, combustion of coal is another source of arsenic because it is found in the generated ashes. The basic properties of Ar includes but not limited to atomic number (33), atomic mass (75) and density ( $5.72 \text{ g cm}^{-3}$ ). The melting and boiling points of Ar are  $817^{\circ}\text{C}$  and  $613^{\circ}\text{C}$ , respectively, while exhibiting a bit complex chemistry with varying oxidation states which are -3, 0, 3 and 5 (Smith et al., 1995). As (V) is dominantly found in aerobic environments, especially in arsenate ( $\text{AsO}_4^{3-}$ ) formed at diverse protonation states like  $\text{H}_3\text{AsO}_4$ ,  $\text{H}_2\text{AsO}_4^-$ ,  $\text{HAsO}_4^{2-}$ , and  $\text{AsO}_4^{3-}$  (Wuana & Okieimen, 2011). In the presence of metal cations, arsenate and other arsenics in ionic forms can be precipitated and exhibit characters typical of chelates (Bodek et al., 1988). Stability of metal arsenate complexes is only possible in certain conditions. In fact, within reasonable reducing and acidic conditions, arsenic (V) have the potential to co-precipitate with or get adsorbed on to iron oxyhydroxides (Wuana & Okieimen, 2011).

Whereas the aforementioned conditions can immobilize coprecipitates, increased pH enhances the mobility of arsenic (Smith et al., 1995). As (III) can exist as arsenite ( $\text{AsO}_3^{3-}$ ) due to its ability to dominate during reducing conditions with the protonated form as  $\text{H}_3\text{AsO}_3$ ,  $\text{H}_2\text{AsO}_3^-$ , and  $\text{HAsO}_3^{2-}$ . Arsenite have the ability to coprecipitate with or adsorb metal sulphides while also exhibiting high affinity towards compounds of sulphur. During severe reducing conditions arsenic may exist in form of arsine and elemental arsenic. Dimethyl arsine  $\text{HAs}(\text{CH}_3)_2$  and trimethylarsine  $\text{As}(\text{CH}_3)_3$  which are methylated arsine derivatives are very volatile and can be created by methylation which is a biotransformation process. However, it is difficult to obtain complexes formations of arsenic with  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  which are simple anions because of the anionic form in which arsenic usually exist in. Similarly, methylarsinic acid  $(\text{CH}_3)\text{AsO}_2\text{H}_2$  and dimethylarsinic acid  $(\text{CH}_3)_2\text{AsO}_2\text{H}$  are other speciations of arsenic which are

organometallic in nature. Due to the ability of As to highly get adsorbed to soils, it makes the transport across surfacewater and groundwater to cover only short distances. Health risks associated to As include damage on the skin, susceptibility to cancer, and impairment of circulatory system (Scragg, 2006).

*Zinc.* It is a transition metal located in group IIB and period IV, of the periodic table. Zinc which naturally occur in soil at approximately  $70 \text{ mg Kg}^{-1}$  within the earth crust is characterized with atomic number 30, atomic mass 65.4, density  $7.14 \text{ g cm}^{-3}$ , melting point  $419.5^\circ\text{C}$ , and boiling point  $906^\circ\text{C}$  (Davies & Jones, 1988). However, its concentrations are anthropogenically increasing as a result of significant daily activities that are industrial in nature especially mining, extraction of coal, combustion of waste, and processing of steel (Wuana & Okieimen, 2011). Zn concentrations can be found in certain foodstuff. Similar situation is obtainable in most drinking water that contains considerable concentrations of Zn but may be influenced by storage especially in the situation of use of metal tanks which may increase the Zn content of the water. Some times increased levels of Zn content in drinking water may be induced by presence of toxic waste sites or other industrial sources, which result in health risks. However, it is worthy to note that as a trace element, Zn remains essential to human health and its deficiency can induce birth defects. Global production of Zn is still on increase; hence it still adds more Zn into the environment. This is even evident with the degree at which water is being polluted with Zn owing to high concentrations that abound in wastewater from industrial plants (Wuana & Okieimen, 2011). It therefore leads to continued deposition of sludge polluted with Zn at river banks. Water acidity increases with increased Zn concentration.

The accumulation of Zn in fish bodies is obtainable when in contact with waterways contaminated with Zn, and can be easily biomagnified as one goes up the food chain (Greany, 2005). Groundwater is also subject to contamination as long as water-soluble Zn exists. There is also a limit for which plants can take up Zn within their systems because Zn easily accumulates in soils (Greany, 2005). Another detrimental effect of Zn is its ability to negatively interfere with soil activities and this in turn hinders the degradation potentials of microorganisms and earthworm especially as relates organic matters (Greany, 2005).

*Cadmium.* Cadmium is another transition element of significant interest. Its atomic number and weight are 48 and 112.4, respectively, whereas the density, melting and boiling points are  $8.65 \text{ g cm}^{-3}$ ,  $320.9^{\circ}\text{C}$ , and  $765^{\circ}\text{C}$ , respectively. Alongside Hg and Pb, Cd is a well known dangerous metal and possesses no known essential biological function. Compounds of Cd often occur in form of divalent Cd (II) ion. However, due to its proximity to Zn in the periodic table (directly below), both share chemical similarity especially in terms of being essential micronutrient for animals and plants. Such may partly be the reason behind the toxicity of Cd; since the metabolic processes tend to malfunction when Zn which is an essential trace element is substituted by Cd (Campbell, 2006). The use of Cd in Ni/Cd batteries is significant because it serves as a source for rechargeable or secondary power, give out high output, less maintenance, long life and high resistance to stress (both electrical and physical). Coatings using Cd offer good corrosion property to vessels and vehicles, especially for the ones in marine and aerospace because of the nature of the environments which is usually high-stressed. Cd can also be used as pigments, stabilizers for polyvinyl chloride (PVC), in alloys and electronic compounds (Wuana & Okieimen, 2011). In phosphate fertilizers, products from refined petroleum and detergents, Cd often exist as impurity. Also, the transport of

Cd can be enhanced geochemically by the acidification of soil and surface water from acid rain, which eventually cause increase in surfacewater concentrations of Cd just as the pH of lake water decreases (Campbell, 2006). Occasionally, Cd can be produced from refining of lead, and can be unavoidably generated as byproduct of Zn as well. Among other ways, the total concentrations of soil Cd can increase due to atmospheric deposition of contaminants, incessant use of pesticides, fertilizers and biosolids (sewage sludge) to serve as agricultural ingredients to soil or the exposure to disposed industrial wastes, which in all influence plant Cd uptake potential due to bioavailability (Weggler et al., 2004). The metal demonstrates little toxicological properties despite its biopersistence ability, but can remain in a host for longer years once it is absorbed.

Dating back to the 1970s, there has been continued concern over human exposure to Cd contamination via food ingestion especially in situations where one consumes some specific vegetables and shellfish. Hence the attention on these perceived source (agro-based products) triggered inquiry into the effect of using sewage sludge which is often in Cd or phosphate fertilizers that contain Cd, on soil for farming of humanly consumed crops (Campbell, 2006). Such scientific investigation gave rise to addressing the concentrations permissible for certain food crops (McLaughlin, 2000).

The presence of Cd in human body is known to inhibit a lot of enzymatic activities. It is assumed that Cd negatively affect kidney tubules by preventing the reabsorption of protein which is the responsibility of certain enzymes, hence causing proteinuria that implies renal damage. Also the activities of alcohol dehydrogenase, delta-aminolevulinic acid synthase, lipoamide dehydrogenase and arylsulfatase, are reduced by Cd, meanwhile it propagates the activities of pyruvate decarboxylase, deltaminolevulinic acid dehydratase and pyruvate dehydrogenase (Manahan, 2003). A remarkable and

widely reported incident of poisoning due to Cd intake was that of dietary situation among the people in the Jintsu River Valley, close to Fuchu, Japan. it was named *itai itai* disease, which Japan means *ouch, ouch* (Manahan, 2003). The situation caused a painful bone disease called osteomalacia in conjunction with malfunctioned kidney (Manahan, 2003). Such poisoning in Jintsu River Valley was traced to production of Pb, Zn and Cd at an upstream mine which eventually contaminated the irrigated rice in the region. Hence, chronic accumulation of Cd in the kidney is its most profound danger to human health since it can lead to kidney dysfunction. Therefore, the most possible entry routes of Cd into human body are food digestion and smoking of tobacco (Manahan, 2003).

*Copper.* This is a group IB transition element and found in period 4 of the periodic table. The atomic number, atomic weight, density, melting and boiling points are 29, 63.5, 8.96 g cm<sup>-3</sup>, 1083<sup>0</sup>C and 2595<sup>0</sup>C, respectively. In crustal rocks, it is found to show 8.1 × 10<sup>3</sup> kgm<sup>-3</sup> and 55 mg kg<sup>-1</sup> as the average density and concentrations, respectively (Davies & Jones, 1988).

Globally, copper (Cu) is commonly utilized, and is needed in plant and animal growth due to its essential micronutrient nature. Haemoglobin production in blood is influenced by Cu as far as human body is concerned, and it similarly plays significant role in plants as it relates resistance to disease, water regulation and production of seed. However, its importance become outlived when it exists in excess, because it is capable of causing health risks such as kidney and liver damages, anaemic, and gastrointestinal irritation (Wuana & Okieimen, 2011). It contaminates drinking water due to the use of pipes made of Cu and also as a result of Cu additive components aimed for algal growth control. Though there might be high complexity when Cu interacts with the

environment, yet studies had portrayed Cu to be relatively fast in being stable when released into the environment and exists in forms that appear not to pose threat to the environment. Buttressing this fact, Cu does not bioaccumulate in the food chain nor get magnified in human body, as opposed to situation with other man-made materials. It is not easy to find much of ionic copper [Cu(II)] in the soil because it easily becomes complexed to organic. At pH 5.5, copper's solubility is enhanced (Martinez & Motto, 2000), and as such makes it ideal for farmland since its pH requirement is 6.0 – 6.5 (Eriksson & Anderson, 1997).

For use by plants, animals, microbes and humans, Cu and Zn are very important and essential metals. Quite a number of physico-chemical factors and some other physiological characteristics of crops determine the discrete interaction between soil and water contamination and uptake of metals by plants. The threats from trace metals contaminated soil may be direct and indirect. A visible adverse impact of the metals on crop is a direct threat, whereas it can be classified as indirect threat when human health is impaired due to entrance of metals into food chain (Wuana & Okieimen, 2011). A pronounced long term production and income loss may be witnessed even at the drop in few percent of crop yield (Bjuhr, 2007). Therefore, most food importers now designate acceptable maximum concentrations so as to drastically reduce the chances of exporting their contaminated crops (Bjuhr, 2007).

*Mercury.* In the periodic table, mercury (Hg) exists alongside Zn and Ca. Hg is the only metal that exist in liquid form at standard temperature and pressure (STP). The atomic number, atomic weight, density, melting and boiling points stand at 80, 200.6, 13.6 g cm<sup>-3</sup>, -13.6<sup>0</sup>C and 357<sup>0</sup>C, respectively. It is often obtained from ore processing as a byproduct (Smith et al., 1995). The major source of Hg contamination is combustion of

coal which in turn releases Hg. Similarly, Hg contamination can come from the manometers used at pressure measuring stations, and gas/oil pipelines (Wuana & Okieimen, 2011). Consequent upon discharge into the environment, Hg can be found in diverse forms such as  $\text{Hg}^{2+}$  (mercuric),  $\text{Hg}_2^{2+}$  (mercurous),  $\text{Hg}^0$  (elemental), or methyl/ethyl mercury (alkylated) (Smith et al., 1995). The presence of stable forms of Hg is decided by pH and redox potential of the system. However, at oxidizing conditions, more stability are accorded to mercuric and mercurous mercury.

Under reducing conditions, inorganic and organic Hg can be reduced to  $\text{Hg}^0$  and subsequently transformed into alkylated forms as a result of biotic and abiotic processes. The alkylated Hg which is both volatile in the air and can dissolve in water, is very toxic (Smith et al., 1995). Yet, Hg(II) when combined with different organic ligands produce strong complexes (Bodek et al., 1988). Hg is mainly removed from solution via soils, humic materials and sediments' sorption. Increase in pH also increases sorption because it depends on pH. In some situations, coprecipitation using sulphides is another way of removing mercury. Also anaerobic situations enhance the transformation of inorganic and organic Hg into alkylated products with the influence of microbial activity especially sulphur-reducing bacteria. Anaerobic situation also avail the formation of elemental mercury via demethylation of methyl mercury or reduction of Hg(II). However, methyl mercury formation can as well occur under  $\text{pH} < 4$ , since increase in pH enhance  $\text{HgS}_{(s)}$  (Smith et al., 1995). Mercury is implicated in damage of kidney (Scragg, 2006).

*Nickel*. This belongs to the group of transition elements and possesses atomic number 28 and atomic weight 58.69. It exists as nickelous ions  $[\text{Ni}(\text{II})]$  within regions of low pH, whereas in slightly alkaline solution it is neutral and precipitates a stable compound

$\text{Ni}(\text{OH})_2$ . Dissolution of the ensuing precipitate in acid solutions gives nickelite ion ( $\text{HNiO}_2$ ) which is water soluble. Yet, Ni can exist in another stable form  $\text{Ni}_3\text{O}_4$  (nickel oxide) under the influence of oxidizing and alkaline situations, thereby making it soluble in solutions that are acidic. However, other forms of nickel oxides exist,  $\text{Ni}_2\text{O}_3$  and  $\text{NiO}_2$ , but show unstable property in alkaline solutions and can decompose by giving off oxygen. When these solids dissolve in acidic regions,  $\text{Ni}^{2+}$  is produced (Pourbaix, 1974).

The occurrence of Ni in the environment is usually at low levels and is important at reduced doses. However, at exceeding the tolerable quantity, Ni can be dangerous. Such situation can be cancerous to different animal body parts and the more vulnerable ones are those that habit near refineries (Wuana & Okieimen, 2011). Ni is commonly used as a component in production of steel and other metals. Nickel can contaminate soil due to release from metal plating industries, electroplating and mining, and burning of fossil fuels (Khodadoust et al., 2004). Once emitted into the air by power plants and trash incinerators, Ni undergoes precipitation before settling on the ground, and the removal of Ni from air consumes a lot of time. Nickel can contaminate surfacewater if it is a component of wastewater streams. Soil particles or sediments can immobilize Ni by mere absorption upon release of large quantity to the environment. The metal can as well leach into adjacent groundwater when present in acidic soils which make it more mobile. Microbial population can be negatively affected with the presence of Ni though there is tendency of development of some resistance by the microbes (Wuana & Okieimen, 2011). However, no record of biomagnification of Ni has been found in food chain considering the fact that it is not known to have any bioaccumulation potential in animals or plants.

Therefore, considering the pollution effect of the aforementioned heavy metals, it becomes imperative to reduce or even totally remove them from the polluted site. Hence, options for restoring contaminated soil to its original state become subject of concern. Remediation technologies tend to embrace either discrete or combined applications of physical, chemical and biological methods.

### **2.19 Remediation options for heavy metal contaminated soil**

The general essence of soil remediation using any approach is to ensure that end solution is achieved and must be capable of protecting human health and the environment (Martin & Ruby, 2004). Therefore, remediation depends on a number of regulatory requirements. In situations where standards are only advisory or non-existing legislative standards, the evaluation of human health and ecological dangers become the basis of remediation. Most regulatory authorities in a bid to adopt remediation strategies give priority to options that decrease the bioavailability of heavy metals on the grounds that the reduced bioavailability is commensurate with the reduced risk, and the bioavailability reductions should be long term effective (Martin & Ruby, 2004).

When soil is contaminated with heavy metals, adopting the suitable remediation technique is highly determined by the physical and chemical forms of the heavy metal contaminant. In order to have a precise evaluation of the contaminated site and to explore remedial alternatives, it is important to obtain data on characteristic of the site in terms of physical characteristics, level and type of contamination. Characterizing the contaminated soil provides idea on level, type and distribution of the pollutant in the soil. Once the site has been characterized, the desired level of each metal in soil must be determined. This is done by comparison of observed heavy metal concentrations with

soil quality standards for a particular regulatory domain, or by performance of a site-specific risk assessment. Remediation goals for heavy metals may be set as total metal concentration or as leachable metal in soil, or as some combination of these.

Several technologies exist for the remediation of metal contaminated soil. Gupta et al (2000) have classified remediation technologies of contaminated soils into three categories of hazard-alleviating measures: (i) gentle *in situ* remediation, (ii) *in situ* harsh soil restrictive measures, and (iii) *in situ* or *ex situ* harsh soil destructive measures. The goal of the last two harsh alleviating measures is to avert hazards either to man, plant, or animal while the main goal of gentle *in situ* remediation is to restore the malfunctionality of soil (soil fertility), which allows a safe use of the soil. At present, a variety of approaches have been suggested for remediating contaminated soils. USEPA (2007) has broadly classified remediation technologies for contaminated soils into (i) source control and (ii) containment remedies. Source control involves *in situ* and *ex situ* treatment technologies for sources of contamination (Wuana & Okieimen, 2011). *In situ* or in place means that the contaminated soil is treated in its original place; unmoved, unexcavated; remaining at the site or in the subsurface. *In situ* treatment technologies treat or remove the contaminant from soil without excavation or removal of the soil. *Ex situ* means that the contaminated soil is moved, excavated, or removed from the site or subsurface. Implementation of *ex situ* remedies requires excavation or removal of the contaminated soil. Containment remedies involve the construction of vertical engineered barriers (VEB), caps, and liners used to prevent the migration of contaminants (Martin & Ruby, 2004).

Another classification places remediation technologies for heavy metal-contaminated soils under five categories of general approaches to remediation: isolation,

immobilization, toxicity reduction, physical separation, and extraction (GWRTAC, 1997). In practice, it may be more convenient to employ a hybrid of two or more of these approaches for more cost effectiveness (Wuana & Okieimen, 2011). The key factors that may influence the applicability and selection of any of the available remediation technologies are: (i) cost, (ii) long-term effectiveness/permanence, (iii) commercial availability, (iv) general acceptance, (v) applicability to high metal concentrations, (vi) applicability to mixed wastes (heavy metals and organics), (vii) toxicity reduction, (viii) mobility reduction, and (ix) volume reduction.

*Immobilization Techniques.* *Ex situ* and *in situ* immobilization techniques are practical approaches to remediation of metal-contaminated soils. The *ex situ* technique is applied in areas where highly contaminated soil must be removed from its place of origin, and its storage is connected with a high ecological risk (e.g., in the case of radio nuclides). The method's advantages are: (i) fast and easy applicability and (ii) relatively low costs of investment and operation. The method's disadvantages include (i) high invasivity to the environment, (ii) generation of a significant amount of solid wastes (twice as large as volume after processing), (iii) the byproduct must be stored on a special landfill site, (iv) in the case of changing of the physicochemical condition in the side product or its surroundings, there is serious danger of the release of additional contaminants to the environment, and (v) permanent control of the stored wastes is required (Wuana & Okieimen, 2011). In the *in situ* technique, the fixing agents' amendments are applied on the unexcavated soil. The technique's advantages are (i) its low invasivity, (ii) simplicity and rapidity, (iii) relatively inexpensive, and (iv) small amount of wastes are produced, (v) high public acceptability, (vi) covers a broad spectrum of inorganic pollutants (Martin & Ruby, 2004). The disadvantages of *in situ* immobilization are (i) its only a temporary solution (contaminants are still in the environment), (ii) the

activation of pollutants may occur when soil physicochemical properties change, (iii) the reclamation process is applied only to the surface layer of soil (30–50 cm), and (iv) permanent monitoring is necessary (Martin & Ruby, 2004; USEPA, 1997).

*Soil Washing.* Soil washing is essentially a volume reduction/waste minimization treatment process. It is done on the excavated (physically removed) soil (*ex situ*) or on-site (*in situ*). Soil washing as discussed in this review refers to *ex situ* techniques that employ physical and/or chemical procedures to extract metal contaminants from soils. During soil washing, (i) those soil particles which *host* the majority of the contamination are separated from the bulk soil fractions (physical separation), (ii) contaminants are removed from the soil by aqueous chemicals and recovered from solution on a solid substrate (chemical extraction), or (iii) a combination of both (Dermont et al., 2008). In all cases, the separated contaminants then go to hazardous waste landfill (or occasionally are further treated by chemical, thermal, or biological processes). By removing the majority of the contamination from the soil, the bulk fraction that remains can be (i) recycled on the site being remediated as relatively inert backfill, (ii) used on another site as fill, or (iii) disposed of relatively cheaply as nonhazardous material.

*Ex situ* soil washing is particularly frequently used in soil remediation because it (i) completely removes the contaminants and hence ensures the rapid cleanup of a contaminated site (Wood, 1997), (ii) meets specific criteria, (iii) reduces or eliminates long-term liability, (iv) may be the most cost-effective solution, and (v) may produce recyclable material or energy (GOC, 2003). The disadvantages include the fact that the contaminants are simply moved to a different place, where they must be monitored, the risk of spreading contaminated soil and dust particles during removal and transport of

contaminated soil, and the relatively high cost (Wuana & Okieimen, 2011). Excavation can be the most expensive option when large amounts of soil must be removed, or disposal as hazardous or toxic waste is required (Wuana & Okieimen, 2011).

Acid and chelator soil washing are the two most prevalent removal methods (Peters, 1999). Soil washing currently involves soil flushing an *in situ* process in which the washing solution is forced through the in-place soil matrix, *ex situ* extraction of heavy metals from the soil slurry in reactors, and soil heap leaching. Another heavy metal removal technology is electroremediation, which mostly involves electrokinetic movement of charged particles suspended in the soil solution, initiated by an electric gradient (Reed et al., 1995). The metals can be removed by precipitation at the electrodes. Removal of the majority of the contaminants from the soil does not mean that the contaminant-depleted bulk is totally contaminant free. Thus, for soil washing to be successful, the level of contamination in the treated bulk must be below a site specific action limit (e.g., based on risk assessment). Cost effectiveness with soil washing is achieved by offsetting processing costs against the ability to significantly reduce the amount of material requiring costly disposal at a hazardous waste landfill (CLAIRE, 2007; Wuana & Okieimen, 2011).

*Phytoremediation.* Phytoremediation, also called green remediation, botanoremediation, agroremediation, or vegetative remediation, can be defined as an *in situ* remediation strategy that uses vegetation and associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless (Cunningham & Ow, 1996; Helmisaari et al., 2007). The idea of using metal-accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years on

wastewater discharges (Chaney et al., 1997; Henry, 2000). Plants may break down or degrade organic pollutants or remove and stabilize metal contaminants. The methods used to phytoremediate metal contaminants are slightly different from those used to remediate sites polluted with organic contaminants. As it is a relatively new technology, phytoremediation is still mostly in its testing stages and as such has not been used in many places as a full-scale application. Phytoremediation is energy efficient, aesthetically pleasing method of remediating sites with low to-moderate levels of contamination, and it can be used in conjunction with other more traditional remedial methods as a finishing step to the remedial process (Wuana & Okieimen, 2011).

The advantages of phytoremediation compared with classical remediation are that (i) it is more economically viable using the same tools and supplies as agriculture, (ii) it is less disruptive to the environment and does not involve waiting for new plant communities to recolonize the site, (iii) disposal sites are not needed, (iv) it is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods, (v) it avoids excavation and transport of polluted media thus reducing the risk of spreading the contamination, and (vi) it has the potential to treat sites polluted with more than one type of pollutant (Wuana & Okieimen, 2011). The disadvantages are as follow (i) it is dependent on the growing conditions required by the plant (i.e., climate, geology, altitude, and temperature), (ii) large-scale operations require access to agricultural equipment and knowledge, (iii) success is dependant on the tolerance of the plant to the pollutant, (iv) contaminants collected in senescing tissues may be released back into the environment in autumn, (v) contaminants may be collected in woody tissues used as fuel, (vi) time taken to remediate sites far exceeds that of other technologies, (vii) contaminant solubility may be increased leading to greater environmental damage and the possibility of leaching (Wuana & Okieimen, 2011).

Potentially useful phytoremediation technologies for remediation of heavy metal-contaminated soils include phytoextraction (phytoaccumulation), phytostabilization, and phytofiltration (Garbisu & Alkorta, 2001).

## **2.20 Bioremediation and associated issues**

Presently, most effort is being put in place to obtain cheap designs and strategies feasible enough for the purpose of cleaning-up contaminated sites. Bioremediation has specifically demonstrated capability to serve as a cheap and veritable clean-up strategy. The use of indigenous microbial population as in-situ bioremediation approach has been an increasing commonly adopted method of contaminated sites clean-up, especially with contaminants that are readily degradable (Jansson et al., 2000; Vogel, 1996).

When a technology implores the metabolic potential of microorganisms for the purpose of cleaning up contaminated environment, then it is known as bioremediation. While most bioproductions or biotechnologies require sterile conditions, bioremediation avail the opportunity of being undertaken in a non-sterile open environment that harbour other organisms. In such situation, whereas the central role of bioremediation is vested on the bacteria anticipated to have the pollutants degrading ability, other organisms like fungi and grazing protozoa also influence the process (Watanabe, 2001).

Bioremediation has attracted increasing attention as an active biotechnological tool to recover a polluted environment, due to its use of microbes to detoxify and breakdown environmental contaminants (Iwamoto & Nasu, 2001). Basically, modification of environment, such as application of nutrient and aeration, and appropriate addition of

degraders by seeding, are the core bioremediation approaches (Iwamoto & Nasu, 2001). This scientific research approach demonstrates edges over the conventional chemical and physical treatment technologies, especially under the conditions of where diluted and widely spread contaminants are involved (Iwamoto & Nasu, 2001).

An on-the-site treatment also known as *in-situ* application is one of the most alluring edges of this technology. Borrowed from Latin, *in-situ* implies “in its original place”. Hence, this aspect of bioremediation avails the remediation of a polluted site devoid of need to physically transport the pollutants, while as the same time minimizing site interferences. In cases of pollution within an actively manufacturing site, bioremediation process can be underway while routine activities of the industry go on. In fact, typical of Japan, residential areas are often situated near contaminated sites, and as such adoption of bioremediation is much beneficial (Iwamoto & Nasu, 2001). Reports on the successful use of bioremediation to recover petroleum-contaminated sites (Iwamoto & Nasu, 2001; Ryan et al., 1991), heavy metals (Barathi & Vasudevan, 2001; Mollea et al., 2005) have been documented.

This is not to imply that bioremediation is devoid of uncertainties and even demerits. Regardless of the fact that in biogeochemical cycles, microorganisms play critical role (Belser, 1979; Wolin & Miller, 1987; Lovely, 1991) and also serve as the primary stimulant while remedying polluted environment, yet the explanation into changes in the microbial community during bioremediation is limited. Hence microbial community tend to be still seen as a “black box”.

The inability of conventional laboratory techniques to culture most environmental bacteria is the reason for the above (Kogure, 1979; Olsen, 1987). Hence, questions arise on, (i) how to clarify the biological contribution to the effectiveness of bioremediation and (ii) how to assess the environmental impact of bioremediation. Due to technical obstacles in monitoring the discrete bacteria that is directly related to the degradation of contaminants, bioremediation is saddled with the task of identifying the cause and developing measures in the case of the failure remediation from a microbiological standpoint.

### **2.21 Bioremediation systems and process**

Technical application of bioremediation technologies can either be ex-situ or in-situ. However, such treatments within the contaminated medium possess several merits over the conventional physical and chemical remediation technologies. Such merits include;

- it can be carried out on site;
- waste is permanently eliminated;
- it attracts zero transportation cost;
- interferences with site is minimized;
- it can be applied even in situations of diluted and widely diffused contaminants;
- and
- the cost is readily.

Hence, bioremediation process can be divided into three different strategies; namely bioattenuation, biostimulation and bioaugmentation.

*Bioattenuation:* It is a simple strategy whereby natural degradation progress is monitored to ensure reduction in a contaminant's concentration with time at desired contaminated site.

*Biostimulation:* consequent upon natural degradation or extremely slow degradation, biodegradation process is then manipulated to stimulate the process and enhance increased rates of reaction. Such condition is referred to as biostimulation as it primarily involves addition of nutrients to the environment in form of nitrogen, phosphorus, and electron acceptors like oxygen (Iwamoto & Nasu, 2001). Sometimes substrates like methane, phenol and toluene are added but should be carefully monitored during biostimulation, especially in the case of toluene and phenol addition because they are popular toxic chemicals. The effectiveness of methane on in-situ biostimulation process was demonstrated in Japan, where methane was injected into TCE-contaminated groundwater in a small-scale field experiment (Iwamoto & Nasu, 2001). However, the interest to introduce desired microbes into polluted environment as an option of bioremediation had attracted significant attention.

## **2.22 Bioaugmentation**

In addition to stimulating the indigenous microbial population to degrade organic compounds, another innovative technique is to add microorganisms with specific metabolic capabilities (Lee & Ward, 1985). This involves the enhancement of the biodegradative ability of polluted sites by inoculation of bacteria that possess intended catalytic capabilities. In situations where bioattenuation and biostimulation are not working properly, this approach is considered the most effective especially when recalcitrant chemicals are involved (Iwamoto & Nasu, 2001). The advent of introducing exogenous microbes into the environment (bioaugmentation) was intended to speed up

the rate of bioremediation. Basically, it is important to assert the fate of an introduced organism in order to pin point its contribution to contaminant degradation and to evaluate its impact on the ecosystem. While some will advocate for inoculation of directly isolated microbes, others may prefer engineered organisms.

However, considering some side effects of bioaugmentation on the ecosystem, much attention is accorded its application. This is because large amounts of degradative bacteria are introduced into the polluted sites; hence the impact of such bacteria on the environment in general and human in particular, need to be clarified beforehand. In most cases, the introduced microbes are designed to perish after the remediation as this is aimed to avoid negative impact on the indigenous microbial community for a long period. The inoculation of bacteria into the subsurface has recorded some success (Walton & Dobbs, 1980; Ohneck & Gardner, 1982; Sikes et al., 1984), yet the technique is besieged by some environmental uncertainties such as adverse effects on human health, survival of the specialized microorganism in the environment and determination of set risk levels acceptable to the public (Joyce, 1983).

While trying to remedy a polluted site in Japan, *Ralstonia eutropha* KT-1, which is phenol-utilizing bacteria was originally isolated from the same polluted site, and was re-injected without addition of any substrate (Iwamoto & Nasu, 2001). Bioaugmentation can be interesting especially in situations where genetically modified microorganisms (GEMs) are utilized. A modified strain, *Burkholderia cepacia* PR1<sub>301</sub> was utilized for field bioaugmentation on Moffett Federal Air-field in the USA after microcosm studies in the laboratory (McCarty, 1998). The result showed that the organism effectively degraded TCE while growing on lactate and as such serves as a replacement for the use

of other substrates like phenol and toluene which are toxic chemicals (Munakata-Marr, 1996; Iwamoto & Nasu, 2001).

However, bioaugmentation is better used when complex recalcitrant compounds are involved (Jansson et al., 2000). As such it serves as a useful alternative considering the use of precisely adopted or designed microbial inoculants (Jansson et al., 2000; Vogel, 1996). Yet, of concern is the fact that microbial bioremediation sometimes lack the ability to degrade every component of chemical mixtures with equal efficiency. Coschigano et al (1994) and Erb et al (1997) pointed out that though xenobiotics are usually recalcitrant to degradation, use of genetic engineering now avails the widening of the substrate range to enhance its degradation. However, knowledge on the inoculum's survival in the environment and the associated effects is another debate over the use of genetically modified microbes (Gaustafsson and Jansson, 1993; Jansson et al., 2000).

Similarly, researchers may differ in applauding bioaugmentation approach because it tend not be clear on whether the added microbes have the ability to thrive long enough in the real harsh environmental conditions, in order to completely perform the desired clean-up task. Hence, the use of traditional cultivation methods to assess the performance, or efficacy of bioremediation is a daunting task. This is because distinction between added inocula and the indigenous microflora requires high specificity (Jansson et al., 2000). Also, it is known that after the introduction of most laboratory grown strains, they always became stressed, hence are not culturable on agar medium (Jansson et al., 2000). Such implies that culture-based monitoring techniques

are devoid of specificity and sensitive requirements for precise monitoring of inocula during bioremediation of contaminated sites.

### **2.23 Bioremediation of toxic metals**

Bioremediation is not limited to restoration of organic compounds contaminated sites but can also be used to treat heavy metals contaminated areas. Under anaerobic condition, some bacteria have reduced the mutagenic and toxic hexavalent chromium to its less toxic form, trivalent (Wang et al., 1989). Bioprecipitation by sulphate-reducing bacteria also abound. Such bacteria convert sulphate present in groundwater to H<sub>2</sub>S which, in turn, will readily react with heavy metals there to form insoluble metal sulphides like ZnSO<sub>4</sub> and CdSO<sub>4</sub> (Iwamoto & Nasu, 2001).

Contamination of environment with heavy metals is a serious problem due to its toxic nature and as such nascent research have used molecular tools to analyze the bacterial (Sandaa et al., 1999a; Brim et al., 1999) and archaeal populations (Sandaa et al., 1999b) with the ability of surviving in metal-contaminated environments. With previous study on microbial community of soil amended with sewage sludge that contained heavy metals, it was observed that two sequence groups affiliated with the  $\alpha$ -Proteobacteria and Actinobacteria were always obtained from clone libraries from the metal-contaminated soil (Watanabe, 2001), in as much as that most Actinobacteria sequences showed low similarity (<85%) to the sequences of any hitherto cultured actinomycete (Watanabe, 2001). The organisms are considered useful for metal bioremediation because of their detoxification machineries (Watanabe, 2001). *Ralstonia eutropha* has been genetically modified to express mouse metallothionein on the cell surface (Valls et al., 2000) and as such it had shown that the inoculation of Cd polluted soil with the

genetically modified *Ralstonia* had significantly reduced the toxic effects of the heavy metal on the growth of tobacco plants (Watanabe, 2001).

Similarly, a number of bacteria, algae and fungi are involved in the catalyzed biomethylation to produce volatile derivatives like dimethylselenide or trimethylarsine (White et al., 1997). Hence, aforementioned mechanisms depict potentials of bioremediation on sites contaminated with heavy metals.

Bioremediation is still considered to be a developing technology. One difficulty is that bioremediation is carried out in the natural environment, which contains diverse uncharacterized organisms. Most pollutants-degrading microorganisms isolated and characterized in the laboratory are now thought to make a minor contribution to bioremediation. Another difficulty is that no two environmental problems occur under completely identical conditions; for example, variations occur in the types and amounts of pollutants, climate conditions and hydrogeodynamics (Iwamoto & Nasu, 2001). These difficulties have caused the bioremediation field to lag behind knowledge-based technologies that are governed by common rationales.

## **CHAPTER THREE**

### **TOXICITY OF LEACHATE**

#### **3.1 Introduction**

Leachate is a heterogeneous substance. This makes it difficult to simply predict and document its toxic impact. However, combination of chemical evaluations and bioassays can provide a better insight into the elucidation of the potential toxic effects of leachate on aquatic life. Methods and associated materials adopted in executing this research are explained in this chapter. Therefore, the major components of the research involve site investigation and characterization, leachate characterization, toxic evaluation of leachate on fish species, and histopathology study. Hence, the results were discussed subsequently.

#### **3.2 Objectives**

This section was designed for the purpose of understanding;

- the characteristics and comparison of leachate from sanitary and non-sanitary (active and non-active) landfills in Malaysia;
- the acute mortality effect of raw leachate on both edible and non-edible fish species which are dominant in Malaysia
- development of a leachate toxicity index in Malaysia
- the possibility of heavy metals uptake in fish exposed to leachate pollution; and
- the evidence of fish tissue impairment due to leachate exposure.

### **3.3 Materials and Methods**

#### **3.3.1 Site Characterization**

Air Hitam Sanitary Landfill (AHL), Jeram Sanitary Landfill (JSL), Bukit Beruntung Landfill (BBL) and Taman Beringin Landfill (TBL) leachate were used in this study. AHL and JSL are sanitary landfills with all requirements for environmental quality assessment (Fauziah & Agamuthu, 2003) while BBL and TBL are non-sanitary ones. The collection of leachate samples from these landfills for detailed biochemical and physico-chemical analysis, toxic assessment, and pollutants removal, meets the investigation aim to document landfill toxicity studies on fish fauna and bioremediation option in Malaysia. The general conditions of the studied landfills are shown in Table 3.1.

The four landfills were adopted to represent active (sanitary & non-sanitary) and closed (sanitary & non-sanitary) landfills. This was designed to serve as key variables potentially affecting the degree of toxicity of leachate in Malaysia. JSL and AHL are active and closed sanitary landfills, respectively, unlike BBL and TBL that are also active and closed, respectively, but are non-sanitary landfills. Such requirements that accorded sanitary status include use of structured perimeter fences and drains, environmental protection facilities, elimination of scavenging activities, enclosing bund, daily cover, separated working area, designated area for unloading, gas removal system, leachate collection system, monitoring of landfill leachate and leachate treatment system (Fauziah & Agamuthu, 2003; MHLG, 2006; Suratman et al., 2011).

Table 3.1 General Conditions of the Landfills Studied

<b>Landfills</b>	<b>Jeram Landfill</b>	<b>Air Hitam Landfill</b>	<b>Bukit Beruntung Landfill</b>	<b>Taman Beringin Landfill</b>
<b>Landfill type</b>	Sanitary (Active)	Sanitary (Closed)	Non-sanitary (Active)	Non-sanitary (Closed)
<b>Location</b>	3 <sup>0</sup> 27.63'N; 101 <sup>0</sup> 02.54'E	3 <sup>0</sup> 07.44'N; 101 <sup>0</sup> 46.22'E	3 <sup>0</sup> 32.14'N; 101 <sup>0</sup> 25.80'E	3 <sup>0</sup> 13.78'N; 101 <sup>0</sup> 39.72'E
<b>Period of landfilling</b>	2007 – date	1995 – 2006	2001 - date	1991 - 2005
<b>Age classification</b>	Young	Stabilized	Mature	Stabilized
<b>Daily Average of waste disposed (tonnage)</b>	2000 and above	1000 – 2000	1500	1800 - 2000
<b>Waste type</b>	Household, Commercial Industrial	Household, Commercial Industrial	Household, Commercial Industrial	Household, Commercial Industrial and others
<b>Form of Leachate</b>	Physical, Chemical and	Physical, Chemical and	Biological	Physical and Biological
<b>Treatment</b>	Biological	Biological		
<b>Distance to river/stream (m)</b>	70	5	5	-
<b>DOE Requirement for Effluent Discharge</b>	Standard B	Standard A	Standard A	Standard B
<b>Fate of Landfill gas generated</b>	Yet to be utilized	Converted to Electricity	No facility	No facility

### **3.3.2 Leachate Sampling and Laboratory Analysis**

For a better understanding of the toxicity study of the leachate, raw leachate samples were collected for 5 times (on different days) from the landfills and duly replicated to ensure coherence in analysis. The raw leachate samples were collected from the pipes directly linked to the landfill cells (AHL & JSL), while the others were scooped from seepages and overflows (BBL & TBL) from designated spots and taken immediately to the laboratory. The leachate analyses were carried out in respect to the physical, biochemical and chemical parameters.

#### **Physical Parameters**

The following parameters were analyzed on-site or less than 2 hours from the time of samples collection, in the laboratory;

- Colour – this was carried out by both visual observation with naked eyes and colorimeter.
- Odour – nose as the sense organ of smell was used to ascertain the odourant quality of the sample.
- Dissolved Oxygen – was measured using the DO 6 Dissolved Oxygen palm-top meter.
- pH – dispensing some volume of the sample into a beaker, the pH meter (HANNA HI 8424) probe was introduced to check the pH level.
- Salinity – probe of a multipurpose Hach Sension 7 was introduced into the sample to take the salinity reading.
- Conductivity – The multipurpose Hach Sension 7 was used to measure the conductivity of the sample.
- Total Dissolved Solid – This parameter was measured with the multipurpose Hach Sension 7.

- Turbidity – 2ml of diluted sample was pipetted into a test tube and placed inside the DR 4000 UV-VIS spectrophotometer and the HACH program was used to take the turbidity reading. Distilled water was used as blank.
- Suspended Solid – 2ml of diluted sample was pipetted into a test tube and placed inside the DR 4000 UV-VIS spectrophotometer; USER program was used to take the suspended solid reading. Distilled water was used as blank (600 nm).

### **Bio-Chemical Parameters**

The following bio-chemical components were analyzed within 2 hours from sample collection time;

- Biochemical Oxygen Demand (BOD) (APHA, 1998)
- Chemical Oxygen Demand (COD) (APHA, 1998)

### **Chemical Parameters**

For the purpose of analyzing the chemical components of the leachate samples from the two landfills, several methods that conform to international standards were adopted. The international standards adopted in this study were;

- APHA- American Public Health Association (APHA, 1998)
- EPA- Environmental Protection Agency (US EPA, 2000)
- AOAC- Association of Official Analytical Chemists (AOAC, 1984; Horwitz, 2000)

Therefore Table 3.2 represents the chemical components that were analyzed in line with their corresponding international test methods.

Table 3.2 Chemical compounds analyzed in the leachate

<b>Parameters</b>	<b>Standard Methods Adopted</b>
Ammoniacal Nitrogen	APHA 4500 NH <sub>3</sub> C
Alkalinity	APHA 2320B
Oil & Grease	APHA 5520B
Chloride	APAH 4500Cl <sup>-</sup>
Total Organic Carbon	APHA 5310B
Nitrate Nitrogen	APHA 4500-NO <sub>2</sub>
Nitrite Nitrogen	APHA 4500-NO <sub>2</sub>
Phosphosphate	APHA 4500 P-B,C
Metals	APHA 3112B
Volatile Fatty Acids	EPA 8270
Alcohols	EPA 8270
Monocyclic Aromatic Hydrocarbons	EPA SW 8260B
Semivolatile Organic Carbon	EPA SW 6010B EPA SW 8081A (for p-Cresol) EPA SW 8260B (for phenol)
Organophosphorus Pesticides	AOAC 974. 22
Organochlorine Pesticides	APHA 6630B
Other Chlorinated Hydrocarbons	EPA 8270

The common principle of Inductively-coupled plasma mass spectrometry (ICP-MS) method was utilized for the metals analysis in the raw leachate, while Gas Chromatography Mass Spectrometry (GC-MS) machine was used to analyze for the volatile fatty acid, alcohols, monocyclic aromatic hydrocarbons, semivolatile organic carbon, organophosphorus pesticides, organochlorine pesticides and other chlorinated hydrocarbons in the raw leachate samples.

### 3.3.3 Fish Test

Fish species used in this research are freshwater types and native to waters of Southeast Asia though a few can thrive well in stagnant waters. Fish species were obtained from commercial aquaculture farms in Malaysia. Ten fish species (Table 3.3) were utilized to carry out the toxicity test of the raw leachate.

Table 3.3 Fish species used for evaluating raw leachate toxicity

Scientific Name	Common/Local Name	Classification
<i>Pangasius sutchi</i>	Patin	Edible
<i>Clarias batrachus</i>	Keli	Edible
<i>Leptobarbus hoeveni</i>	Jelawat	Edible
<i>Mystus nemurus</i>	Baung	Edible
<i>Oreochromis mossambicus</i>	Tilapia Merah	Edible
<i>Xiphophorus maculatus</i>	Red Coral Platy	Non-edible/Ornamental
<i>Poecilia sphenops</i>	Black Molly	Nonedible/Ornamental
<i>Hyphessobrycon eques</i>	Serpae tetra	Non-edible/Ornamental
<i>Devario aequipinnatus</i>	Seluang	Non-edible/Ornamental
<i>Danio rerio</i>	Zebra	Non-edible/Ornamental

### Acclimatization

Before putting the fish into the aquaria, 20 g of aquarium salt was added into each of the aquarium and aerated continuously for 1 week with air pump of 0.04 MPa. This was to remove any trace of chlorine from the tap water. The fish was later put into the aquarium maintained at pH 7.2 – 8.0, dissolved oxygen concentration 7.0 – 8.2 mg/L, and temperature 27.3 ° - 28.2 °C. The fishes were acclimated for 7 days without further aeration to resemble the natural aquatic environment for the fish (Emenike et al., 2012a). There was much unfilled air space in the aquarium that allows for moderate natural aeration (Plate 3.1).



Plate 3.1 Fish aquarium used for acclimatization

The test organisms were fed with commercial fish feed (pellets). Feeding was stopped 48 hours prior to initiating the acute toxicity testing (APHA, 1998). This was to reduce metabolic wastes from the fish during the experiment. The photoperiod was set at 12 hours of light and 12 hours of dark throughout the duration of the experiment. Proper measures were taken to ensure that mortality of less than 5% was maintained 5 days prior to toxicity testing. The fish used for toxicity were fingerlings (ranged from 2 – 5cm).

### **Acute toxicity testing**

Standard recommendations (APHA, 1998; USEPA, 2000; OECD, 1993) were considered while carrying out the acute toxicity test on the aforementioned fish species. Laboratory Static Test was carried out to determine the median Lethal Concentration ( $LC_{50}$ ) of the raw leachate sample on each fish species. Specifically the acute toxicity test in this study was based on the ISO 7346/1 water quality- determination of acute

lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (*Teleostei, Cyprinidae*) - Part 1: Static method of 1984] (APHA, 1998).

For each fish species, a group of 10 fish (in triplicates) of same weight were selected randomly and transferred from the acclimatization tank using small hand net into suitable test aquarium of 25 L capacity. This was to avoid any occurrence of mechanical injury to the test fish. The fishes' weight to water ratio in the aquarium was maintained at an average of 1.0 g/L. A range finding test was carried out with widely spaced concentrations before definitive ranges were chosen for the acute tests. A control group of equal number of fish was set up without addition of leachate sample. Dissolved oxygen level of not less than 5 mg/L was observed despite the absence of air pump throughout the experiment (control maintained 7.8 mg/L without use of air pump). The water temperature was maintained at  $28 \pm 1^\circ\text{C}$  which is optimal for most fish in aquatic environment. All experiments were conducted on 96 hours basis and no feeding was done during the experiment. The mortality and behavioural changes of both control and exposed groups were recorded daily at 12 hours intervals. Zero mortality was maintained in the control group thereby allaying fear of experimental interference due to starvation. Dead fish were immediately removed from the experiment in order to prevent DO depletion which is typical of static bioassay and also preservation for tissue analysis.

A 12 hourly record of the mortality result against every used raw leachate concentration was taken. Due to observed partial mortality in some replicates during the 96 hours test, median lethal concentration ( $\text{LC}_{50}$ ) of the raw leachate on the fish species for each of the landfills was calculated using Finney's probit method (Finney's Probit Analysis

version 1.5) (Peltier & Weber, 1985). Graphical plot of discrete percentage mortality of test organism against test time was generated.

Similarly, the data (mortality response) was also fitted to generate a predictive model that can be used to calculate degree of mortality with time upon introduction of varying concentrations of leachate. Datafit version 9.0 (Oakdale Engineering software) was used to generate the model to fit all leachates' acute toxicity tests by integrating the discrete % mortality of each fish species along the exposure time to generate one plot model for all the landfills' leachate;

$$Y = a^*X1 + b^*X2 + c^*X3 + d^*X4 + e^*X5 + f \dots\dots\dots \text{(Eq.1) (Emenike et al., 2013)}$$

Where Y = discrete % mortality that occurs with the varying leachate concentrations

a\* - e\* = corresponding variables to leachate concentrations (mortality coefficients)

f\* = a constant mortality coefficient

X1 - X5 = varying leachate concentrations.

### **3.3.4 Fish Lethal Leachate Toxicity Index (FLLTI)**

A FLLTI proposed in this study was to describe the potential toxicity of landfill leachate in Malaysia to fish species. The FLLTI is derived and modified from the Pesticide Toxicity Index (PTI) developed by Munn and Gillion (2001). It depends on the model concentration addition which presume that the toxicity of multiple compounds is additive. But it does not account for possible antagonistic or synergistic interactions. While PTI in United States was adopted for comparison of relative toxicity of pesticide mixtures among sites, this study is proposing FLLTI as a comparative

model for relative toxicity of leachate and over time and to identify the risk distribution on fish species upon contact with surface water.

Therefore, PTI was modified to obtain FLLTI as shown below;

$$FLLTI = \sum_{i=1}^n \frac{L_i}{MTC_{x,i}} \dots\dots\dots \text{(Eq. 2)}$$

Where

$L_i$  = concentration of leachate i

$MTC_x$  = Medium toxicity concentration of leachate i for fish x

n = number of landfills leachate

L and MTC are expressed in same units.

Hence, to calculate FLLTI on any of the fish species, it is obtained as stated herein;

-to calculate FLLTI on *Clarias batrachus* (Cb)

$$FLLTI_{Cb} = \frac{L_{ASL}}{MTC_{CbASL}} + \frac{L_{NASL}}{MTC_{CbNASL}} + \frac{L_{ANSL}}{MTC_{CbANSL}} + \frac{L_{NANSL}}{MTC_{CbNANSL}} \dots \text{(Eq. 3)}$$

Where

ASL = Active Sanitary Landfill (e.g. JSL)

NASL = Non-Active Sanitary Landfill (e.g. AHL)

ANSL = Active Non-Sanitary Landfill (e.g. BBL)

NANSL = Non-Active Non-Sanitary Landfill (e.g. TBL)

$L_{ASL}$  = Concentration of leachate from JSL

$L_{NASL}$  = Concentration of leachate from AHL

$L_{ANSL}$  = Concentration of leachate from BBL

$L_{NANSL}$  = Concentration of leachate from TBL

$MTC_{CbASL}$  = Median toxicity concentration of JSL for *Clarias batrachus*

$MTC_{CbNASL}$  = Median toxicity concentration of AHL for *Clarias batrachus*

$MTC_{Cb}ANSL$  = Median toxicity concentration of BBL for *Clarias batrachus*

$MTC_{Cb}NANSL$  = Median toxicity concentration of TBL for *Clarias batrachus*

Therefore, FLLTI is the sum of toxicity quotients for each leachate over a fish species as measured from the  $LC_{50}$  (the adopted toxicological endpoint in this study).

### **3.3.5 Tissue Analysis (Histology Method)**

In order to further examine and evaluate the effect of the leachate on the test organisms other than mortality rate, laboratory analysis of gills of the dead fish were conducted using histology approach. Sample abstraction and fixation was done on the  $LC_{50}$  concentrations (2 of each fish species per concentration) and dissected accordingly to obtain the liver and gills. Care was taken to avoid pinching or pressing hard on the gills and liver samples that were chosen for fixation. Sharp blade was used to cut the tissue samples to 2mm thick and 19mm long. These were rinsed with normal saline solution.

The tissue samples were placed into specimen tubes containing 30 ml of fixative solutions (10% formalin [neutralized with  $MgCO_3$ ] for the liver and Bowin Solution for the gills). The composition of the 10% formalin was 10 ml formalin with 90 ml distilled water, while the Bowin solution was 75 ml saturated picric acid, 25 ml formalin and 5ml glacial acetic acid. This was allowed to stand for 24 hours. Caution was taken where labelings were done with dark pencil only. This stage was repeated for another 2 days.

## **Dehydrations and Paraffin Infiltration**

The fixatives were poured out from the specimen tubes into the waste bottle. After this, the specimen tubes were filled with 10ml of 70% alcohol solution to start the dehydration process.

### **Immersion**

In order to ensure dehydration, the following were carried out;

- Tissue samples were transferred into specimen tubes with 85% alcohol solution and kept for 30 minutes
- This was followed by another transfer into 95% alcohol for another 30 minutes before being transferred into the last specimen tube with 95% alcohol and kept for 30 minutes

### **Alcohol Removal**

After the dehydration process, two immersions in Terpeneol solution were done. Terpeneol solution is a clearing agent and it was used to clear out the alcohol in the samples. The samples were immersed twice in Terpeneol at intervals of 30 minutes.

### **Paraffin Infiltration**

The last step in this stage was the transfer of the tissue samples from the second Terpeneol solution into a tube that contained a mixture of Terpeneol-paraffin in a 1:1 ratio. The Terpeneol-paraffin mixture has been melted in the oven (60°C) enabling it to infiltrate the tissue. The tissue samples were immersed in the mixture and kept in the oven for 30 minutes. In order to complete the paraffin infiltration process, two more immersions in the paraffin were carried out.

## **Embedding**

Paper boxes were prepared for embedding of the tissue samples. The paraffin boxes were filled with melted paraffin and the tissue samples were immediately transferred into them. Caution was taken in the placement orientation of tissue samples as this affects the direction of sectioning to be done during preparation of histological slides. Once the surface of the paraffin solidified, the paper block containing the embedded tissue sample was placed into a container filled with water to cool and harden the paraffin block quickly.

## **Tissue Sectioning and Affixing of Tissue Sections onto Slides**

- The paraffin blocks containing the embedded tissue samples were trimmed to suitable size according to types of section of interest (longitudinal for liver and transverse for gills). These were then attached firmly to block stage.
- the block stage was fixed on the microtome holder for sectioning (cutting) the tissue sample
- a diamond pencil was used to mark the histological slide in order to define the face of the slide mounted with the tissue section
- A drop of Mayer's Albumin at the center of the slide was immediately spread evenly with a clean finger in order to make a thin layer of the Mayer's Albumin covering the slide.
- This was followed by a drop of distilled water at the center of the slide and slightly glided to allow even spread to  $\frac{3}{4}$  part of the slide.
- The tissue section was then lowered on the slide and ensured that there was no air bubble trapped under the paraffinized tissue section.
- Ensuring there was enough water to warm the paraffin section, it was placed on a slide warmer at 40 - 45°C for about 7mins.

- Tissue sections were allowed to spread a bit and the slides were temporarily removed from the slide warmer in order to remove excess water using a paper towel.
- It was replaced on the slide warmer to ensure complete drying and kept in readiness for staining.

### **Haematoxylin and Eosin Staining Technique**

The slides were transferred into caplin jars for the staining process. The slides were then stained as below;

- Placed in Xylol solution for 3 minutes (for deparaffinization or dewaxing).
- Transferred to another Xylol solution for 3 minutes.
- Placed in 95% alcohol for 3 minutes (for dehydration).
- Transferred into another 95% alcohol for 3 minutes.
- Transferred again into 70% alcohol container for 3 minutes.
- Put in a distilled water basin to rinse the slides for 3 minutes.
- Put in another distilled water basin for 3 minutes to ensure proper rinsing.
- Placed in a container of Alum Harris Haematoxylin Solution for 45 second only (for nuclei staining).
- Transferred to 0.2% HCL Solution for 2 second (for reducing excessive staining).
- Placed under running tap water for 3 minutes.
- Transferred to 0.2% NaHCO<sub>3</sub> solution for 2 minute.
- Placed under running tap water for 3 minutes.
- Viewed under microscope to confirm that only the nuclei were stained.
- Transferred to distilled water container for 3 minutes.

- Placed in container of Eosin for 1 minute (for Cytoplasm Staining).
- Transferred to 95% alcohol for quick rinsing.
- Then to another 95% alcohol for second quick rinsing.
- Placed in absolute alcohol (100%) solution for 3 minutes (for dehydration).
- Repeated the above in another absolute alcohol solution for 3 minutes.
- Transferred to Xylol solution for 3 minutes.
- Repeated the above in another Xylol solution for 3 minutes.

### **Mounting the Coverslip**

The slides from the last Xylol solution were placed on the paper towel with the surface having the tissue sections facing upwards.

- Canada balsam (which dissolves in Xylene) was used as the mounting medium. A drop of it was placed onto the tissue sections.
- The coverslip was slowly placed onto the sections so that air bubble was not trapped underneath. This was done immediately to prevent tissue sections from drying.
- Each slide affixed with written label.

### **Microscopy**

Each slide was viewed under light microscope while alternating the magnifications to obtain clear image. An external lens (Dinoeyes, 1.3 M/ Resolution 1280 x 1024, AM-423 model) was inserted into the microscope and attached to a Personal Computer to obtain enhanced images of the stained tissues as shown in Plate3.2.



Plate 3.2 Tissue view using Dinoeyes attached to computer and microscope.

### **3.3.6 Determination of heavy metals in fish**

Dead fish exposed to the  $LC_{50}$  from the leachate samples (after 96 hours) were randomly selected for heavy metal analysis. Dry-ashing method was adopted for acid-digestion of the fish samples (Tuzen, 2003). At  $110^{\circ}\text{C}$ , the selected samples were dried to constant weight. One gram of each sample was placed in porcelain crucible and heated for 4 hours at  $450^{\circ}\text{C}$  until grey ash was obtained. The discrete ash was acidified by dissolving 5 ml of  $\text{HNO}_3$  (65% v/v). The ensuing solution was transferred into a volumetric flask (25 ml) and made up to volume (Tuzen, 2003; Vaidya & Rantala, 1996). Similarly, a blank was prepared as all metals were determined against aqueous standards. Optima 5300 DV was used to determine the heavy metals accumulated by the fish due to exposure to raw leachate, and concentrations were recorded in  $\mu\text{g/g}$ .

### **3.4 RESULTS AND DISCUSSIONS**

#### **3.4.1 Leachate Characterization**

Based on the description of the study areas in terms of the take off date of landfill operation, JSL is the youngest while TBL is the oldest. The properties of the raw leachate samples in this study differed among the landfills in quality based on varying concentrations across the measured parameters. Detailed characteristics of the leachate samples from the four different landfill types obtained in the present study are itemized in Tables 3.4 – 3.13.

Colour is an important parameter in water quality and effluent discharge considerations. The apparent black colour of JSL leachate was accompanied with a slightly ammoniac odour whereas raw leachate samples from AHL, BBL and TBL were brownish (clearer) with more ammoniac odour especially in the case of AHL (stench ammoniac) (Table 3.4). In as much as colour may not be a significant factor in toxicity of leachate, the variation across the different landfill types studied herein, depend on their operational status. However, colouration can be a reflection of dissolved components of the waste (Lagerkvist, 2003). JSL and BBL still receive MSW and are subjected to deposition of water soluble compounds; hence higher leachate colour (>540 for both landfills) and turbidity (4150 and 1765 FAU, respectively) values. Detection of oil and grease was pronounced in two landfills (JSL & AHL) leachate but the concentration was very high in JSL with 48 mg/L as against 7 mg/L in AHL. The value for oil and grease in JSL may not be unconnected with the fact that it is still operational landfill and as such the daily fresh waste composition may have influenced on the leachate quality in terms of oil and grease concentration.

Table 3.4 Physico-chemical Properties of the Landfill Leachate Samples

Component	Unit	JSL	AHL	BBL	TBL	Standard Limits (EQA/ EPA)
<b>Apparent colour</b>	-	Black	Bright Brown	Dark Brown	Bright Brown	-
<b>Odour</b>	-	Slightly ammoniac	Stench ammoniac	Ammoniac	Ammoniac	-
<b>pH</b>	-	7.35	8.2	7.1	6.8	6.0-9.0
<b>Temperature</b>	<sup>0</sup> C	27.5	29.5	28	27	40
<b>Salinity</b>	<sup>0</sup> / <sub>00</sub>	5.7	8.3	4.2	12	NA
<b>Conductivity</b>	mS/cm	10.04	20	14	34.6	NA
<b>Turbidity</b>	FAU	4, 150	108	274	130	NA
<b>Dissolved Oxygen</b>	mg/L	5.8	5.8	5.2	5.30	NA
<b>BOD</b>	mg/L	27,000	3,500	259	127	20
<b>COD</b>	mg/L	51,200	10,234	985	482	400
<b>BOD/COD</b>	mg/L	0.53	0.34	0.26	0.26	NA
<b>Total Dissolved Solid</b>	mg/L	1,730	830	860	2146	NA
<b>Suspended Solid</b>	mg/L	688	97	87	14	50
<b>Total Organic Carbon</b>	mg/L	380	110	70	42	NA
<b>Oil &amp; Grease</b>	mg/L	48	7	3	4	5

NA - not available

\*data represents mean values from n=5

The aggressiveness of the leachate samples were evaluated by measuring their pH values. It was observed that the measured pH values across the landfills were 7.35, 8.2, 7.1 and 6.8 for JSL, AHL, BBL and TBL, respectively. This implied that all landfills were under methanogenic stage of degradation characterized by neutral pH. It agrees with the pH 8, 7.5 and 7.1 obtained in landfills in Germany (Ehrig, 1983), Great Britain (Robinson & Gronow, 1993) and Sweden (Lagerkvist, 1994) which were established to be within their methanogenic stages (interval pH 6.8 – 9). Yet, the pH values may not be enough for the classification of landfills because according to Kjeldsen et al (2002) landfill is at an acidogenic phase if the pH range is 4.5 – 7.5. Therefore evaluation of other components of leachate become important in assessing the phase at which degradation is occurring in a landfill.

A very important aspect of leachate characterization is the dissolved organic matter. This is often evaluated in form of BOD<sub>5</sub>, COD and TOC. Table 3.4 indicates that JSL recorded the highest COD concentration (51,200 mg/L), and while it was approximately five times less (10,234 mg/L) in AHL, BBL and TBL showed much reduced concentrations at 985 mg/L and 514 mg/L, respectively. Such findings were also observed for BOD<sub>5</sub> and TOC, depicting higher organic contents in active sanitary landfills than as obtainable in older or closed landfills. This is agreeable with findings by Yusof et al (2009) in a study of three different landfills in Malaysia. Yet the present study also partly disagreed with the Yusof et al research in terms of classifying the active sanitary landfill as being in the acidogenic phase based on the BOD/COD. This is because all the studied landfills recorded BOD/COD range of 0.26 – 0.53, indicating methanogenic phase (Christensen, 2001; Calli et al., 2005; Fan et al., 2006) with the lesser value reflecting older landfill. Therefore, the difference in BOD/COD between the previous study and the present may be due to the differences in age of the landfill as at the time of conducting both studies [in as much as, that JSL is the active sanitary landfill in this study with 0.53 BOD/COD, the previous (0.67) did not state the name of the sanitary landfill].

The values of BOD<sub>5</sub> and COD of all leachate samples exceeded the allowable discharge limit in Malaysia EQA Act, 1974 (2007) and by USEPA (2000). So regardless of operational status of landfills, some may share close similarity in leachate composition. This concurs with landfill study which showed that the anaerobic environment in landfills is due to biodegradability of organic components in the MSW and the associated compaction of waste layers which in turn avail similarities to the leachate composition among different landfills (Kjeldsen et al., 2002). Therefore BOD and COD levels in the studied landfills become source of concern if the leachate finds way into

nearby watercourses because such concentrations can possibly deplete oxygen concentration in aquatic system (Emenike et al., 2013).

Ammonical-nitrogen (NH<sub>3</sub>-N) observed in AHL (880 mg/L), BBL (720 mg/L), TBL (630 mg/L) and JSL (600 mg/L) were very high (Table 3.5). High NH<sub>3</sub>-N is potentially due to biotransformation especially the hydrolysis and fermentation of organic nitrogen from the deposited MSW in the landfills. Basically, as the biotransformations continue, there is an increase in soluble nitrogen solubilisation which increases the resultant concentration of ammonia nitrogen in mature leachate (Jokela & Rintala, 2003; Yusof et al., 2009).

Table 3.5 Anionic Components of the Landfill Leachate Samples (mg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard limits (EQA/EPA)</b>
<b>Chloride</b>	4,150	4150	4830	2780	250
<b>Sulphate</b>	54.89	37.1	92.3	65.3	250
<b>Phosphate</b>	113	70.2	100	92	5
<b>Nitrate</b>	38.6	29.1	40.1	35.2	10
<b>Nitrogen</b>					
<b>Nitrite</b>	4.8	2.7	23.3	20.1	1
<b>Nitrogen</b>					
<b>Alkalinity</b>	1980	9000	120	120	NA
<b>Ammonical nitrogen</b>	600 ± 599	880	720	650	5

NA – not available

This might possibly be a major toxic pollutant. In fact, excluding depletion of oxygen in surface water body and other alterations in the stream bottom flora and fauna, the major potential impact of leachate release to surface water is ammonia toxicity (Kjeldsen et al., 2002). All leachate qualities varied on NH<sub>3</sub>-N due to age and operational status disparity; hence the degree of biodegradation and formation of complex compounds

must have taken place in AHL, BBL and TBL over the years. It is expected that the TBL being the oldest should show the highest NH<sub>3</sub>-N concentration while BBL should record a closer concentration to JSL. However, the reason for less value of NH<sub>3</sub>-N in TBL compared to AHL (both are closed landfills) might be due to the variance in deposited waste type over time and the quantity of nitrogenous compounds (though not evaluated in this study). Similar reason may apply to the case of BBL and JSL (both are active landfills) too. In fact the current level of NH<sub>3</sub>-N in JSL is in contrast to 0.09 mg/L previously reported by Emenike et al (2011), but the reason is that the landfill had rapidly moved into a methanogenic phase; hence it's now being considered an old landfill by concerned authorities. Investigations had shown that NH<sub>3</sub>-N concentrations show no decreasing trend with time and may range from 500 – 2000 mg/L in old landfills (Kjeldsen et al., 2002; Emenike et al., 2013). Reduction in NH<sub>3</sub>-N concentration in landfill is difficult unless through leaching because there is no mechanism for its degradation under methanogenic conditions (Kjeldsen et al., 2002; Burton & Watson-Craik, 1998). Based on the foregoing, studies had tagged NH<sub>3</sub>-N as the most significant long-term component of leachate (Robinson, 1995; Kurniawan et al., 2006a; Kruempelbeck & Ehrig, 1994).

The levels of soluble metal (Table 3.6) in the active sanitary landfill in this study were higher than those in the older landfills and such had been reported of similar young landfills (Lagerkvist, 2003; Yusof et al., 2009; McBean et al., 1995; Calli et al., 2005; Agamuthu, 2001). Whereas this is supposed to be due to an increase in metal solubilisation brought about by low pH that results of production of acid during the acidogenic phase, it is easy to question this in the case of JSL which showed high metal concentrations despite the neutral pH. However, the reason may be associated with the fact that though all the landfills studied are considered to be mature landfills, yet JSL is

the youngest and may have just transformed from acidogenic to methanogenic phase which avails the high level of metal solubilisation as corroborated by Lagerkvist (2003). The concentrations of Cr was high in all the landfills and exceeded the Malaysia discharge standard limit (0.05 mg/L); JSL (25.27 mg/L), AHL (0.11 mg/L), BBL (17.3 mg/L) and TBL (6.2 mg/L).

Table 3.6 Metal Components of the Landfill Leachate Samples (mg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EQA/EPA)</b>
<b>Mercury</b>	0.05	0.12	0.04	0.03	0.005
<b>Cadmium</b>	<0.001	<0.001	0.4	0.4	0.01
<b>Chromium</b>	25.27	0.11	17.3	6.2	0.05
<b>Copper</b>	3.59	<0.001	2.62	0.5	0.2
<b>Nickel</b>	19.50	0.29	12	0.85	0.2
<b>Zinc</b>	827.7	0.1	236	24.3	2.0
<b>Lead</b>	<0.001	<0.001	0.1	0.01	0.1
<b>Manganese</b>	540.6	0.12	5.1	3.1	0.2
<b>Iron</b>	97.76	3.10	7.13	4.89	5.0
<b>Calcium</b>	20.17	25.6	91.2	72.3	NA
<b>Potassium</b>	530	440	530	390	NA
<b>Magnesium</b>	11.4	20.3	25.5	20.4	NA
<b>Sodium</b>	58.7	48.6	40.3	35.2	NA

NA – not available

Yet, the closed sanitary landfill had the least concentrations in heavy metal distribution especially in terms of Ni, Zn, Mn and Fe, with the means for the active sanitary landfill being 19.50, 827.7, 540.6, 97.76 mg/L, for the active non-sanitary landfill being 12, 236, 5.1, 7.13 mg/L and for the closed non-sanitary landfill being 0.85, 24.3, 3.1, 4.89 mg/L, respectively. Such high concentrations of Fe and Mn were confirmed with the result of studies conducted by Abdul Aziz et al (2004) and Yusof et al (2009). Also, the higher concentration of metals in JSL leachate is of great concern considering the potential impact on the environment and as such it disagrees with the landfill heavy

metal studies (Christensen et al., 2001; Robinson, 1995; Reinhart & Grosh, 1998; Revans et al., 1999; Kjeldsen & Christophersen, 2001; Kjeldsen et al., 2002).

Detailed chemical parameters were studied in order to fully characterize the leachate from the landfills. Chemical groups included were monocyclic aromatic hydrocarbon, semivolatile organic carbon, organophosphorus pesticides, organochlorine pesticides, volatile fatty acids, alcohols and other chlorinated hydrocarbons. The study revealed low concentrations of the aforementioned parameters (though might have exceeded standard limits), which might be due to waste type deposited in the landfills. Benzene (Table 3.7) was detected in all landfills; 0.01, 0.22, 0.6 and 0.4 mg/L for JSL, AHL, BBL and TBL, respectively (all exceeded allowable limit). Similarly, 1.2 – 1.5 mg/L of toluene and 0.8 – 0.86 mg/L of ethyl benzene were other monocyclic aromatic compounds found in AHL, BBL, and TBL. While it is not clear on whether such concentrations depicts or reflects the age or phase of any landfill, the compounds have been documented to be among the over 250 compounds associated to leachates from municipal landfills. Previous landfill study by Staubitz et al (1989) recorded benzene range of 0.001 – 7.4 mg/L.

Table 3.7 Monocyclic Aromatic Hydrocarbon Components of the Landfill Leachate Samples (mg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>Benzene</b>	0.01	0.22	0.6	0.4	0.005
<b>Toluene</b>	<0.01	1.2	2.1	1.5	1.00
<b>Ethyl Benzene</b>	<0.01	0.86	0.85	0.8	0.70
<b>m+p-Xylene</b>	<0.01	<0.01	<0.001	<0.001	0.001
<b>o-Xylene</b>	<0.01	<0.001	<0.001	<0.001	0.001

The monocyclic aromatic hydrocarbons especially benzene is often associated with toxicity; under section 112 of the US Clean Air Act, benzene is a hazardous air pollutant and the National Institute for Occupational safety and Health (NIOSH) recommends an exposure limit of 0.1ppm as 10-hour Time Weighted Average (TWA) (ATSDR, 1990).

Table 3.8 shows the results of the analysis of semi-volatile organic carbon components in the studied leachate samples. Though most of the parameters were less than 0.01 mg/L, including phenol, the presence of o-cresol and p-cresol at 0.09 and 0.06 mg/L, respectively, is of significant concern in AHL.

Table 3.8 Semi-volatile Organic Carbon Components of the Landfill Leachate Samples (µg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>1,8-Cineol</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>Alpha Thujone</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>Camphor</b>	<0.01	<0.01	<0.01	<0.01	2
<b>n-Butyl-Benzenesulfamide</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>o-Cresol</b>	<0.01	0.09	<0.01	<0.01	NA
<b>p-Cresol</b>	<0.01	0.06	<0.01	<0.01	50
<b>Phenol</b>	<0.01	<0.01	<0.01	<0.01	0.5

NA - not available

The presence of both forms of cresol in AHL leachate may be attributed to the type of waste deposited in the landfill. O-cresols are used as solvents, disinfectants and chemical intermediate (ATSDR, 1990). Also p-cresol is used in the formulation of antioxidants and in the fragrance and dye industries (ATSDR, 1990). This is to imply that deposition of waste containers of disinfectants, pesticides, perfumes and deodorants may have led to the presence of o-cresol and p-cresol in the AHL leachate. The reason

might be due to the lack of disposal options for household hazardous waste which led to such materials being discarded into the MSW stream.

Table 3.9 showed the distribution of concentrations of organophosphorus pesticides in the landfills leachate studied. With results showing that concentrations detected were less than 1 µg/L. This is possibly due to the disposal of used pesticide cans or some other associated containers were regulated especially with the help of waste scavengers at landfill sites.

Table 3.9 Organophosphorus Pesticides Components of the Landfill Leachate Samples (µg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>Chlorpyrifos</b>	<1	<1	<1	<1	0.1
<b>Diazinon</b>	<1	<1	<1	<1	0.022
<b>Ethion</b>	<1	<1	<1	<1	100 – 14000
<b>Malathion</b>	<1	<1	<1	<1	NA
<b>Bromophos</b>	<1	<1	<1	<1	NA
<b>Phenamiphos</b>	<1	<1	<1	<1	NA

NA - not available

Basically, all the organochloride pesticides such as DDT, Dieldrin, 4,4-DDE and Chlorpyrifos that are notable toxic pesticides (Alabaster & Lloyd, 1980) have concentrations less than 0.01 µg/L (Table 3.10). This makes their presence insignificant in acute toxicity testing. However, it cannot be ruled out that they have the build-up potential in the environment to cause unsuspected side-effects. Therefore the insignificant concentration of such chemical components may be due to the regulated use and disposal of products with such chemical components.

Table 3.10 Organochlorine Pesticides Components of the Landfill Leachate Samples (µg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>Aldrin</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>α-BHC</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>β-BHC</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>χ-BHC</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>δ-BHC</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>4,4-DDE</b>	<1	<1	<1	<1	NA
<b>4,4-DDT</b>	<1	<1	<1	<1	NA
<b>DDT</b>	<0.01	<0.01	<0.01	<0.01	0.044
<b>Dieldrin</b>	<0.01	<0.01	<0.01	<0.01	NA
<b>Endosulfan I</b>	<1	<1	<1	<1	0.08
<b>Endosulfan II</b>	<1	<1	<1	<1	0.08
<b>Endosulfan Sulfate</b>	<1	<1	<1	<1	0.08
<b>Endrin</b>	<0.1	<0.01	<0.01	<0.01	2
<b>Endrin Aldehyde</b>	<0.1	<0.01	<0.01	<0.01	NA
<b>Hepatchlor Epoxide</b>	<0.1	<0.01	<0.01	<0.01	0.2
<b>Heptachlor</b>	<0.01	<0.01	<0.01	<0.01	0.4
<b>Lindane</b>	<1	<1	<1	<1	0.2
<b>Methoxychlor</b>	<1	<1	<1	<1	40

NA - not available

The volatile fatty acids analyzed in the leachate samples are shown in Table 3.11. All the acids values were less than 5 µg/L except propanoic acid which was 2,100 µg/L (AHL only). This might be attributed to the waste type deposited in the landfill and it might be a by-product of degradation in the landfill. Propanoic acid is a potential toxicant.

Table 3.11 Volatile Fatty Acids Components of the Landfill Leachate Samples ( $\mu\text{g/L}$ )

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>Ethanoic acid</b>	<5	<5	<5	<5	20
<b>Propanoic acid</b>	<5	2,100	<5	<5	200
<b>Iso-Butyric acid</b>	<5	<5	<5	<5	NA
<b>Butanoic acid</b>	<5	<5	<5	<5	NA
<b>Iso-Pentanoic acid</b>	<5	<5	<5	<5	NA
<b>Pentanoic acid</b>	<5	<5	<5	<5	NA
<b>Hexanoic acid</b>	<5	<5	<5	<5	NA

NA - not available

The alcoholic concentrations were less than  $5 \mu\text{g/L}$  (Table 3.12). As demonstrated in the studies by Farquahar and Rover, (1973), Chian et al (1976), Ehrig (1983) and Harmsen, (1983), alcoholic components, volatile fatty acids, pH and bicarbonate, of leachate composition are affected by the dominant biochemical processes occurring in a landfill. Therefore such low concentrations of alcohols in this study may be a reflection of such processes.

Table 3.12 Alcoholic Components of the Landfill Leachate Samples ( $\mu\text{g/L}$ )

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>TBL</b>	<b>BBL</b>	<b>Standard Limits (EPA)</b>
<b>Methanol</b>	<5	<5	<5	<5	200000
<b>Ethanol</b>	<5	<5	<5	<5	100000
<b>2-Butanol</b>	<5	<5	<5	<5	200000
<b>Propanol</b>	<5	<5	<5	<5	400000
<b>Butanol</b>	<5	<5	<5	<5	100000

Analysis of all the landfills leachate in Table 3.13, showed the significant absence of other chlorinated hydrocarbons.

Table 3.13 Other Chlorinated Hydrocarbon Components of Landfill Leachate Samples (µg/L)

<b>Component</b>	<b>JSL</b>	<b>AHL</b>	<b>BBL</b>	<b>TBL</b>	<b>Standard Limits (EPA)</b>
<b>Dichloromethane</b>	<5	<5	<5	<5	5
<b>1,1-Dichloromethane</b>	<5	<5	<5	<5	5
<b>1,2-Dichloromethane</b>	<5	<5	<5	<5	NA
<b>1,1,1-Trichloromethane</b>	<5	<5	<5	<5	NA
<b>1,2-Dichloropropane</b>	<5	<5	<5	<5	5
<b>Chloroform</b>	<5	<5	<5	<5	5
<b>Trichloroethylene</b>	<5	<5	<5	<5	5
<b>Tetrachloroethylene</b>	<5	<5	<5	<5	5
<b>Carbon Tetrachloride</b>	<5	<5	<5	<5	5
<b>Chlorobenzene</b>	<5	<5	<5	<5	5
<b>1,4-Dichlorobenzene</b>	<5	<5	<5	<5	NA
<b>1,2-Dichlorobenzene</b>	<5	<5	<5	<5	NA

NA – not available

This might be due to the sanitary nature of both landfills. In as much as such little concentrations might have effect in bioaccumulation, the concentrations are insufficient to be of much concern in acute toxicity testing.

In general, the chemical constituents of all leachate samples in this study were compared to those identified in other leachate obtained from the methanogenic phases of landfills of other Asian countries like Hong Kong (Pillar Point and Ma Yau Tong), Thailand (Lat Krabang) and Indonesia (Leuwigadja) (Robinson, 2007; Agamuthu, 2011). Leachate generated from the sanitary landfills shared similarity in organic contents (TOC) with landfill in Thailand, though AHL was slightly less. The COD and BOD<sub>5</sub> levels in both sanitary landfills were similar to both Pillar Point and Lat Krabang, whereas BBL and TBL values were close to concentrations obtained in Ma Yau Tong.

Other parameters, such as NH<sub>3</sub>-N were related to value obtained in Hong Kong and Indonesia. Heavy metals concentrations across the reference landfills were similar to levels obtained in AHL, BBL and TBL, but differed significantly with JSL concentrations. Figure 3.1 demonstrates the potential concentration of components of leachate that may find way into the environment on daily basis.

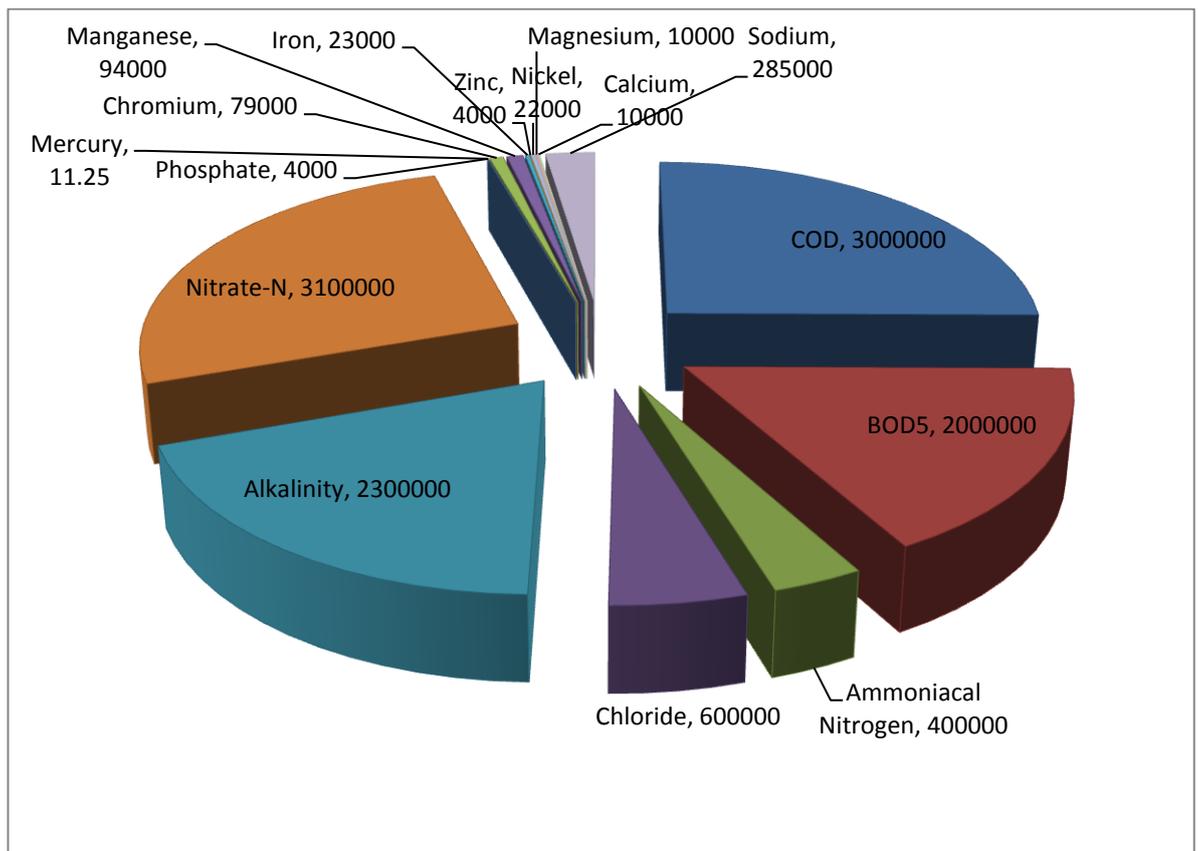


Figure 3.1 Estimated raw leachate components (mg/L) released daily from landfills in Malaysia

This was calculate based on the fact that leachate is generated at the rate of 150 L/tonne of waste, and most Asian landfills receive an average of 2000 tonnes of waste per day (Agamuthu et al, 2011). However, the potential of such leaching into nearby water courses excludes landfills that have proper liners and leachate treatment facilities. As

leachate composition depends on a number of conditions which include climatic and economic situations, results of this study had demonstrated the importance of studying leachate characteristics for each class of landfill in order to identify peculiarities pivotal to selection of treatment methods and containment procedures that will prevent seepage into water courses. An idea of the possible toxicants in the leachate as obtained from the aforementioned characteristics of the leachate samples, paved way for conducting acute toxicity test on fish species as a way of understanding the potential impact of different landfills leachate on aquatic life.

#### **3.4.2 Impact of Acute Toxicity Test**

The impact of the studied landfill leachates on the survival of ten fish species was characterized of diverse degree of mortality; both across the different fish species and the four landfill types. Therefore, this section will analyze the effects of the leachate as it affects discrete fish species with intermittent comparison across landfill types.

##### ***Xiphophorus maculates***

*X.maculates* varyingly responded to the raw leachate samples used in this study as observed in Figures 3.2 – 3.5. The variation was not just limited at the different concentrations that were maintained at common difference in each test; rather it also differed across type of landfill leachate used. This is to imply that after range finding test that was conducted on the fish before definitive acute test under static condition, 1.2 – 2.8% v/v of leachate was the exposure range for the active sanitary landfill (JSL) (Figure 3.2) and the closed sanitary landfill (AHL) (Figure 3.3) as against 6.25 – 11.25 and 4 – 12% v/v, utilized for active non-sanitary landfill (BBL) (Figure 3.4) and closed non-sanitary landfill (TBL) (Figure 3.5), respectively.

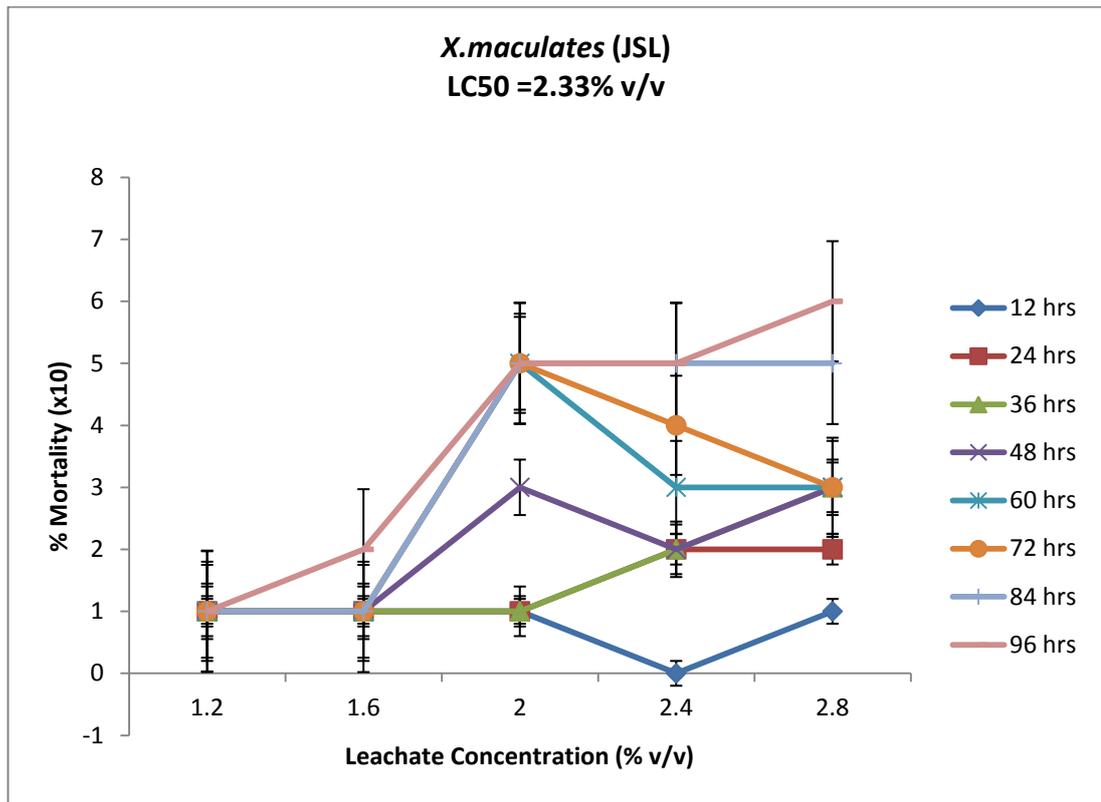


Figure 3.2 Mortality impact of JSL on *X.maculates* for 96 hours exposure

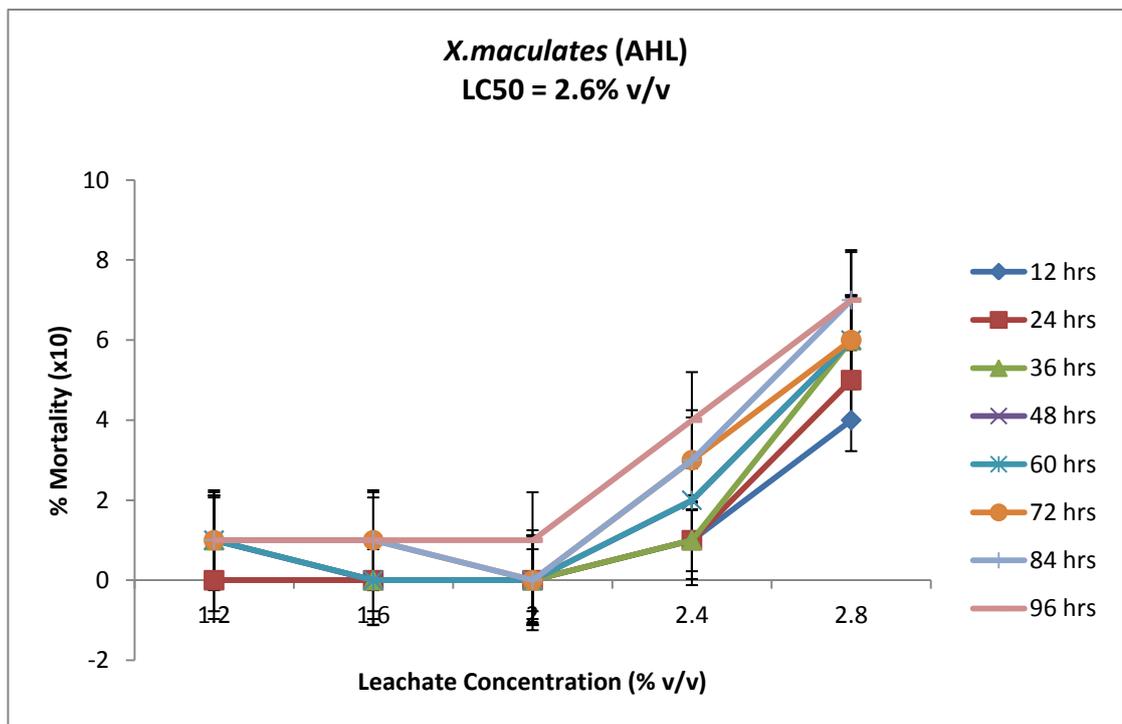


Figure 3.3 Mortality impact of AHL on *X.maculates* for 96 hours exposure

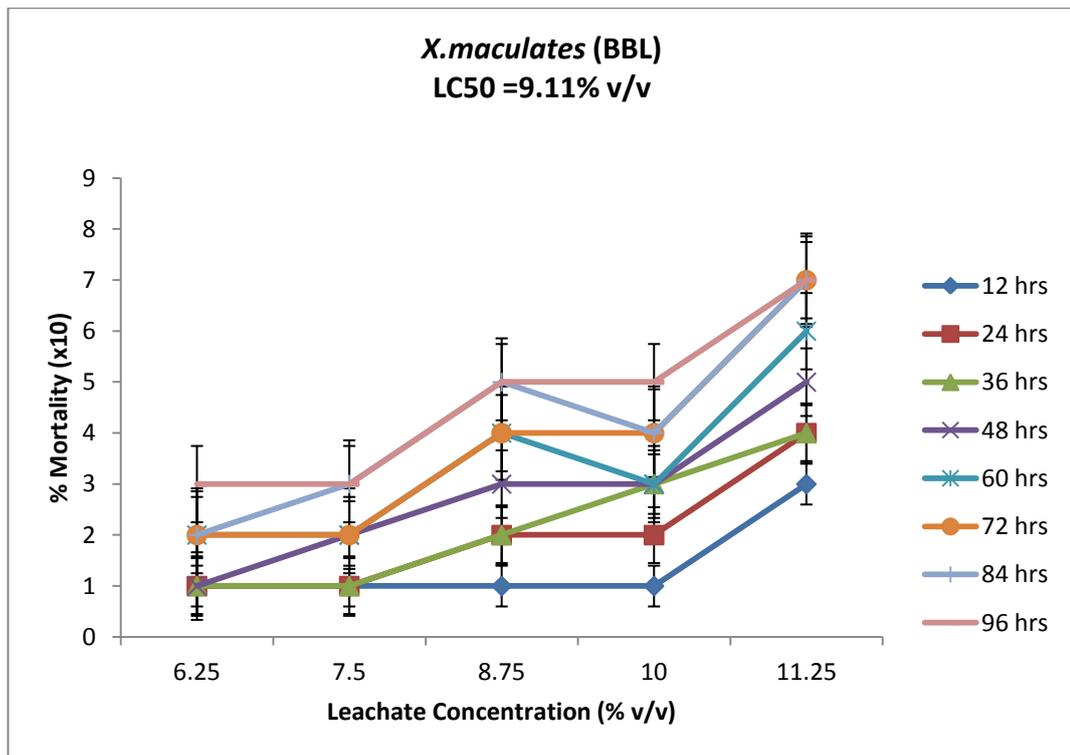


Figure 3.4 Mortality impact of BBL on *X.maculates* for 96 hours exposure

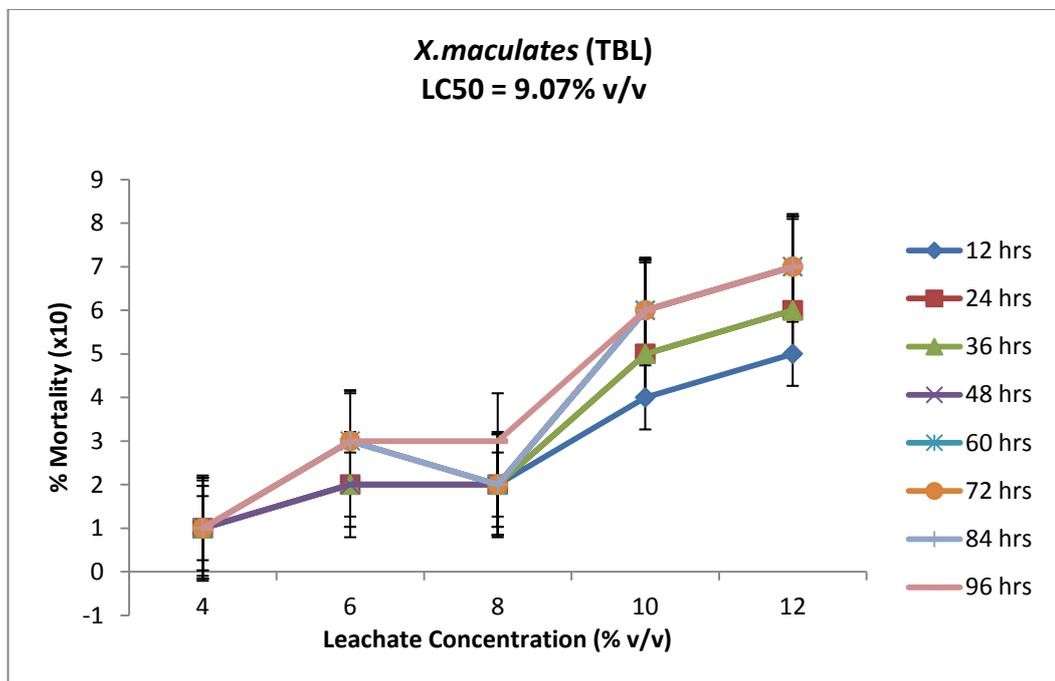


Figure 3.5 Mortality impact of TBL on *X.maculates* for 96 hours exposure

A simple implication of the aforementioned observation is that *X.maculates* was more vulnerable to leachate from landfills with sanitary than non-sanitary landfill leachate.

This reflected in the mean lethal concentrations (LC50) that was calculated on the *X.maculatus* exposure to the four landfill leachates under review. The result showed that 50% survival for *X.maculatus* upon exposure to JSL was at 2.33% v/v whereas 2.6, 9.11 and 9.07 % v/v, were calculated on exposure to AHL, BBL and TBL, respectively. Such effect can be attributed to the varying leachate properties (Jaffar et al, 2009; Emenike et al., 2012a). However, the fact that JSL showed higher toxicity effect than AHL tend to contrast the study by Emenike et al (2013) which concluded that closed sanitary landfill have more mortality effect on fish than the active landfill. But the contrast may be due to the type of fish species which might have some peculiarity to toxicants that will initiate mortality. Regardless of the fact that JSL was the most toxic on *X.maculatus*, a close look on the discrete Figures shown earlier, indicate that the highest rate of mortality was noticed with AHL at 0.73%/hr against 0.63%/hr observed in JSL (note that concentration of leachate used in AHL and JSL was considered because such was far below concentrations used in BBL and TBL tests).

Apart from the LC50 generated, which distinguish leachate types based on toxic levels, the mortality data of each test response was fitted into a predictive model plot as shown in Figures 3.6 – 3.9. The model plot generated corresponding variables (a-e) (Table 3.14) which can be integrated into model equation (Eq. 1) to calculate degree of mortality with time upon introduction of varying concentrations of leachate.

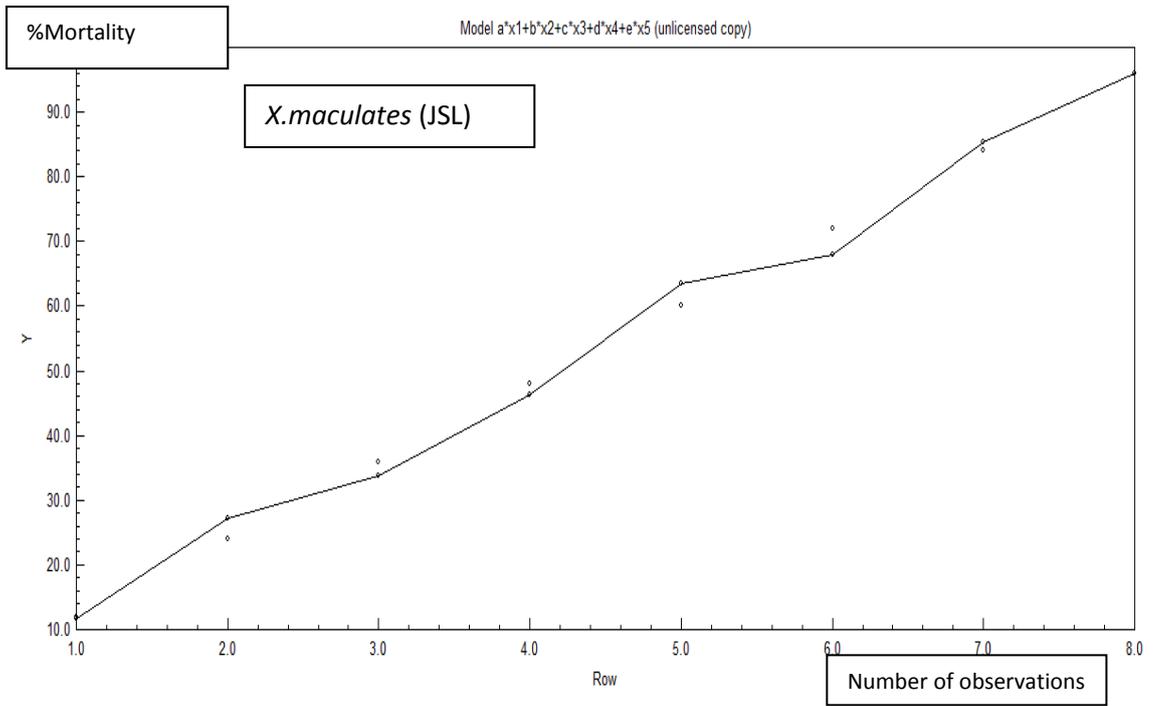


Figure 3.6 Model of JSL leachate toxicity test on *X.maculates* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

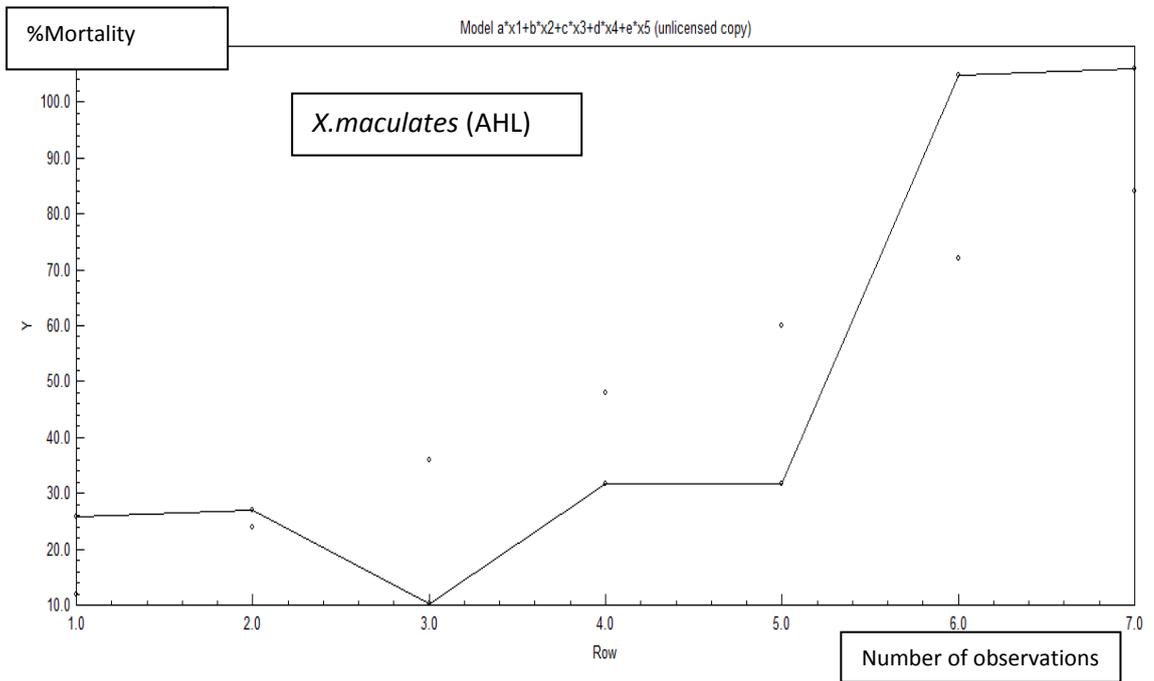


Figure 3.7 Model of AHL leachate toxicity test on *X.maculates* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

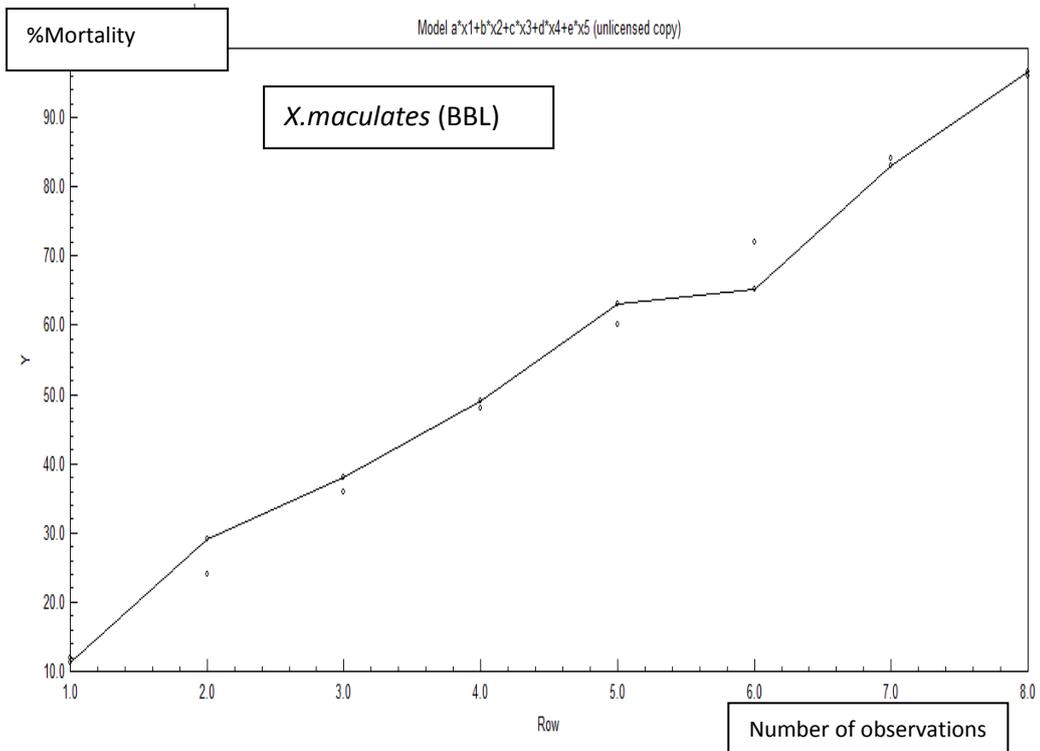


Figure 3.8 Model of BBL leachate toxicity test on *X.maculates* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

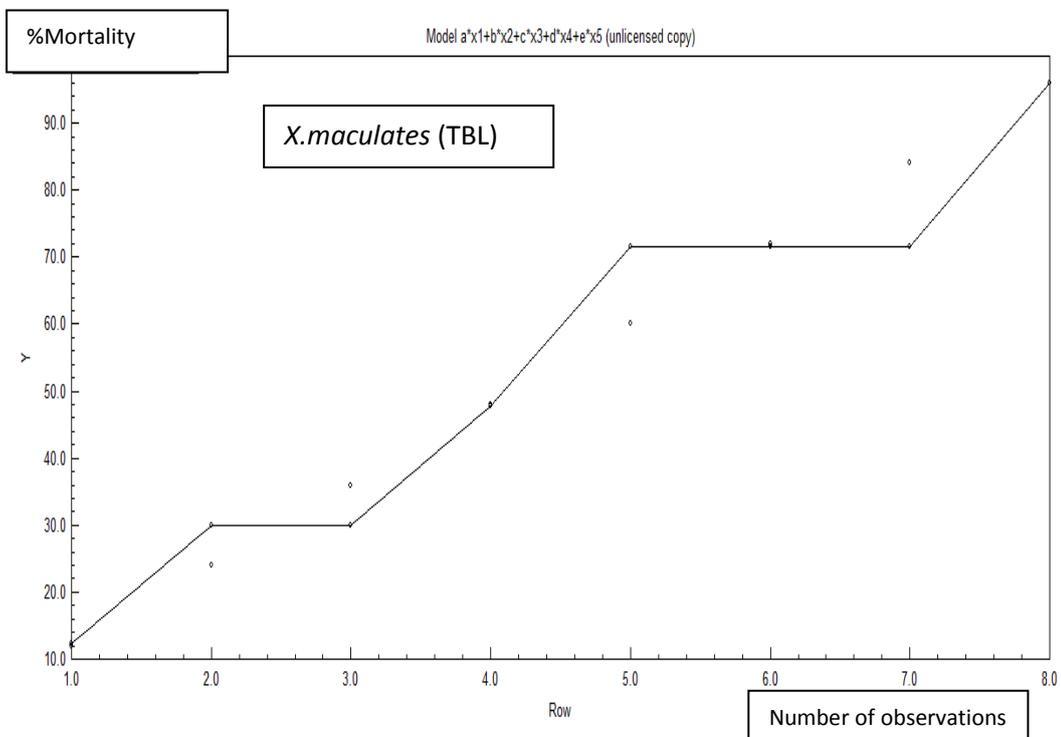


Figure 3.9 Model of TBL leachate toxicity test on *X.maculates* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.14 Predictive Model plot values for *X.maculates* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	6.508	2.085	1.079	0.086	-6.756	-2.49	-9.9E+14	-0.036
<b>b</b>	4.474	1.49	21.54	0.354	8.823	2.15	9.91E+14	0.036
<b>c</b>	6.305	4.24	1.76E+17	0.202	15.831	2.64	24.294	1.609
<b>d</b>	4.067	0.59	51.63	0.238	1.966	0.25	23.743	1.761
<b>e</b>	-5.084	-0.96	-17.80	-0.098	4.941	0.85	9.91E+14	0.036

All plots fitted linearly in partial agreement with Emenike et al (2013) where the predictive model plot fitted both linearly and non-linearly while also comparing effect on active and closed sanitary landfills on a fish species. The variation may be attributed to the use of the same range of leachate concentrations for exposure, which in turn did not allow for partial mortality in higher concentrations, whereas in this study partial mortality occurred across all leachate concentrations.

*Mystus nemurus*

Figures 3.10 – 3.13 represent the mortality responses of *M.nemurus* to the raw leachate samples under review. After conducting a range finding test for all the leachate samples, it was observed that JSL (Figure 3.10) and AHL (Figure 3.11) recorded same test range on *M.nemurus* (0.4 – 2% v/v) whereas approximately 3.13 – 5.63 (Figure 3.12) and 3 – 5% v/v (Figure 3.13), were obtained on exposure to BBL and TBL, respectively.

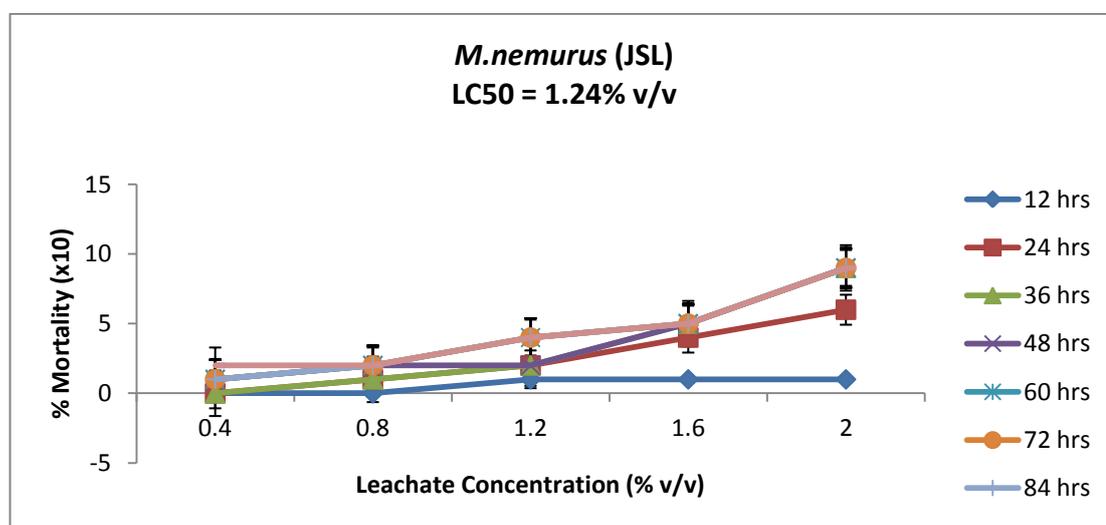


Figure 3.10 Mortality impact of JSL on *M.nemurus* for 96 hours exposure

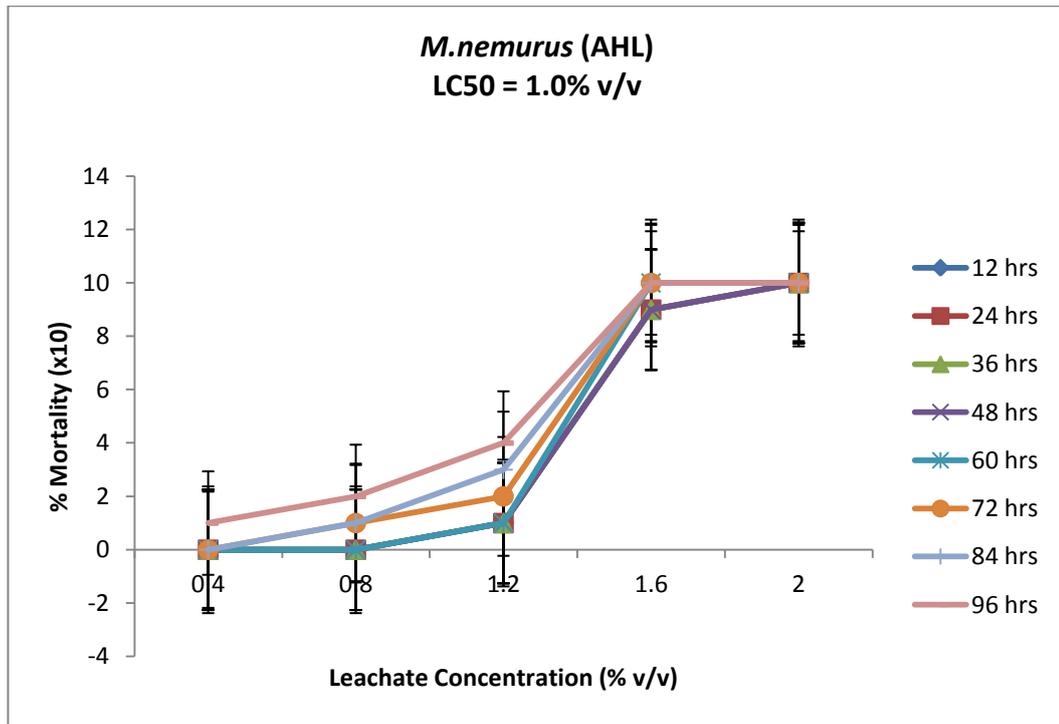


Figure 3.11 Mortality impact of AHL on *M.nemurus* for 96 hours exposure

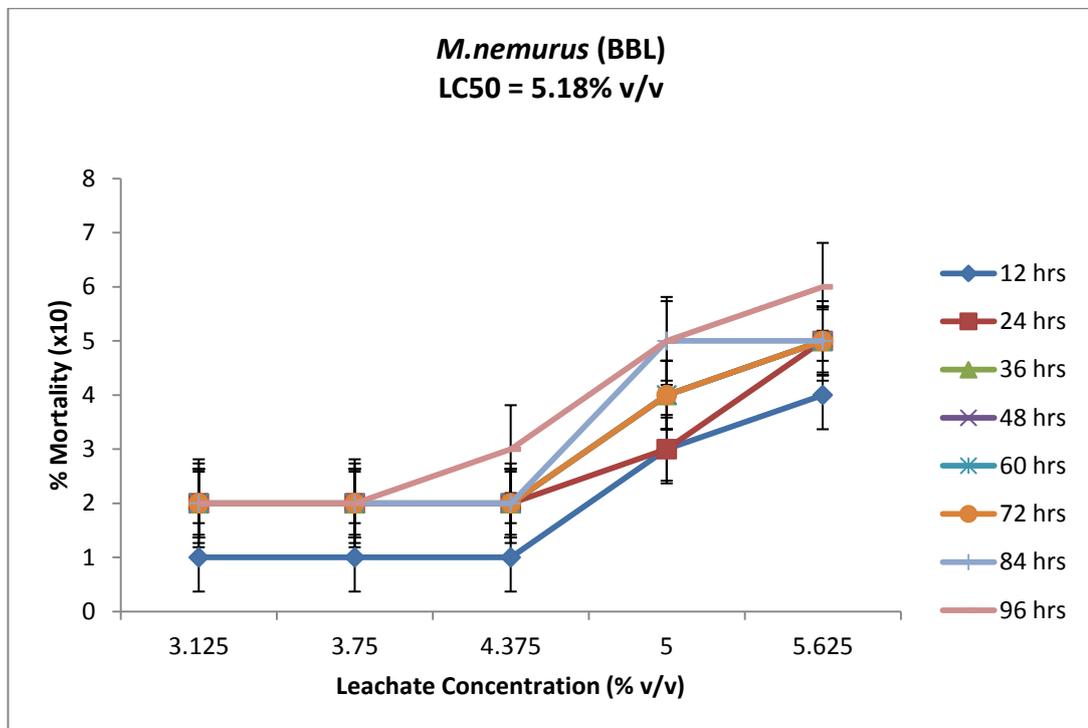


Figure 3.12 Mortality impact of BBL on *M.nemurus* for 96 hours exposure

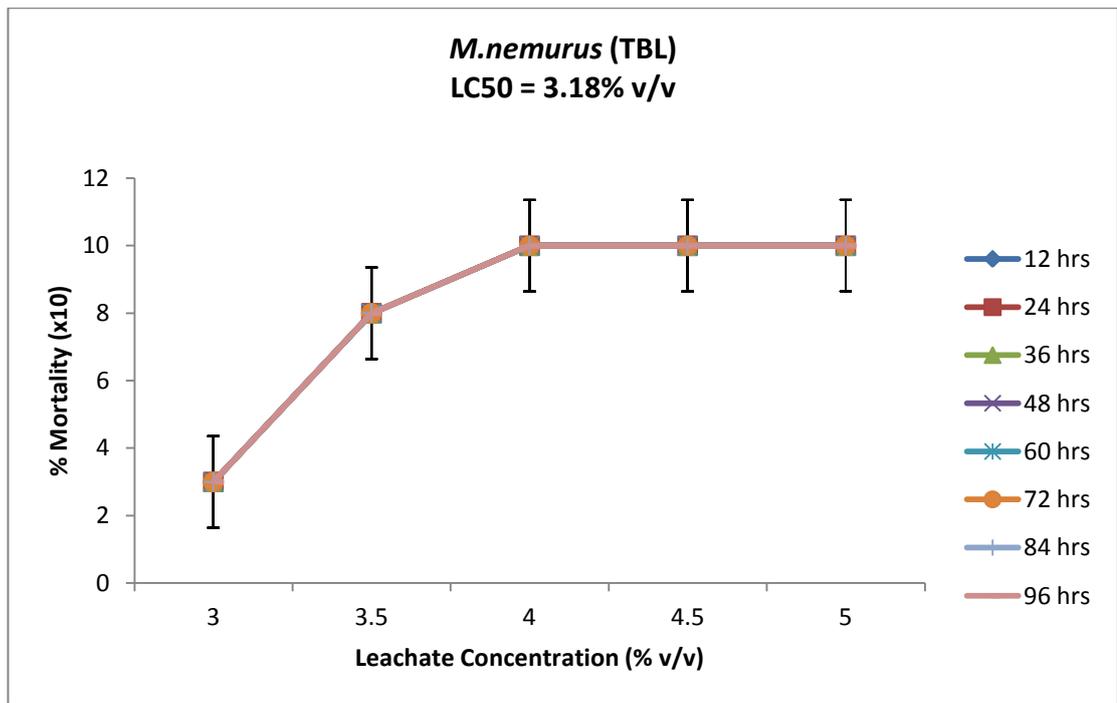


Figure 3.13 Mortality impact of TBL on *M.nemurus* for 96 hours exposure

The calculated LC<sub>50</sub> revealed that *M.nemurus* will have equal mortality and survival upon exposure to 1.0, 1.24, 3.18 and 5.18% v/v of AHL, JSL, TBL and BBL, respectively. Recall that less concentration in this respect imply higher toxicity. Therefore, it means that the trend of toxicity on *M.nemurus* was increasing from exposure to non-sanitary landfill leachate towards landfill leachate with sanitary status. This is in agreement with Jaffar et al (2009) on exposure of *C.carpio* to leachate from a sanitary landfill and two non-sanitary landfills in Malaysia. As against the observation on *X.maculatus* where JSL was the most toxic, AHL turned out to be the most toxic on *M.nemurus*. While considering that the variation may be due to the difference in types of fish, the variation in leachate properties is also highly suspected. The varying degree of mortality was best demonstrated in the deaths recorded across the discrete leachate exposure concentration. By comparing exposure to AHL and JSL, 100% mortality was recorded at 2 and 1.6% v/v of AHL exposure within 12 hours, as against 90 and 50% deaths, respectively seen at 36<sup>th</sup> hour of exposure to same concentrations of JSL. Such

result raises the concern that slight difference in LC50 value may not depict insignificant difference in degrees of mortality.

Figures 3.14 – 3.17 showed that the exposure trend fitted into the predictive model (Eq. 1). However, there was high variation in the generated values of the variables (Table 3.15). This may be attributed to varied response of the fish across the exposure concentrations. The model plot also showed “paired similarity” situations; Figures 3.14 and 3.15 showed that mortality expectations across time were fitted within the positive Y-values, whereas Figures 3.16 and 3.17 were best fitted within the negative Y-values. Therefore this may depict trend of difference between toxicity impacts of sanitary and non-sanitary landfill leachate.

Table 3.15 Predictive Model plot values for *M.nemurus* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	6	0.687	-25.2	-1.565	-30	-0.101	1.57E+18	1.82E-35
<b>b</b>	-6	-0.407	30	1.732	-28.09	-0.013	-1.08E+50	-3.70E-35
<b>c</b>	12	2.214	12	0.548	-162.279	-0.022	-4.16E+50	-3.70E-35
<b>d</b>	-12	-0.719	4.30E-15	1.13E-16	-1.78E+18	-0.027	6.55E+50	3.70E-35
<b>e</b>	24	2.191	2.19E-14	7.06E-16	1.78E+18	0.027	8.53E+34	3.70E-35

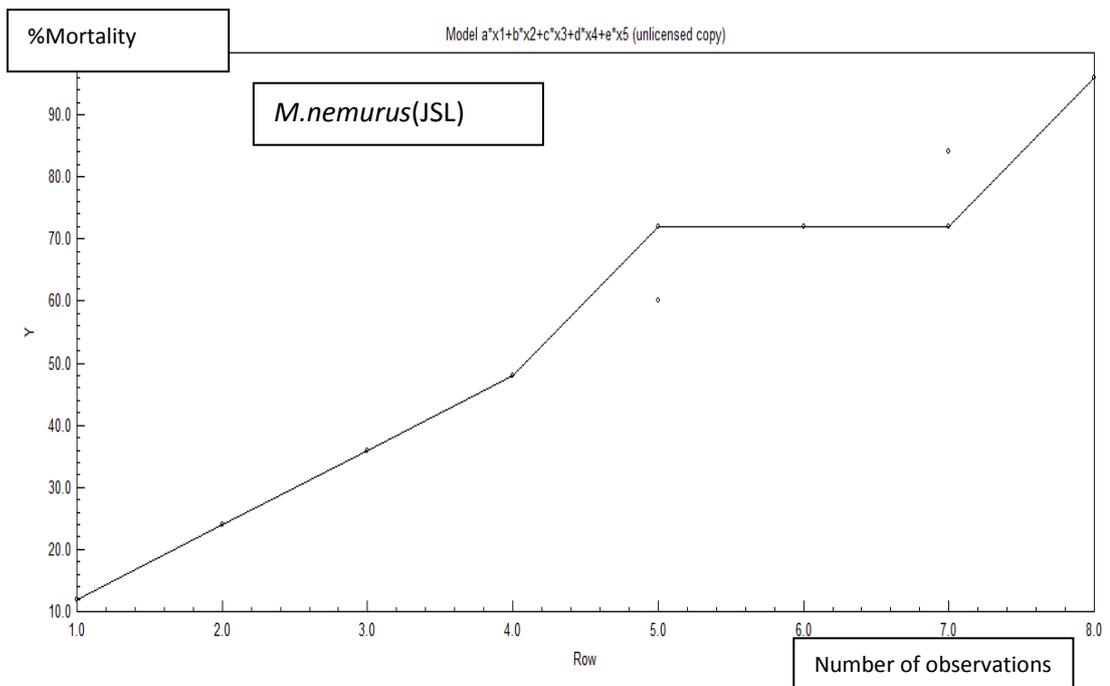


Figure 3.14 Model of JSL leachate toxicity test on *M.nemurus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

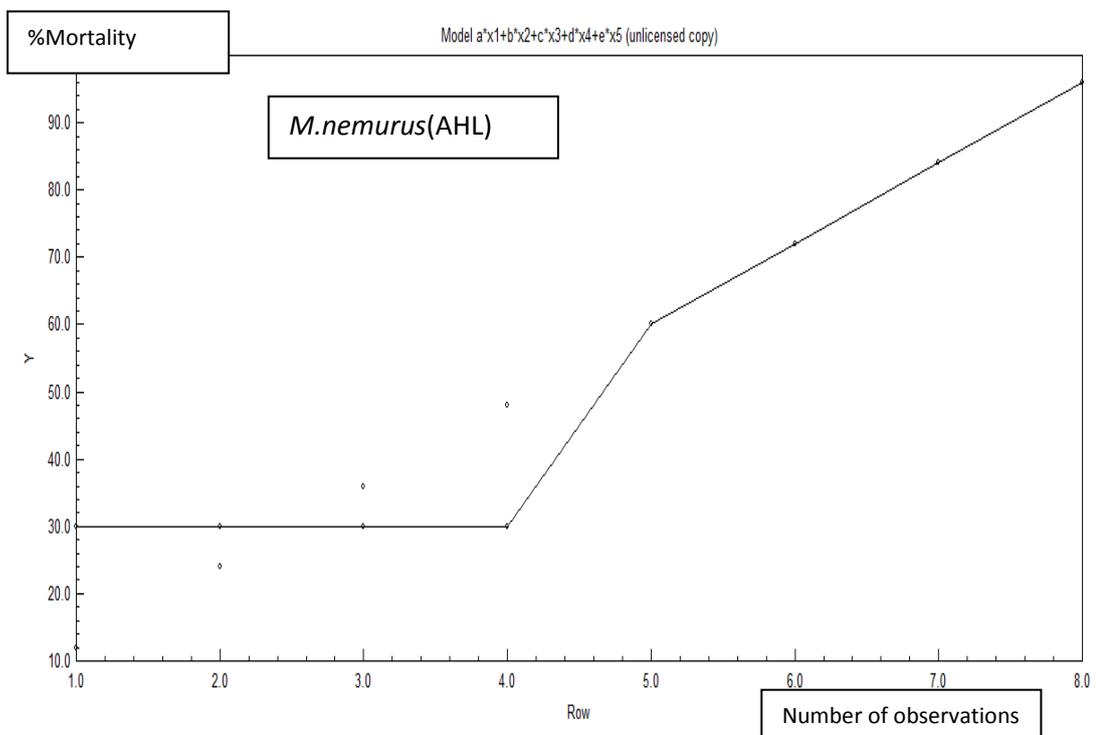


Figure 3.15 Model of AHL leachate toxicity test on *M.nemurus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

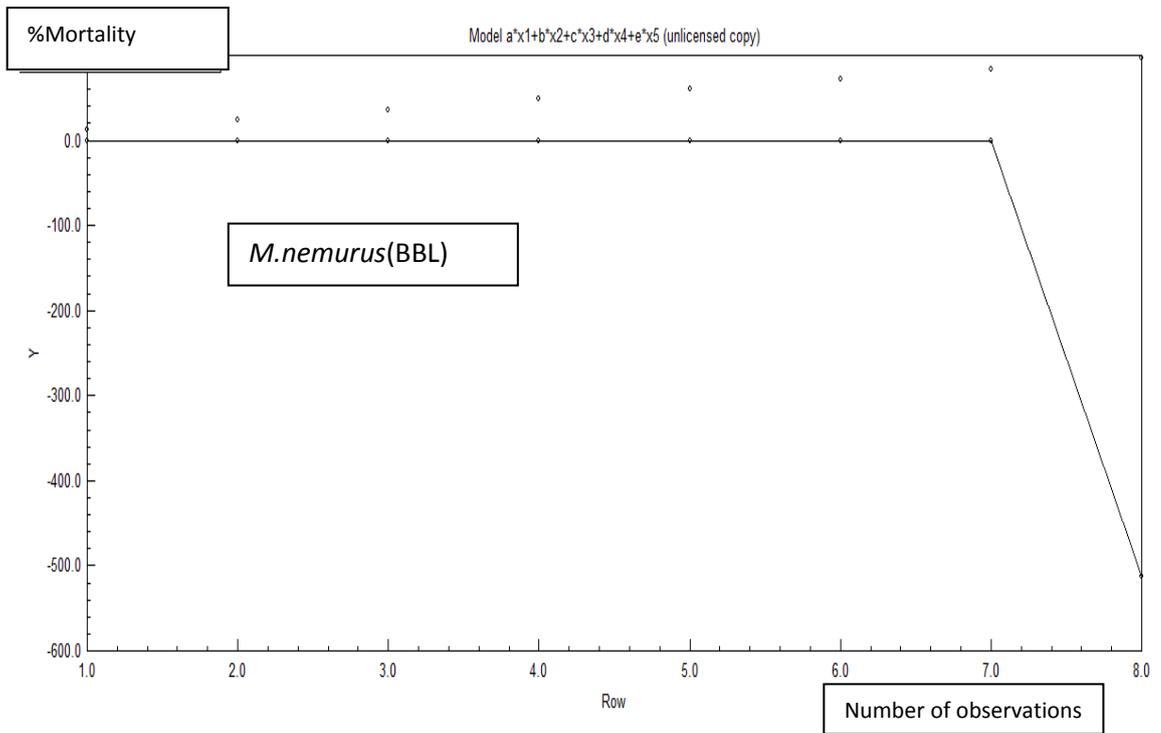


Figure 3.16 Model of BBL leachate toxicity test on *M.nemurus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

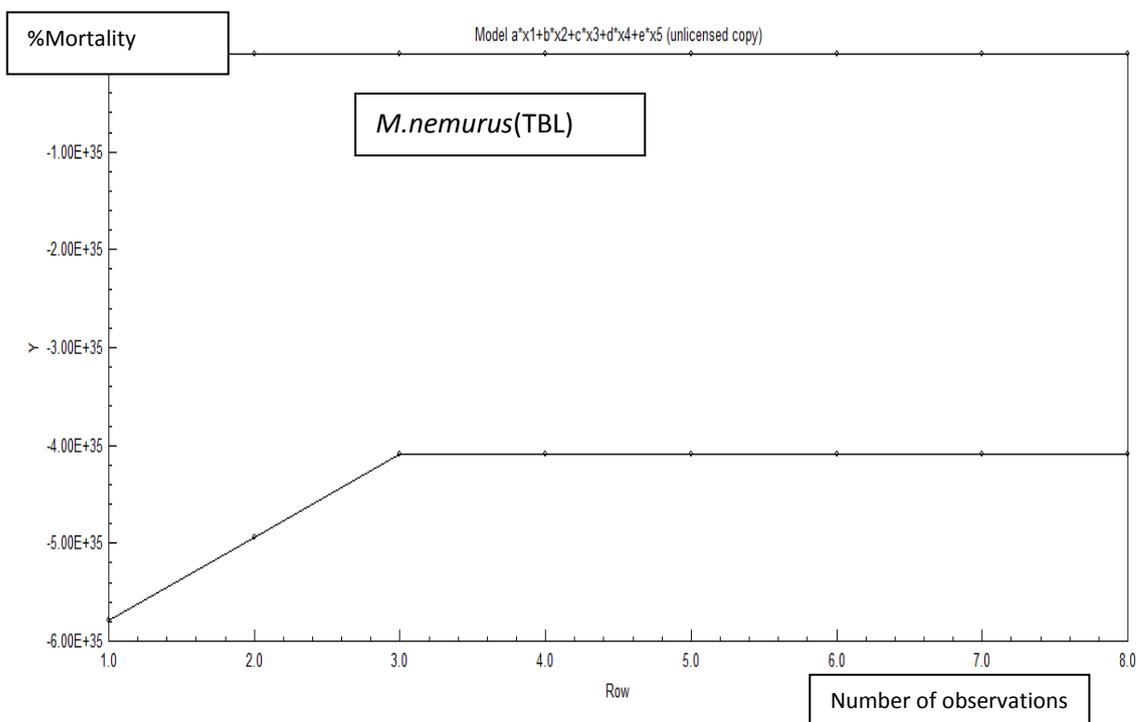


Figure 3.17 Model of TBL leachate toxicity test on *M.nemurus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

**Danio rerio**

Exposure of *D.rerio* to raw leachate samples from the four landfills for 96 hours is shown in Figures 3.18 – 3.21. Unlike in the exposure of *X.maculates* and *M.nemurus* to same raw leachate samples, there were no same leachate concentration ranges across the landfills. LC<sub>50</sub> obtained upon exposure to JSL was 3.14% v/v (Figure 3.18) while 1.25% v/v was recorded from AHL exposure (Figure 3.19). This means that the toxicity potential of AHL to *D.rerio* was almost at triple strength when compared to JSL.

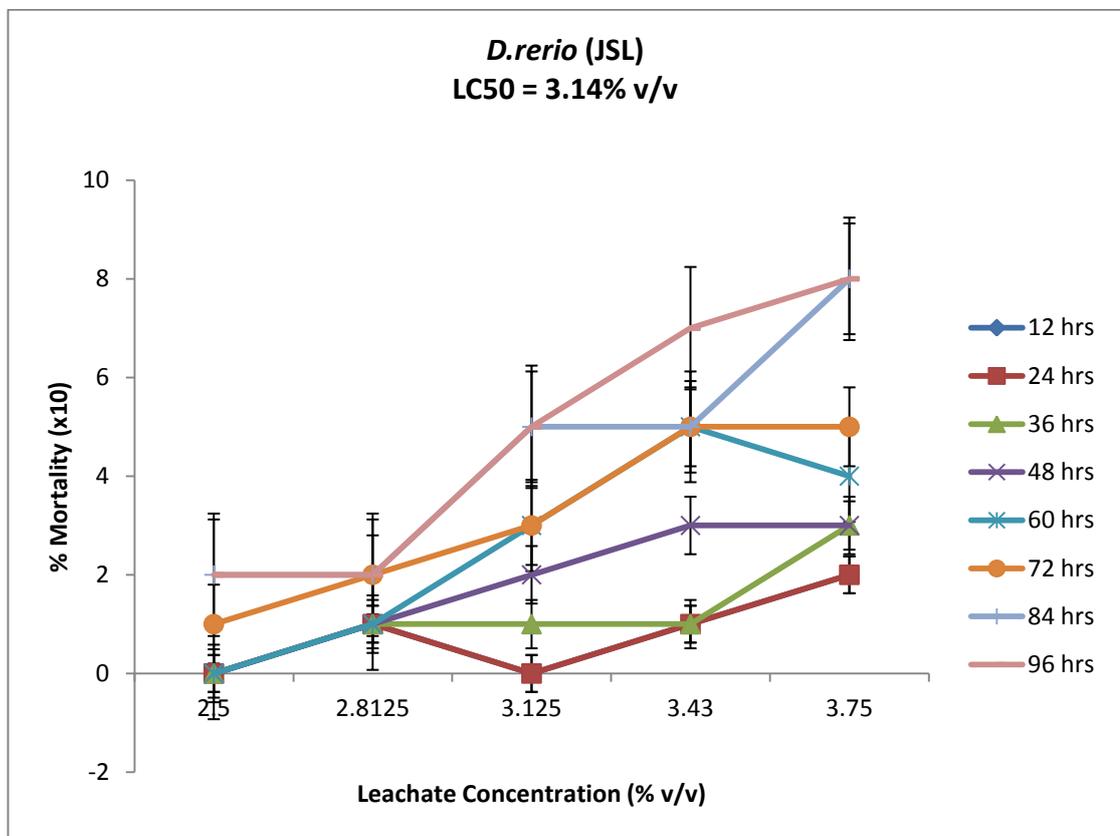


Figure 3.18 Mortality impact of JSL on *D.rerio* for 96 hours exposure

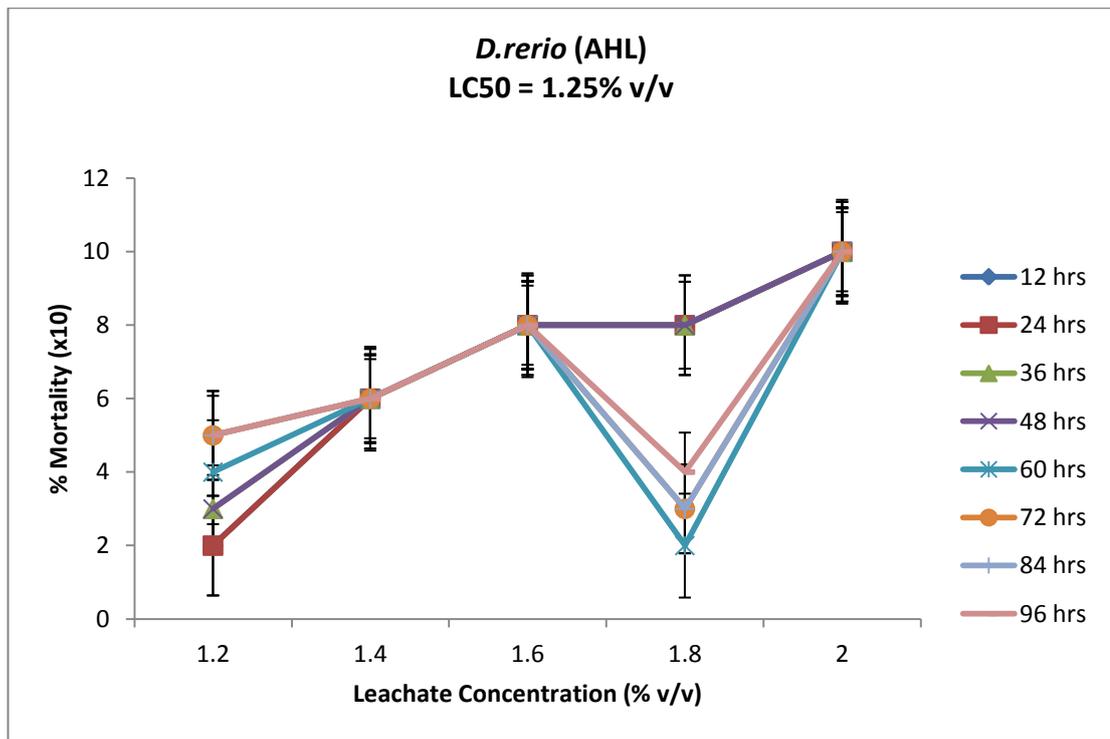


Figure 3.19 Mortality impact of AHL on *D. rerio* for 96 hours exposure

It was noticed that the mortality of *D. rerio* on exposure to AHL was more instant especially within 1.4 – 2% v/v; hence those that survived early hour mortality (12 – 48 hours), almost survived the entire duration of experiment. Such may reflect response pattern of *D. rerio* to very toxic environment and resistive potential.

Contrary to the trend observed from the exposure of *X. maculatus* and *M. nemurus*, *D. rerio* turned out to be more vulnerable to BBL than TBL. With LC<sub>50</sub> at 7.5% v/v, BBL (Figure 3.20) proved to be more toxic than TBL (Figure 3.21) that recorded 10.25% v/v. To buttress this, 90% mortality was recorded at 11.25% v/v of BBL whereas at 12% v/v of TBL only 70% death was observed.

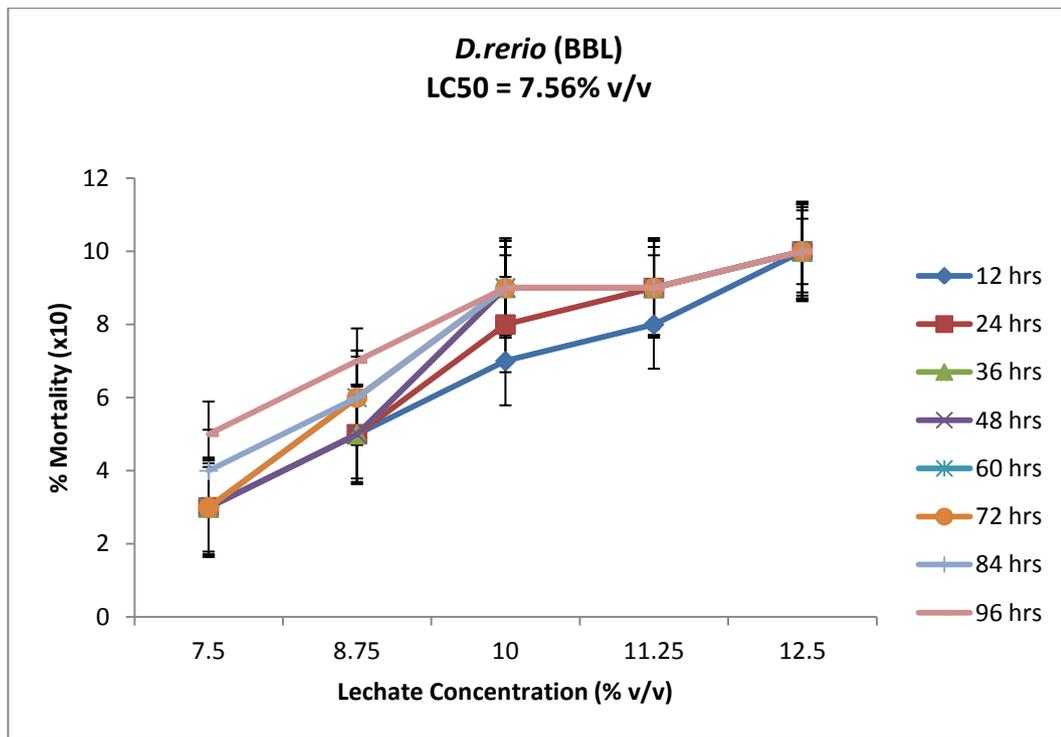


Figure 3.20 Mortality impact of BBL on *D. rerio* at 96 hours exposure

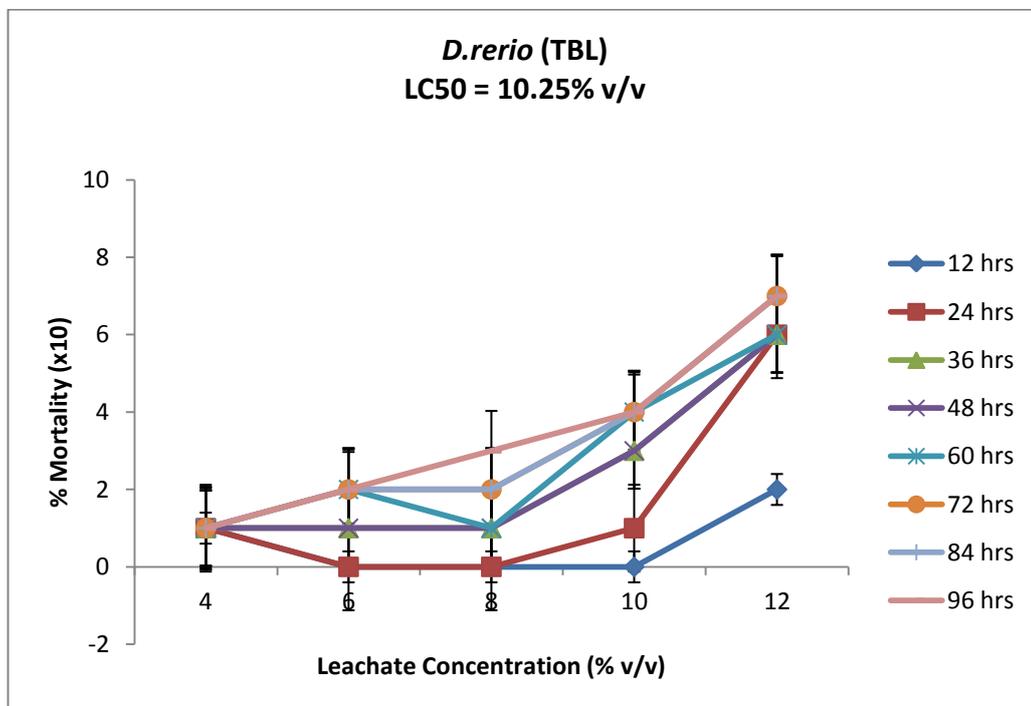


Figure 3.21 Mortality impact of TBL on *D. rerio* for 96 hours exposure

Similar to exposure of other fish species, the model plot of leachate toxicity on *D. rerio* (Figures 3.22 – 3.25) was fitted into Equation 1. Hence, values of concentration coefficient variables were obtained (Table 3.16) for prediction of mortality trend along varying concentrations of leachate. Results from *D. rerio* exposure are important because of the sensitive nature of the fish and its use in most bioassays (Sisinno et al, 2000).

Table 3.16 Predictive Model plot values for *D. rerios* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	1.41	0.240	-7.13E+16	-0.026	-19.09	-1.42	5.85	1.50
<b>b</b>	2.53	0.671	23.84193	0.025	-8.73	-0.37	-12.31	-1.22
<b>c</b>	9.9	1.26	4.39E+16	0.017	20.73	1.70	15.08	2.78
<b>d</b>	14.8	1.19	6.03E+16	0.026	21.27	2.15	28.46	1.81
<b>e</b>	-7.64	-1.28	49.46776	0.052	7.09	0.80	0.62	1.50

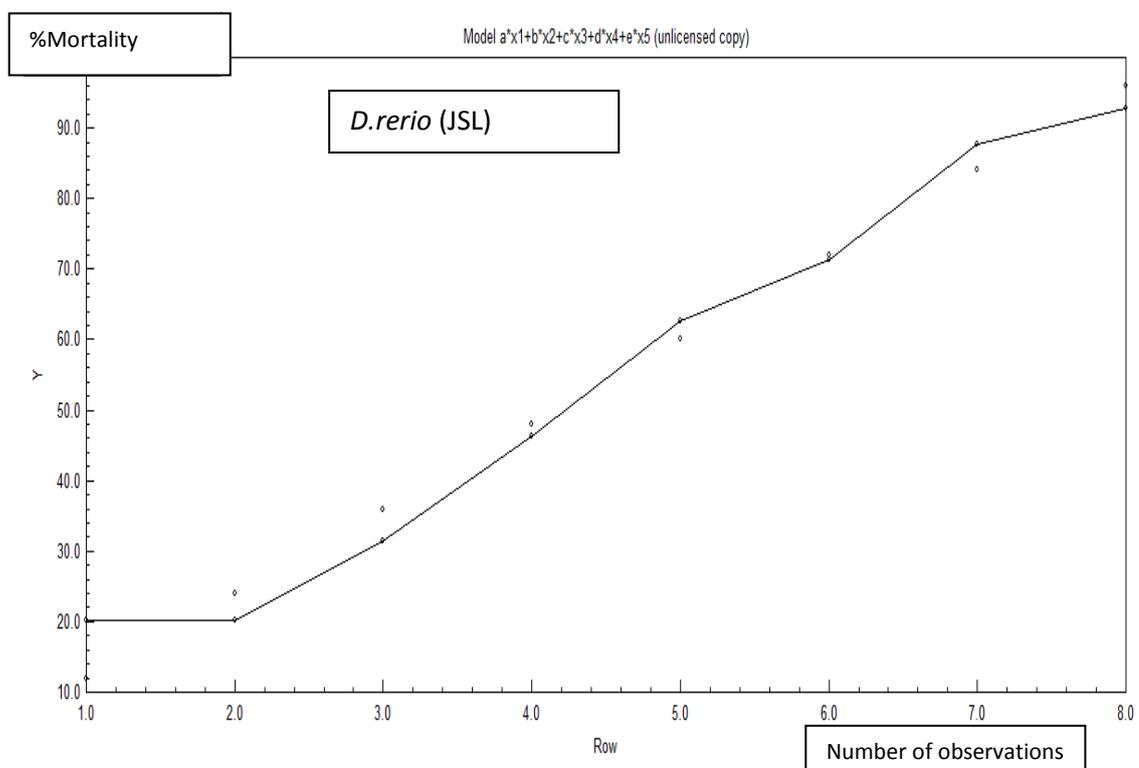


Figure 3.22 Model of JSL leachate toxicity test on *D. rerio* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

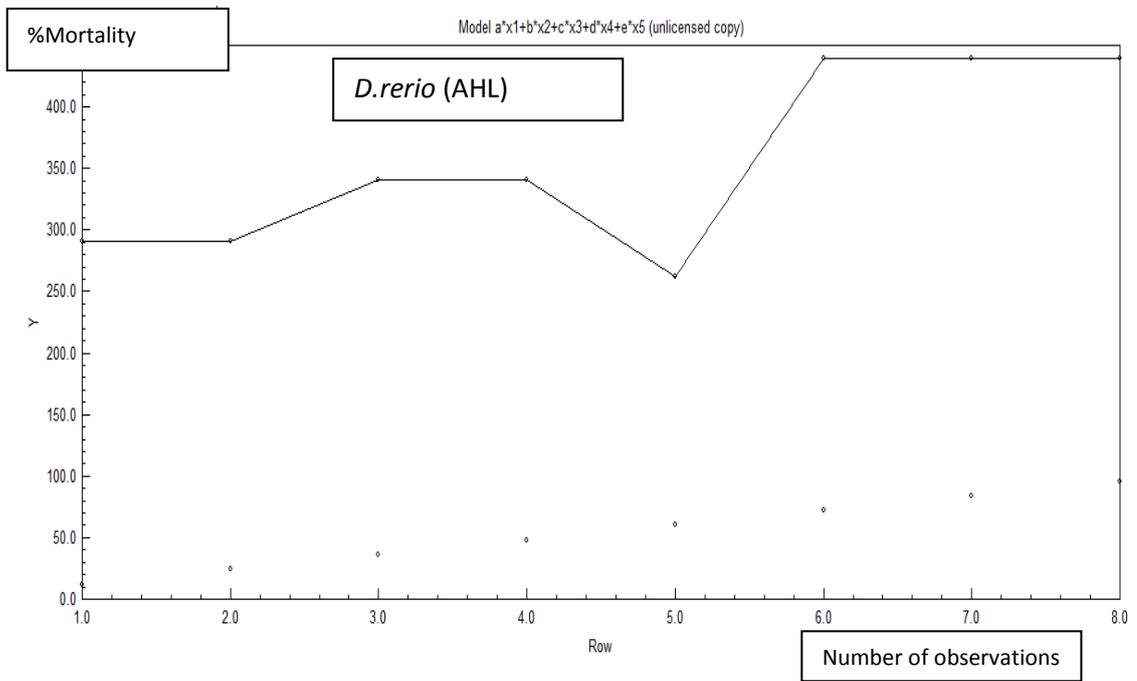


Figure 3.23 Model of AHL leachate toxicity test on *D. rerio* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

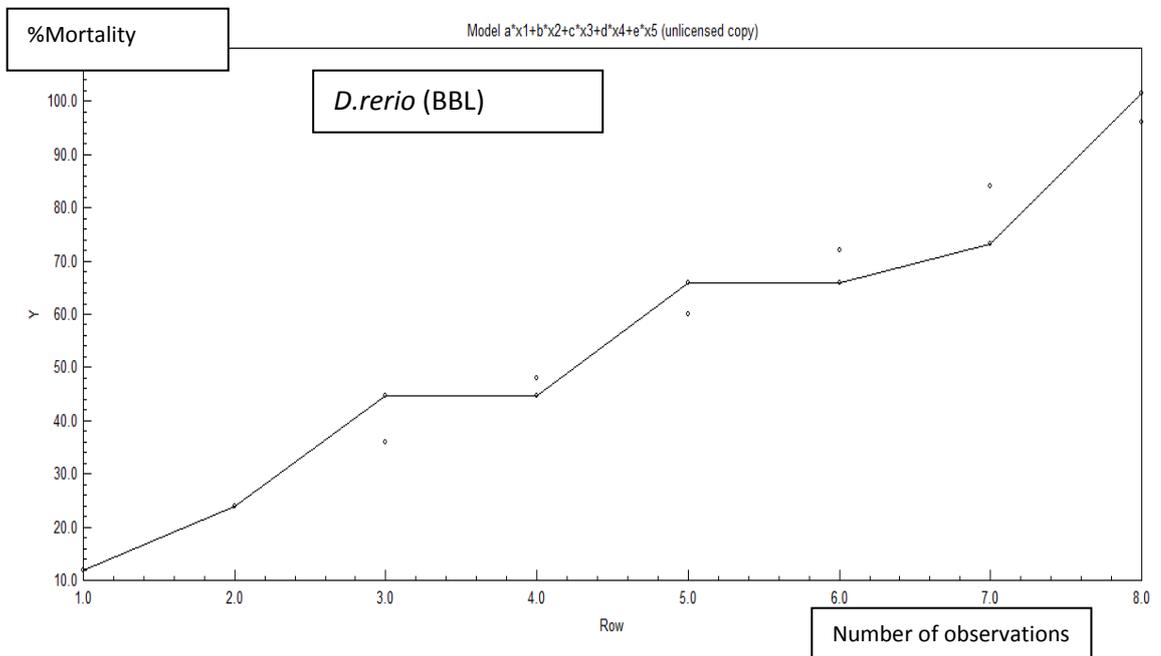


Figure 3.24 Model of BBL leachate toxicity test on *D. rerio* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

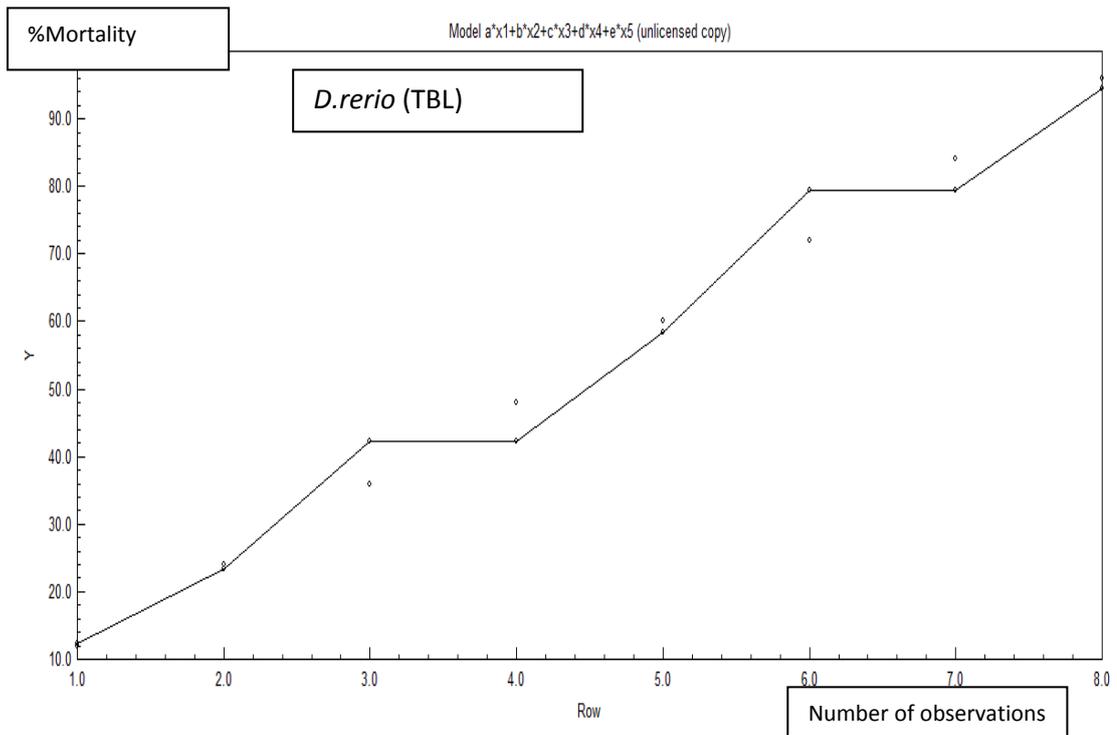


Figure 3.25 Model of TBL leachate toxicity test on *D. rerio* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

**Devario aequipinnatus**

*D. aequipinnatus* varied in response to the raw leachate samples from JSL (Figure 3.26), AHL (Figure 3.24), BBL (Figure 3.28) and TBL (Figure 3.29). The variation was limited to the different concentrations with a common difference as exhibited in each test. Rather it also differed from one landfill leachate used to another. Based on the range finding test, there was no similar test ranges across the landfills; hence the definitive test experiment varied among the leachate types, with AHL showing the least range (1.1 – 1.5%; common difference = 0.1).

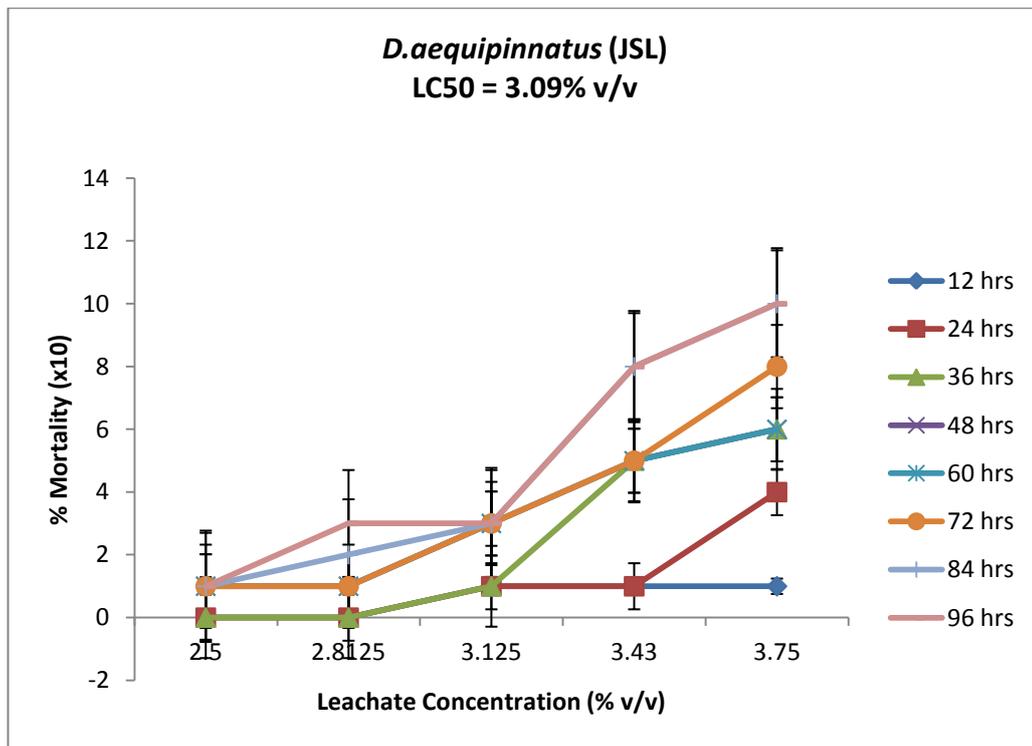


Figure 3.26 Mortality impact of JSL on *D. aequipinnatus* for 96 hours exposure

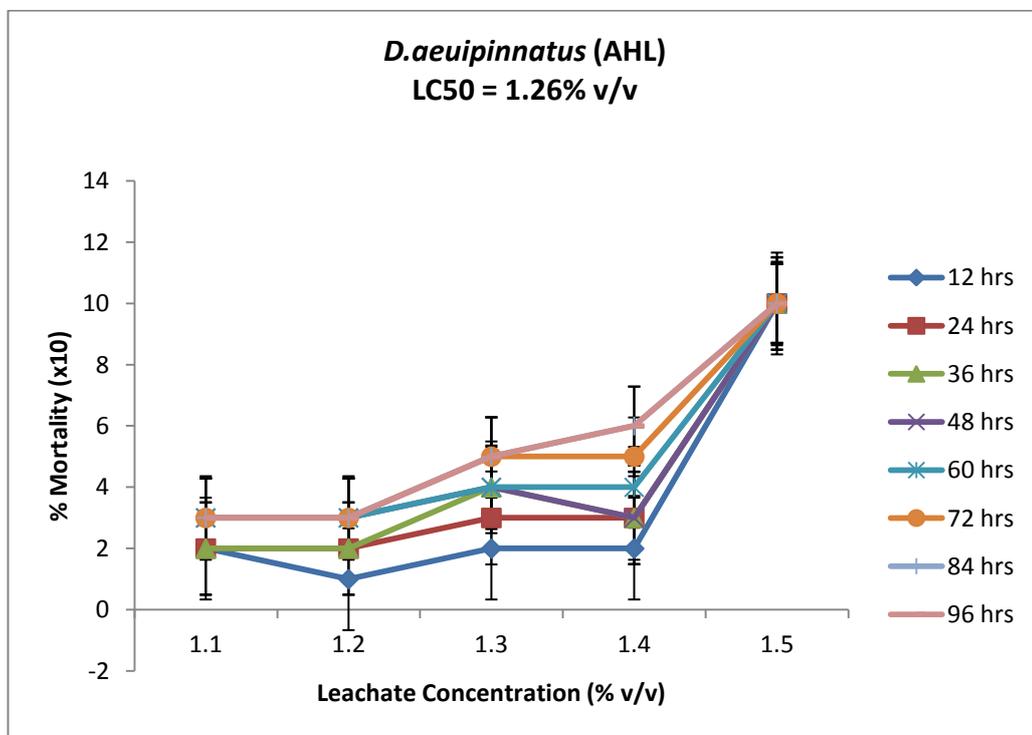


Figure 3.27 Mortality impact of AHL on *D. aequipinnatus* for 96 hours exposure

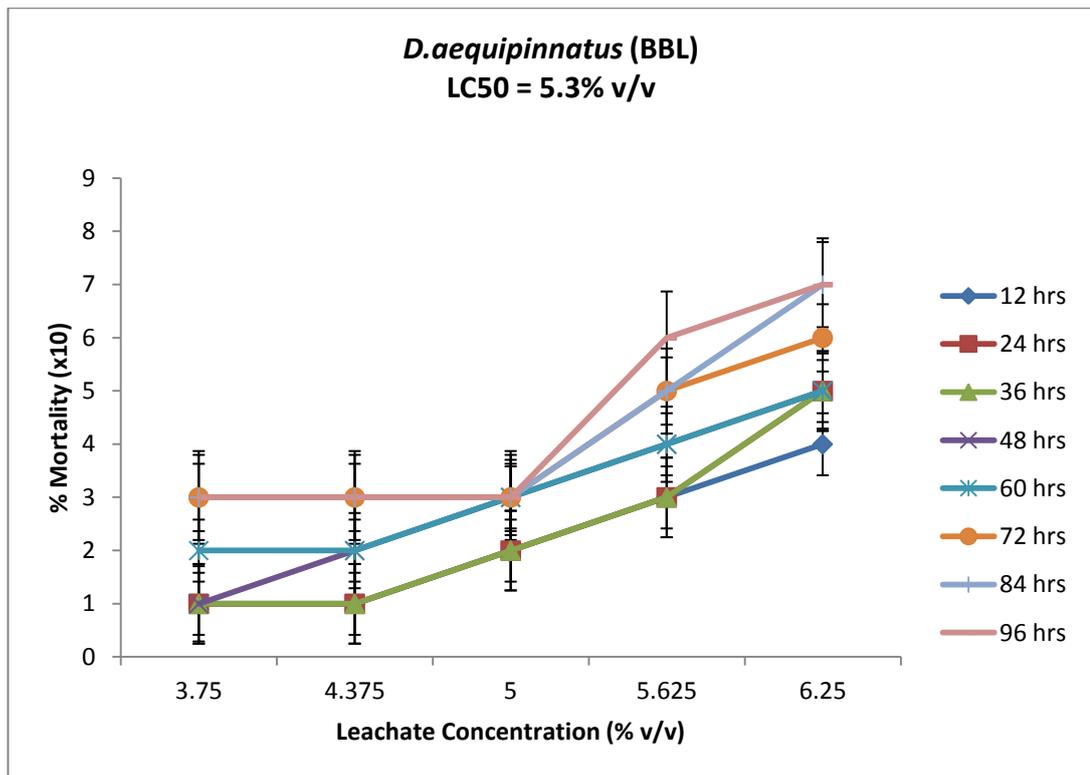


Figure 3.28 Mortality impact of BBL on *D. aequipinnatus* for 96 hours exposure

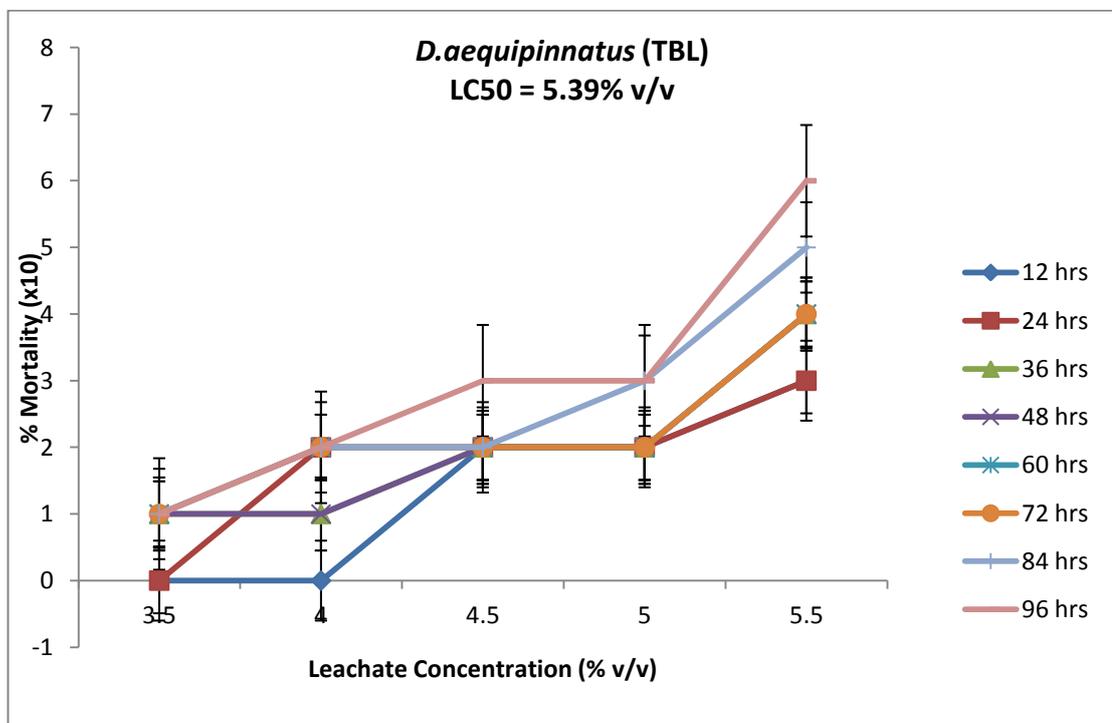


Figure 3.29 Mortality impact of TBL on *D. aequipinnatus* for 96 hours exposure

The result showed that very high mortality potential was characteristic of leachate from the closed sanitary landfill (LC50 = 1.26% v/v) as against least obtained at BBL and TBL wherein the LC50 values were 5.5 and 5.39% v/v, respectively. Considering the high level of disparity in exposure range concentrations of leachate samples, it may not be plausible to make a direct comparison of mortality impact among landfills in relation to discrete leachate concentrations. Yet, in as much as it is a fact that significant mortality for *D.aequipinnatus* was observed with the increase in concentration and exposure time for the four leachates. But, the trend of mortality across time was not uniform. For example while mortality across different concentrations within 24 hours of exposure to BBL showed perfect linearity; it was not the same in other exposures. This may be attributed to fish species and inherent response ability to pollutants.

Expectedly, the proposed predictive model (Equation 1) was used to linearly fit the varying concentrations of each leachate type across 96 hours exposure time (Figure 3.30 – 3.33). Table 3.17 contains variable values of leachate concentration coefficients. The best fitting was obtained in exposure to TBL (Figure 3.33) because only three input data were outside the line of best fit.

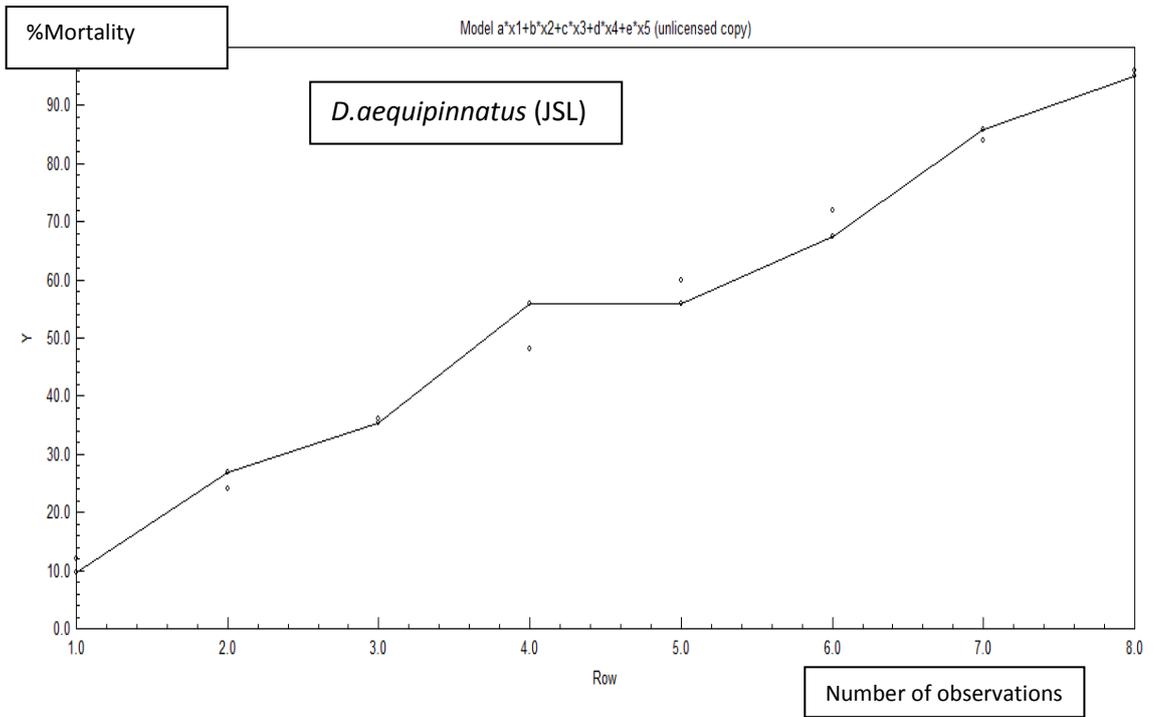


Figure 3.30 Model of JSL leachate toxicity test on *D. aequipinnatus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

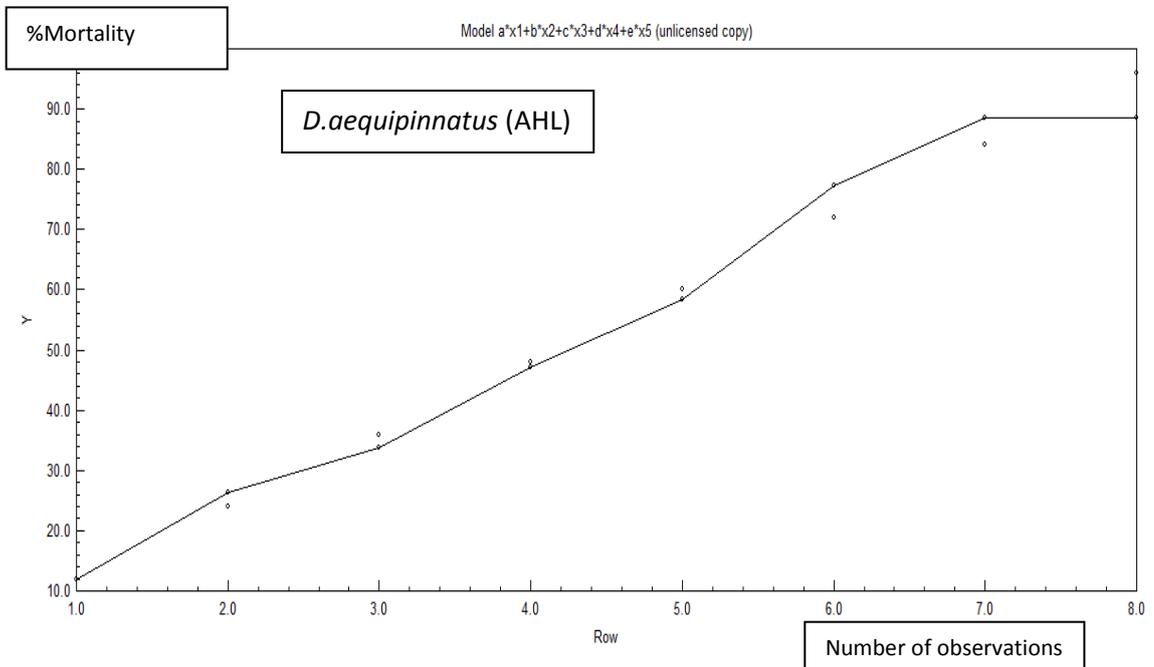


Figure 3.31 Model of AHL leachate toxicity test on *D. aequipinnatus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

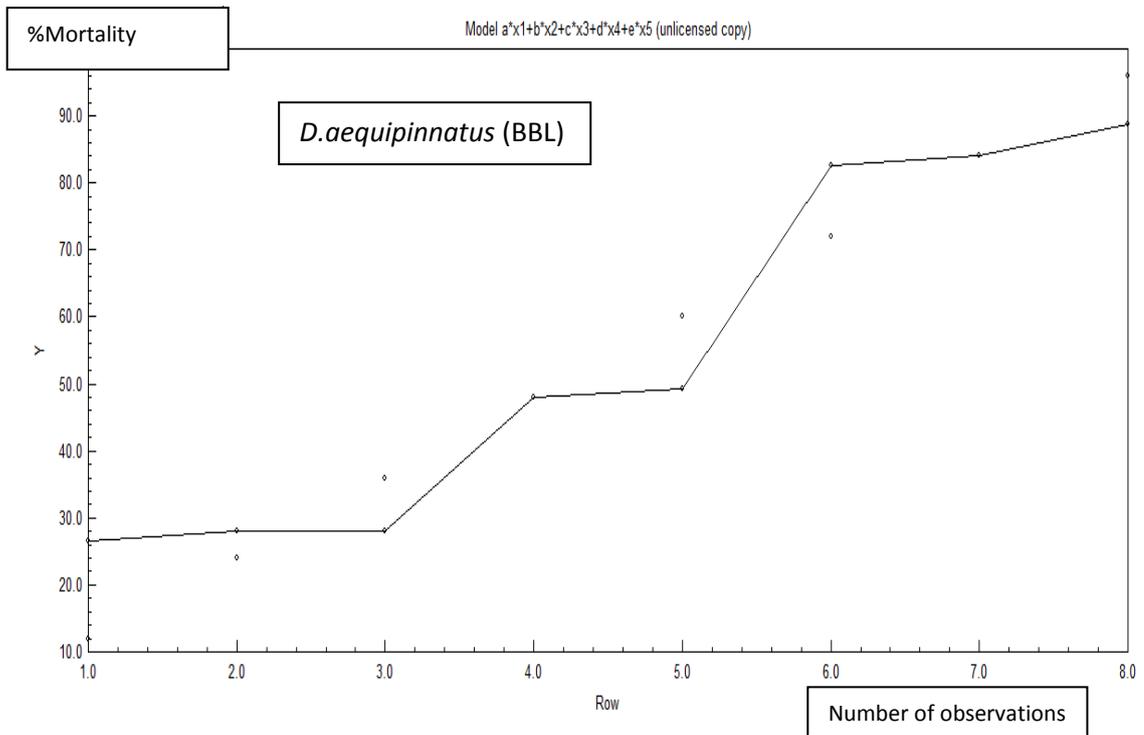


Figure 3.32 Model of BBL leachate toxicity test on *D. aequipinnatus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

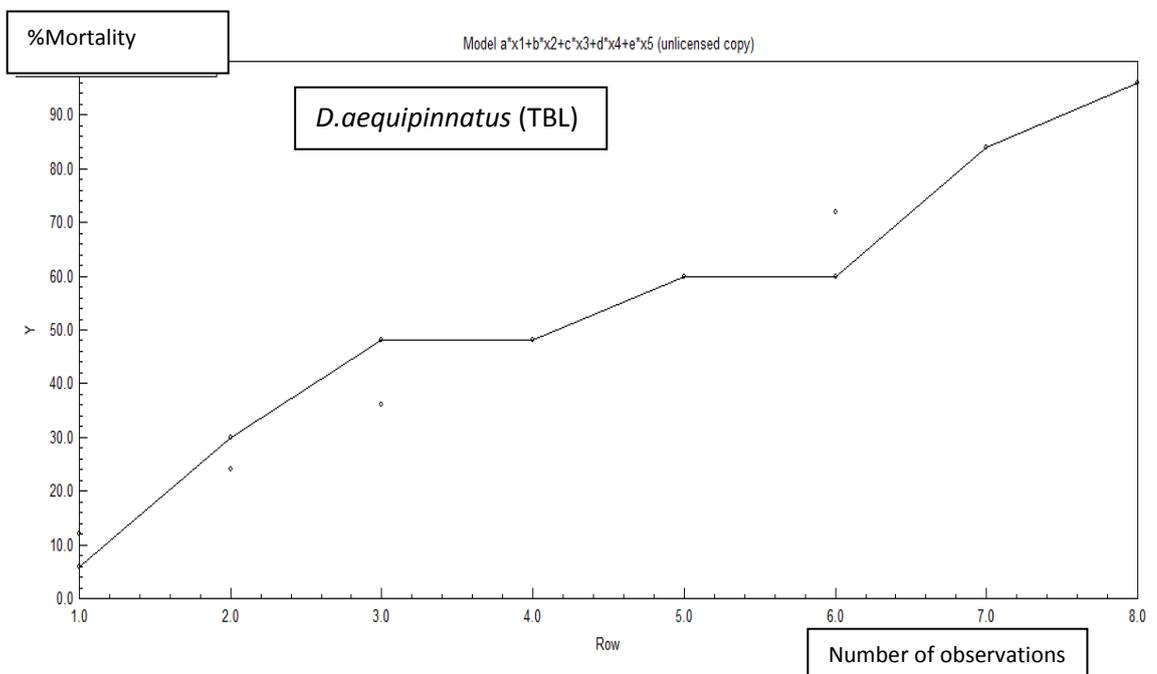


Figure 3.33 Model of TBL leachate toxicity test on *D. aequipinnatus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.17 Predictive Model plot values for *D.aequipinnatus* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
a	5.77	2.47	-5.7	-3.10	1.63	0.17	66	2.57
b	-0.80	-0.29	11.25	3.03	4.66	0.26	-42	-1.59
c	4.72	0.71	7.5	1.01	-10.51	-0.62	-54	-2.63
d	9.29	1.97	-4.5	-0.39	25.72	1.08	12	1.90
e	1.81	0.12	18	1.44	1.27	0.07	-36	-1.31

**Oreochromis mossambicus**

The exposure of *O.mossambicus* to leachate from the active sanitary landfill increased in mortality with time along 2.5 – 3.75% v/v (Figure 3.34). This led to generation of 3.22% v/v LC<sub>50</sub>. The rate of mortality was gradual; even at the highest exposure concentration (3.75% v/v) only 10% mortality was recorded between 12<sup>th</sup> and 60<sup>th</sup> hour of exposure (approximately 0.2% death/hour).

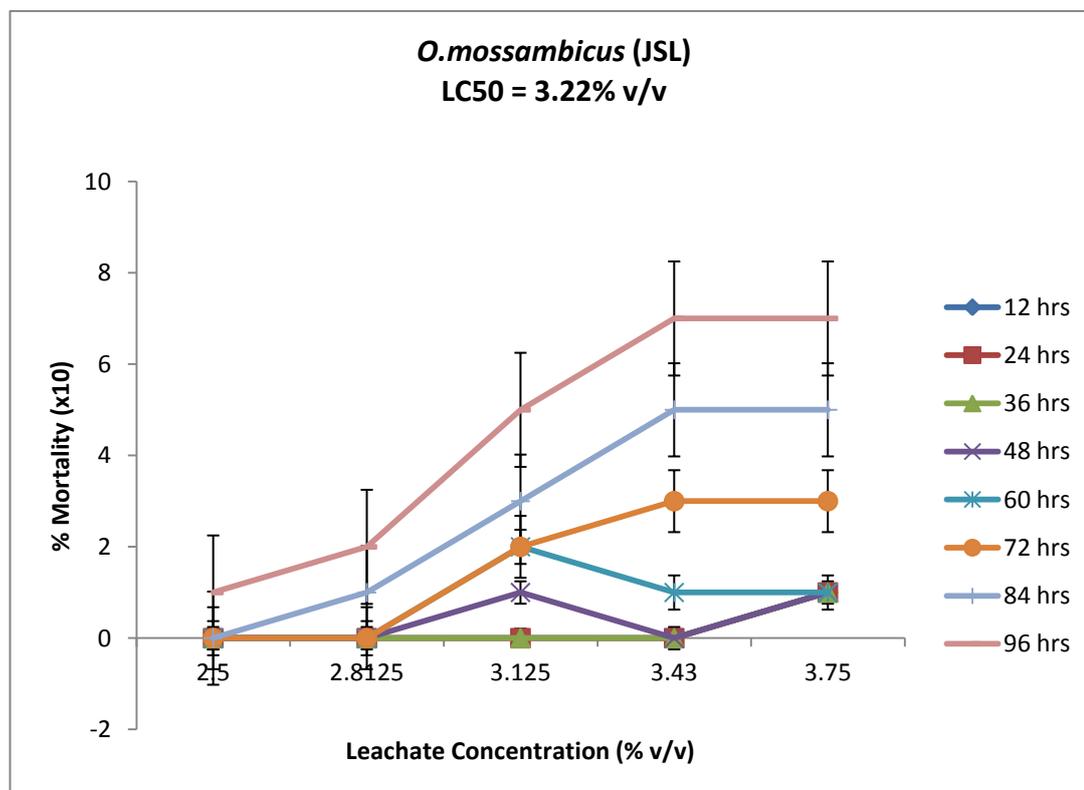


Figure 3.34 Mortality impact of JSL on *O.mossambicus* for 96 hours exposure

Similar exposure to closed sanitary landfill revealed same trend of increased mortality but in a more severe manner (Figure 3.35). Generated  $LC_{50}$  was 1.78. Total mortality (100%) observed at 36<sup>th</sup> hour exposure was a huge difference from only 10% recorded at 1% v/v. Though the highest exposure concentration in JSL was 3.75% v/v, yet the degree of mortality observed was less than the mortality at 2.5% v/v of AHL. This makes AHL more toxic to *O.mossambicus* as compared to JSL and others.

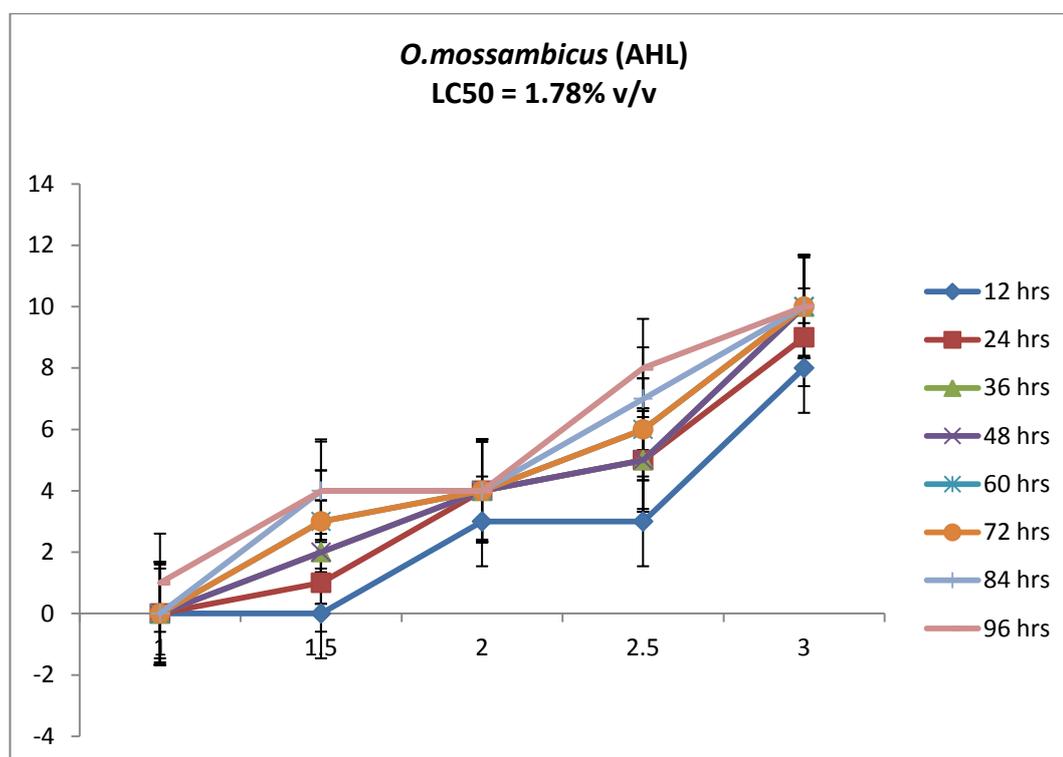


Figure 3.35 Mortality impact of AHL on *O.mossambicus* for 96 hours exposure

On the other hand, BBL exhibited more toxicity than TBL.  $LC_{50}$  analysis of *O.mossambicus* exposure to both raw leachate samples indicated that while 7.92% v/v (Figure 3.36) was obtained on BBL exposure, TBL recorded 9.84% v/v (Figure 3.37). This trend concurs with exposure of *D.rerio* to BBL and TBL.

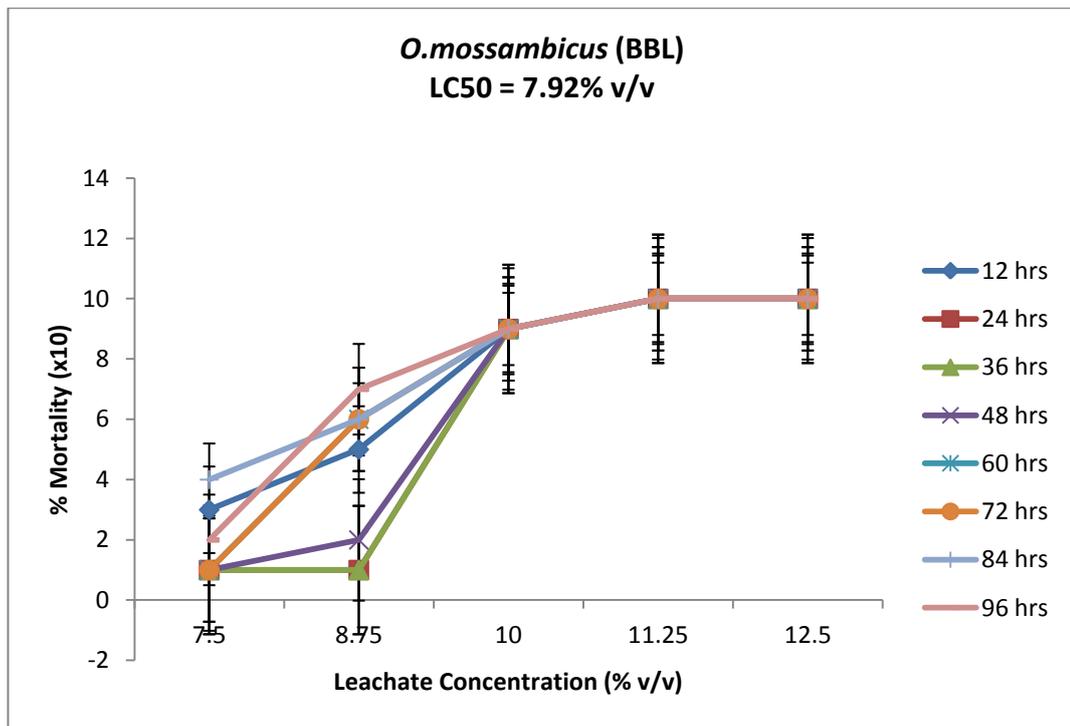


Figure 3.36 Mortality impact of BBL on *O. mossambicus* for 96 hours exposure

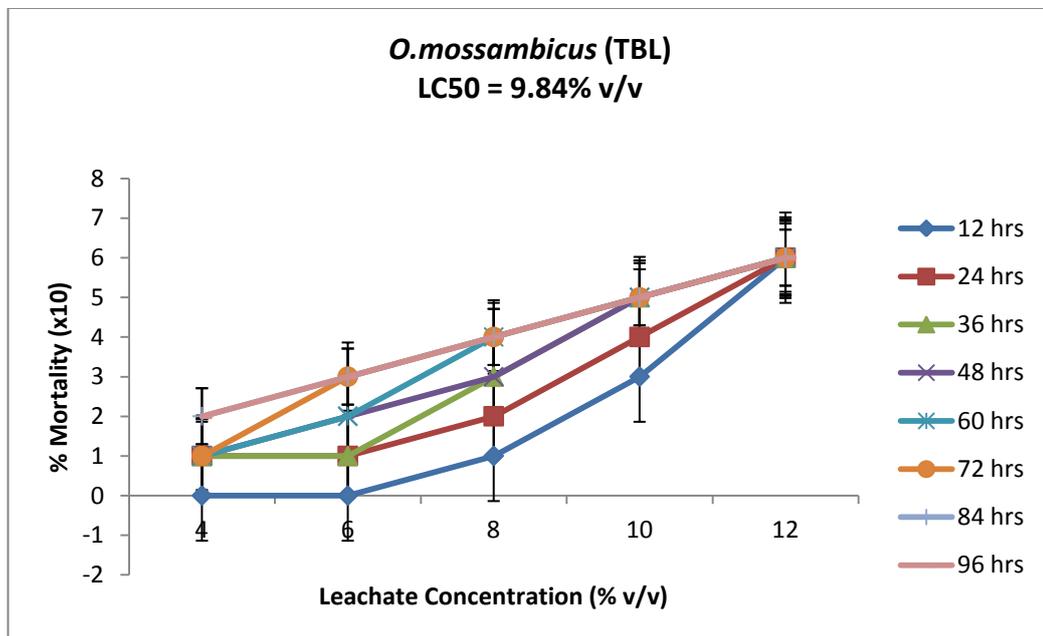


Figure 3.37 Mortality impact of TBL on *O. mossambicus* for 96 hours exposure

In general, AHL showed highest toxic impact on *O. mossambicus* before JSL, BBL and TBL, respectively. Leachate properties which varied across the landfills definitely

enhance the varying degree of toxicity. This reflected in the model plot (Figures 3.38 – 3.41). In as much as it is clear that all exposures fitted into the proposed model equation. Yet the distances of the input data from the line of best fit is a reflection of varying trend of mortality across discrete leachate concentrations with time. Values of the variables are shown in Table 3.18. Results are similar to previous studies on *O.mossambicus* and *Sarotherodon mossambicus* exposures to raw leachate (Emenike et al., 2011; Wong, 1989).

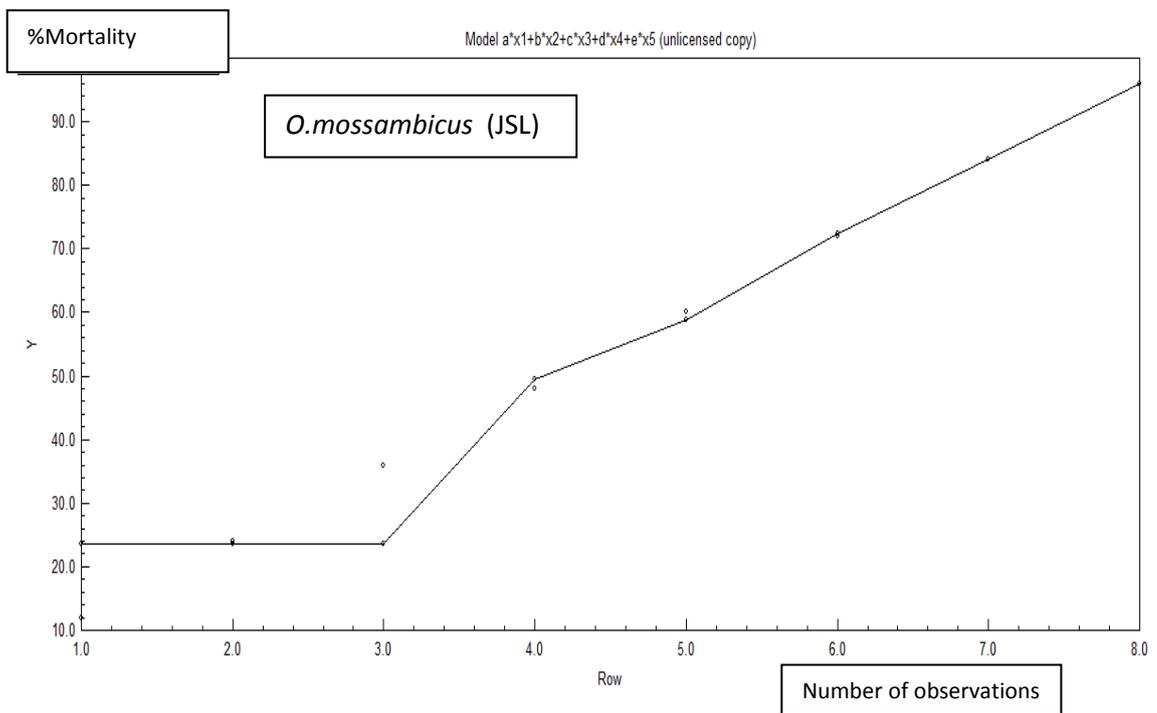


Figure 3.38 Model of JSL leachate toxicity test on *O.mossambicus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

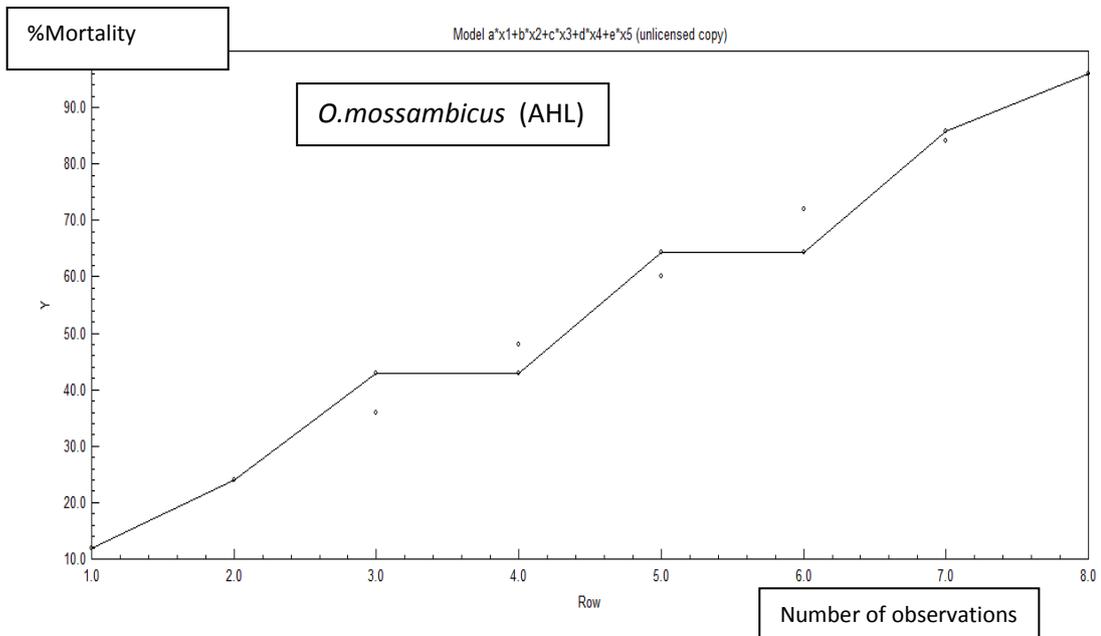


Figure 3.39 Model of AHL leachate toxicity test on *O. mossambicus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

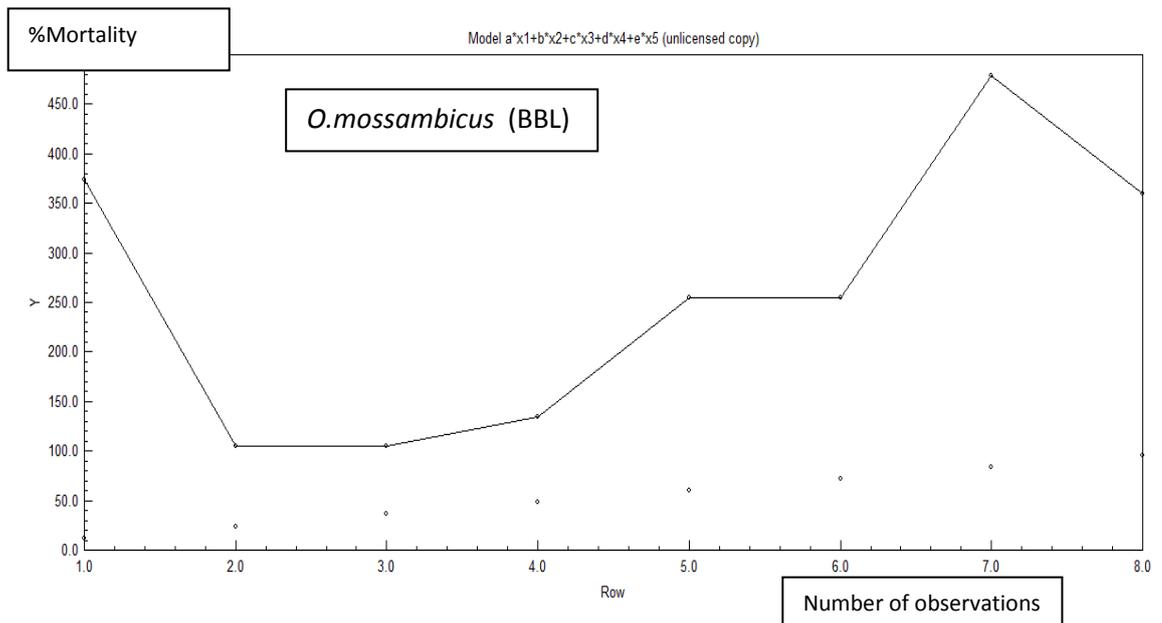


Figure 3.40 Model of BBL leachate toxicity test on *O. mossambicus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

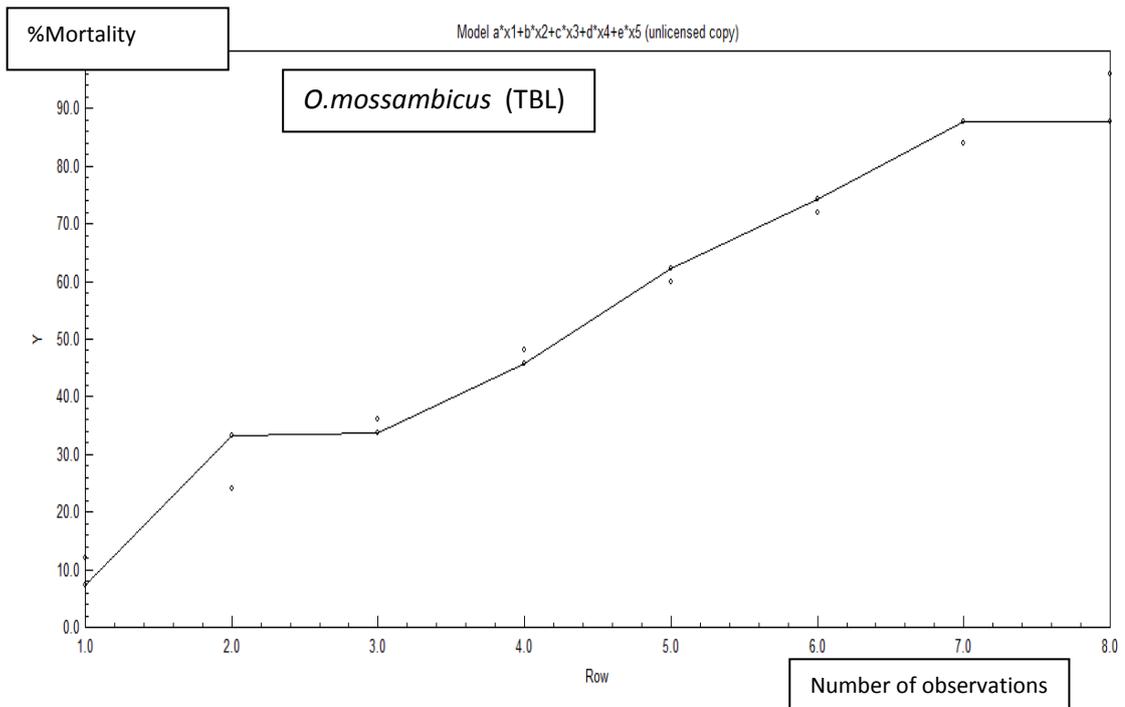


Figure 3.41 Model of TBL leachate toxicity test on *O.mossambicus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.18 Predictive Model plot values for *O.mossambicus* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	23.49	4.52	8.06	1.057	-9.6E+14	-0.0006	6.54	1.27
<b>b</b>	-16.72	-2.45	10.63	0.85	-1.97E+17	-0.09	-16.15	-1.41
<b>c</b>	26.04	4.04	-28.1	-1.04	2.20E+17	0.09	16.62	1.46
<b>d</b>	-27.96	-1.43	10.8	1.16	29.92858	0.08	12	1.43
<b>e</b>	-25.66	-1.00	-0.34	-0.02	74.79864	0.09	13.38	1.53

**Hyphessobrycon eques**

Figures 3.42 – 3.45 represent the mortality responses of *H.eques* to the raw leachate samples under review. At the end of range finding tests for all leachate samples, it was observed that none of the leachate sample had similar toxicity range; hence the variations in the definitive test range with AHL at 1.1 – 1.5% v/v, JSL at 2.5 – 3.75% v/v, BBL at 6.25 – 11.25% v/v and TBL at 3.125 – 5.625% v/v. Such ranges easily gave an insight on the direction of severe toxicity from the studied landfill leachates.

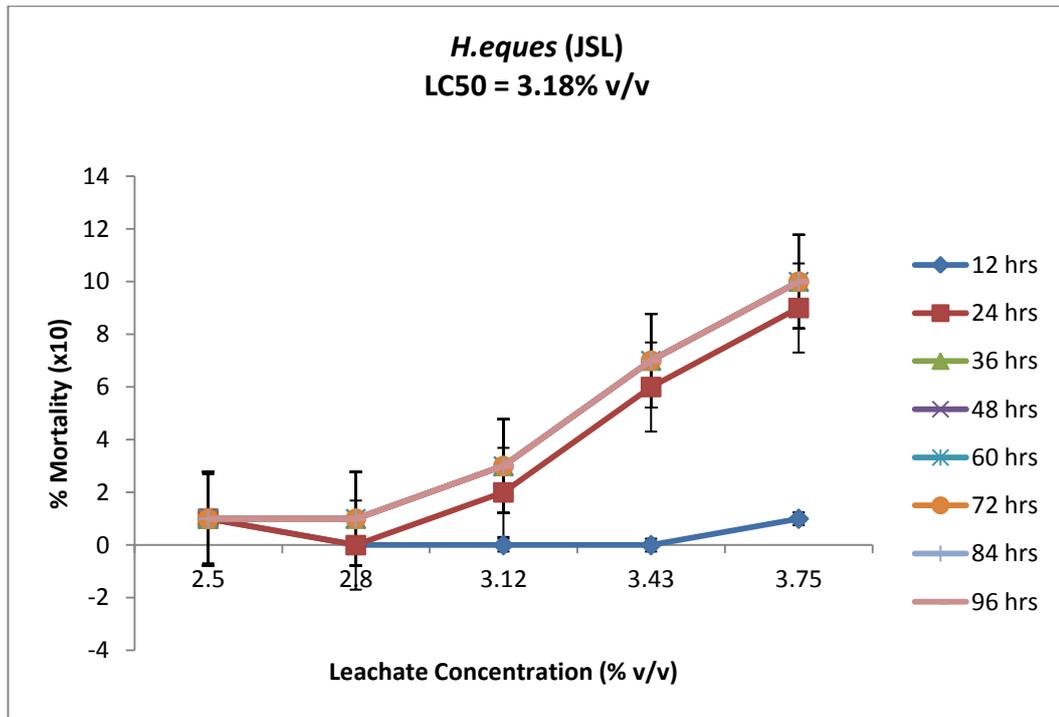


Figure 3.42 Mortality impact of JSL on *H. eques* for 96 hours exposure

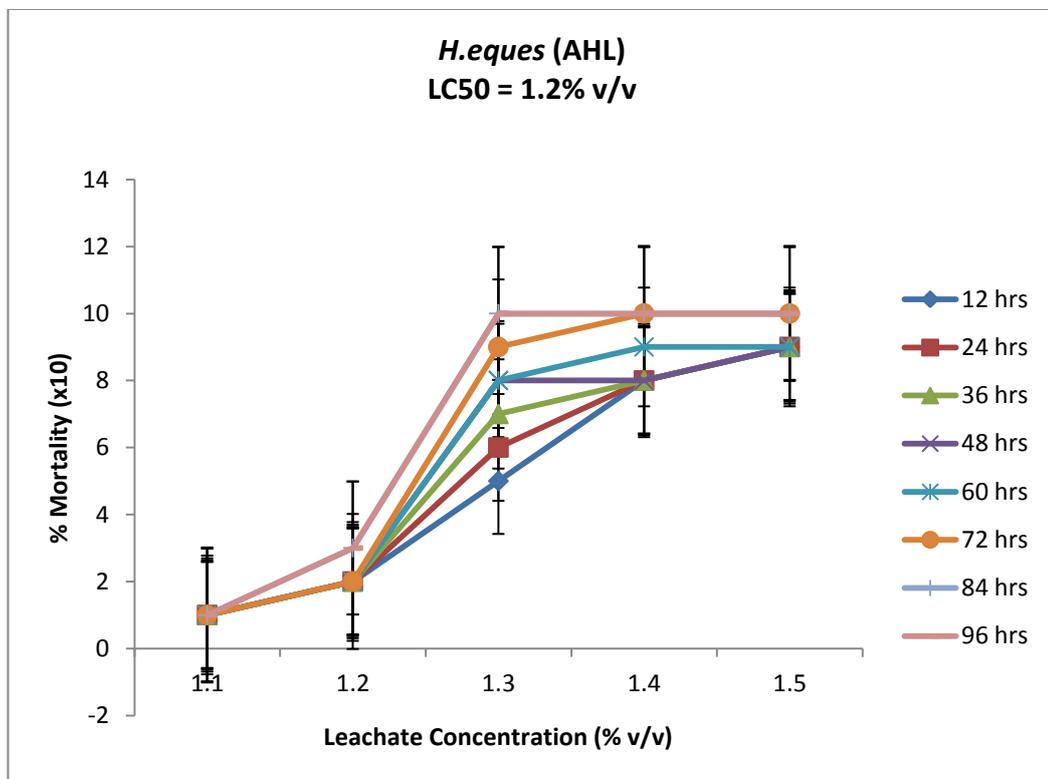


Figure 3.43 Mortality impact of AHL on *H. eques* for 96 hours exposure

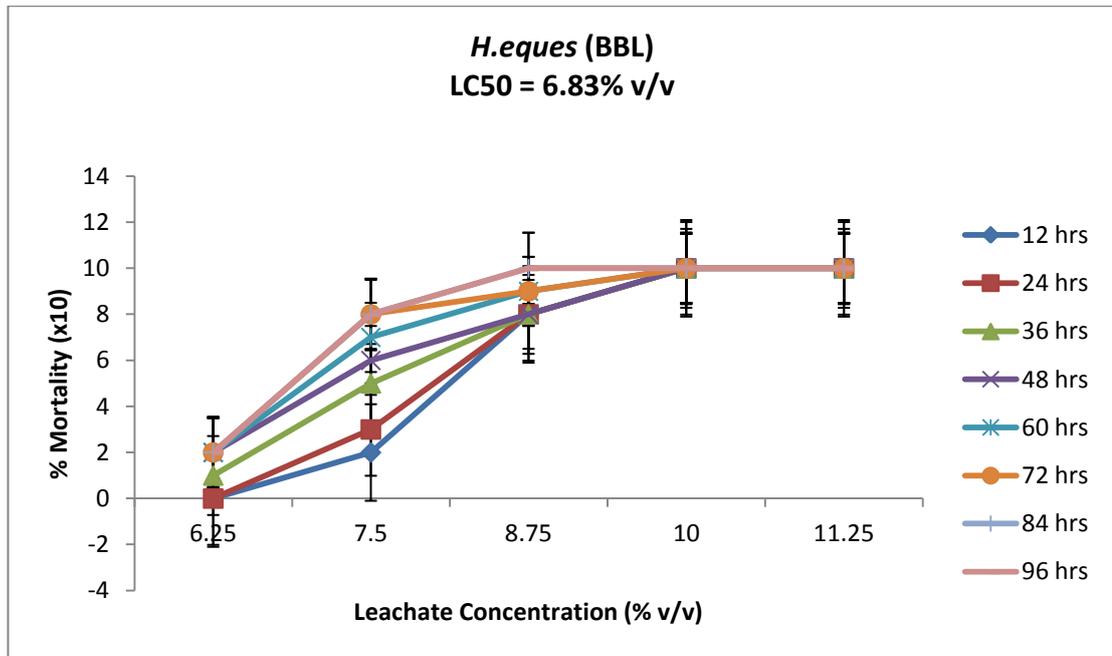


Figure 3.44 Mortality impact of BBL on *H.eques* for 96 hours exposure

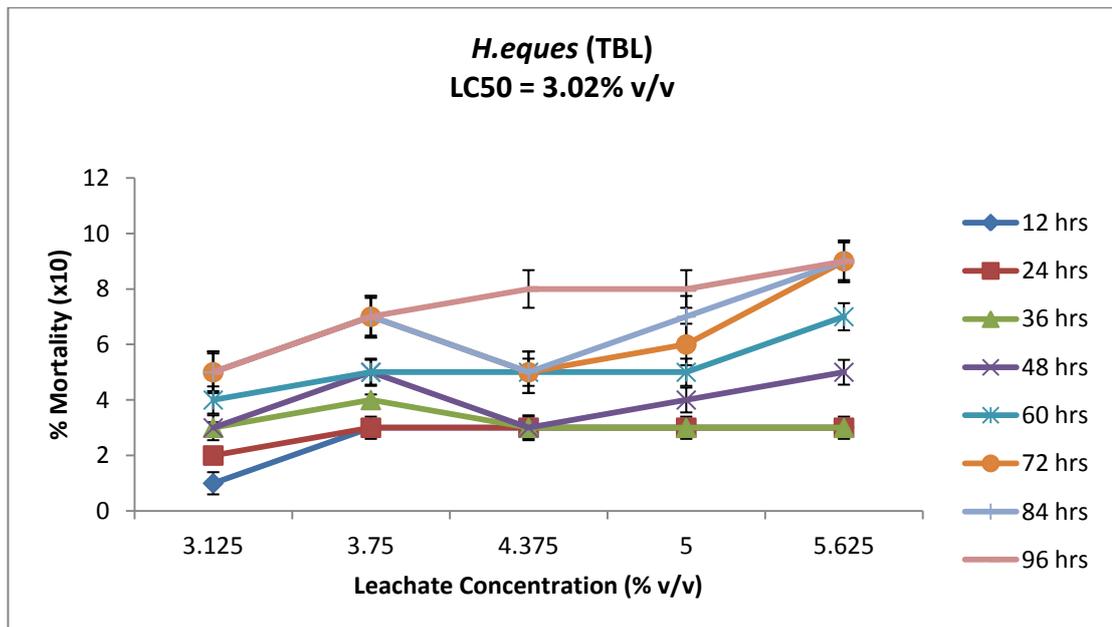


Figure 3.45 Mortality impact of TBL on *H.eques* for 96 hours exposure

The calculated LC<sub>50</sub> revealed that *H.eques* have equal mortality and survival upon exposure to 1.2, 3.18, 3.02 and 6.83% v/v of AHL, JSL, TBL and BBL, respectively. This implies that the trend of toxicity on *H.eques* was increasing from the non-sanitary

landfill leachate exposure towards landfill leachate with sanitary status. As reported by Jaffar et al (2009), closed sanitary landfill showed more toxic effect than leachate from non-sanitary landfill. Reason may be associated to the leachate collection system; considering difference in characterized leachate quality from landfills at different levels of classification (Agamuthu, 2001; Fauziah & Agamuthu, 2003; Emenike et al., 2013). At 12 hours of exposure, fish mortality in JSL exposure was gradual unlike in the other leachates, yet 100% was attained by the end of 96 hours. This is a proof that toxicity increases with time; hence a delayed effect should not be mistaken to be a measure of resistance by fish species.

Figures 3.46 – 3.49 showed that the exposure trend fitted into the predictive model while Table 3.19 shows the generated values of the leachate concentration variables. However, there were better fitting with AHL, BBL and TBL, respectively, than with JSL. This may be attributed to varied responses of the fish across the exposure concentrations. Hence, it might depict trend of difference between active sanitary landfill and the other landfill classes.

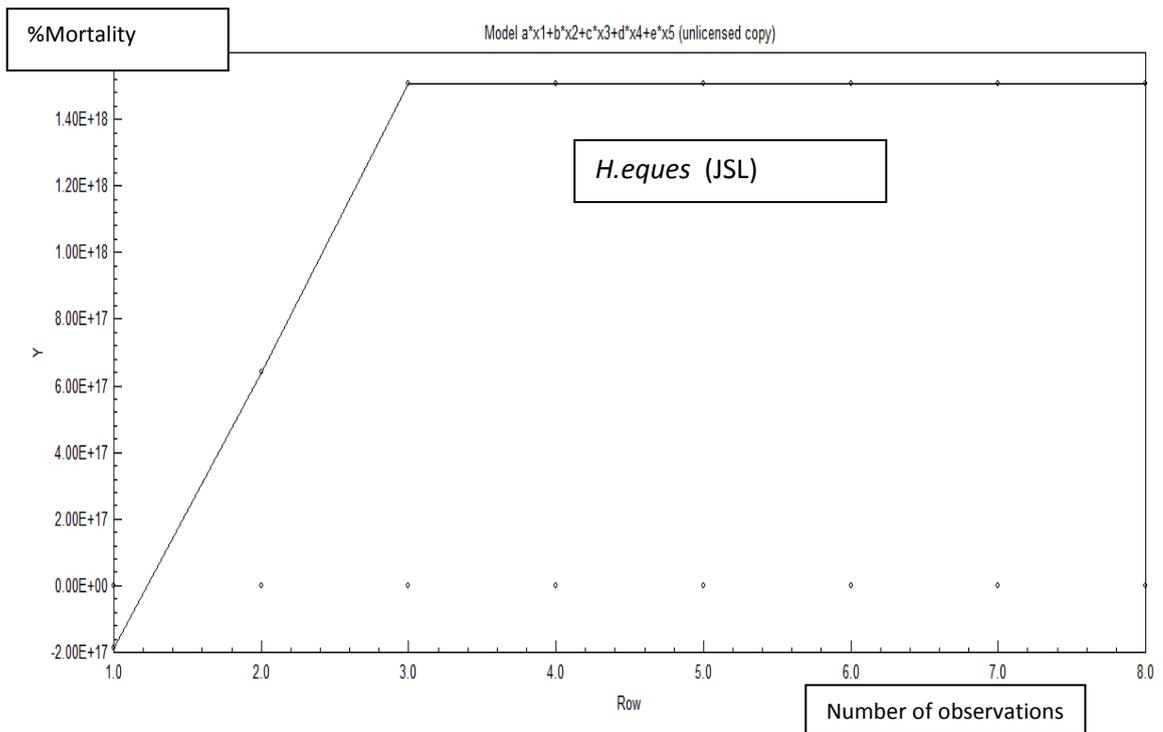


Figure 3.46 Model of JSL leachate toxicity test on *H. eques* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

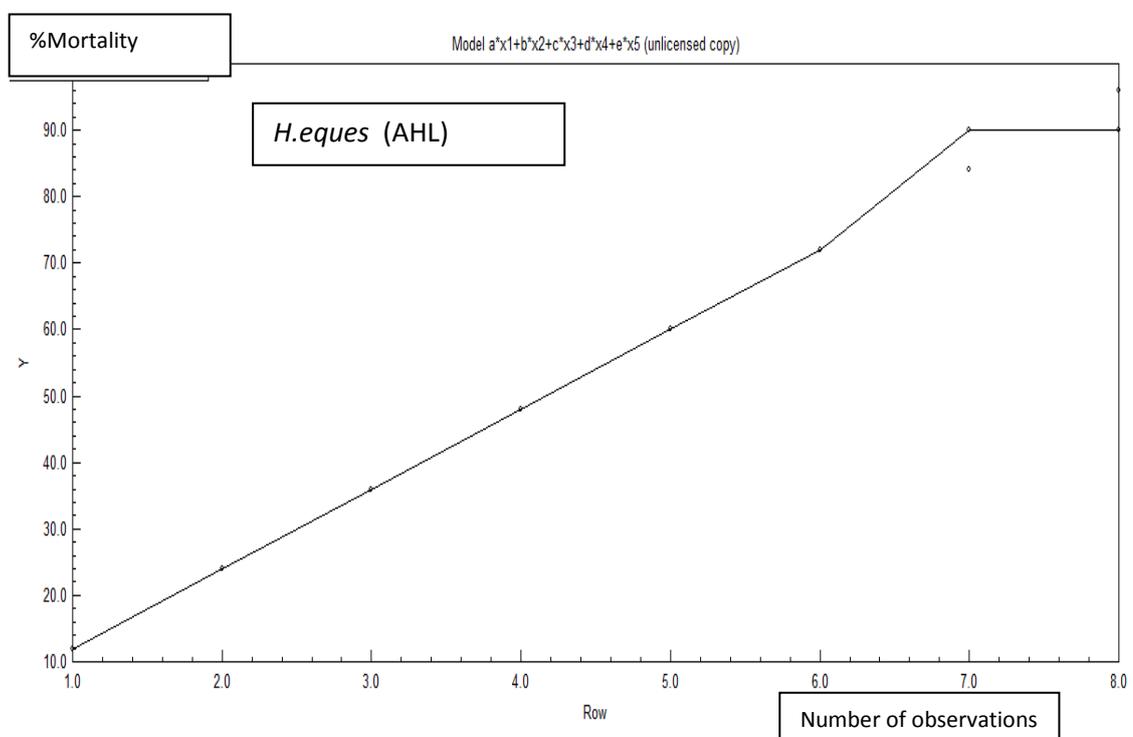


Figure 3.47 Model of AHL leachate toxicity test on *H. eques* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

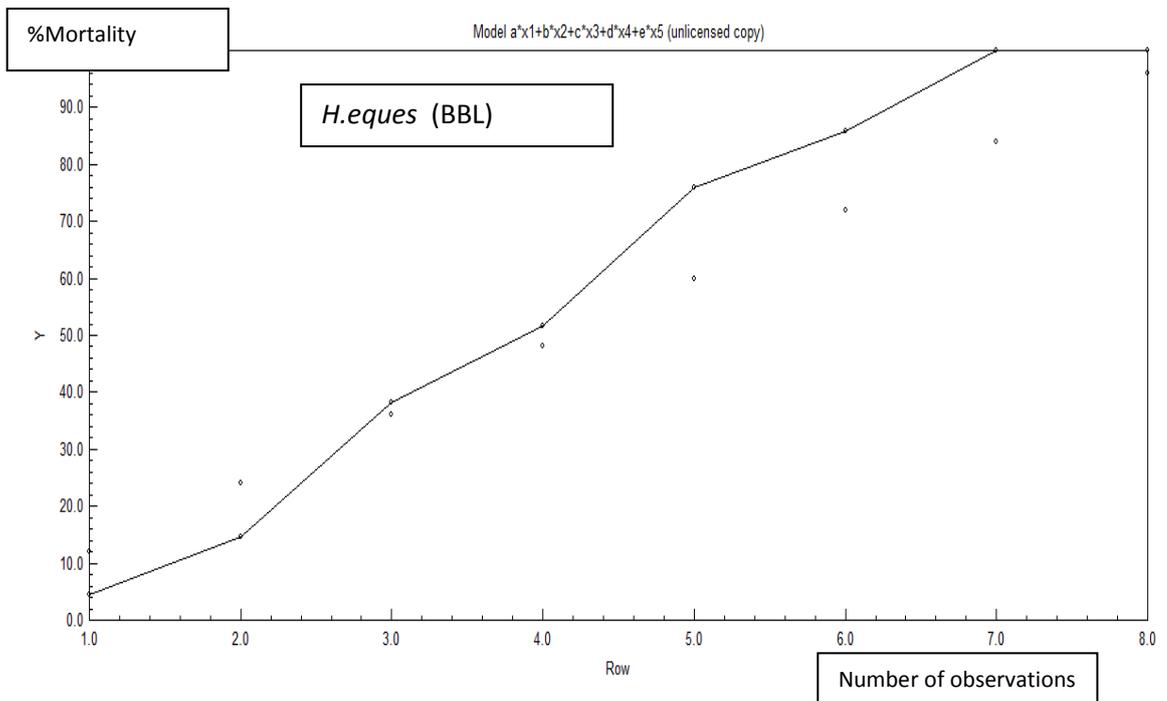


Figure 3.48 Model of BBL leachate toxicity test on *H.eques* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

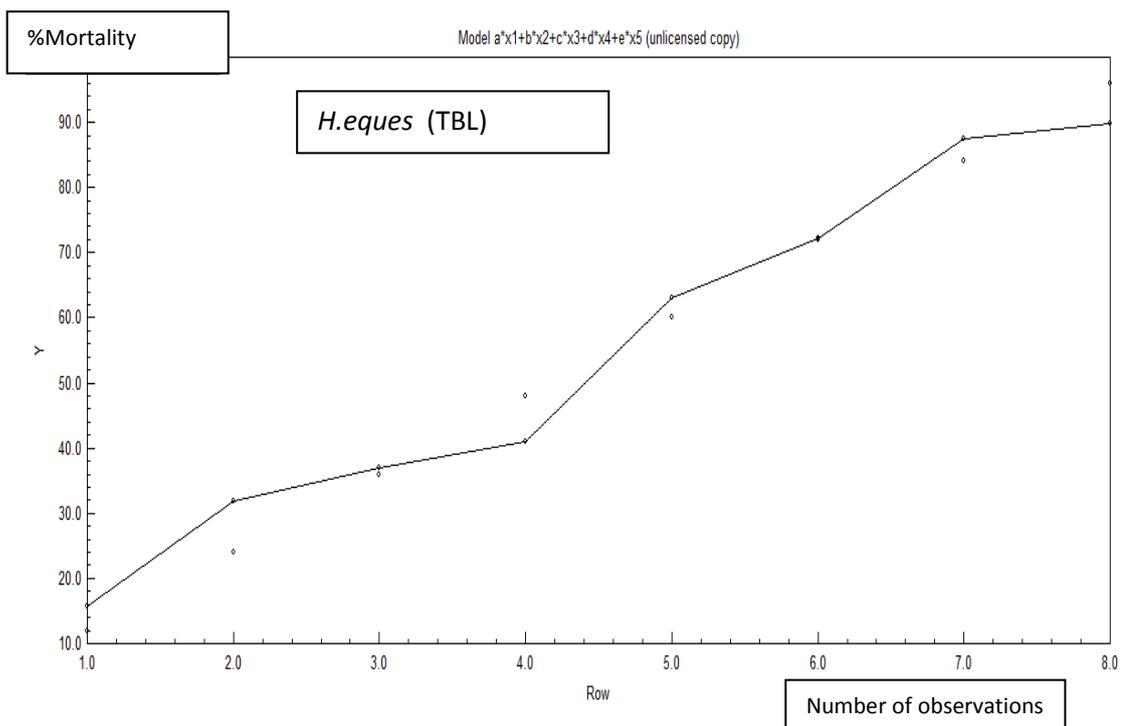


Figure 3.49 Model of TBL leachate toxicity test on *H.eques* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.19 Predictive Model plot values for *H.eques* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
a	1.24E+19	1.52E-17	-12	-1.06	-1.37E+16	-0.23	-0.30	-0.068
b	7.22E+32	1.24E-17	12	1.88	1.37E+16	0.23	15.41	1.625
c	-2.17E+33	-1.24E-17	12	5.48	14.03718	0.93	-4.34	-0.84
d	1.44E+33	1.24E-17	6	0.94	10.07036	0.58	-10.81	-1.86
e	-1.26E+19	-1.54E-17	-48	-0.79	3.506818	0.11	15.95	2.33

### *Leptobarbus hoeveni*

Exposure of *L.hoeveni* to raw leachate samples for the purpose of assessing the acute toxicity effect of the four landfill types demonstrated a continued variation of mortality across the designated leachate concentrations. Figures 3.50 – 3.53 show the relationship between the definitive test concentrations and the time of exposure (96 hours). A quick glance at the LC<sub>50</sub> results showed a sharp difference between sanitary landfills (active and closed) and non-sanitary types (active and closed). Figures 3.50 and 3.57 recorded 1.55 and 1.15% v/v LC<sub>50</sub> for JSL and AHL, respectively. It means that while JSL and AHL may show higher LC50 values than BBL and TBL, the fact still remains that AHL is more toxic to *L.hoeveni* than any other leachate sample.

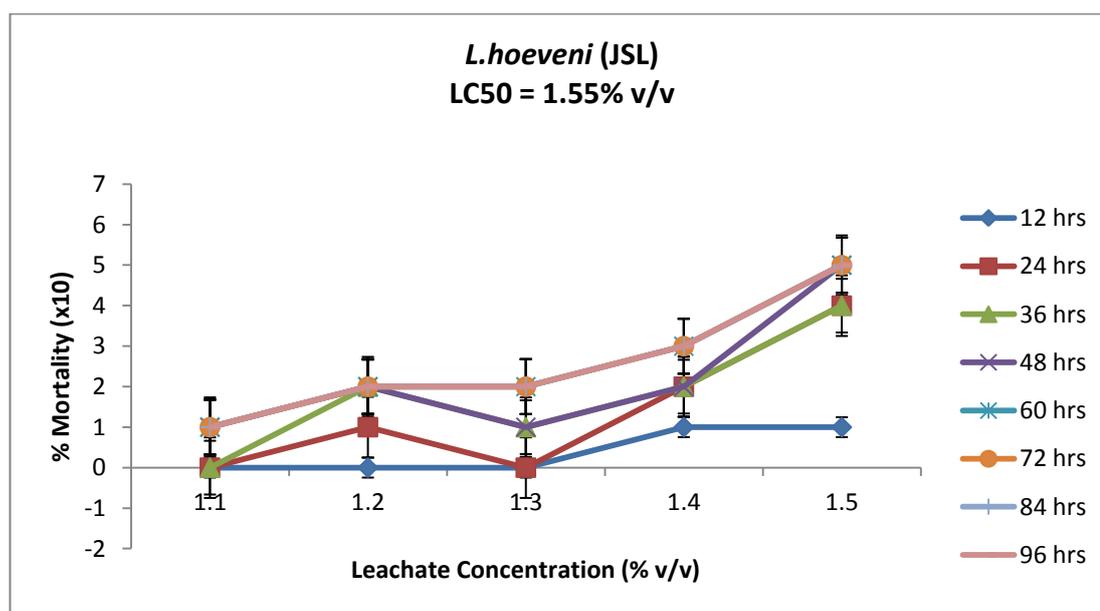


Figure 3.50 Mortality impact of JSL on *L.hoeveni* for 96 hours exposure

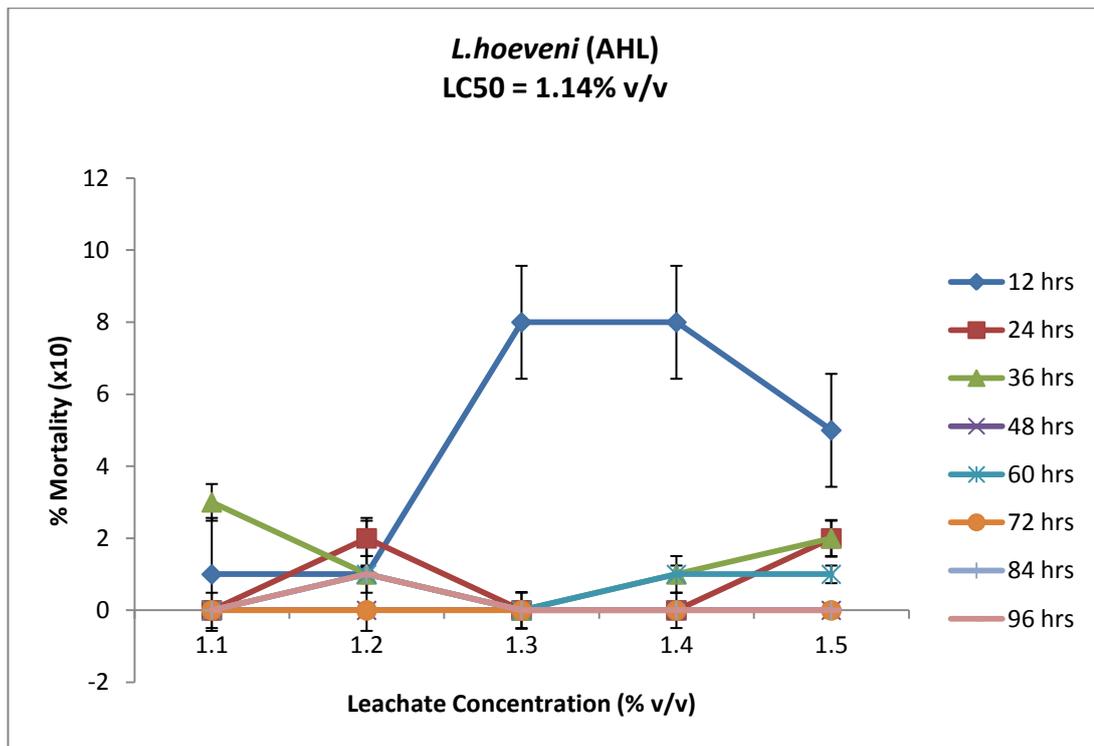


Figure 3.51 Mortality impact of AHL on *L. hoeveni* for 96 hours exposure

A direct comparison can be made between JSL and AHL exposure because they have same definitive test concentration range. The degree of mortality at 12<sup>th</sup> hour of exposure was extremely severe at AHL than in JSL. This goes a long way to depict the toxic potential of the leachate in relations to time; hence giving an insight on the colossal impact it will have in situation of leachate pollution of nearby surface water. The study may not rule out oxygen depletion in water upon immediate contact with the heterogeneous pollutant (Emenike et al., 2012a; Jaffar et al., 2009; Svobodova et al., 1993), while at the same time suspecting high level of induced metabolic interference in the fish.

Again, BBL turned out to be more toxic than TBL considering their LC<sub>50</sub> values (BBL = 5.83% v/v; TBL = 7.09% v/v) as shown in Figures 4.52 and 4.53. This may be depending on fish species in terms of resistive ability.

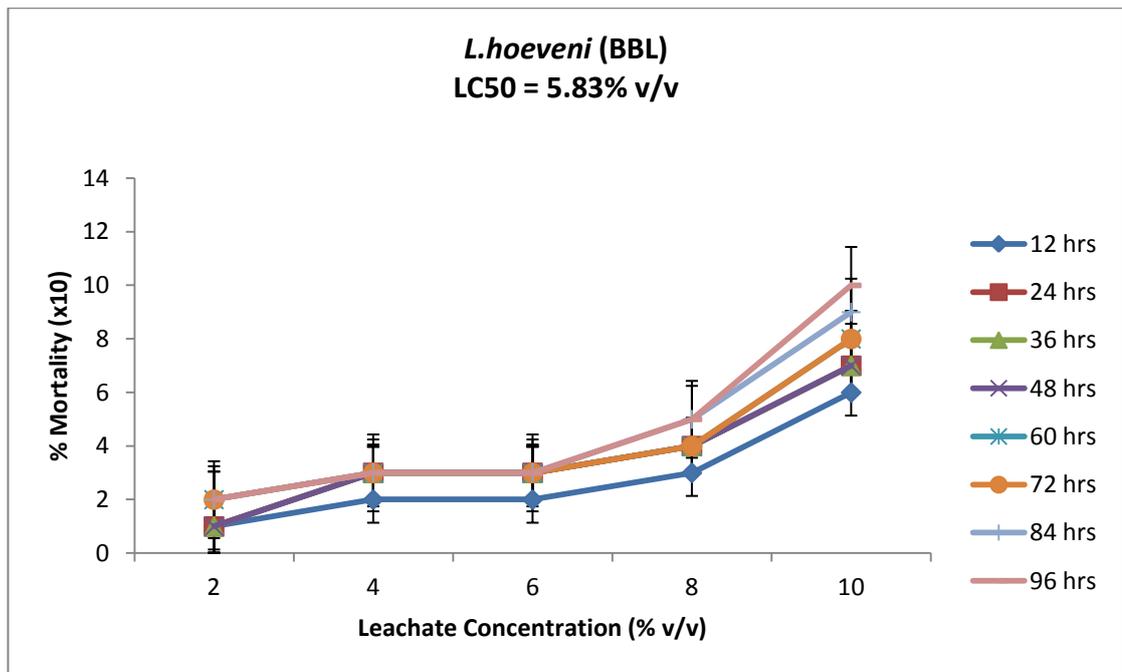


Figure 3.52 Mortality impact of BBL on *L.hoeveni* for 96 hours exposure

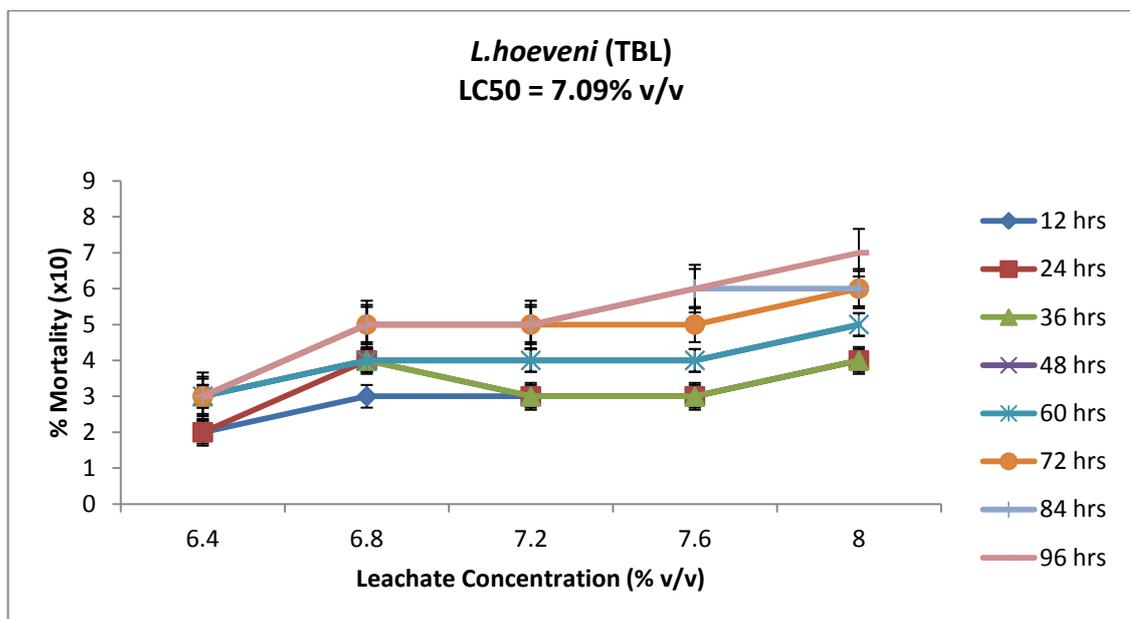


Figure 3.53 Mortality impact of TBL on *L.hoeveni* for 96 hours exposure

Based on the distances between the input data and line of best fit, it can be inferred that best fitting was obtained from JSL exposure (Figure 3.54). The high level of mortality of *L.hoeveni* towards exposure to AHL may have reflected in the pattern it showed

while fitting into the predictive model (Figure 3.55); hence the big gap between the lowest value of the coefficient variable and the highest. Figures 3.56 – 3.57 showed the fit patterns of BBL and TBL exposure, whereas Table 3.20 contains the concentration coefficient variables.

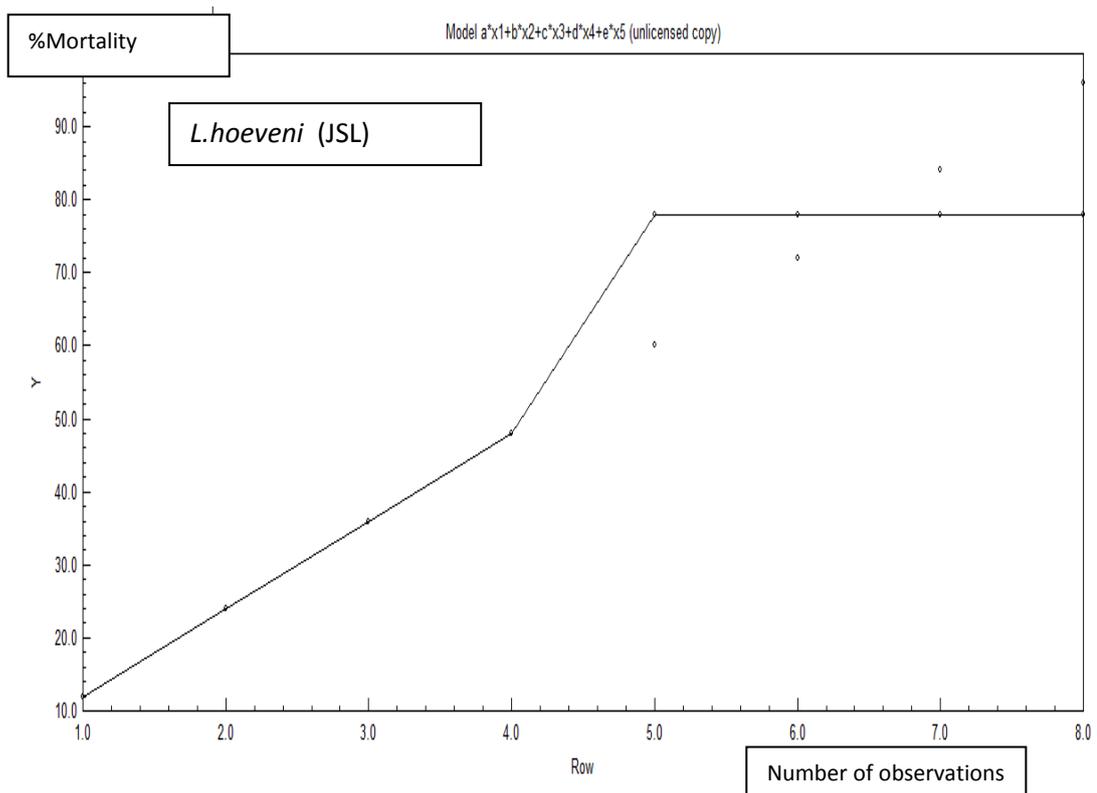


Figure 3.54 Model of JSL leachate toxicity test on *Lhoeveni* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

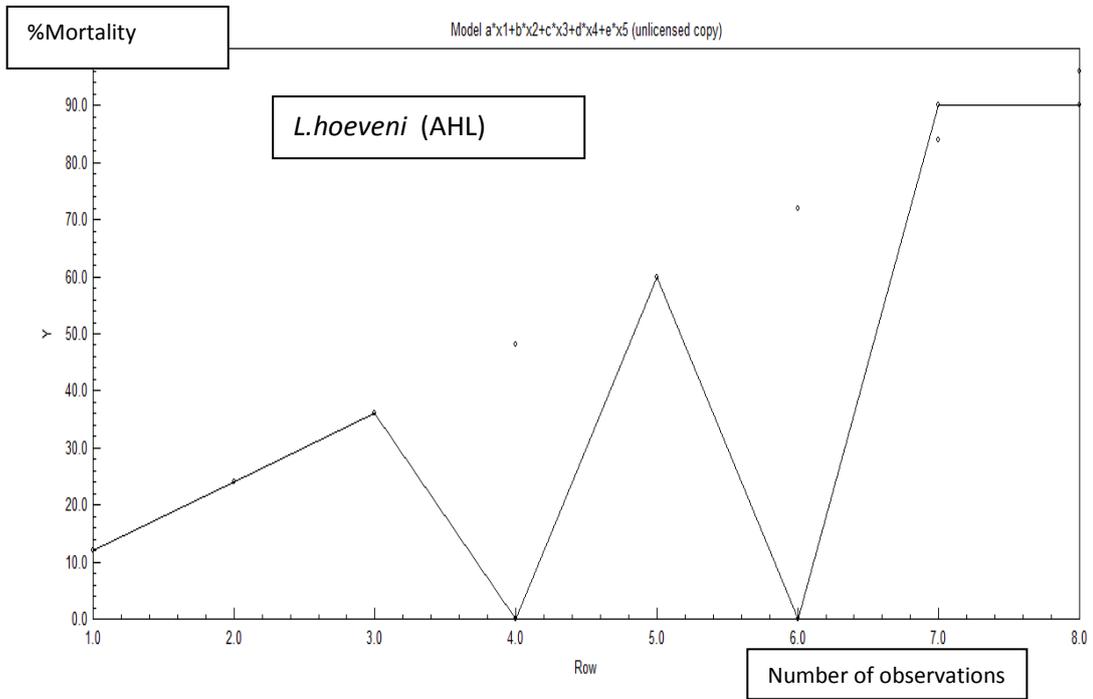


Figure 3.55 Model of ASL leachate toxicity test on *L.hoeveni* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

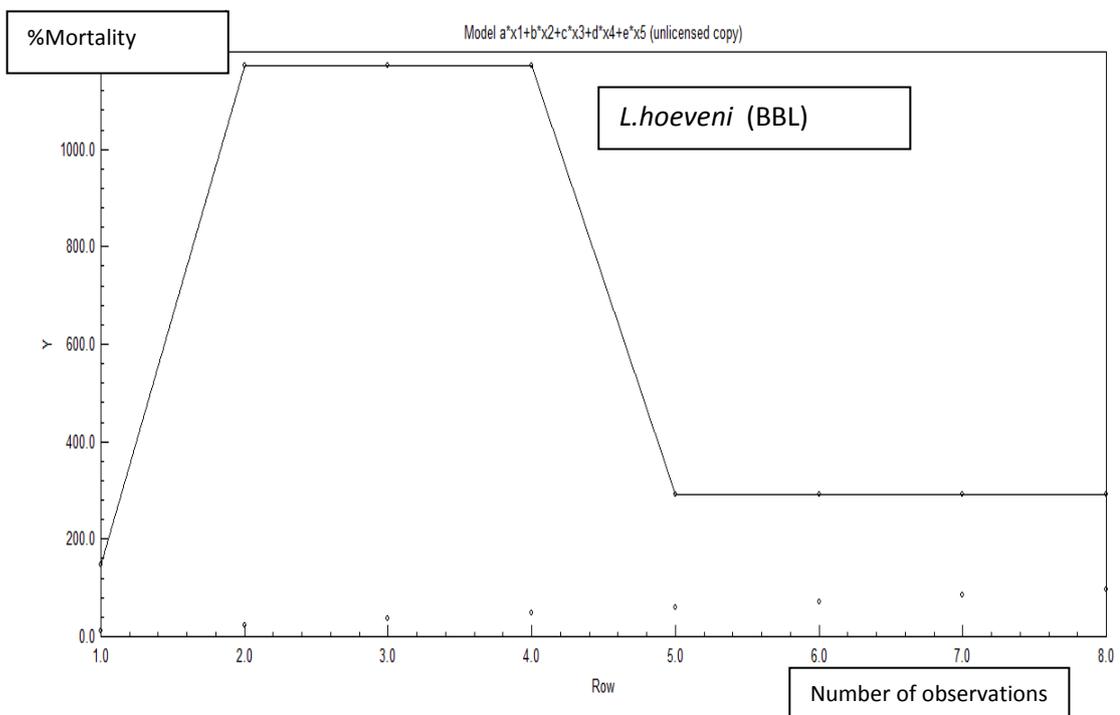


Figure 3.56 Model of BBL leachate toxicity test on *L.hoeveni* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

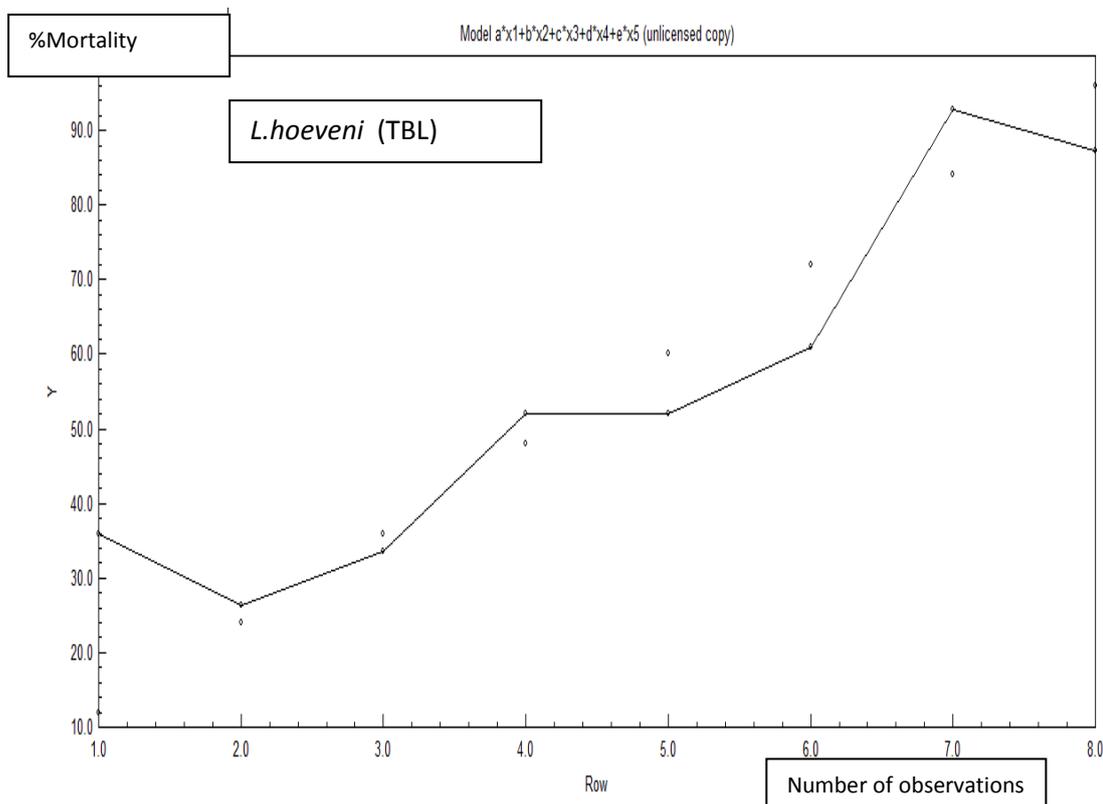


Figure 3.57 Model of TBL leachate toxicity test on *L.hoeveni* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.20 Predictive Model plot values for *L.hoeveni* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	6	0.099	-78	-1.794	-10.605	-0.008	-5.455	-0.242
<b>b</b>	6	0.082	48	0.855	355.993	0.029	31.818	1.60
<b>c</b>	24	0.3038	-11.25	-0.215	-3.63E+18	-0.027	-8	-0.25
<b>d</b>	-12	-0.1328	90	2.535	3.63E+18	0.027	-9.45	-0.54
<b>e</b>	6	0.0932	18	0.649	-877.602	-0.026	7.272	0.370

### *Poecillia sphenops*

The exposure of *P.sphenops* to leachate from the active sanitary landfill increased in mortality with time; 1.2 – 2.8% v/v (Figure 3.58). An LC<sub>50</sub>, 3.28% v/v was generated from the exposure. The rate of mortality was gradual; even at the highest concentration

(2.8% v/v) only 10% mortality was recorded within 24 hours of exposure (approx 0.2% death/hour) as against 90% mortality observed with AHL exposure (Figure 3.59).

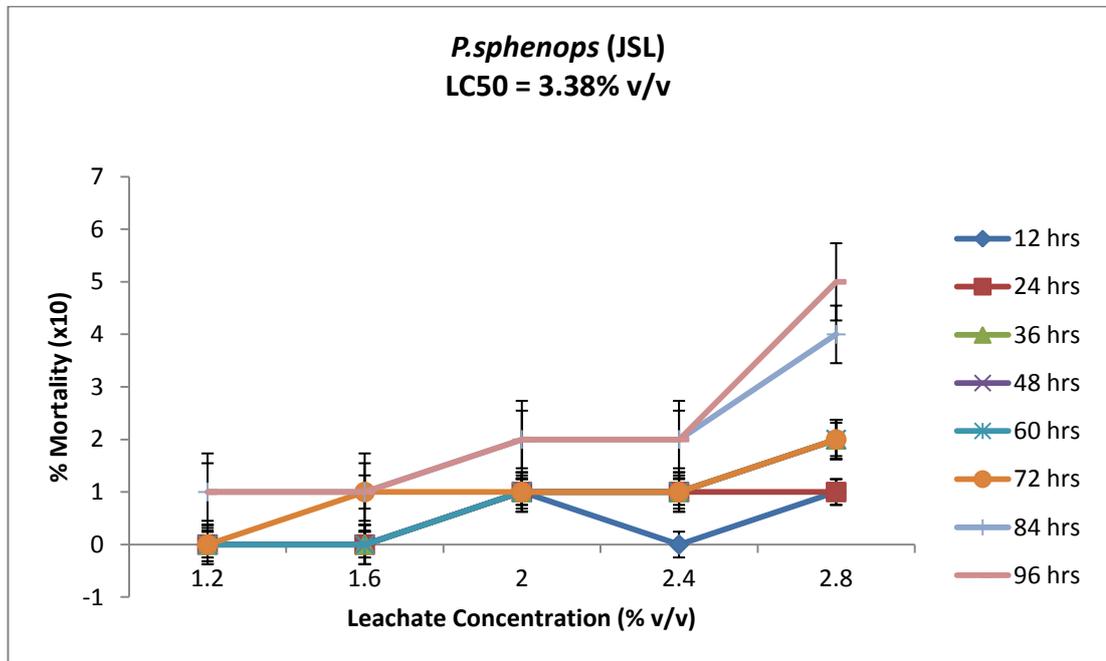


Figure 3.58 Mortality impact of JSL on *P.sphenops* for 96 hours exposure

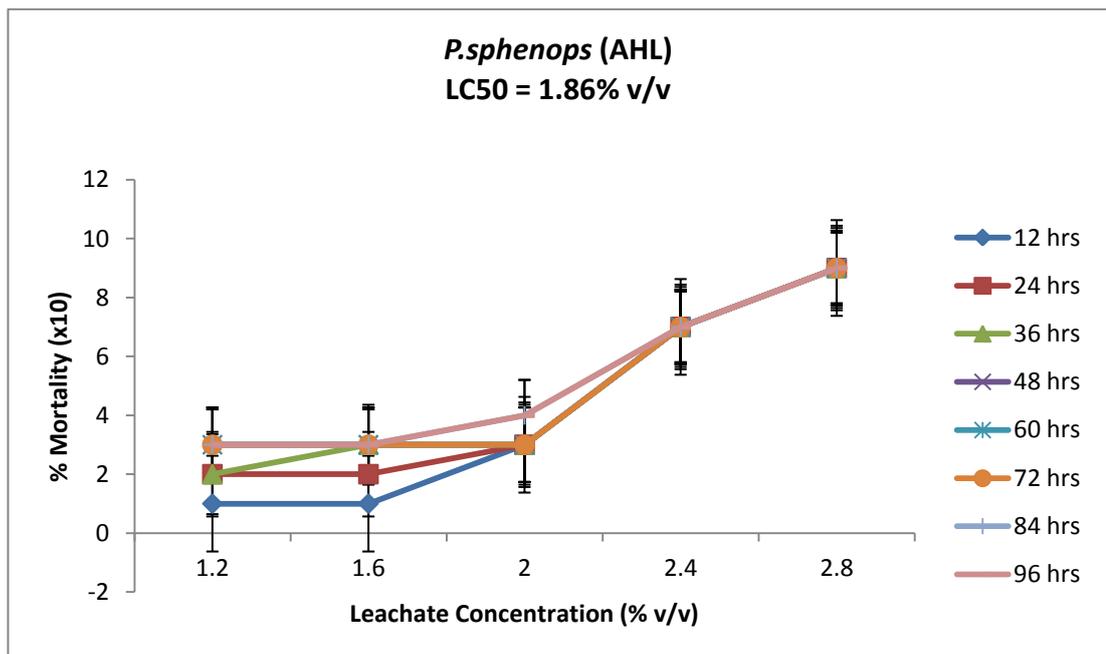


Figure 3.59 Mortality impact of AHL on *P.sphenops* for 96 hours exposure

Though a similar trend of mortality was observed at AHL exposure, death response was more severe; hence the generated LC<sub>50</sub> value was 1.86% v/v. A direct comparison with JSL exposure can be made since both were conducted under the same test range (1.2 – 2.8% v/v). *P.sphenops* could not tolerate AHL easily especially within the 12 hours of exposure. Result showed that 90%, 70% and 30% of the fish test population died within 12 hours of exposure to 2.8, 2.4 and 2% v/v, respectively. However, it appeared that the discrete degree of mortality became gradual after 12 hours; in fact zero mortality occurred from 24 hours in 2.8 and 2.4% v/v exposures. Reason may be that after the instantaneous mortality, the ones that survived became more adapted to the polluted environment (at least for the duration of the experiment).

On the other hand, TBL exhibited more toxicity than BBL. LC<sub>50</sub> analysis of *P.sphenops* exposure to both raw leachate samples indicated that while 10.01% v/v (Figure 3.60) was obtained on BBL exposure, TBL recorded 8.08% v/v (Figure 3.61). The result is similar to the exposures of *X.maculates* and *H.eques*, and as such the study may assume that the non-edible fish were more susceptible to closed non-sanitary landfill leachate than when exposed to leachate from active non-sanitary landfill.

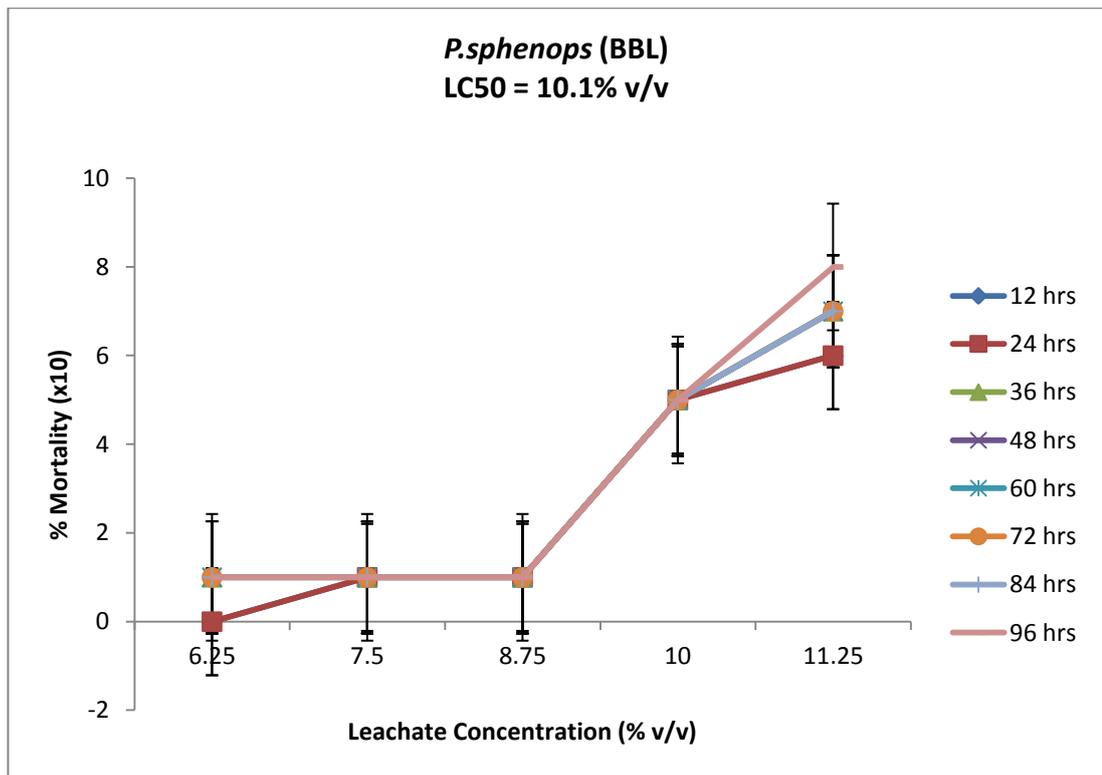


Figure 3.60 Mortality impact of BBL on *P.sphenops* for 96 hours exposure

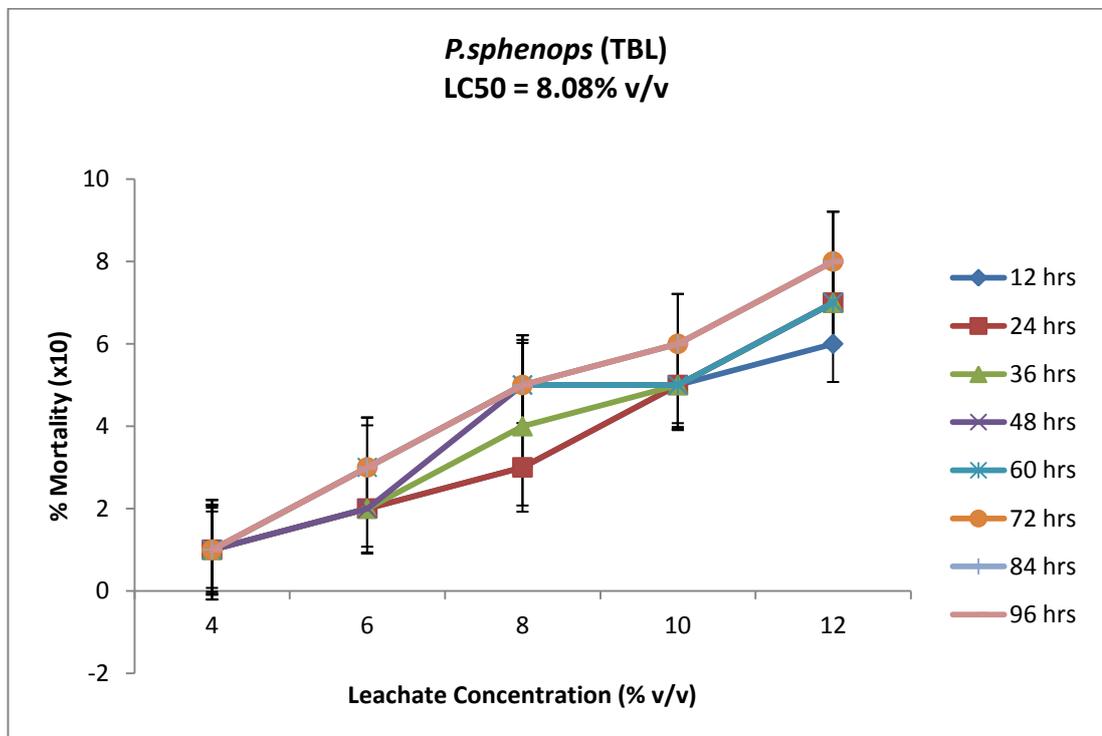


Figure 3.61 Mortality impact of TBL on *P.sphenops* for 96 hours exposure

In general, AHL showed highest toxic effect on *P.sphenops* before JSL, TBL and BBL, respectively. Varying leachate properties affect the toxicity potentials of landfills. Figures 3.62 – 3.65 may have reflected this in the model plot. Excluding BBL exposure (Figure 3.41), others were fitted within the positive Y-values. Corresponding a-e values were recorded in Table 3.21.

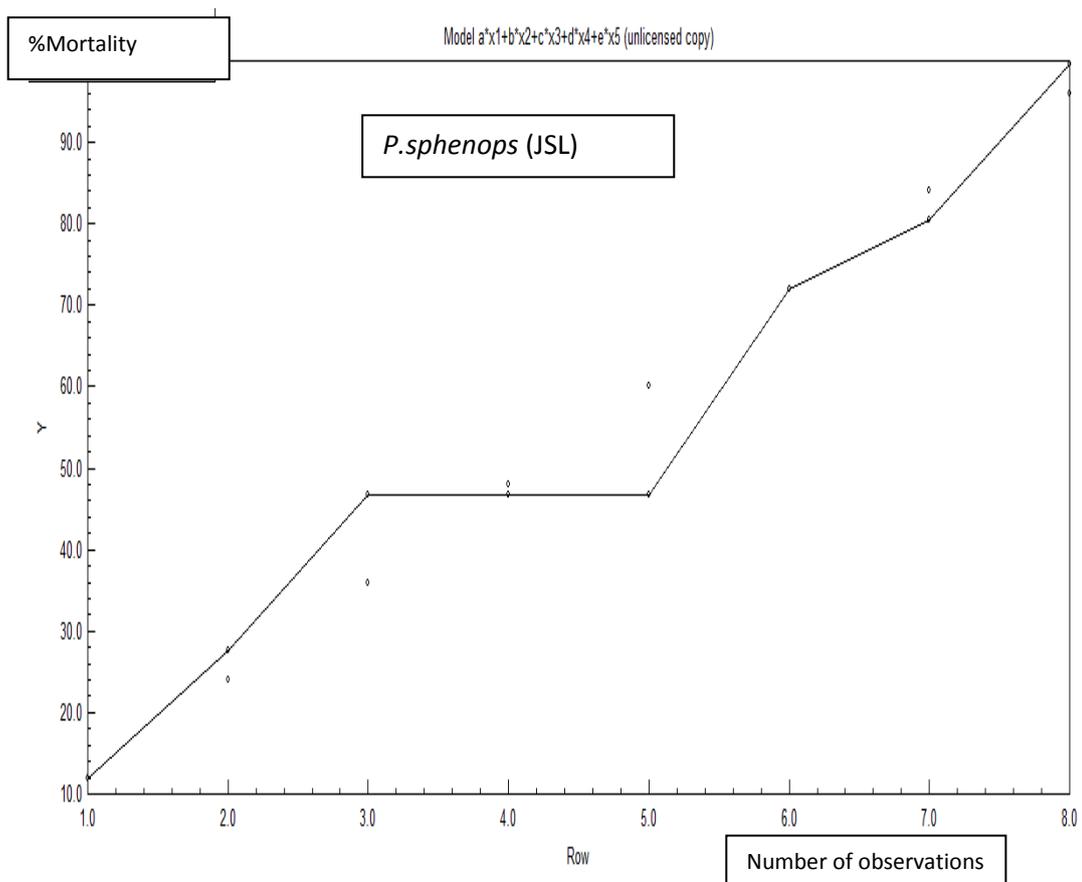


Figure 3.62 Model of JSL leachate toxicity test on *P.sphenops* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

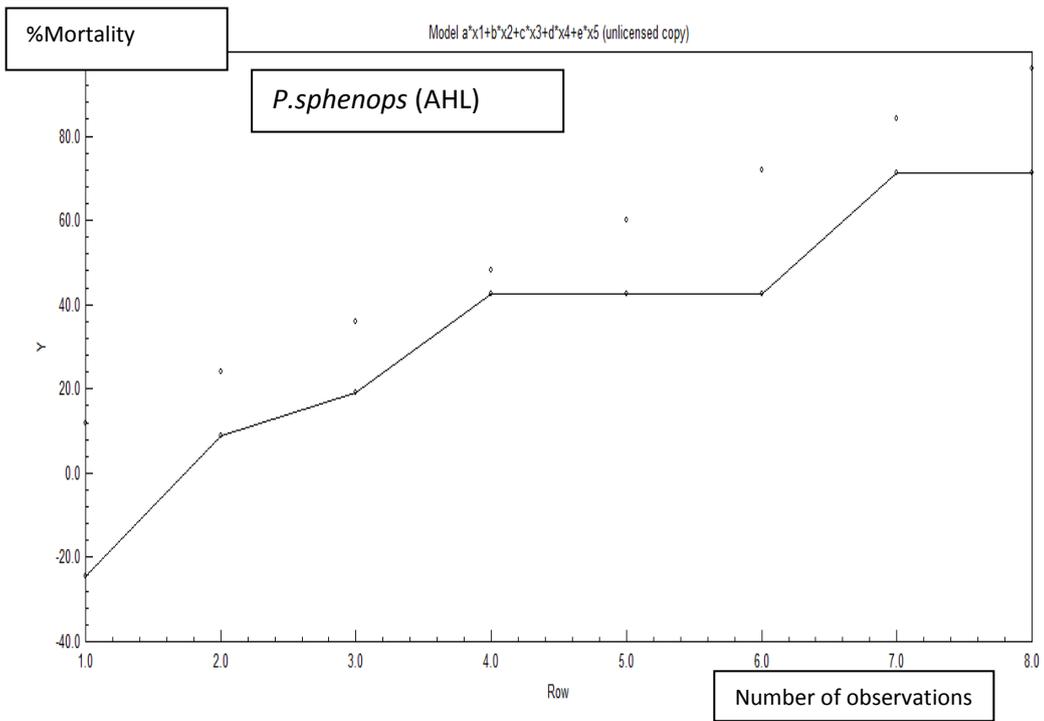


Figure 3.63 Model of AHL leachate toxicity test on *P.sphenops* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

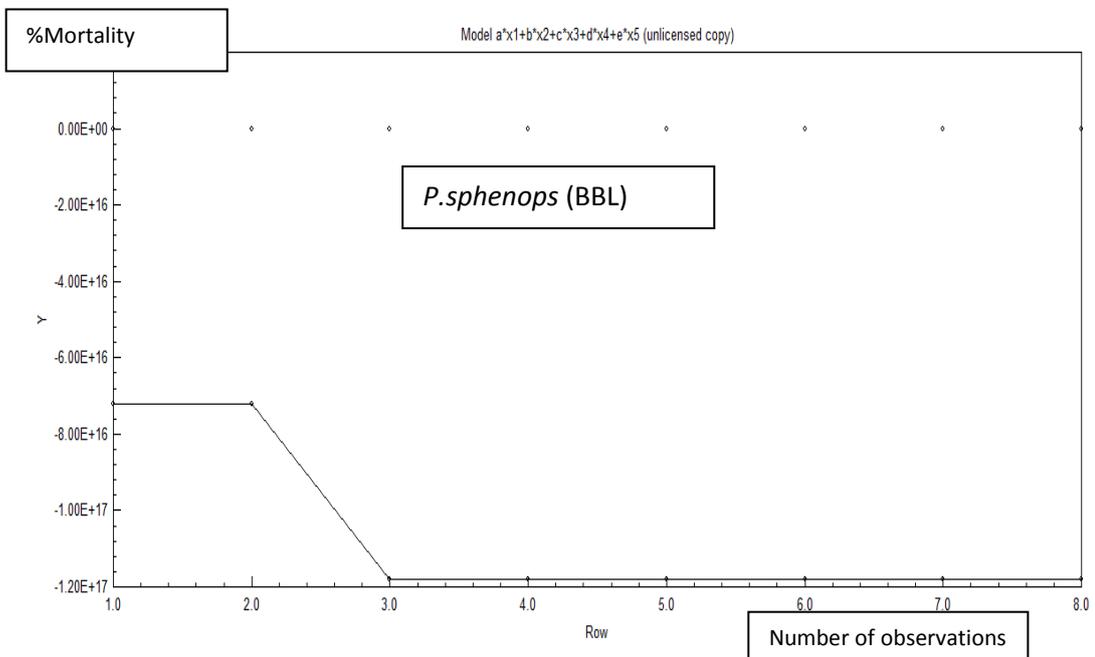


Figure 3.64 Model of BBL leachate toxicity test on *P.sphenops* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

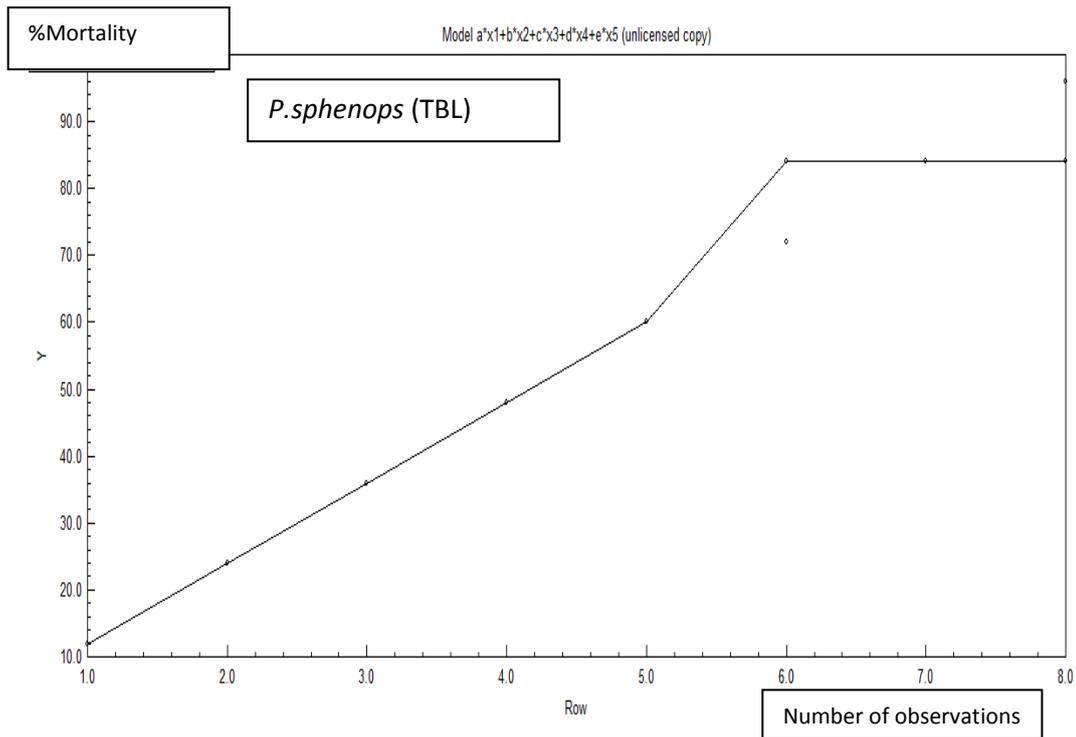


Figure 3.65 Model of TBL leachate toxicity test on *P.sphenops* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.21 Predictive Model plot values for *P.sphenops* across the leachate types

	<b>JSL</b>		<b>AHL</b>		<b>BBL</b>		<b>TBL</b>	
<b>Variable</b>	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	19.2	2.043	-6.45E+15	-0.289	36	1.86E-16	12	0.905
<b>b</b>	15.6	1.139	8.29E+15	0.289	7.89E+31	1.45E-16	12	0.688
<b>c</b>	-7.2	-0.511	28.73163	0.855	-9.65E+32	-1.45E-16	12	1.732
<b>d</b>	25.2	2.104	10.18376	0.216	5.70E+32	1.45E-16	12	0.904
<b>e</b>	-38.4	-2.464	23.22196	0.566	-4.60E+16	-1.45E-16	-180	-3.947

### *Pangasius sutchi*

*P.sutchi* exposure to JSL and AHL pointed in the direction that an increase in concentration enhances the mortality rate of a pollutant. Figures 3.66 and 3.67 explained the observable mortality distribution of *P.sutchi* when exposed to different

concentrations of leachate from the active and non-active sanitary landfills in this study.

LC<sub>50</sub> of JSL exposure was 3.5% v/v as against 3.28% v/v recorded at AHL exposure.

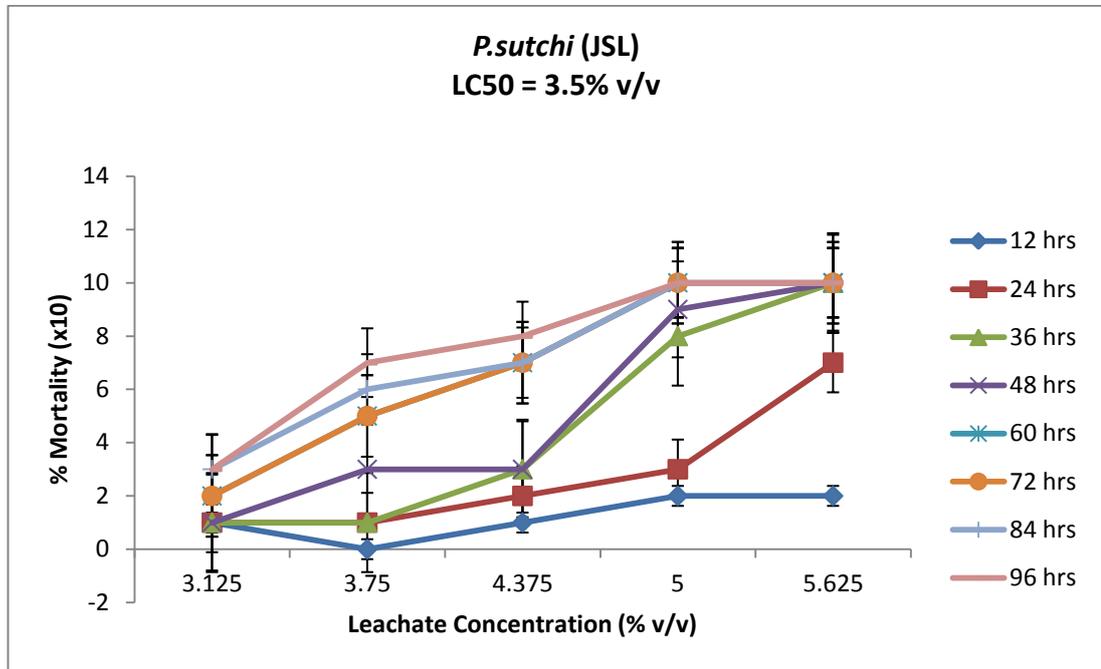


Figure 3.66 Mortality impact of JSL on *P.sutchi* for 96 hours exposure

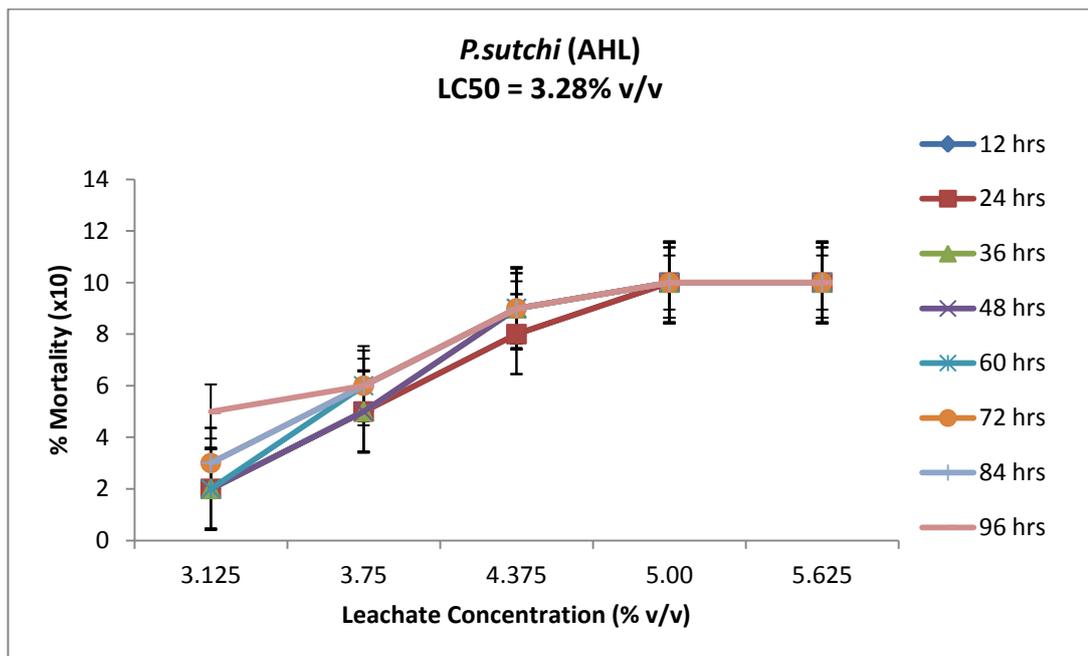


Figure 3.67 Mortality impact of AHL on *P.sutchi* for 96 hours exposure

The pattern of mortality in both exposures (JSL & AHL) was similar which probably led to obtaining close LC<sub>50</sub> values from both. However, Figure 3.67 showed a very steep trend in the rate of mortality upon AHL exposure and might have caused the instantaneous mortality potential of the leachate within 12 hours of acute toxicity testing. Therefore AHL showed more toxic potential than JSL in relation to effect on *P.sutchi*. This still upholds the study by Emenike et al (2012) when assessing the effect of closed sanitary landfill on *P.sutchi*.

But the mortality effect of BBL on *P.sutchi* was far less than the impact from TBL exposure. BBL recorded an LC<sub>50</sub> 16.68% v/v (Figure 3.68) while 3.5% v/v (Figure 3.69) was obtained at TBL exposure. Reason for such disparity could not easily be pointed out but the study can associate it to fish type and possibly some peculiarity in respect to degree of resistivity of the fish to certain levels of toxicant concentration. Bias based on seasonal variation and leachate sample collection system can be ruled out considering the fact that all toxicity testing were done the same period across the landfills and fish types.

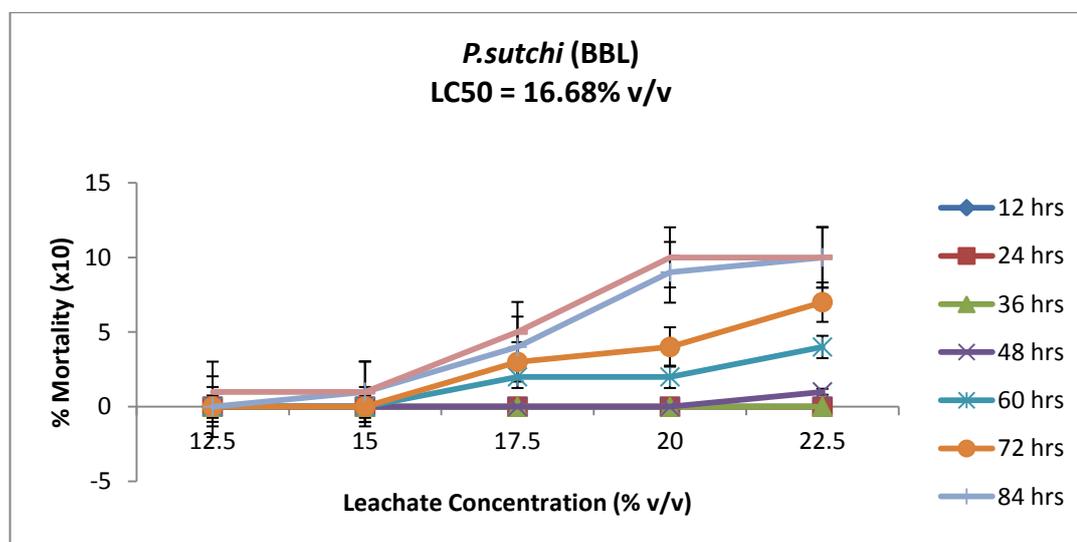


Figure 3.68 Mortality impact of BBL on *P.sutchi* for 96 hours exposure

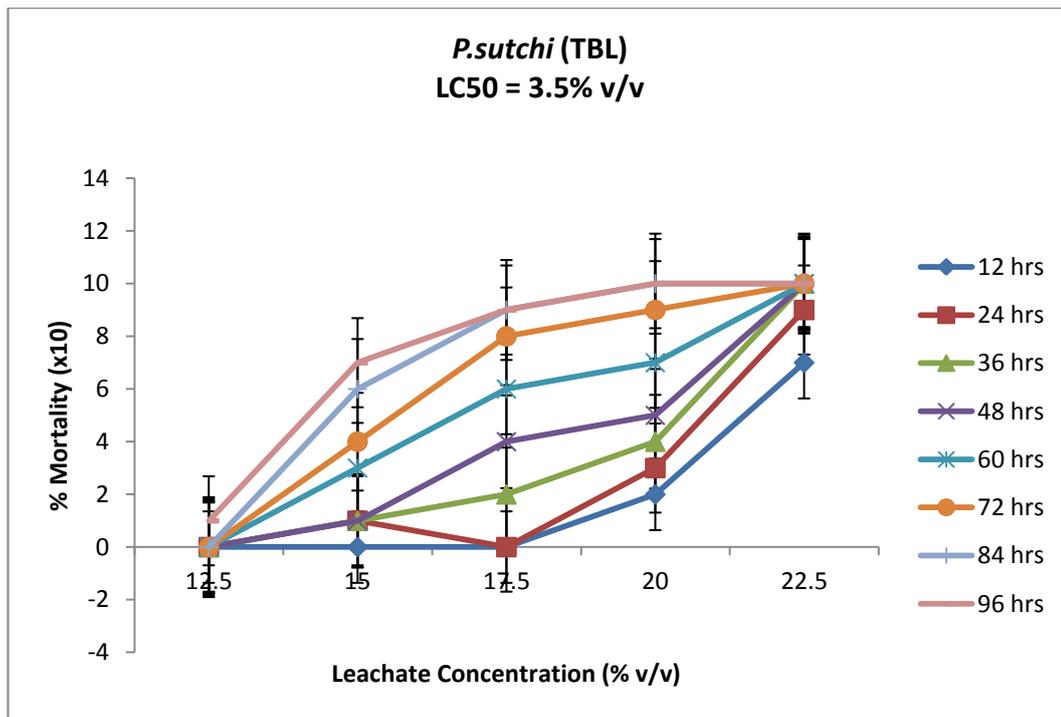


Figure 3.69 Mortality impact of TBL on *P.sutchi* for 96 hours exposure

Figures 3.70 – 3.73 explains the patterns of model plots across the leachate types while trying to fit the toxicity trend into the proposed model (Eq. 1). Whereas JSL (Figure 3.70) was fitted in the positive Y-values, AHL (Figure 3.71) showed a reverse order. The observable degree of mortality is the reason for such distance between the input data and model points, though it still obeyed the linear order. Variables' values are shown in Table 3.22. Generated values serve as coefficient values to corresponding leachate concentrations which combine linearly to calculate the mortality time.

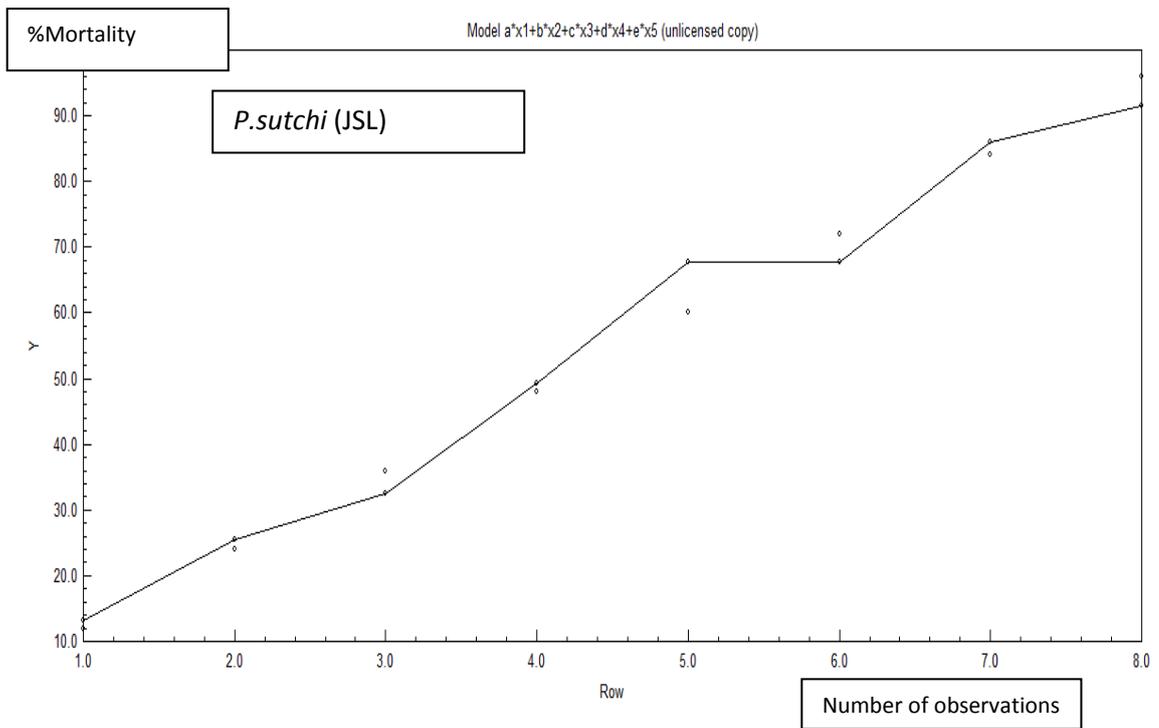


Figure 3.70 Model of JSL leachate toxicity test on *P.sutchi* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

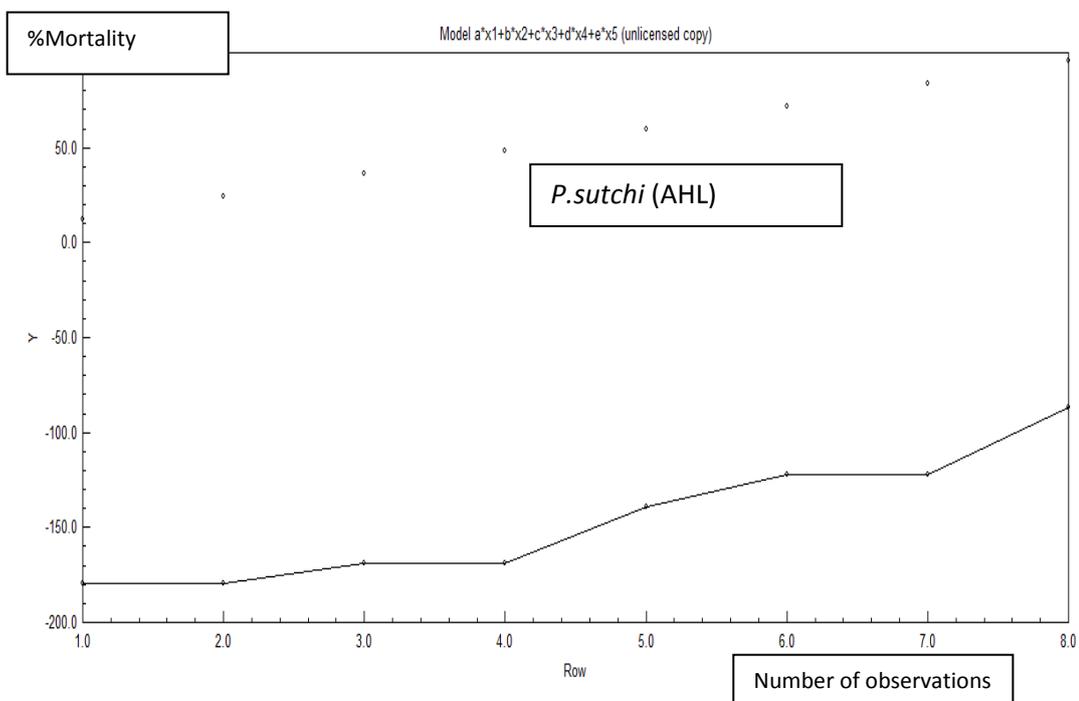


Figure 3.71 Model of AHL leachate toxicity test on *P.sutchi* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

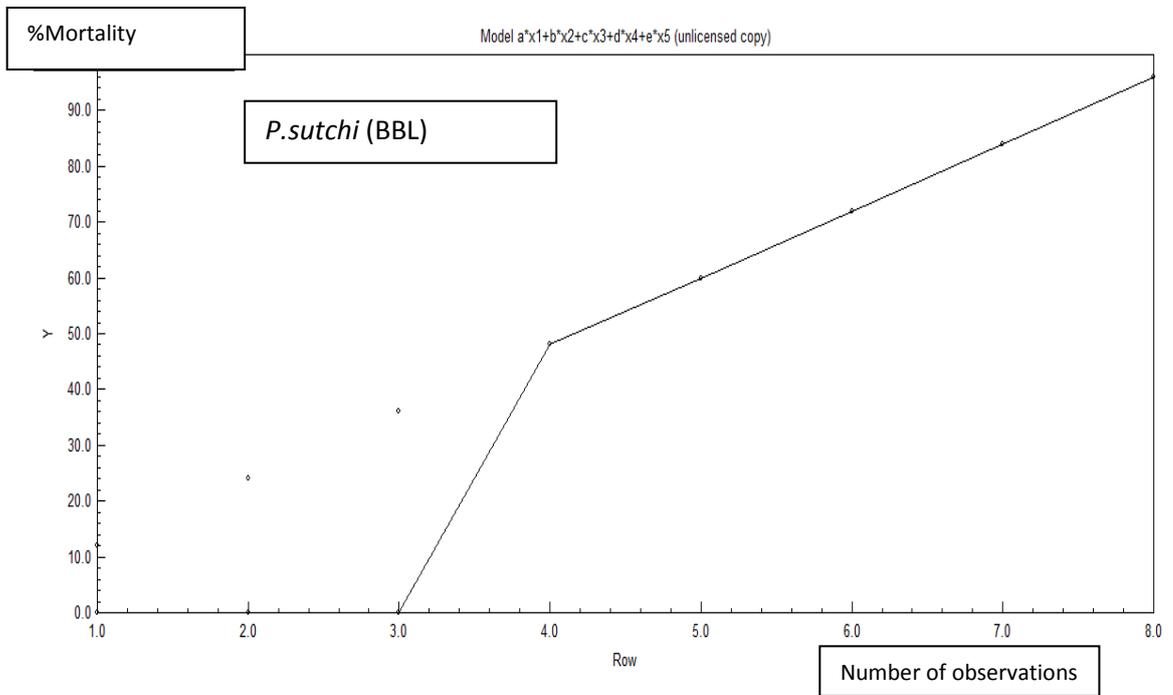


Figure 3.72 Model of BBL leachate toxicity test on *P.sutchi* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

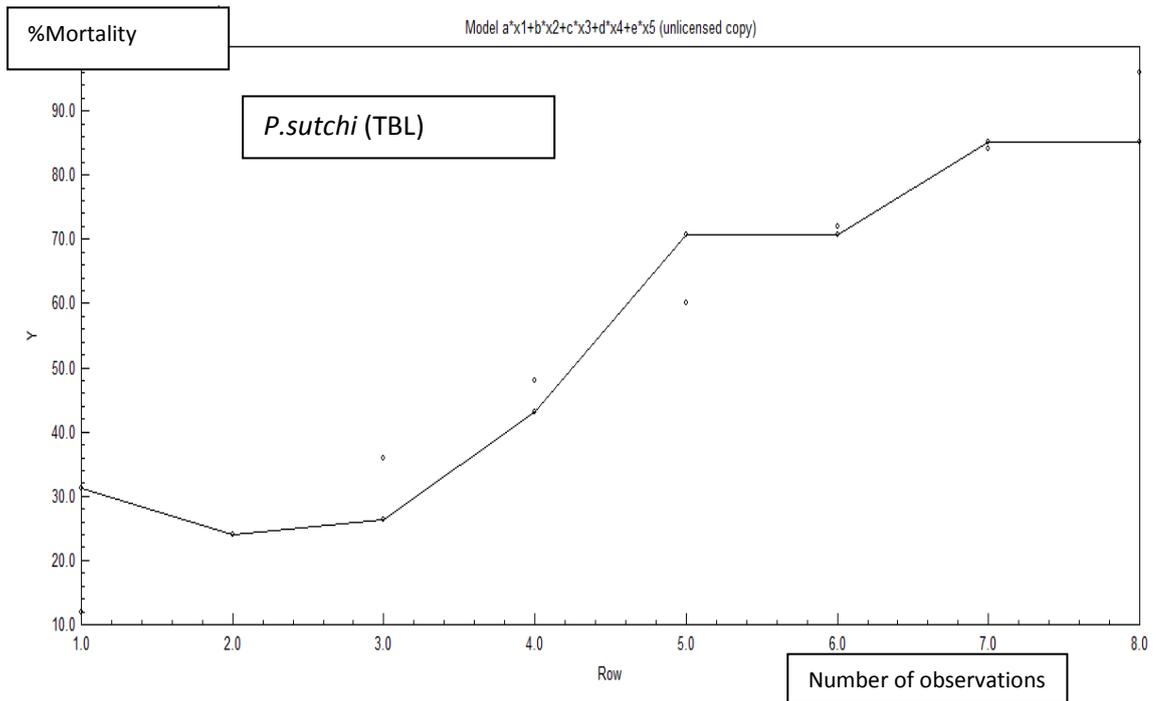


Figure 3.73 Model of TBL leachate toxicity test on *P.sutchi* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.22 Predictive Model plot values for *P.sutchi* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
a	1.124	0.632	-3.13E+16	-0.028	48	1.852	-25.2	-0.962
b	1.194	0.486	3.13E+16	0.028	-66	-1.235	27.6	1.539
c	-2.213	-0.599	11.01453	0.019	4.48E-14	7.05E-16	-7.2	-0.399
d	7.731	2.262	29.07791	0.068	198	0.946	14.4	1.014
e	10.669	1.812	17.47253	0.066	78	1.204	42	1.278

### *Clarias batrachus*

Similar to other fish species, *C.batrachus* was also exposed to the four leachate sources and the distributions of corresponding toxicity effect are shown in Figures 3.74 – 3.77. *C.batrachus* varyingly responded to the raw leachate samples. There were some similarities in the test ranges used in exposure of *C.batrachus* and *P.sutchi*. On exposure to JSL, the LC<sub>50</sub> value was 7.99% v/v (Figure 3.74) while AHL showed more toxicity; hence the LC<sub>50</sub> value was 5.99% v/v (Figure 3.75).

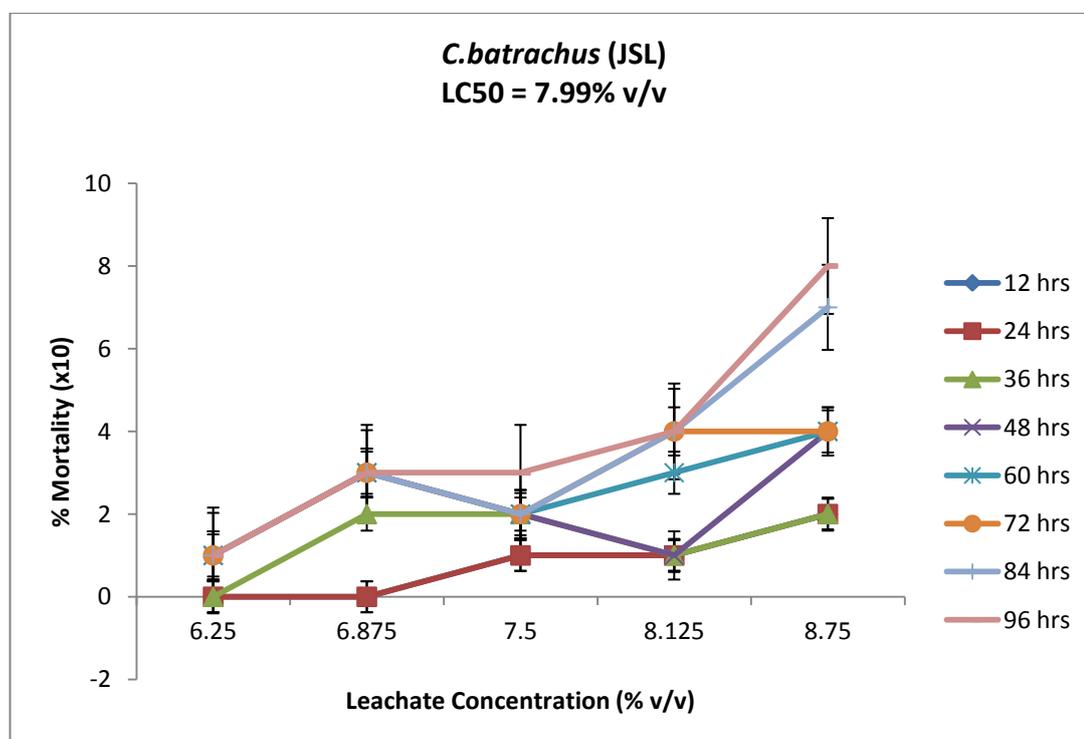


Figure 3.74 Mortality impact of JSL on *C.batrachus* for 96 hours exposure

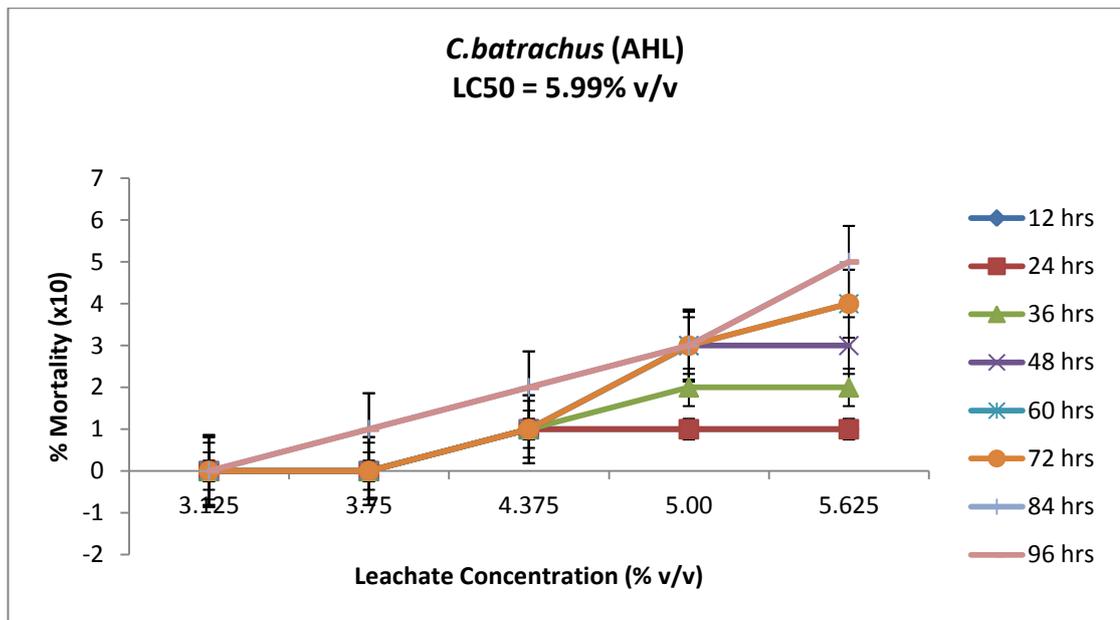


Figure 3.75 Mortality impact of AHL on *C. batrachus* for 96 hours exposure

Unlike the much disparity observed in *P. sutchi*, toxic effects of BBL and TBL, the LC<sub>50</sub> values were close with 20.01% for BBL (Figure 3.76) and 19.86% v/v for TBL (Figure 3.77). Both exposures (*C. batrachus*) observed a near similar mortality trend all through the exposure duration excluding at the 25% v/v exposure concentration in BBL.

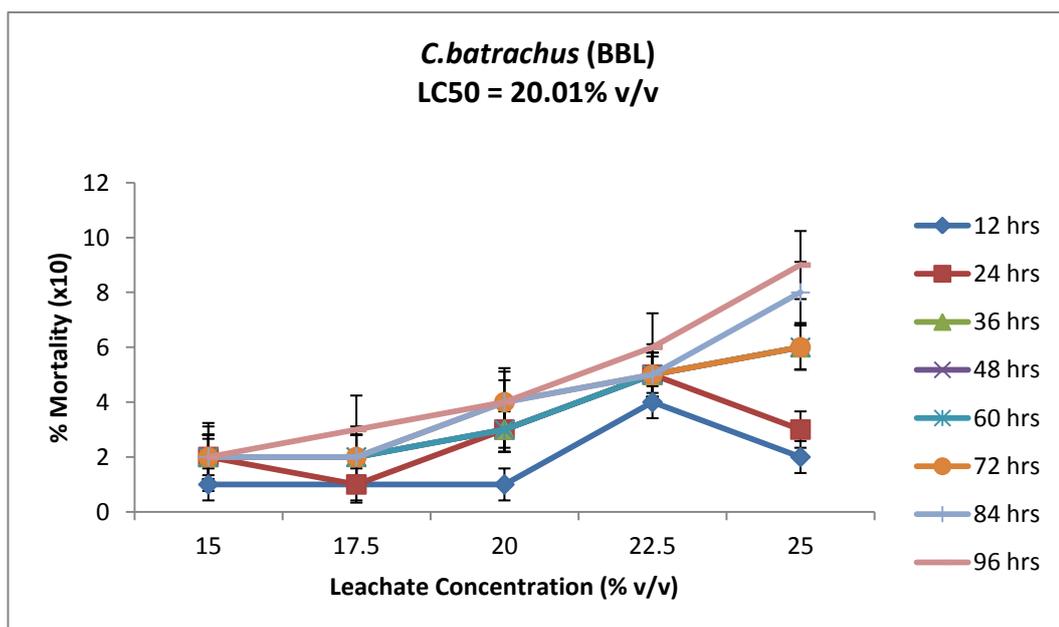


Figure 3.76 Mortality impact of BBL on *C. batrachus* for 96 hours exposure

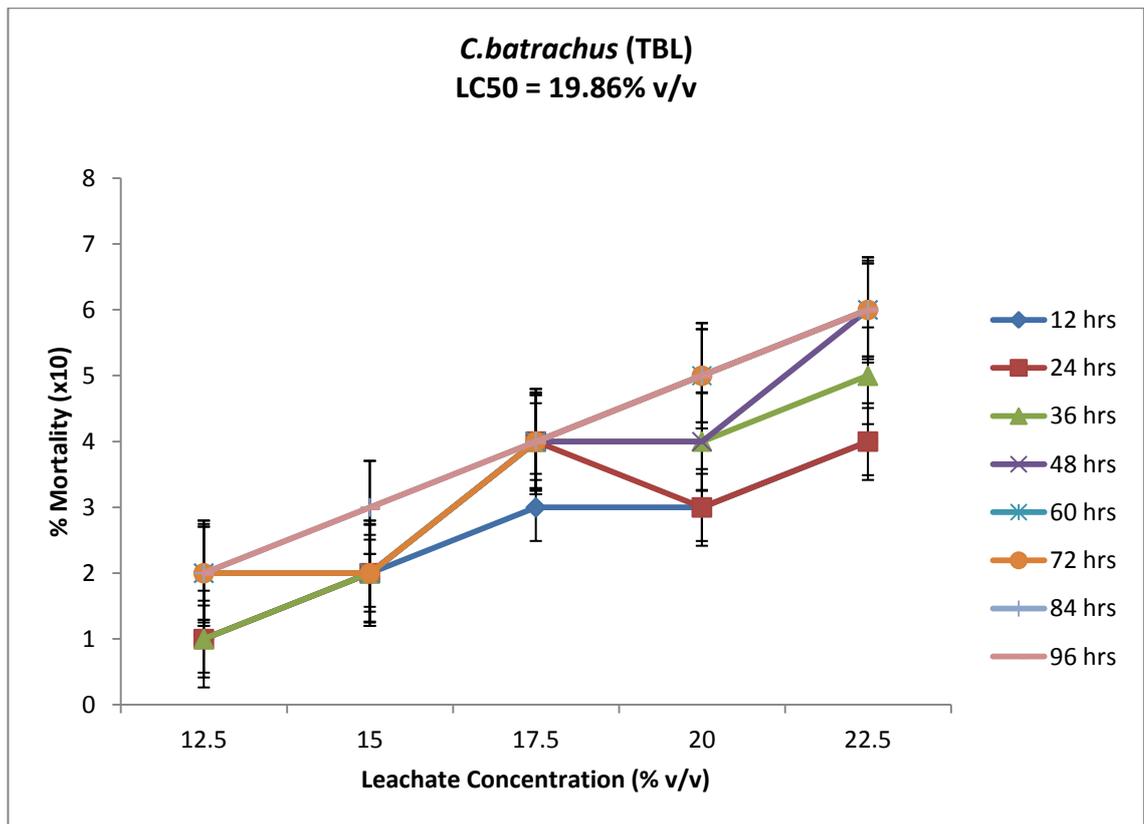


Figure 3.77 Mortality impact of TBL on *C. batrachus* for 96 hours exposure

Figures 3.78 – 4.81 and Table 3.23 show the exposure trend as fitted into the predictive model. All the model plots were fitted with the positive Y-values. However, AHL and JSL pattern showed more similarity by having same points of origin in the plots. Results had shown that AHL was most the toxic leachate source to *C. batrachus*. This most probably is due to peculiar leachate property of AHL that avails more toxic potential than others, especially the  $\text{NH}_3\text{-N}$  concentration (880 mg/L) (Emenike et al., 2012a; Emenike et al., 2013). Also *C. batrachus* is the most resistive fish species out of the ten species used in this study since it can withstand tougher changes in the habitat than other species.

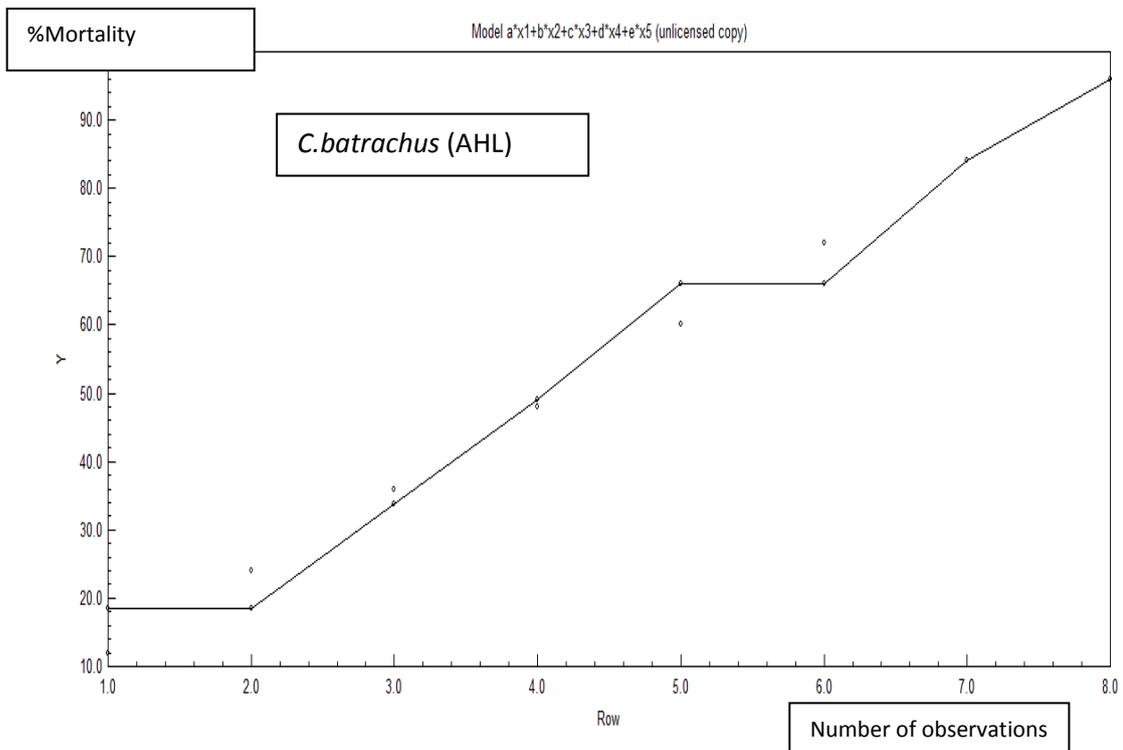


Figure 3.78 Model of AHL leachate toxicity test on *C. batrachus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

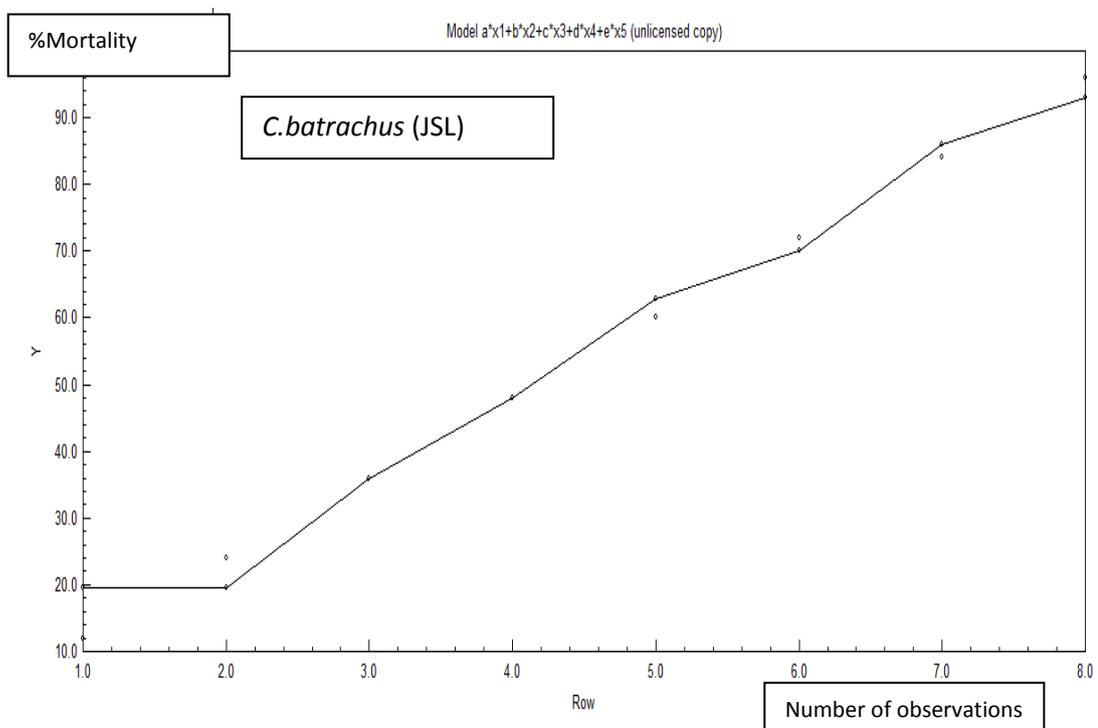


Figure 3.79 Model of JSL leachate toxicity test on *C. batrachus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

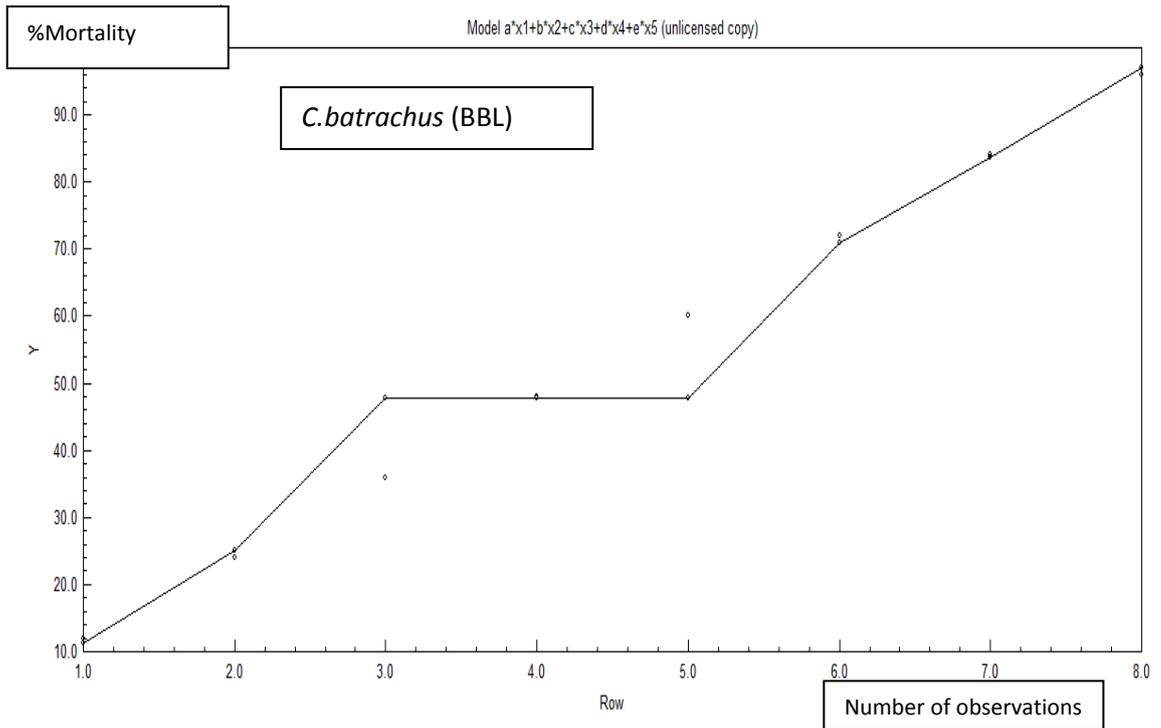


Figure 3.80 Model of BBL leachate toxicity test on *C. batrachus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

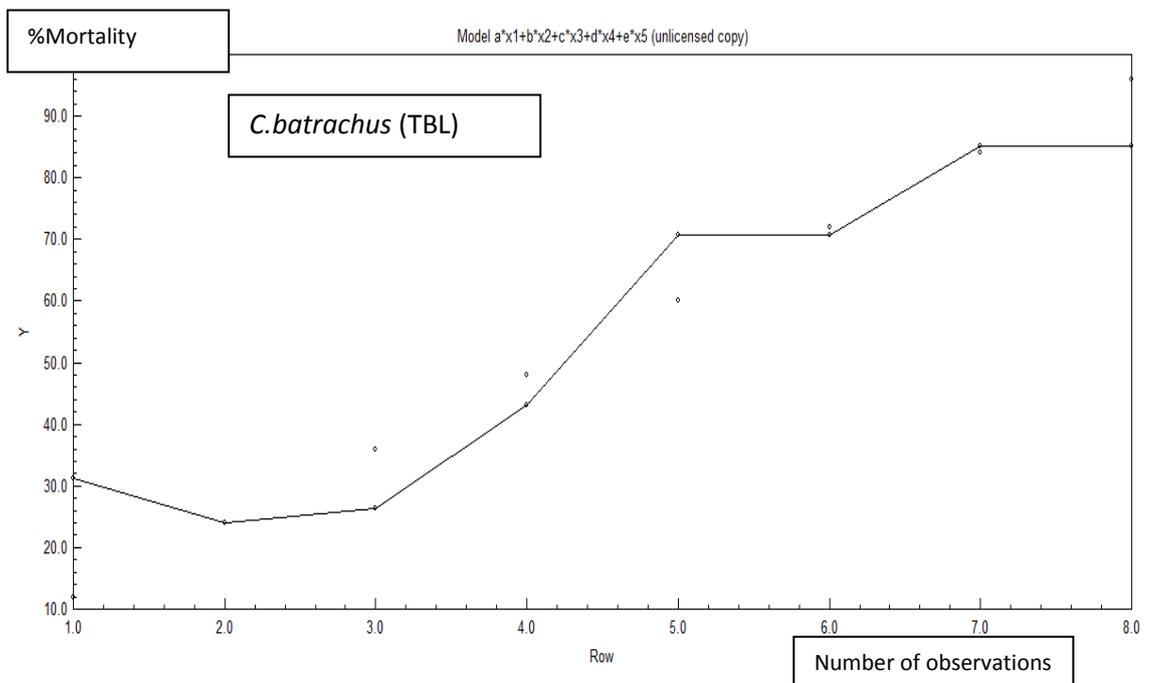


Figure 3.81 Model of TBL leachate toxicity test on *C. batrachus* in order to correlate and predict the relationship between varying concentrations with degree of mortality along 96 hr exposure

Table 3.23 Predictive Model plot values for *C.batrachus* across the leachate types

Variable	JSL		AHL		BBL		TBL	
	Value	t-ratio	Value	t-ratio	Value	t-ratio	Value	t-ratio
<b>a</b>	5.302	2.258	16.909	2.079	6.371	0.942	-25.2	-0.962
<b>b</b>	7.349	2.773	-1.636	-0.144	3.464	0.439	27.6	1.539
<b>c</b>	1.581	0.261	3.273	0.396	23.010	2.209	-7.2	-0.399
<b>d</b>	7.442	1.389	-2.182	-0.123	3.649	0.172	14.4	1.014
<b>e</b>	-5.953	-0.448	12	1.198	-42	-1.814	42	1.278

Therefore, it is observed that the ten fish species used for the bioassay in this study responded to mortality as initiated by the components of the raw leachate samples. The responses were similar amongst some of the fish species but still differed widely across the landfills. Hence, such observation combined with the interest for understanding the discrete potentials of the landfills to initiate toxicity, to pave way for the enrichment of toxicity data bank while at the same time proposing and generating the Fish Lethal Leachate Toxicity Index (FLLTI) that is proposed for leachate pollution assessment component in Malaysia.

### 3.4.3 Fish Lethal Leachate Toxicity Index (FLLTI)

While pollution levels can be evaluated via measuring the landfill pollution index (LPI), the use of FLLTI across landfills should be equally considered. LPI only take into count the measured concentrations of various prescribed physico-chemical parameters of the ecosystem (Chong et al., 2004). But, just as Enu-ah et al (2009) stated, chemical knowledge of pollutants are not sufficient for understanding their potential impact, rather toxicological evaluations via bioassays should be considered.

Result showed that BBL had the least FLLTI value (1.28) as against the highest value obtained in AHL (5.46) (Table 3.24). Considering the fact that pollution index is an indication of the amount of severity of the pollution generated during the life cycle of an

item, such as leachate, then the FLLTI values across the landfill is an implication of differences in their potential impact on the aquatic system. The higher the number in the index number implies more pollution, and as such it means that AHL can initiate high level of toxicity upon contact with surface water. This can be attributed to the leachate characteristics (Emenike et al., 2013).

Table 3.24 Values of calculated FLLTI (Index Values across Landfills)

<b>Landfill</b>	<b>Type</b>	<b>Value</b>
<b>AHL</b>	Non-Active Sanitary	5.46
<b>JSL</b>	Active Sanitary	3.87
<b>TBL</b>	Non-Active Non-Sanitary	1.76
<b>BBL</b>	Active Non- Sanitary	1.28

The result had shown that sanitary landfills pose higher lethal potential than the non-sanitary ones. This can be attributed to landfill leachate collection system installed in the landfills (sanitary). Collection process of the raw leachate from sanitary landfills is a cell-to-pond traffic via installed pipes which minimize contact with any other medium like soil; hence the collected raw leachate tend to possess intact concentrations of the components since they did not pass through any form of dilution, filtration and even adsorption before getting to the leachate ponds. This was collaborated by Lagkervist (2003) wherein the study stated that landfill design and leachate collection system influences the leachate quality. Yet, regardless of similarity in status (active sanitary and closed sanitary; non-active sanitary and closed non-sanitary), the FLLTI values varied across the four leachate sample types. It is crystal clear that toxicity impact was much higher upon exposures to closed sanitary landfill than with the active sanitary landfill, just as TBL recorded higher value than BBL. The reason for such will definitely bother

on the varying leachate quality rather than differences in species since all fish species used in this study were most vulnerable to AHL exposure than with JSL.

The calculated  $LC_{50}$  from the fish exposed to the various concentrations of leachate when pooled into Equation 2 as mentioned earlier, generated the FLLTI as shown in Table 3.25. It represents the index values across the fish species and individual landfills. *C.batrachus* had the least FLLTI value (0.4) while the highest was associated to *M.nemurus* (2.31). Considering the fact that the higher an FLLTI values is, the more susceptible a fish species can be, it means that *M.nemurus* was the most lethally affected species in the experiment, whereas *C.batrachus* turned out to be the least mortally affected. Though the fish species used in the experiment were of freshwater origin, some might even thrive in slightly stagnant waters. It is obvious that *M.nemurus* had the least tolerance to water pollutions (though this experiment was limited to leachate study) as clearly observed with the  $LC_{50}$  calculations across the four studied landfill leachates. Considering the consistency in maintaining the least mortality responses to all the landfills used in the experiments, the study depicts that *C.batrachus* was very resistant than any other fish species. A relative comparison between *C.batrachus* and *M.nemurus* showed that *M.nemurus* was approximately six times ( $2.31/0.4$ ) more susceptible to leachate mortality than *C.batrachus*.

While only *C.batrachus* and *P.sutchi* showed FLLTI values  $>1$  in the experiment, the rest were  $<1$ . The value between *C.batrachus* and *P.sutchi* may be attributed to the similarity of fish species (often regarded to as catfish) which reflected in their degrees of survival from the leachate exposure unlike with other fish species. Therefore, in as much as the study is not really to evaluate individual fish characteristics, yet the FLLTI

values may be used to classify the species in relation to water pollution tolerance ability (Table 3.25).

Table 3.25 Values of calculated FLLTI (Index Value across Fish Species)

<b>Species</b>	<b>Value</b>	<b>Weight Classification</b>
<i>C.batrachus</i>	0.4	Ht
<i>P.sutchi</i>	0.96	Ht
<i>X.maculates</i>	1.03	Mt
<i>P.spehnops</i>	1.06	Mt
<i>O.mossambicus</i>	1.1	Mt
<i>D.rerio</i>	1.35	Mt
<i>D.aequipinnatus</i>	1.49	Mt
<i>H.eques</i>	1.62	Mt
<i>L.hoeveni</i>	1.84	Mt
<i>M.nemurus</i>	2.31	Lt

Ht - high tolerance; Mt- mild tolerance; Lt- low tolerance

The result showed that since *C.batrachus* and *P.sutchi* had FLLTI values of 0.4 and 0.96 respectively, they were classified under the high tolerant group whereas others with the exception of *M.nemurus* (2.31) were placed under the medium resistant group.

Table 3.25 showed that *D.rerio* with other six fish species were classified in the medium group, and considering the significance of *D.rerio* in most bioassays, it may be necessary to suspect that similarity in toxicity response may be an evidence of relative similarity of different fish species. *D.rerio* showed FLLTI value of 1.35 such that suspecting *O.mossambicus* (1.1) and *D.aequipinnatus* (1.49) organisms similar to *D.rerio* in reference to bioassay relevance may be plausible. *D.rerio* which is predominantly resident in Europe is considered an important freshwater fish for most bioassays that even involves wastewater (APHA, 1998). Therefore, a closer similarity in FLLTI value on *D.rerio* with those of *O.mossambicus* and *D.aequipinnatus* may suggest that *D.rerio*

shared similar trend of metabolic responses and defense mechanism with the later species. Though additional bioassays may be required, but *O.mossambicus* and *D.aequippinnatus* can be suggested as alternative species to *D.rerio* for most related bioassays in Asia considering their dominance in Asia waters, hence scarcity of bioassay fish species that do arise sometimes will be highly minimized. However, *D.aequippinnatus* may be preferred ahead of *O.mossambicus* considering the importance of the later as direct source of food for humans.

The index values across the fish species had also shown that while edible fish species were the most resistant to the leachate toxicity (*C.batrachus*, 0.4; *P.sutchi*, 0.96), yet some edible ones were also the most affected (*L.hoeveni*, 1.84; *M.nemurus*, 2.31). Though most of the non-edible/ornamental species were in the mid range of the toxic responses, yet result may be dependent on the fish species used in this study. Therefore, more bioassays is required to incorporate more vulnerable species and the very tolerant ones as this will enhance the generation of comprehensive leachate toxicity data based on minimal level of leachate contamination (1%).

Lethal concentrations adopted helped to evaluate a near worst scenario effect of raw leachate on the fish species. Unlike in the assessment of the potential effects of pesticides on the aquatic environment in a controlled manner (Munn & Gillion, 2001), standardized laboratory bioassays did not expose the fish species to a single compound, rather it evaluated the heterogeneous concentration of leachate. This may be part of the reasons for slight variation and inconsistencies of some of the fish species in terms of mortality responses across the four landfills. The  $LC_{50}$  and FLLTI values generated on each fish species might be pivotal to assessment of leachate danger, though the bioassay

still raise some queries. Such can be explained by the fact that most of the species used in the bioassays tend to be rarely the same species that reside in a particular system (like the test aquarium, rivers and lakes), the stage of test-life history never included all the exposed life stages. Again, the test duration may not match the predicted exposure duration; just as the physico-chemical test conditions are not the same as the expected field conditions. Basically, reported responses do not include all the responses of concern and t-endpoints are at different level of biological organisation (organism) than the assessment endpoints (population to ecosystem) (Sutter, 1995). It is still worthy of note that regardless of the aforementioned limitations, bioassays remain a useful tool for quantifying toxicological effects of specific contaminants on aquatic life in a consistent reproducible manner (Munn & Gillion, 2001), and its relevance on FLLTI cannot be over emphasized.

#### **3.4.4 Relationship between leachate quality and fish mortality**

Considering the fact that zero mortality was observed within the control group (non-leachate exposed) of all the fish species all through the experiment (96 hours in each condition), it become necessary and easy to implicate the leachate quality as the root cause of the fish mortality. Therefore with leachate being a heterogeneous compound, it is a daunting task to ascertain a precise pollutant in isolation as being the sole cause of mortality of any of the fish species. Whereas this study did not investigate individual component of leachate quality, the overall/combined effect become paramount.

Presence of organic matters/ or loading in any given waste pile is very important, as it plays significant role in assessing the states of degradation, microbial distribution and potential pollution ability. However, its toxic relevance seem not to be a major concern

in the past, but the varying trend of mortality observed in this study suggest otherwise. In general, all the organic related components of the leachate as analysed based on the physico-chemical leachate characterization revealed that regardless of the landfill status, BOD and COD values from each seriously exceeded the standard discharge limits, precisely 50 and 100 mg/L, respectively as enshrined in the Environmental Quality Act of Malaysia (2007). Such BOD and COD levels are capable of enhancing the reduction of oxygen level required for survival of fish species with any aquatic system (Emenike et al., 2012a), especially on freshwater fish. The engineered nature of JSL and AHL did accord them advanced and coordinated method of collecting the raw leachate which in turn revealed that they had the highest BOD and COD levels, as against the anticipated trend of a decreasing order in respect to organic contents; JSL < BBL < AHL < TBL because landfills that still receive waste should show higher organic loading just as a younger landfill also follow similar trend in organic content disparity (Kjeldsen et al., 2002; Lagerkvist, 2003; Emenike et al., 2013). Therefore, mortality across the fish species upon exposure to the various leachates can be associated to the BOD<sub>5</sub> and COD contents. Yet, both cannot be referred to as the main toxicant sources considering the fact that the severity of mortality impact did not increase in the order of lowest value (BOD<sub>5</sub> & COD) to the highest. Rather, AHL which was less than JSL in value for the aforementioned parameters demonstrated the highest toxic effect (death). Then this imply that some other component(s) of the leachate may be the leading toxicant(s) capable of according higher mortality impact to a particular leachate sample regardless of its corresponding BOD<sub>5</sub> and COD values.

Hence, ammonia was suspected considering its concentration values across the landfills. Since all the leachate samples recorded values above 500 mg/L of ammoniacal-nitrogen (NH<sub>3</sub>-N), the aquatic condition was expected to be under survival threat. Ammonia and

alkalinity are strong indicators of toxicity in the aquatic environment. The study may infer that a more severe degree of toxicity associated to AHL was due to the highest concentrations of  $\text{NH}_3\text{-N}$  (880 mg/L) and alkalinity (9000 mg/L) it recorded. To add as support to this observation, Kurniawan et al (2006a) confirmed that of the toxic pollutants that are present in landfill leachate,  $\text{NH}_3\text{-N}$ , resulting from the decomposition process of organic nitrogen cannot only be identified as a major long-term pollutant, but also as the primary cause of acute toxicity (Kurniawan, 2009). This maybe be agreeable with this present study, considering the degree of mortality initiated by ASL on all fish species than as seen from all other leachate samples despite containing lower concentrations in most other identified parameters (especially as obtained in JSL). Due to anaerobic conditions required for the stability of  $\text{NH}_3\text{-N}$ , it typically accumulates in the leachate which makes it harmful. Exposure of *D. rerio* across the leachate samples showed mortality that can be related to the study by Kurnawan et al (2006b) where death of *D. rerio* was obtained upon exposure to ammonia polluted water. This pointed out that untreated  $\text{NH}_3\text{-N}$  is highly toxic to aquatic organisms especially when it exceeds 100 mg/L.

The toxic effect of the leachate may have occurred due to the penetration of ammonia across the fish tissues. While cell membranes of organisms are comparatively impermeable to ammonia ion ( $\text{NH}_4^+$ ), molecular ammonia ( $\text{NH}_3$ ) can readily diffuse across the tissue in the presence of a concentration gradient (Alabaster & Lloyd, 1980). This is to imply that upon leachate exposure, an alteration might have occurred at the water-tissue interface, hence affecting the acid-base balance such that any side with the lower pH will attract molecular ammonia. Therefore, the molecular ammonia passed from the polluted water through the epithelium of the gills to the blood and vice-versa. In most situations, ammonia blows have toxic effect on the brain; hence reason for

nervous symptoms that are so pronounced in cases of ammonia toxicity of fish (Alabaster & Lloyd, 1980).

Nitrite may not really be the cause of mortality of the test organisms in this study. It agrees with Emenike et al (2012a). This is because Lewis and Morris (1986), have shown that nitrite toxicity to fish can be affected by certain water quality characteristics. Their study showed that 96h LC<sub>50</sub> for rainbow trout ranged from 0.24 to 12.2 mg/L, depending on the chloride content of the dilution water (in their study the chloride content ranged from 0.35 to 40.9 mg/L). Svobodva et al, (1993) therefore, suggested that for the estimation of safe nitrite concentration for particular locations, it is necessary to measure the ratio of chloride to nitrite. These ratios (expressed as mg/L Cl<sup>-</sup> : mg/L N-NO<sub>2</sub><sup>-</sup>) are recommended to be no less than 17 for rainbow trout and 8 for fish of low economic importance (Svobodva et al., 1993). But in this study, the chloride concentration for JSL and AHL was 4,150 mg/L each with corresponding nitrite concentration of 4.8 mg/L and 2.7 mg/L, respectively. BBL and TBL contained 4830 mg/L and 2750 mg/L, with corresponding 23.3 mg/L and 20.1 mg/L of nitrite, respectively. Therefore the chloride to nitrite ratios calculated were approximately 865, 1,537, 204 and 138 for JSL, AHL, BBL and TBL, respectively; and as such may not have contributed significantly to the acute toxicity of the studied leachate.

Propanoic acid is a potential toxicant. The concentration in AHL leachate was 2,100 µg/L. Outside the fact that toxic effects of propanoic acid are expressed in enzyme disruption of liver enzymes in humans (Scarlett et al., 2012), it had induced mortality on *Cyprinus carpio* at mean toxic dose of 96,000 µg/L within 24 hours (<http://www.pesticideinfo.org>, accessed 8<sup>th</sup> July, 2010). However the study did not show

the minimum or maximum dose; hence this might vary depending on the type of fish used.

Toxicity in most leachate from various landfills in the world like Sweden, Lithuania and Brazil have been attributed to ammonia (Svensson et al., 2005; Silva et al., 2004; Pivato and Gaspari, 2006), yet the presence of phenolic compounds which is a semivolatile organic carbon in leachate sample cannot be ignored. Phenols can occur as either monobasic (cresol, naphthol, xylenol e.t.c.) or polybasic (pyrocatechol, hydroquinone, pyrogallol et.c.). This study revealed the presence of o-cresol and p-cresol at 0.09 and 0.06 mg/L, respectively in AHL leachate only. The maximum concentrations admissible for fish culture are 0.001, 0.003, 0.004 and 0.001 mg/L of chlorophenol, cresol, resorcinol and hydroquinone, respectively (Svobodva et al, 1993). Though there is no study of o-cresol and p-cresol effect on fish, animal studies have reported effects to the liver, kidney and central nervous system (CNS) from acute inhalation of mixed cresols (ATSDR, 1990). Whereas acute animal tests in rats have shown mixed cresols to have moderate acute toxicity, o-cresol, m-cresol and p-cresol have been shown to have high acute toxicity from oral exposure (U.S Department of Health and Human Services, 1993). Phenols causes unacceptable taint to water and fish, and can affect the fish central nervous system. Signs of phenol intoxications are characterized by increased activity and irritability, leaping out of water, loss of balance and muscular spasms (Svobodva et al, 1993). Hence the degree of toxicity observed on exposure of all species to AHL may also be connected to some of the semi-volatile organic carbon components of the raw leachate. However, the presence of monocyclic aromatic hydrocarbons at varying concentrations may not be the major cause of fish mortality. This is because in JSL which is considered the second most toxic leachate in this study contained only benzene 0.01 mg/L (though above standard limits) whereas

the rest contained not just benzene (0.22 – 0.6 mg/L), but also toluene (1.2 – 2.1 mg/L) and ethyl benzene (0.8 – 0.86 mg/L), hence showing that their presence may be more implicated in cyto- and neuro-toxicity effects which were not detailed in the present study. .

Definitely, the presence of metals can pose significant threat to the fish environment. It seemed the operational status of JSL and BBL enabled higher concentrations of metals in both landfills. The highest concentrations of Zn (827 mg/L), Ni (19.5 mg/L), Mn (540 mg/L), Cr (25 mg/L) as founded in the study exceeded standard limits and as such can play significant role in the toxic impacts of the leachate. For example, Zn has a lethal concentration of 0.1 mg/L for salmonids and 0.5-1.0 mg/L for Cyprinids (Svobodva et al., 1993). Toxicity of JSL leachate might be influenced by presence of metals such as Zn especially when Ca content was not correspondingly high unlike in the case of BBL where the Zn concentration was 236 mg/L but with a corresponding Ca concentration that was almost five times higher (91 mg/L) than what was obtained in JSL. Study by Svobodva et al. (1993), found that the toxicity of Zn to fish was influenced by chemical characteristics of water; in particular, decreasing Ca concentrations increase the toxicity of Zn. The presence of mercury across the landfills suggests some deposition of toxic leaching substances in the landfills and hence may have the potential of bioaccumulation in fish.

Another suspected toxicant in this study is Fe especially in JSL leachate (97.76mg/L). In surface waters, Fe occurs in ferrous state II (soluble compounds) or ferric state III (mostly insoluble compounds). The ratio of these two forms of Fe depends on the oxygen concentration in the water, the pH and on other chemical properties of the water. Fish may be harmed by Fe compounds in poorly oxygenated waters with a low pH

where the Fe is present mainly in the form of soluble compounds (Alabaster & Llyod, 1980).

Nickel compounds are of medium toxicity to fish. With short periods of exposure, the lethal concentration is between 30 and 75 mg/L (Alabaster & Lloyd, 1980). But in this study the Ni concentration ranged from 0.85 - 19.5 mg/L. However, in soft waters with low calcium concentrations, the lethal concentrations of nickel compounds for the stickleback were less than 10 mg/L (Svobodva et al., 1993). In such cases Ni can be regarded as highly toxic to fish. After toxic exposure to nickel compounds, the gill chambers of the fish are filled with mucus and the lamellae are dark red in colour. Therefore this led to determination of heavy metals accumulation in fish as a way of assessing the possible effect of leachate due to pollutant's penetration of fish tissue.

However, it is worthy to note that this present study completely disagrees with the fact that landfills that still receive MSW produce more toxic leachate than non-active one (Atwater et al., 1983; Plotkin & Ram, 1984; Schrab et al., 1993). Their arguments might be founded only on characterization of leachate whereas mortality bioassay in this research has shown otherwise.

#### **3.4.5 Accumulation of heavy metals in fish**

Most studies in the past, ultimately concluded that heavy metals in landfill leachate should not be a major concern with the assertion that average metal concentrations at landfills are fairly low (Christensen et al., 1994; Revans et al., 1999; Kjeldsen et al., 2002). However, this study stand to disagree with such, pointing out to the fact that bioaccumulation can be gradual but significant with time. Table 3.26 represents the concentrations of heavy metals identified in the *P. sutchi* after exposure to both LC<sub>50</sub>

(3.2% & 3.8% v/v) of the raw leachate samples (AHL & JSL, respectively). Exposures to just JSL and AHL were selected considering the fact that they were the most toxic in this study whereas *P.sutchi* was just selected to represent the fish community. The fish was selected to evaluate the impact of the leachate on a more resistant fish which will give an idea of what will be the effect on less tolerant fish species.

Table 3.26 Heavy metal concentrations accumulated in the fish after 96 hours leachate exposure

Analyte	Sample	Sample Conc. ( $\mu\text{g g}^{-1}$ )	Std. Dev	RSD (%)
<b>Mn</b>	<b>AHL</b>	0.202	0.001	0.51
	<b>JSL</b>	0.169	0.001	0.63
	<b>Ct</b>	0.002	0.001	0.82
<b>Cu</b>	<b>AHL</b>	0.158	0.001	0.87
	<b>JSL</b>	0.130	0.002	1.82
	<b>Ct</b>	ND	-	-
<b>Zn</b>	<b>AHL</b>	3.264	0.007	0.21
	<b>JSL</b>	1.806	0.005	0.27
	<b>Ct</b>	0.061	0.001	0.40
<b>Fe</b>	<b>AHL</b>	2.112	0.168	0.80
	<b>JSL</b>	1.854	0.010	0.55
	<b>Ct</b>	0.492	0.006	0.85
<b>Cr</b>	<b>AHL</b>	0.242	0.000	0.01
	<b>JSL</b>	0.242	0.002	0.60
	<b>Ct</b>	ND	-	-
<b>Al</b>	<b>AHL</b>	0.250	0.002	0.59
	<b>JSL</b>	0.438	0.009	2.06
	<b>Ct</b>	ND	-	-

Ct – control, ND- not detected

Comparison of the heavy metals accumulated in both leachate samples-exposed fish with the control experiment (Ct) showed high variations. As shown in Table 3.26, the concentrations of Mn, Zn and Fe were considerably low in the control experiment. Similarly, Cu, Cr and Al were not even detected in the non-leachate exposed *P.sutchi*.

The result indicated that regardless of the status of the studied landfills (non-active or active), the *P. sutchi* was able to accumulate some of the available heavy metals within 96 hr exposure. Cu varied from 0.13 – 0.15  $\mu\text{g g}^{-1}$  as the least accumulated element, while Zn ranged from 1.8 – 3.2  $\mu\text{g g}^{-1}$ . Though based on a study by Tuzen (2003), metal contents in fish depend on the analyzed species, yet it is clear that metals from leachate can still penetrate fish tissues no matter the concentration. With Zn concentration being the highest, it also concurs with previous studies by Cid et al (2001) and Tuzen (2003) where metals accumulation (Pb, Zn, Cd, Fe, Mn & Cu) were compared. Zn concentration as observed from this analysis is considered too high because it is established that Zn has a lethal concentration of 0.1  $\mu\text{g g}^{-1}$  for salmonids and 0.5 - 1.0  $\mu\text{g g}^{-1}$  for Cyprinids (Svobdova et al., 1993). Also in the event of decline in Ca concentration, Zn toxicity will be elevated.

Similarly, Fe is another element of importance. This study has shown that the characterized leachate samples exceeded the available discharge limits for various substances which include Fe (5  $\mu\text{g g}^{-1}$ ) (USEPA, 2000). Hence for *P. sutchi* to accumulate 1.8 – 2.1  $\mu\text{g g}^{-1}$  of Fe within 96 hours is high as fish can be harmed in poorly oxygenated waters with low pH and soluble Fe (Svobdova et al., 1993). Considering the fact that the gills of fish have alkaline surface, soluble ferrous iron can become oxidized to insoluble ferric compounds. This covers the gills lamella and inhibits respiration. With Fe and under decreased water temperature, bacteria (iron-depositing) will increase on the gills and further oxidize ferrous compounds. Precipitated iron compounds and tufts of the iron bacteria reduce the gill area available for respiration, damage the respiratory epithelium and may eventually suffocate the fish. While studies have shown that it is not easy to measure lethal concentration of Fe as it

depends more on the other physico-chemical properties of the water, yet within Cyprinid culture, the concentration of soluble ionized forms of Fe should not exceed  $0.2 \mu\text{g g}^{-1}$  while for Salmonids, the limit is  $0.1 \mu\text{g g}^{-1}$  (Alabaster & Lloyd, 1980).

Though this study did not look at the heavy metal accumulation at various tissue levels, the result has shown that *P. sutchi* easily accumulated the heavy metals. This can be explained by the fact that fish usually have the ability to concentrate heavy metals in their muscles (Rahman et al., 2012). Therefore tissue analysis conducted was to obtain a cellular evidence of the impact of leachate pollution on fish organisms.

#### **3.4.6 Tissue Analysis (Histopathology)**

##### Gills

Results of the stained gills of the exposed fish showed same appearance (Plate 3.3). The result reflects a proper detection of nucleus and cytoplasm. This was characterized by the deep blue and pink colouration which represents the nucleus and cytoplasm, respectively. The gills of exposed fish (extreme situation) showed significant difference when compared to the stained gills of the control group. Plate 3.4 represents the stained gills of exposed fish species. The result was characterized with poor colouration or sparse colour distribution. Colouration of the lamella with deep blue and pink is typical of nucleus and cytoplasm of any tissue, respectively, identified to be in a normal condition. It was observed under microscopic view that the lamella was much lifted. The sparse colouration showed much denaturation of the nucleus and cytoplasm. This showed severe effect of exposure when compared with the gills from the control group.

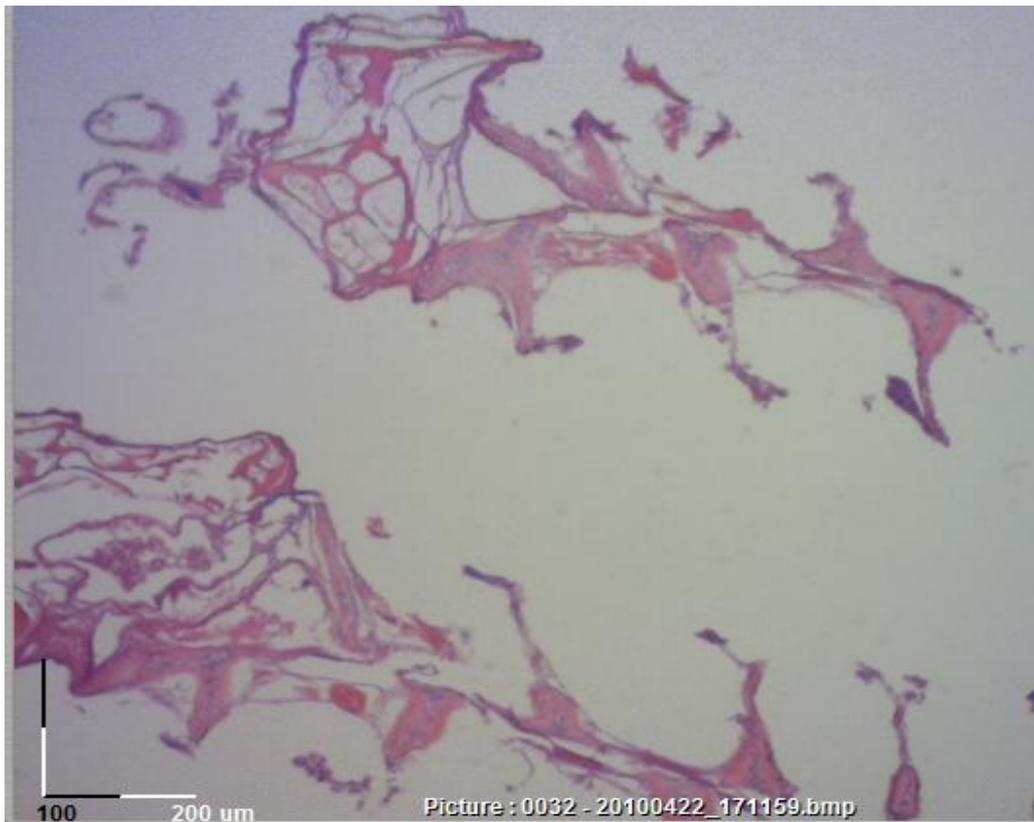


Plate 3.3 Stained gills of the exposed fish species

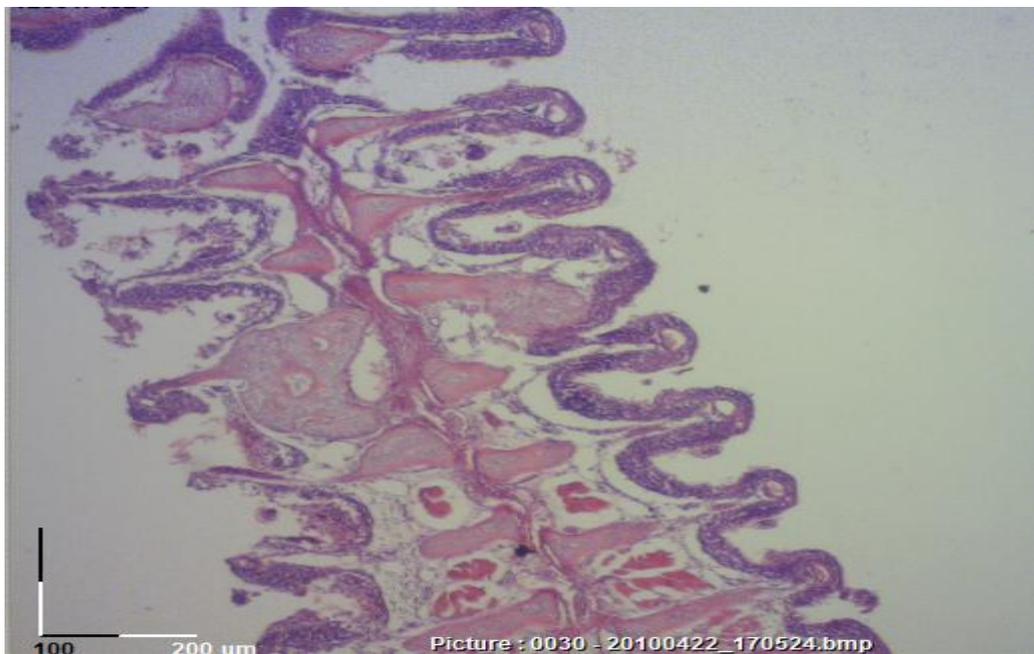


Plate 3.4 Stained gills of the non-exposed fish species

The stained gills of the control group showed deep blue and pink colouration of the lamella which reflects a normal tissue condition for nucleus and cytoplasm,

respectively, which will allow proper metabolic activities. Since the gills were in contact with only normal water in the aquaria, the stain showed absence of cellular impairment. However, this was the reverse in case of the exposed group as it was characterized of poor or sparse colour distribution. This may be due to the penetration of the components of the leachate into the internal system of the fish. It might have occurred consequent to the fact that fish utilizes the gills for breathing; hence it becomes a target site for any contaminant. Therefore, it is possible that the toxic components like dissolved ions, propanoic acid and other compounds had initiated complex reactions on the cells of the gills. This then caused lysis in the tissue might imply attack on the nucleus and cytoplasm. Therefore, it depicts the internal toxicity potential of the leachate and might indicate some degree of bioaccumulation.

Limited study has been carried out on tissue analysis of fish as it relates landfill leachate involvement. However, gills of Atlantic Salmon exposed to Skerries Brook water contaminated with leachate from nearby landfill, exhibited histopathological changes. The study revealed a lamella lifting which was a lesion not found in the control group (Mathieu et al., 2007). The fish gill is a morphologic and physiologic complex organ involved in respiration, acid-base regulation, osmoregulation and excretion (Evans et al., 2005). Its complexity and constant contact with the external environment makes the gill the first target of waterborne pollutants. This was typical of the fish gills exposed in this study. The fish species demonstrated lifting of the lamella (Plate 3.3) when compared to the control group. This may be due to much accumulation of the toxic constituents in the leachate onto the gills as a result of its level of contact with the contaminated water in the aquaria.

## Liver

Results of stained liver of the exposed fish showed same appearance, hence all were also represented as one (Plate 3.5). Whereas the stained liver of non-exposed *P. sutchi* was emphatically stained deep blue and pink (Plate 3.6), the pale stain as seen in Plate 3.5 characterized the liver of the fish exposed to raw leachate.

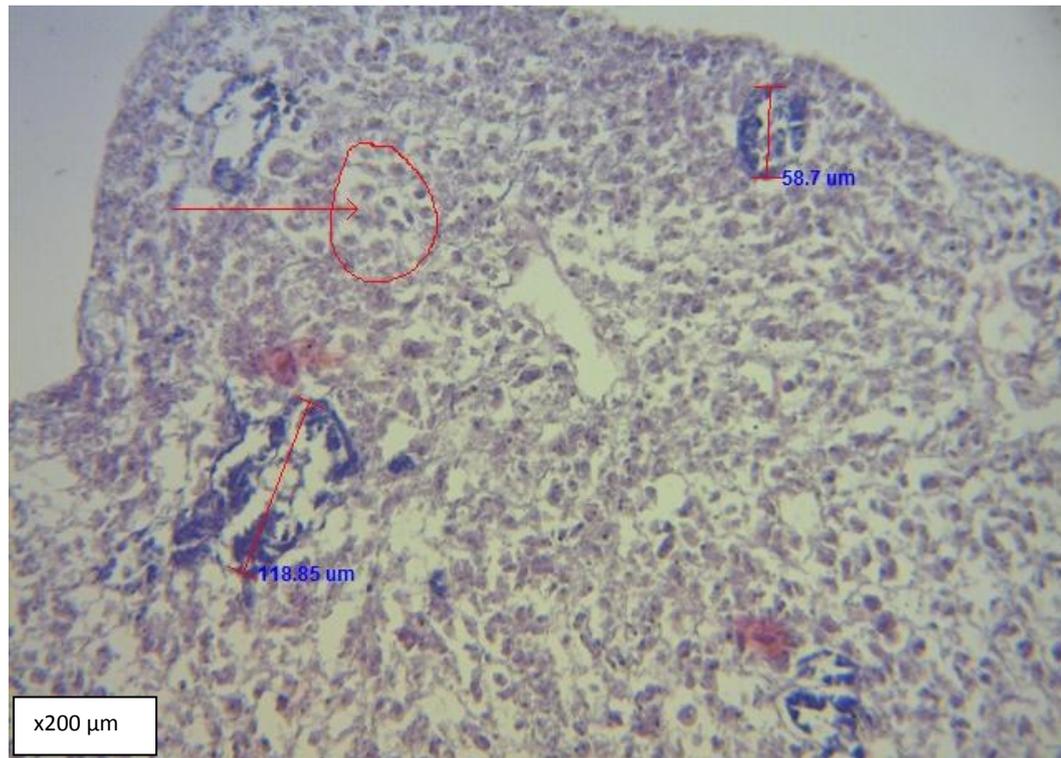


Plate 3.5. H&E stain on excised liver of the leachate-exposed fish. The arrow points to evidence of structural lesion or cellular disruption.

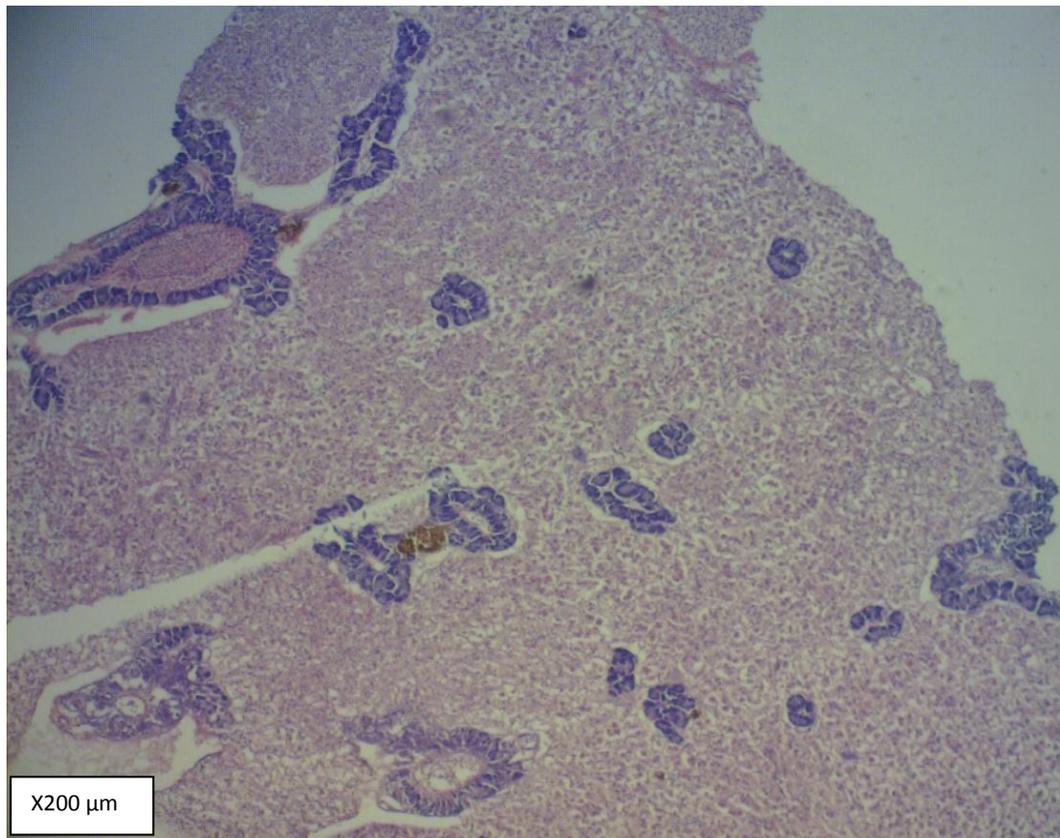


Plate 3.6. H&E stain on excised liver of the non-leachate-exposed fish.

The emphatic deep blue and pink colourations depict a normal tissue condition for the nucleus and cytoplasm that can allow proper metabolic activities within the cells of the organism. This implied that there was cellular disruption within the liver of the exposed *P. sutchi* (Emenike et al., 2012a; Emenike et al., 2012b) which agrees with the study by Mathieu et al. (2007) where the lifting of the gill lamella (lesion) was observed after fish exposure to Brook water that was contaminated with leachate flow from a nearby landfill. Nervous symptoms are often pronounced in situations of ammonia toxicity on fish (Alabaster & Lloyd, 1980; Emenike et al., 2012b). This might have caused such lysis to the fish liver because in as much as ammonia ion ( $\text{NH}_4^+$ ) cannot penetrate cell membrane of organisms, molecular ammonia ( $\text{NH}_3$ ) can readily diffuse across the tissue especially as concentration gradient exists. Study on *A. anguilla* (Eel) showed that liver

was the most damaged organ in the fish due to bioaccumulation of heavy metals from a nature reserve in France (Ribeiro et al., 2005).

The characterization of raw leachate samples across the four landfills in this study had not only shown the presence of potential toxicants in MSW landfill leachates, rather the bioassays had demonstrated lethal effect consequent upon exposure to fish species due to leachate pollution. Regardless of the landfill status toxicity potential abound in all the studied landfills though at varying degrees of mortality effect. However, considering the liquid nature of leachate, it will definitely find its way into watercourses if not properly checked. Yet, soil is the medium which in most cases, it has to pass through before seeping/flowing into groundwater or surface water. This will imply that such soil will be polluted and have the potential of trapping some of the pollutants like heavy metals, hence assessment of microbial distribution on such leachate contaminated soil is necessary with the view of utilizing them for the purpose of potential bioremediation of such polluted soil. Such approach may reclaim leachate polluted site while at the same time help in minimizing heavy metals that might be washed into nearby surface water or those that might seep into groundwater.

## CHAPTER FOUR

### Bioremediation of leachate contaminated soil

#### 4.1 Introduction

Contamination of soil with heavy metals as a result of different anthropogenic activities that include metal mining, landfilling and other industrial activities has posed a serious environmental concern. Humans and the environment are exposed to significant risk due to high metal toxicity and the potential to infiltrate the food chain (Boyd, 2010; Ma et al., 2011). Therefore, in regards to such risk, scientists and engineers tend to be saddled with the onerous task of developing removal or immobilization methods for metal contaminated soil (Plociniczak et al., 2013). Bioaugmentation of contaminated soil with desired microbial strains is one of the methods adopted for tackling heavy metal menace in the environment. Among other contaminants present in leachate at varying concentrations, heavy metals were of interest in this aspect of the study. Reason for this is based on the bioaccumulation of heavy metals in the fish species consequent upon exposure to leachate pollution in aquatic system.

#### 4.2.Objectives

This section was designed for the purpose of understanding;

- potential distribution of bacteria in leachate contaminated soil
- the effect of bioaugmenting leachate contaminated soil (heavy metals) with leachate-site persistent microbes
- the generation of removal rate of heavy metal from a bioremediated leachate contaminated soil using a kinetic model.

### **4.3 Materials and Methods**

Whereas some techniques were adopted and modified from previous studies, a number of procedures were designed according to researcher's discretion. Hence, sample collection, heavy metal characterizations, microbial isolations and formulation, and eventual experimental setup were the various components of the bioremediation process in this study. Bioaugmentation was adopted as against biostimulation and bioattenuation.

#### Collection of soil samples

Soil samples used in the study were classified into two; originally contaminated and non-contaminated soil. Originally contaminated soil was obtained from a closed non-sanitary landfill. The soil had been soaked with leachate due to seepage from the waste-cells. Such soil was carefully scooped (0-20 cm from surface) into soil bag and immediately transferred to the laboratory for the purpose of isolation of persistent bacteria that were to be used for bioaugmentation. Similarly, non-contaminated soil was collected from the University of Malaya garden which was used to setup the bioremediation microcosm. Upon conveyance to the laboratory, the soil samples were air-dried whereas portions of the soil were immediately sacrificed for heavy metal analyses.

Collection of samples and subsequent contaminations were carried out according to 2004 ASTM E-1197 Standard guidelines for conducting terrestrial soil-core microcosm test (Sprocati et al., 2011). Worthy of note is also the fact that all sample collections were replicated to accommodate variability and ensure homogeneity. Sequel to sample collections, microbial study became a routine component of the study.

### Microbial isolation from originally contaminated soil

Since bioaugmentation was the preferred method of bioremediation adopted in this study, therefore microbes (bacteria only) present in the originally contaminated soil was of significant interest. It is assumed that microbes that were found in the contaminated soil should be considered persistent and might have potential role in the biochemical transformation in the contaminated environment and as such enhanced concentrations of such organisms might be beneficial; hence enhance microbial bioaugmentation process.

Therefore, 1 g of soil was mixed with saline water (0.9% NaCl) and the suspension vortexed for 2 hours at 150 rpm using Lab-Line 3521 orbit shaker (LabLine Instruments, Inc, Maharashtra, India). Serial dilutions were plated (Kauppi et al., 2011) on nutrient agar (NA) and subsequently incubated for 48 hours at 33<sup>0</sup>C. Single colonies were re-streaked separately on freshly prepared NA to obtain discrete pure culture suitable for identification.

Isolated bacteria were identified using the Biolog GEN III MicroPlate protocol. The GEN III MicroPlate<sup>TM</sup> test panel provided a standardized micromethod using 94 biochemical tests (Bochner, 1989a; Bochner, 1989b). Omnilog<sup>®</sup> Data Collection which is Biolog's microbial identification system software was used to identify each bacterium from its phenotypic pattern in the GEN III MicroPlate.

For the identification purpose, the cells were freshly regrown (16-24 hours) in order to avoid lose of viability and metabolic vigor which is typical of most organisms at stationary phase. Using inoculation fluid (IF), inoculum of each target cell was prepared using Protocols A (IF-A catalog no. 72401) and B (IF-B catalog no. 72403) at turbidity range of 95 – 98% T. This was done using a cotton-tipped inoculatorz swab (Catalog

no. 3321) to pick up about 3 mm diameter area of cell growth from the surface of the agar plate, and eventually dipping it into the desired IF. To ensure uniform suspension, any cell clumps was carefully crushed against the tube wall. Ensuing cell suspensions were poured into a multichannel pipette reservoir.

An 8-channel automated pipettor was used to dispense 100  $\mu$ L of the suspension into each of the wells in the MicroPlate (Catalog no. 1030). The wells (Table 4.1) contain 71 carbon source utilization assays (Columns 1-9) and 23 chemical sensitivity assays (Columns 10-12), hence they can be identified at the species levels based on the “Phenotypic Fingerprint” of the microorganisms provided by the test panel. These MicroPlates were placed in Omnilog reader wherein they were read using Biolog’s Microbial Identification Systems software, and the identified microbes were recorded. The isolated/identified microbes were formulated for the bioaugmentation process required for remediation of soil contaminated with heavy metals due to leachate pollution.

Table 4.1 Layout of Assays for MicroPlate (GEN III)

A1 Negative control	A2 Dextrin	A3 D-Maltose	A4 D- Trehalose	A5 D- Cellobiose	A6 Gentibiose	A7 Sucrose	A8 D-Turanose	A9 Staychose	A10 Positive control	A11 pH 6	A12 pH 5
<b>B1</b> D-Raffinose	B2 α-D-Lactose	B3 D- Melibiose	B4 β-Methyl- D- Glucoside	B5 D-Salicin	B6 N-Acetyl-D- Glucosamine	B7 N-Acetyl-β- Mannosamine	B8 N-Acetyl-D- Galactosamine	B9 N-Acetyl Neuraminic acid	B10 1% NaCl	B11 4% NaCl	B12 6% NaCl
<b>C1</b> α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D- Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
<b>D1</b> D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 Myo- inositol	D5 Glycerol	D6 D-Glucose-6- PO <sub>4</sub>	D7 D-Fructose-6- PO <sub>4</sub>	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
<b>E1</b> Gelatin	E2 Glycl-L- Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyrogutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
<b>F1</b> Pectin	F2 D- Galacturonic Acid	F3 L- Galactonic Acid Lactone	F4 D- Gluconic Acid	F5 D- Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
<b>G1</b> p-Hydroxy- Phenlyacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto- Gglutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo- Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
<b>H1</b> Tween 40	H2 γ-Amino- Butyric Acid	H3 α-Hydroxy- Butyric Acid	H4 β- Hydroxy- D,L- Butyric Acid	H5 α-Keto- Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

### Microbial formulation

Microbial formula used in the bioaugmentation experiment was composed of 9 strains isolated from a leachate contaminated site in Malaysia. Each strain was grown as a pure culture in NA plates at 33<sup>0</sup>C for 2 days before being inoculated in nutrient broth E and grown to stationary phase in a rotating shaker at 29<sup>0</sup>C and 150 rpm. Discrete suspensions at the same physiological phase (1.3 ABS at 600nm) were then pooled in equal proportions to set-up inocula for bioaugmentation.

### Bioaugmentation setup

Soil microcosm contamination was done according to the ASTM guidelines: characterized leachate was evenly dispersed into each microcosm to attain 10% v/w concentrations. Four microcosms in triplicates were set up under different conditions: A, contaminated soil amended with 6 bacteria strains from the isolated 9; B, soil amended with 3 strains; C, amended with the 9 isolated strains, and D, contaminated soil without any amendment. Bioaugmentation with the microbial formula was performed 3 days after the contamination (Sprocati et al., 2011) to mark the start of the bioremediation experiment.

Microcosms A, B and C were watered with 100 mL of the inocula (obtained from equal volumes of pooled discrete strains) each containing about  $3 \times 10^9$  CFU/g. Soil moisture content was maintained by regular watering with distilled water. Caution was applied to prevent excess watering since leaching was not required in the experiment in order to avoid loss of metals contents. Portions of soil microcosms were sacrificed every 20 days (until 100 days) for onward metal analysis and assessment of microbial density.

### Chemical analyses

For the purpose of analyzing the heavy metal content of the several soil samples in this study, acid digestion was first done. To 0.5 g of soil sample, HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> was added (Hseu et al., 2002) before using Multiwave 3000 microwave digester (Perkin Elmer/Anton Paar) for sample digestion. The elemental concentrations were measured using Optima 5300 DV (Perkin Elmer, Massachusetts, USA). Evaluation of a procedure blank was always carried out. Every labware utilized in the experiment was soaked with diluted nitric acid overnight before being rinsed in double deionised water. Experiments were duly replicated.

### Determination of heavy metal degradation

Concentrations of the heavy metals in the discrete microcosms were recorded after analyses at 20 days interval, for period of 100 days. Results were evaluated for significance using ANOVA at P < 0.05. The data were processed to calculate the percentage of heavy metal removal from each treatment as stated below;

$$\% \text{ of heavy metal removal} = \left( \frac{C_{0(x)} - C_{F(x)}}{C_{0(x)}} \right) \times 100\% \quad (\text{Eq. 4})$$

Where

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

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

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

$$K = -\frac{1}{t} \left( \ln \frac{C}{C_0} \right) \quad (\text{Eq. 4})$$

Where;

K= First order rate constant for metal uptake per day

t= time in days

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

C<sub>0</sub> = initial concentration of metal in the soil (mg/Kg)

#### **4.4 Results and Discussions**

The results of the heavy metals removal from the leachate contaminated soil is discussed in this section. This ranged from the microbial isolation from the originally contaminated soil to its use in bioaugmentation as a method of bioremediation in a sustainable manner.

##### **4.4.1 Microbial isolation from originally contaminated soil**

Considering the condition of bioremediation type required in this study, the isolation of microbes needed for the process became inevitable. The soil which was originally contaminated by leachate that seeps from waste cells was characterized of microorganisms (limited to bacteria) based on isolation and identification. Table 4.2 contains the list of nine bacteria isolated from the contaminated soil.

The list showed diverse genera of bacteria that included both gram-positive and – negative *Bacillus*. In as much as literature may not portray some of the identified microbes as enhancers of any sort of bioremediation (heavy metals or any other organic

and inorganic pollutant), yet their presence in the leachate polluted soil raises the interest on their existence and function in the contaminated environment. It is not out of way to state that the ability of some microbes to tolerate heavy metals polluted sites and even enhance transformations that reduce the toxicity, project such microbes as potentially useful in bioremediation.

Table 4.2 Bacterial species isolated and the distribution in microcosms for bioaugmentation

Treatment A	Treatment B	Treatment C	Treatment D (Control experiments)
NU	<i>Bacillus thuringiensis</i>	<i>Bacillus thuringiensis</i>	NU
<i>Pseudomonas putida biotype B</i>	NU	<i>Pseudomonas putida biotype B</i>	NU
<i>Stenotrophomonas maltophilia</i>	NU	<i>Stenotrophomonas maltophilia</i>	NU
<i>Flavimonas oryzihabitans</i>	NU	<i>Flavimonas oryzihabitans</i>	NU
NU	<i>Lysinibacillus sphaericus</i>	<i>Lysinibacillus sphaericus</i>	NU
<i>Acinetobacter schindleri</i>	NU	<i>Acinetobacter schindleri</i>	NU
<i>Brevundimonas vesicularis</i>	NU	<i>Brevundimonas vesicularis</i>	NU
<i>Microbacterium maritypicum</i>	NU	<i>Microbacterium maritypicum</i>	NU
NU	<i>Rhodococcus wratislaviensis</i>	<i>Rhodococcus wratislaviensis</i>	NU

NU means not use (such bacteria in treatment C was not used)

Table 4.2 showed that *Bacillus thuringiensis* led the pack of gram-positive microbes alongside others that included *Lysinibacillus sphaericus*, *Microbacterium maritypicum* and *Rhodococcus wratislaviensis*. While in the gram-negative class were *Pseudomonas putida biotype B*, *Stenotrophomonas maltophilia*, *Flavimonas oryzihabitans*, *Acinetobacter schindleri*, and *Brevundimonas vesicularis*.

The presence of *B.thuringiensis* may not be unconnected to the fact that they are common soil microbes and *B.cereus* is often seen as a biochemical indicator for concealed mineralisation (Reith et al., 2005). While *B.thuringiensis* share high similarity with *B.cereus*, the former widely known to be insecticidal and nematicidal (Feitelson, 1993; Damgaard et al., 1996); hence it is frequently used for production of biological control agents for the suppression of pests of agricultural and medical importance (Schnepf et al., 1998; Glare & O'Callaghan, 2000; Choi et al., 2003). Having being isolated in the leachate contaminated soil, its relevance in bioremediation may not be farfetched considering the fact that *Bacillus species* from biodegraded medieval wall paintings of seven historical churches in Campania. Though, also found to be involved in such degradation were *Brevundimonas sp.* and *Staphylococcus sp.*, but the most frequently occurring taxa during the isolation were closely related to *Bacillus cereus/thuringiensis/anthracis* (Pepe et al., 2010). Its relevance in heavy metal removal from contaminated soil were reported (Luo et al., 2011; Babu et al., 2013), and the presence of *Bacillus species* in soil has often been related to the presence of heavy metals there off (Avidano et al., 2005; Viva et al., 2006; Fabiani et al., 2009).

Similarly, *Pseudomonas putida biotype B* as found in the leachate contaminated soil is also suggestive of the organism as a common microbe in polluted soil (Rani et al., 2009; Valls et al., 2000). Though the organism may have some clinical implication especially in the area of pathogenicity, yet its resistive nature to pollution especially to some toxic metals from aqueous (Chen et al., 2005) solutions may have influenced its presence in the leachate contaminated soil. Genetic modification of *Pseudomonas* strains had enhanced its biodegradative activities, and as such, potentially introduced for bioremediation applications in metal polluted sites (Rani et al., 2009).

Also found in the leachate contaminated soil is *Stenotrophomonas maltophila* which is a bacterium with aerobic and non-fermentative gram-negative nature. It is widespread in the environment (Pages et al., 2008). It is a dominant rhizosphere inhabitant, frequently isolated from the rhizosphere of wheat, oat, cucumber, maize, oilseed and potato (Berg et al., 1994; Debette et al., 19980; Heuer et al., 1999; Lambert et al., 1989), but its presence in the leachate contaminated soil may not be connected to the mentioned habitat. *S.maltophila* as against its moscomial pathogen characteristics, also have the ability to degrade xenobiotic compounds (Binks et al., 1995; Lee et al., 2002), can detoxify high molecular weight polycyclic aromatic hydrocarbons (Pages et al., 2008) and is seen to have a decontamination (bioremediation) potential; such attribute may be relative to its presence in the contaminated soil.

*Flavimonas oryzihabitans* is another soil microbe isolated from the leachate contaminated site. A motile gram-negative aerobic bacillus that thrives in moist environments, *F.oryzihabitans* is non-fermenting and commonly known to be pathogenic with high susceptibility to a number of antibiotics (Sanchez-carillo et al., 1996; Iglesias & Martinez, 2004). Literature has not specified its presence in heavily contaminated soil especially in situations of heavy metals involvement. However, the organism has been partially implicated in the degradation of pesticides (DDT) but require a nutrient source, bean coffee, as a support for growth (Barrangan-Huerta et al., 2007).

Another important bacteria isolated was *Lysinibacillus sphaericus*. Also known to be insecticidal, it is another gram-positive bacteria identified in this study. Its insecticidal effect may be highly limited to mosquito control since it has little or no adverse effects on a range of other organisms (Brown et al., 2004; Lacey, 2007; Berry, 2012).

However, its presence in contaminated sites and wastewater had been reported (Bahuguna et al., 2011; He et al., 2011). Its presence and importance in this study can be collaborated with Tang et al (2009) wherein isolated *L.sphaericus* showed a bioremediation effect by providing a metal binding site, while Cerrato et al (2010) demonstrated the reduction and oxidation properties of the organism on Mn in water.

A group IV member of the genus *Pseudomonas*, *Brevundimonas vesicularis* was also isolated from the leachate polluted soil. Previously known as *Corynebacterium vesicularis*, it is a gram-negative *bacillus*, aerobic, nonsporulating and glucose-non-fermenting microbes that is often isolated from both environmental and clinical specimens (Shang et al., 2012). Its soil bioremediation potential has not been documented, however, the non-living biomass of *B.vesicularis* had enhanced the removal of Ni(II) and Cu(II) from wastewater (Singh & Gadi, 2012).

Much of soil bioremediation relevance of *Microbacterium maritypicum* may not have been documented as well but some *Microbacterium* species are known to have some degradative potential (Li et al., 2005). This gram-positive bacterium was isolated from the leachate contaminated soil and is primarily a clinical microbe (Ko et al., 2007).

Last of the gram-negative microbe isolated from the soil in review of *Acinetobacter schindleri*. Though not widely investigated, but most genus of *Acinetobacter* are strictly aerobic and non-fermentative. It can commonly occur in soil but can also survive on moist and dry surfaces, including hospital; hence it can initiate noscomial infection (Pantophlet et al., 2002). The ability to survive moist environment may have aided its survival in the leachate contaminated soil. In as much as literature is yet to document complete degradation potential of *A.schindleri*, it was found among the microbial

community of a uranium mine in Limpopo, South Africa (Chabalala & Chirwa, 2010). Therefore it may have a role within a heavy metal rich environment, hence its isolation in this study.

The bacterial community isolated from the leachate soaked soil also had *Rhodococcus wratislaviensis* which is gram-positive and strictly aerobic with irregular rod shaped. It is widely distributed in soils (Warhurst & Fewson, 1994). Strain of this organism was isolated from forest soil and was found to degrade nitroaromatic compounds (Navratilova et al., 2005). While the microbe may not have been directly implicated in remediation of heavy metal contaminated soil, its isolation in this study may have significance in terms of direct degradation or symbiotic potential to enhance degradation.

In as much that the use of more selective media may have aided isolation and identification of some other organisms, but nutrient agar which is known to be a more general media can detect a wider spectrum of organisms. Therefore it is significant to note that while only nine bacteria species were isolated, it might imply the high resistive nature of the organisms to leachate polluted site in general and heavy metals in particular. Hence considering this potential relevance of the organisms, the study further sorted them into three categories as shown in Table 4.2 in order to serve as the different treatment types adopted for the bioaugmentation of the heavy metal contaminated soil due to leachate pollution. Treatment A comprised all the gram-negative microbes (except *M.maritypicum*) whereas treatment B contained the gram-positive ones and treatment C was made up of all the isolated microbes.

#### 4.4.2 Characterisation of leachate contaminated soil and its remediation (heavy metal removal)

The spiked soil was characterised of heavy metals as shown in Table 4.3. It is worthy of note that the concentrations of the heavy metals were viewed for remediation just based on the availability in the soil after being spiked with 10% v/w raw leachate.

Table 4.3 Initial and residual/final concentrations of heavy metals from the bioremediation of leachate contaminated soil

Heavy metals	Initial Concentrations (mg/kg)	Mean Residual Concentrations (mg/kg)			
		Treatment A	Treatment B	Treatment C	Treatments D
<b>Pb</b>	2.068	0.79 ± 0.16	0.60 ± 0.17	0.84 ± 0.18	1.21 ± 0.2
<b>Cd</b>	0.017	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.006
<b>Al</b>	512	110.75 ± 42.42	141.43 ± 71.32	145.72 ± 59.97	209.67 ± 47.08
<b>Mn</b>	1.29	0.48 ± 0.13	0.45 ± 0.17	0.46 ± 0.13	0.98 ± 0.049
<b>Cu</b>	0.241	0.07 ± 0.015	0.03 ± 0.015	0.09 ± 0.015	0.11 ± 0.015
<b>Zn</b>	2.71	1.09 ± 0.016	0.72 ± 0.09	1.16 ± 0.15	1.41 ± 0.08
<b>Fe</b>	154	63.74 ± 17.8	51.13 ± 4.00	67.33 ± 21.39	80.33 ± 8.96
<b>Ni</b>	0.091	0.02 ± 0.006	0.02 ± 0.006	0.04 ± 0.006	0.03 ± 0.06
<b>Cr</b>	0.265	0.05 ± 0.017	0.05 ± 0.017	0.09 ± 0.02	0.10 ± 0.012

Consequent upon the bioaugmentation of the leachate polluted soil for the purpose of heavy metals removed, reductions in the various heavy metals concentrations were observed across both different days of biomonitoring and the various treatments (A-D). Table 4.3 also represents the initial concentrations of the heavy metals in the contaminated soil (same across all treatments) and the final mean concentrations after 100 days of biomonitoring. Discrete concentrations of the metals across the various 20 day biomonitoring intervals showed similar trend in variations. Therefore to have a better evaluation of the biodegradation effect of the study, each studied metal is discussed separately across time and treatments.

### Lead (Pb)

Lead (Pb) is an environmental pollutant of great concern due to its degree of toxic effect. This study while bioremediating leachate polluted soil had considered Pb as one of the heavy metals to be remedied. One-way ANOVA for Pb (Table 4.4) took into account the concentration of the heavy metal in the leachate spiked soil at initial day denoted as “0” and the intermittent monitoring for the next 100 days at 20 days intervals; hence the last monitoring was represented as “5”. Therefore, the analysis of variance indicated significant differences with  $p < 0.05$  between initial day of contamination and final monitoring periods; hence denoted as mean levels 0 and 5 across the treatments. Though significant differences were obtained, the degree of significance were higher with inoculated treatments than the control experiment ( $p = 0.002$ ). Reason may be the influence of the microbes as introduced into the soil microcosms and can be correlated with the fact that the bioremediation of polluted soils using microbes has been widely reported (Watanabe, 2001; Sprocati et al., 2011).

However, Figure 4.1 showed the extent to which Pb was removed from the individual treatments. It showed that while about 71% Pb was removed in treatment B in 100 days, only 42% was removed in the control experiment. Basically, natural bioremediation cannot be ruled out in the control experiment since the soil was not autoclaved nor sterilized in any form, hence normally existing microbes may have some effect but in less extent when compared to other treatments. The difference in Pb removal between the treatments with highest removal (B) and the least (D = control) was significant at  $p (0.017) < 0.05$  as  $F = 15.566$ . Therefore, Pb concentration showed a significant reduction with the introduction of inocula into the leachate polluted soil.

Table 4.4 One-way ANOVA of levels and mutual for bioremediation of Pb

		<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>A</b>	Between Groups	2.437	1	2.437	286.164	.000
	Within groups	0.34	4	0.009		
	Total	2.471	5			
<b>B</b>	Between Groups	3.233	1	3.233	215.502	.000
	Within groups	.060	4	.015		
	Total	2.262	5			
<b>C</b>	Between Groups	2.262	1	2.262	134.242	.000
	Within groups	.067	4	.017		
	Total	2.329	5			
<b>D</b>	Between Groups	1.113	1	1.113	54.373	.002
	Within groups	.082	4	.020		
	Total	1.195	5			
<b>AB</b>	Between Groups	.056	1	.056	2.384	.197
	Within groups	.094	4	.024		
	Total	.150	5			
<b>AC</b>	Between Groups	.003	1	.003	.129	.738
	Within groups	.101	4	.025		
	Total	.105	5			
<b>AD</b>	Between Groups	.256	1	.256	8.842	.041
	Within groups	.116	4	.029		
	Total	.372	5			
<b>BC</b>	Between Groups	.086	1	.086	2.713	.175
	Within groups	.127	4	.032		
	Total	.214	5			
<b>BD</b>	Between Groups	.552	1	.552	15.566	.017
	Within groups	.142	4	.035		
	Total	.694	5			
<b>CD</b>	Between Groups	.202	1	.202	5.404	.081
	Within groups	.149	4	.037		
	Total	.351	5			

Yet, it may be considered a surprise that the highest removal was recorded in treatment B. One would expect that soil inoculated with all the nine isolated bacterial species (treatment C) will show highest percentage of Pb removal than any other because it is easy to hypothesize that with increased diversity of microbes, bioremediation potential will increase, but unfortunately it was not observed that way in this study. Considering the fact that no previous bioremediation research had utilized the pattern of bacterial combinations used in this study, therefore it is difficult to make any comparison to this

result. However, the mechanism behind the result may be found in interactions that exist among microbes when concentrations and diversity are manipulated. *B.thuringiensis* which can be found in two of the inocula-treated microcosms (B & C) is known to have heavy metal removal capacity and has shown 77% removal of Pb from a mine tailing extract medium that contained 100 mg/L of Pb (Babu et al., 2013). Hence, this ability is enough to have enhanced the Pb removal ability of the inocula in treatments B & C, but seemed that the best interaction that yielded the optimal removal of Pb existed among *B.thuringiensis*, *L.sphaericus* and *R.wratislaviensis*. Despite the presence of *B.thuringiensis* in treatment C, removal was more pronounced in treatment A; hence the order of Pb removal across the treatments was  $D < C < A < B$ .

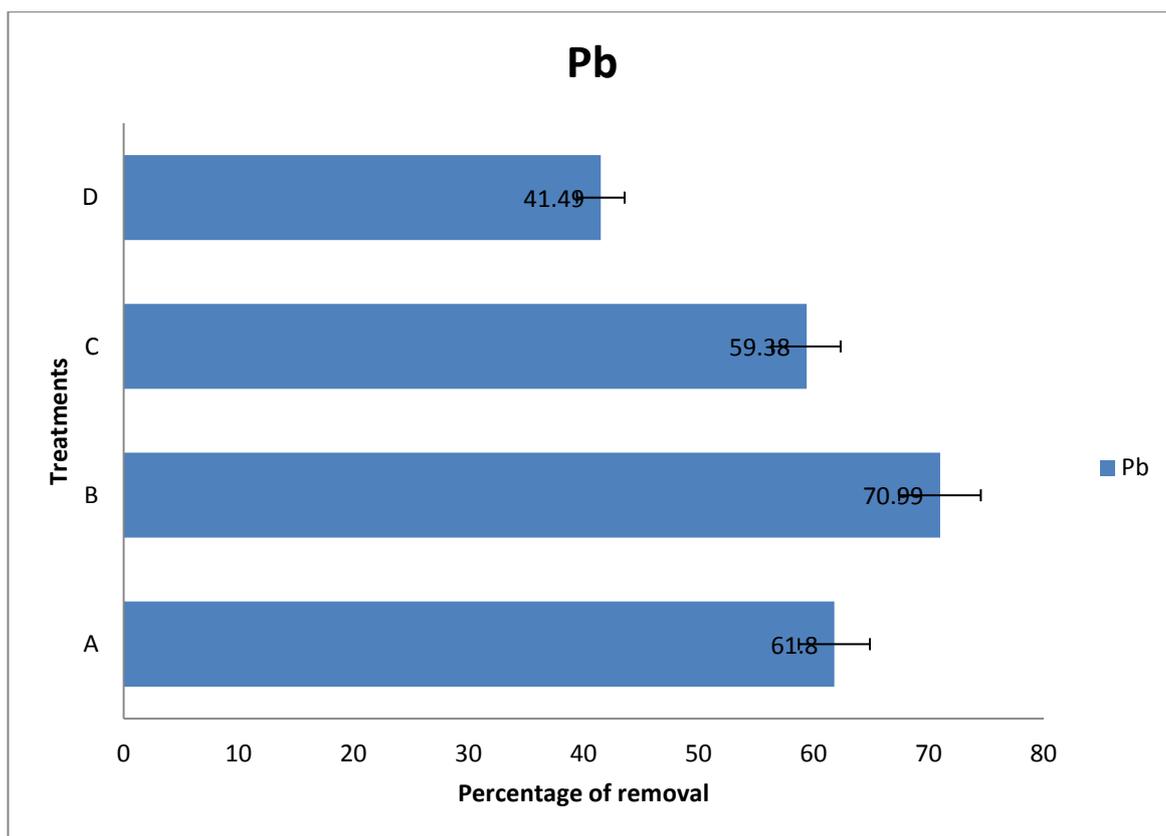


Figure 4.1 Percentage of Pb removed during bioremediation

### Cadmium (Cd)

Table 4.3 showed the mean values of Cd that remained after 100 days of experimental set-up was 0.01 mg/kg all through the different treatments. Cd was considered in this experiment due to the fact that it is often found in Malaysia leachate and pose significant threat to the environment. Therefore, Figure 4.2 which represents the extent of Cd removed from the treatments showed a flat percentage of removal (41.18%). Reason for this may be that resident microbes that are in the soil had the capability to degrade the Cd content equally.

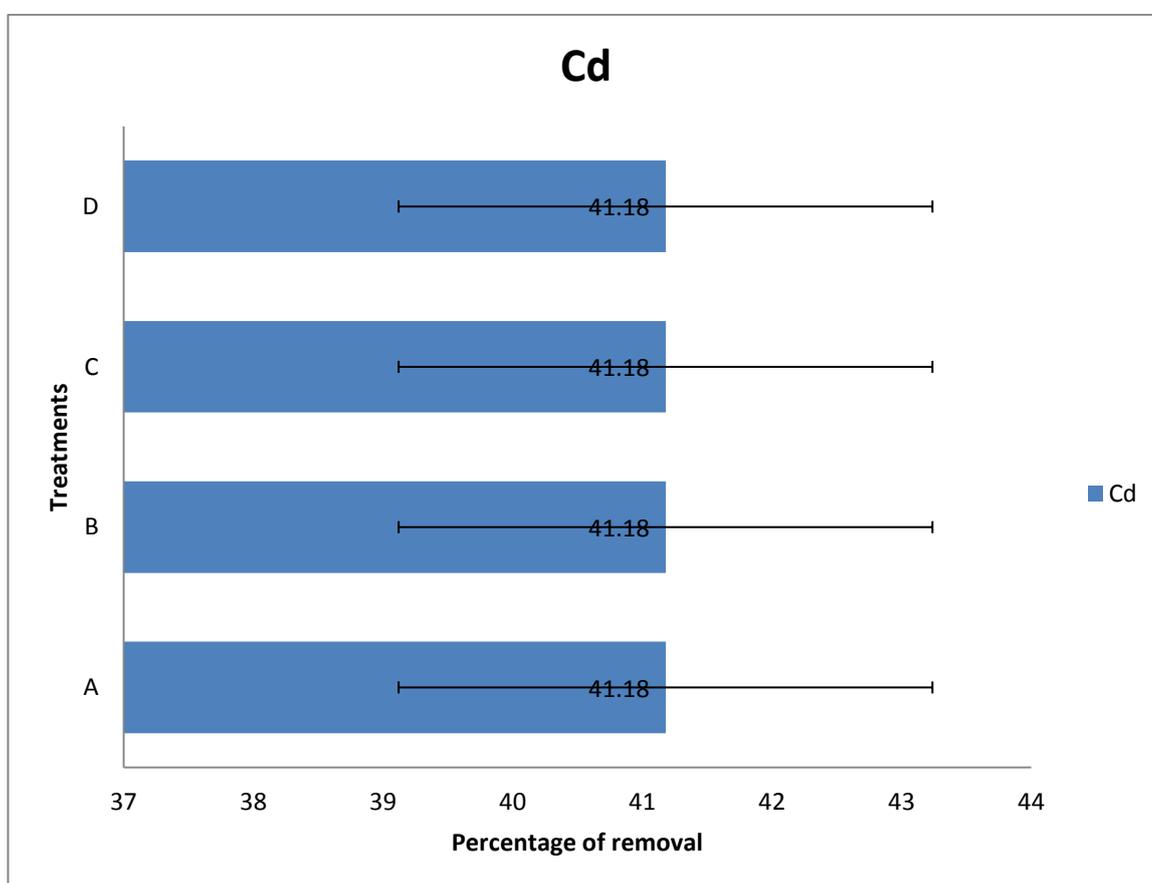


Figure 4.2 Percentage of Cd removed during bioremediation

Also, considering the concentration of the bioavailable Cd in the microcosms, then the study can imply that its bioremediation may not show much variation in terms of total removal. It can be better explained on the grounds that each of the microcosms with the

exception of the control, had at least an organism with reported ability to degrade Cd. *M.maritypicum* and *P.putida* biotype B in treatments A & C may influence the Cd degradation because with the addition of glucose, *M.maritypicum* isolated from mangrove was used to remove Cd from hazardous industrial residue (Aniszewski et al., 2010), while strains of *Pseudomonas*, *P.putida* 62BN and *P.monteilli* 97AN were active in the reduction of soil Cd content as required to improve plant growth on a once toxic environment (Rani et al., 2009). Yet, treatment B, though an inoculants with only three bacterial strains contains *B.thuringiensis* and *Bacillus* sp had accounted for the removal of 80% of Cd after isolation from cadmium hyper accumulator *Solanum nigrum* L (Luo et al., 2011).

However, in as much as the percentage of Cd removal was the same in all treatments for 100 days, yet analysis of variance found significant differences in the degradation, not only across the different biomonitoring intervals but also across the microcosms. Hence  $p < 0.05$  was obtained while comparing the efficiency of the treatment (Table 4.5). This might imply that multiple microbial approach exist which can still give the same degradation output at the end of monitoring period, but the synergistic effect expected in some microcosms (especially in A & B that posses more than one organism with reported Cd removal potential) may have conditions that deter it.

Table 4.5 One-way ANOVA of levels and mutual for bioremediation of Cd

		<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>A</b>	Between Groups	6.353	1	6.353	.	.
	Within groups	.000	4	.000		
	Total	6.353	5			
<b>B</b>	Between Groups	6.353	1	6.353	.	.
	Within groups	.000	4	.000		
	Total	6.353	5			
<b>C</b>	Between Groups	6.353	1	6.353	.	.
	Within groups	.000	4	.000		
	Total	6.353	5			
<b>D</b>	Between Groups	6.353	1	6.353	.	.
	Within groups	.000	4	.000		
	Total	6.353	5			
<b>AB</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			
<b>AC</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			
<b>AD</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			
<b>BC</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			
<b>BD</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			
<b>CD</b>	Between Groups	.000	1	.000	.	.
	Within groups	.000	4	.000		
	Total	.000	5			

### Aluminium (Al)

Aluminium (Al) in addition to some other metalloids is known to have detrimental effect to the ecosystem (Bernhoft, 2012; Auger et al., 2012). The high concentration of Al in the leachate contaminated soil is a simple confirmation that this trivalent metal is the most abundant of all metallic elements in the earth's crust (Verstraeten et al., 2008). Its interaction with microbes seems to be complex and its exclusion from all living

systems definitely raises questions about its toxicological significance (Auger et al., 2012).

Yet, a look at Table 4.3 showed that Al was reduced in all the treatments though at varying concentrations unlike the type of uniform concentration obtained in Cd removal. This can mean that microbes in this study had influenced the removal or immobilization of the trivalent element. Based on ANOVA results, the p values of the treatments across the intervals of biomonitoring were 0.011, 0.028, 0.119 and 0.002 for A, B, C and D, respectively. Hence, it implied that while the degradation changes across A, B and D were significant, it was not the same situation in C. Such may be attributed to the interaction of the microbes because regardless of the number of strains introduced, their specific interactions along time may be a factor influencing degradation or metal uptake.

However, a clearer idea of the Al removal is shown in Figure 4.3 wherein it can be observed that non-significant difference obtained across the biomonitoring time in treatment C is not a complete reflection of the extent of Al removal from the contaminated soil especially when compared with other microcosms. The chart showed that in as much as more than 50% of Al was removed in all microcosms, yet the control experiment which purely rely on natural microbial niche recorded the least degree of removal (59.05%) as against 78% obtained in treatment A. Therefore, treatment A was the highest place of Al removal though statistical evaluation did not indicate any difference between the two (A & D) and even across the treatments, hence the p values 0.55 (AB); 0.67 (AC); 0.054 (AD); 0.96 (BC); 0.24 (BD); 0.46 (CD).

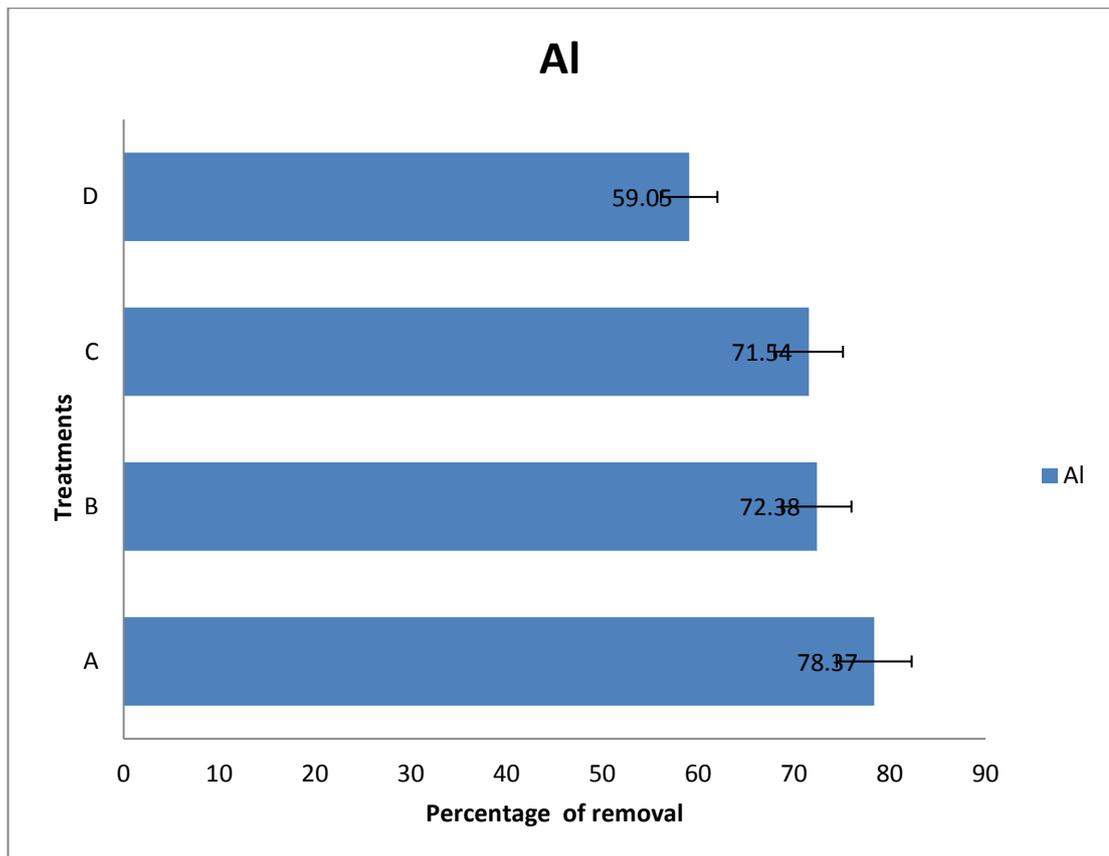


Figure 4.3 Percentage of Al removed during bioremediation

Still, it is important to note that unlike the situation in Pb removal where the association of the three bacterial strains seemed to give edge in metal removal, the consortium of nine microbes turned out to show best removal capability when Al is involved. A precise study to nail the actual causative microbe to this effect may not be readily available in literature, hence comparison became limited. However, the ability of *P.fluorescenes* to shuttle metabolites toward the production of organic acids and lipids play key roles in chelating, immobilizing and exuding Al (Auger et al., 2012) in this study. Treatment A contained *P.putida biotype B* as part of the inoculants and being of the same genus with *P.fluorescenes*, sharing similarities in metabolic properties may be the reason for the microcosm's ability to remove more Al. But this may still require further investigation because for the fact that *Pseudomonas species* can be found in treatment C as well, then it is expected that both treatments (A & C) will show the

highest percentage of Al removal. However, various actions of Al on microbes may have influenced the variations in the degree of removal. Studies had reported such actions to include but not limited to growth inhibition (Guida et al., 1991), interference in the ability of microbe to capture essential micronutrient (El Hage Chahine et al., 2012) and regulation of bacterial motility (Appanna, 1989).

### Manganese (Mn)

The result of heavy metal removal from the leachate contaminated soil showed that the amended soils reduced the Mn content thrice times more than the non-amended soil (Table 4.3). It is very clear that the inocula played significant role in the removal of Mn from the contaminated soil. The result along the days of biomonitoring were all statistically significant ( $p = 0.00$ ). Therefore, the microbial activities were capable of initiating metabolic reactions sequel to the biodegradation of Mn, a divalent metal.

However, Figure 4.4 represents the percentage of Mn removal/reduction across the A-D microcosms. While the treated soils recorded higher percentage of removal than in treatment D, the percentage of removal among the amendments when compared gave the order of Mn reduction to be  $A < C < B$ . Though the ANOVA evaluation of the data set did not find any significant difference among comparisons of AB ( $F = 0.58$ ,  $p = 0.82$ ), AC ( $F = 0.68$ ,  $p = 0.80$ ), BC ( $F = 0.01$ ,  $p = 0.98$ ), yet each of the treatments recorded significant difference with D; hence AD ( $F = 39.97$ ,  $p = 0.03$ ), BD ( $F = 24.92$ ,  $p = 0.08$ ), CD ( $F = 44.9$ ,  $p = 0.03$ ). The slightest variations among them may have direct link to the bacterial strains contained in each microcosm. This is because it is possible that microbes in all the amended soils combined to initiate active binding site for Mn. Therefore, it enhanced the ability to reduce it more than what was observed in treatment

D. It is possible that *L.sphaericus* may have combined better with *B.thuringiensis* and *R.wratislaviensis* to initiate a stronger synergistic effect typical of Mn reduction in this experiment. Reason for such suspicion is that *L.sphaericus* was found to reduce and oxidize Mn in water (Cerrato et al., 2010) and as such may have given the microcosm B such slight edge over microcosm C which also contained *L.sphaericus* but might not have optimal performance in the presence of some other contained microbes.

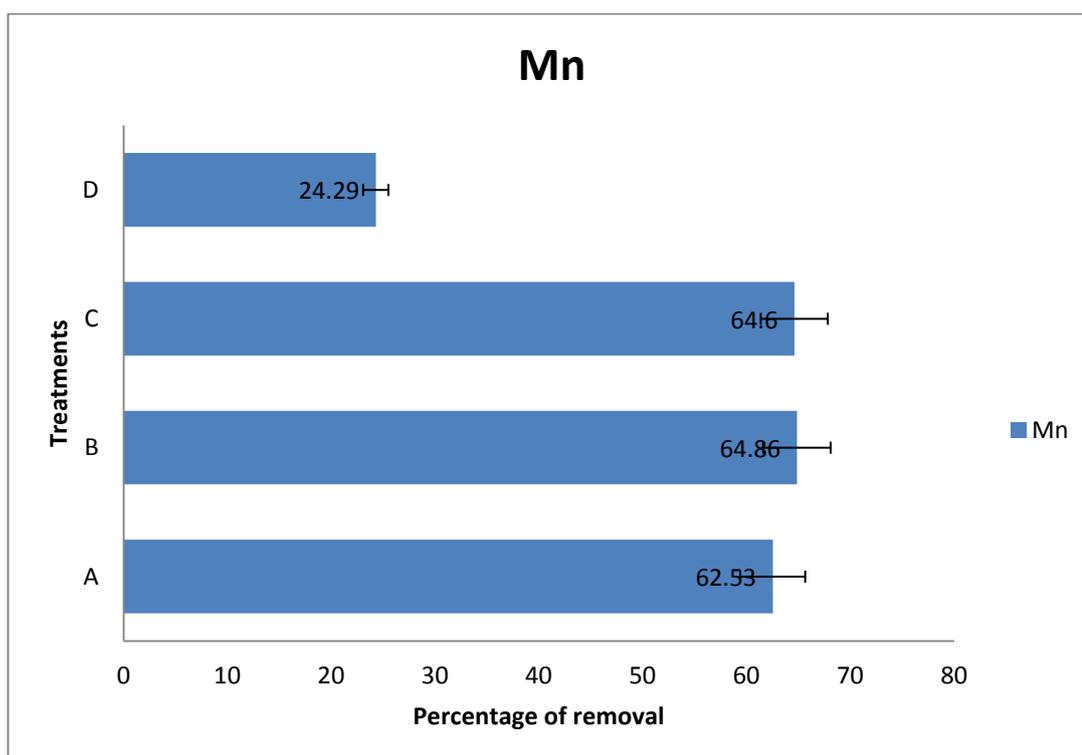


Figure 4.4 Percentage of Mn removed during bioremediation

#### Copper (Cu)

After 100 days of biomonitoring of leachate contaminated soil for the removal of Cu, the mean concentrations (mg/kg) of the metal remaining in the treatments A, B, C, and D were 0.07, 0.03, 0.09 and 0.11, respectively (Table 4.3). Though Cu might be needed in minute concentrations as a trace dietary mineral but its toxic effect in the environment led to the interest for its reduction via bioremediation in this study. Based

on the bioavailable Cu concentration in this study, it is not out of way to state that both natural remediation and bioaugmentation approaches showed significant impact in the reduction since more than 50% of the original 0.24 mg/kg was removed in all treatments including the control experiment. This observation is supported by the fact that significant differences were confirmed at levels “0” and “5” across all the treatments as evaluated in One-way ANOVA ( $p < 0.05$ ). It appeared that naturally existing microbial species had in one way or the other influenced the Cu removal, but whether such natural removal can take place in situation of higher concentrations of pollution and time required for the degradation made the result of the inoculated soil more significant.

Figure 4.5 showed that treatment B removed about 86% of Cu as against 69%, 64% and 52% removed from treatments A, C and D, respectively. The difference between B and D was significant ( $p = 0.003$ ) and even with the other treatments; AB ( $p = 0.033$ ) and BC ( $p = 0.013$ ). *B.thuringiensis* may have played a major role in such degree of reduction especially in treatments B and C because study by Oves et al (2013) while working on industrial effluent contaminated soil affirmed Cu removing potential of a bacterial strain OSM29 found to be *B.thuringiensis*. Similar removal was also confirmed in an enhanced phytoremediation of heavy metal contamination study where *B.thuringiensis* GDB-1 removed 8% Cu from a mine tailing extract medium (Babu et al., 2013). Yet, it will be easy to hypothesize that treatment C would have shown the optimal Cu removal considering the implication of *P.putida*, *B.vesicularis* and *S.maltophilia* in the studies on Cu removing potential (Chen et al., 2005; Choudhary & Sar, 2009; Plocinizack et al., 2013; Singh & Gadi, 2012; Gosh & Saha, 2013). Hence, this study will continue to suspect that a special form of interaction exist among *B.thuringiensis*, *L.sphaericus* and *R.wratislaviensis*. This must have caused the optimal

removal of Cu from contaminated soil, because the same microbes did not initiate the same degree of Cu degradation when combined with the other six bacterial species.

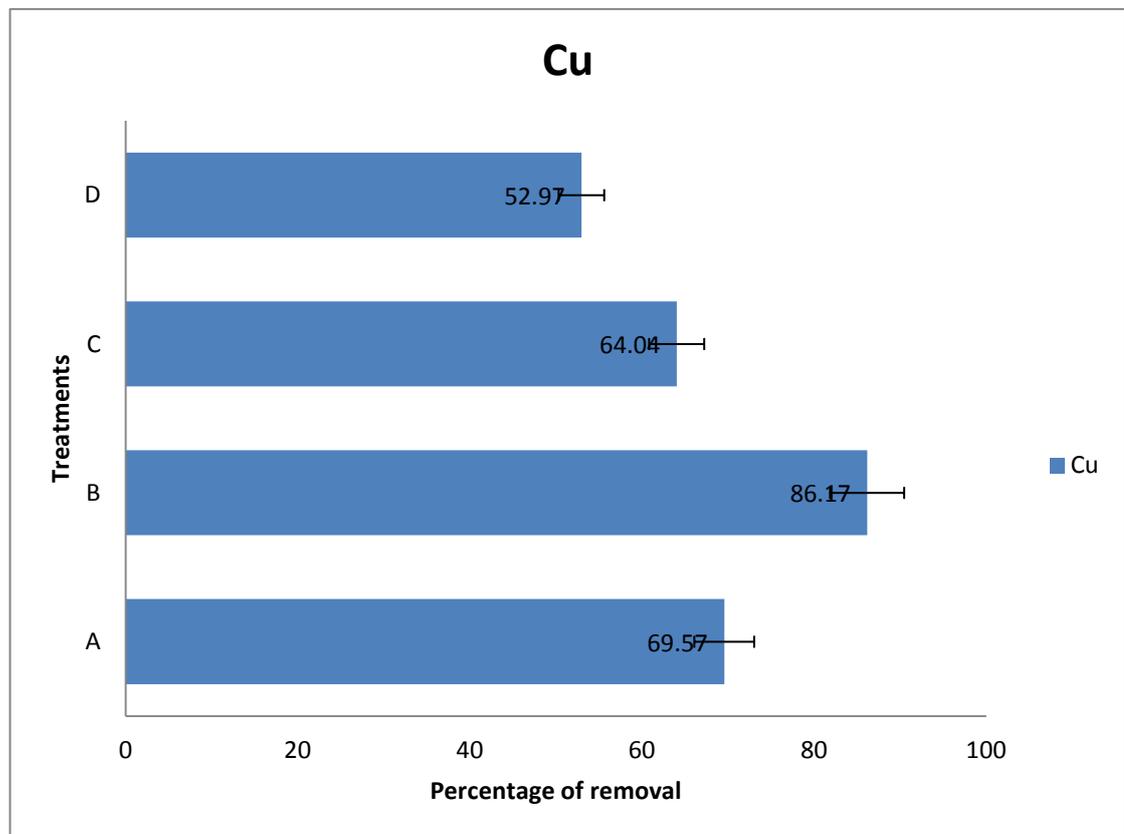


Figure 4.5 Percentage of Cu removed during bioremediation

### Zinc (Zn)

Results for Zn degradation from Table 3.34 showed similar trend in heavy metal removal as in the case of Cu removal. However, less than 50% namely, 1.41 mg/kg of Zn removal was obtained in the control experiment. It means that natural removal was lesser for the Zn contaminated soil (Figure 4.6) than in situation of Cu removal. Again, treatment B with 73% removal proved to be more effective than treatments A and C. When all treatments showed that significant difference existed along the days of biomonitoring ( $p < 0.05$ ), it was not the same when comparing between inoculated treatments and control experiment for the purpose of statistical assessment of better

effectiveness. Treatments A and B showed significant differences when each was compared with the control, hence the corresponding p values were 0.033 and 0.001, respectively. There was no significant difference between treatments C and D.

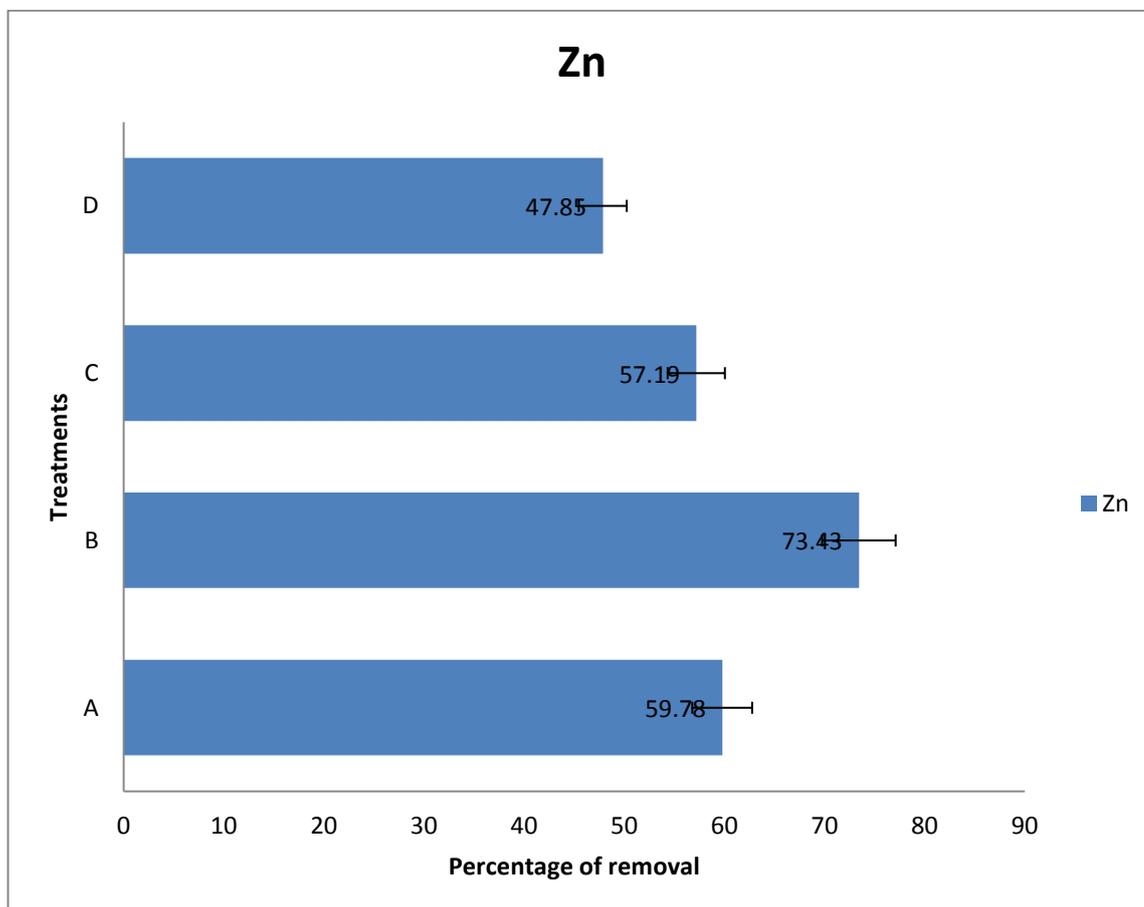


Figure 4.6 Percentage of Zn removed during bioremediation

Explaining the reason for optimal performance among the inoculated microcosms, *B.thuringiensis*, *P.putida* biotype B and *M.maritopicum* may have played more significant role. Based on study by Aniszewski et al (2010), 14 – 68% of Zn was removed from hazardous residue using *Microbacterium* sp. Similarly, *P.putida* strains, MH3, MH6 and MH7 were actively altered the uptake of Zn in a bioaugmented soil (Plociniczak et al., 2013). Again, *B.thuringiensis* GDB-1 was found to also remove 64% Zn in a heavy metal contaminated site (Babu et al., 2013). This is to mean that all the

inoculated soils should be expected to show higher degree of Zn removal than the control experiment.

However, it is important to note that treatment B which contains only three bacterial species out of the isolated nine still exhibited Zn removals of 20% more than in treatments A and C. This will imply that beyond the insecticidal nature of *L.sphaericus* and the normal soil flora nature of *R.wratislaviensis*, some other metal binding properties may be attached to the microbes but the conditions that allow for their optimal performances will require further investigation.

#### Iron (Fe)

Treatments A, B, C and D as used for the bioremediation of leachate contaminated soil, recorded residual concentrations (mg/kg) of Fe at 63.74, 51.33, 67.33 and 80.33, respectively (Table 4.3). Just like Cu and Zn, Fe plays vital role in the environment as nutrient supplier, yet the associated risk is embedded in the fact that Fe ions possess high potential for biological toxicity (Nanami et al., 2005). Based on the behaviour of Fe in the environment and the extent of its bioavailability in this study, it may be inferred that both natural remediation and bioaugmentation approaches make significant impacts on Fe reduction to the tune of close to 50% and above, of both the initial 154 mg/kg in the control experiment and inoculated soils. The changes along the duration of the experiment were significant as shown in all treatments (Table 4.6). As expected, some degree of natural remediation occurred in the non-inoculated soil; hence impact of naturally existing microbes cannot be over ruled.

Table 4.6 One-way ANOVA of levels and mutual for bioremediation of Fe

		<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>A</b>	Between Groups	5705.153	1	5705.153	36.017	.004
	Within groups	633.606	4	158.401		
	Total	6338.759	5			
<b>B</b>	Between Groups	3611.110	1	3611.110	449.889	.000
	Within groups	32.107	4	8.027		
	Total	3643.217	5			
<b>C</b>	Between Groups	6389.346	1	6389.346	27.942	.006
	Within groups	914.667	4	228.667		
	Total	7304.012	5			
<b>D</b>	Between Groups	9188.194	1	9188.194	228.752	.000
	Within groups	160.667	4	40.167		
	Total	9348.860	5			
<b>AB</b>	Between Groups	238.392	1	238.392	1.432	.297
	Within groups	665.712	4	166.428		
	Total	904.104	5			
<b>AC</b>	Between Groups	19.368	1	19.368	.050	.834
	Within groups	1548.272	4	387.068		
	Total	1567.640	5			
<b>AD</b>	Between Groups	413.008	1	413.008	2.080	.223
	Within groups	794.272	4	198.568		
	Total	1207.280	5			
<b>BC</b>	Between Groups	393.660	1	393.660	1.663	.267
	Within groups	946.773	4	236.693		
	Total	1340.433	5			
<b>BD</b>	Between Groups	1278.960	1	1278.960	26.538	.007
	Within groups	192.773	4	48.193		
	Total	1471.733	5			
<b>CD</b>	Between Groups	253.500	1	253.500	.943	.387
	Within groups	1075.333	4	268.833		
	Total	1328.833	5			

However, Figure 4.7 depicts the degree of variation of Fe removal when compared among the treatments. Despite the fact that the trend of reduction was similar to that observed for Cu, Zn and Pb ( $D < C < A < B$ ), statistical difference was only obtained at the comparison of treatments B and D ( $F = 26.54$ ,  $p = 0.007$ ). Little is known of the role of the isolated microbes on Fe removal from contaminated soil. However, considering the divalent nature of Fe as well, the study can assume that the type of microbial interaction that took place while remedying Cu, Zn and Pb might have occurred in the

case of Fe removal as well. The edge which treatment B had over others may also be linked to metal binding ability of *L.sphaericus* as reported by Tang et al (2009) since it contains hexa-histidine tag (His<sub>6</sub> – tag) that possess metal binding property. Yet, the optimal binding effect may have specific interaction in isolation with *B.thuringiensis* and *R.wratislaviensis* only, as this will reduce the bias of anticipating similar effectiveness in treatment C which contained all the microbes including *L.sphaericus*.

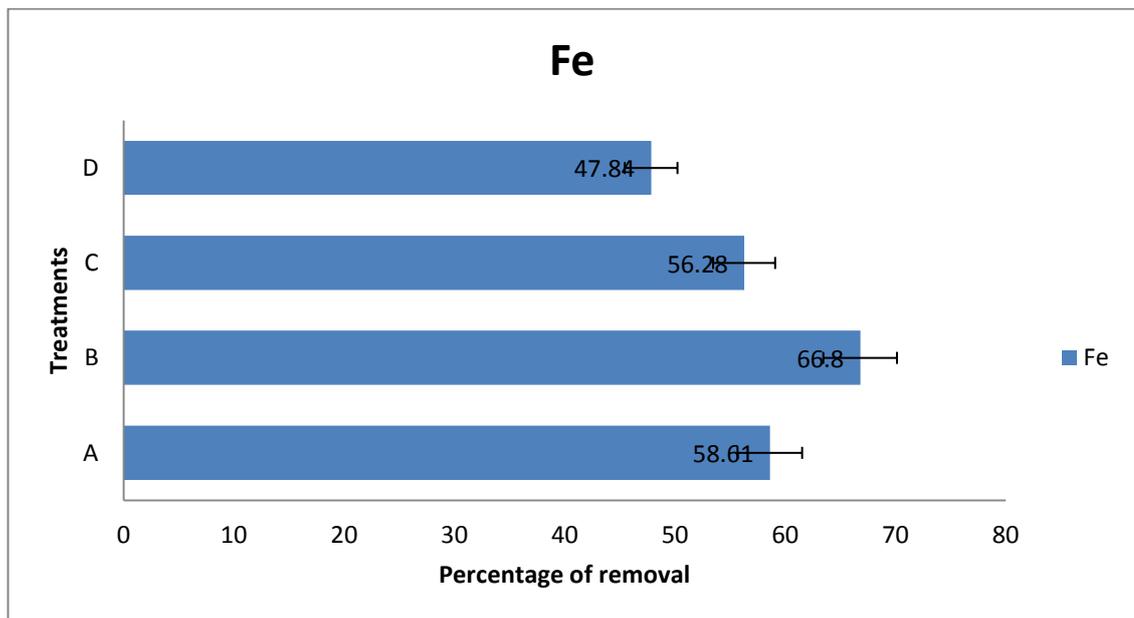


Figure 4.7 Percentage of Fe removed during bioremediation

### Nickel (Ni)

The result of heavy metal removal from the microcosms showed that all the soil treatments reduced Ni content in relation to the bioavailable concentration, but with higher removal recorded in the control experiment than in treatment C (Table 4.3). It seemed that the inocula played an apparent role in the removal of Ni from the contaminated soil. Biomonitoring based on the assessment of corresponding residuals along the stipulated days showed significant differences ( $p < 0.005$ ) in all the treatments

at levels “0” and “5” (initial and final readings of Ni). Hence, the biodegradation of Ni, a divalent metal can be associated to microbial interactions.

From Figure 4.8, it is clear that the control experiment (D) removed an additional 10% of Ni more than treatment C that contained all microbes. This is to imply that natural remediation gave a better effectiveness than the use of nine microbes (together) isolated from leachate contaminated soil. Similarly, with higher effectiveness recorded in treatments A and B over treatment C, may be an indication that while the pool of the nine isolated organisms reduce Ni concentrations, selection seem to be more ideal if optimal removal is expected.

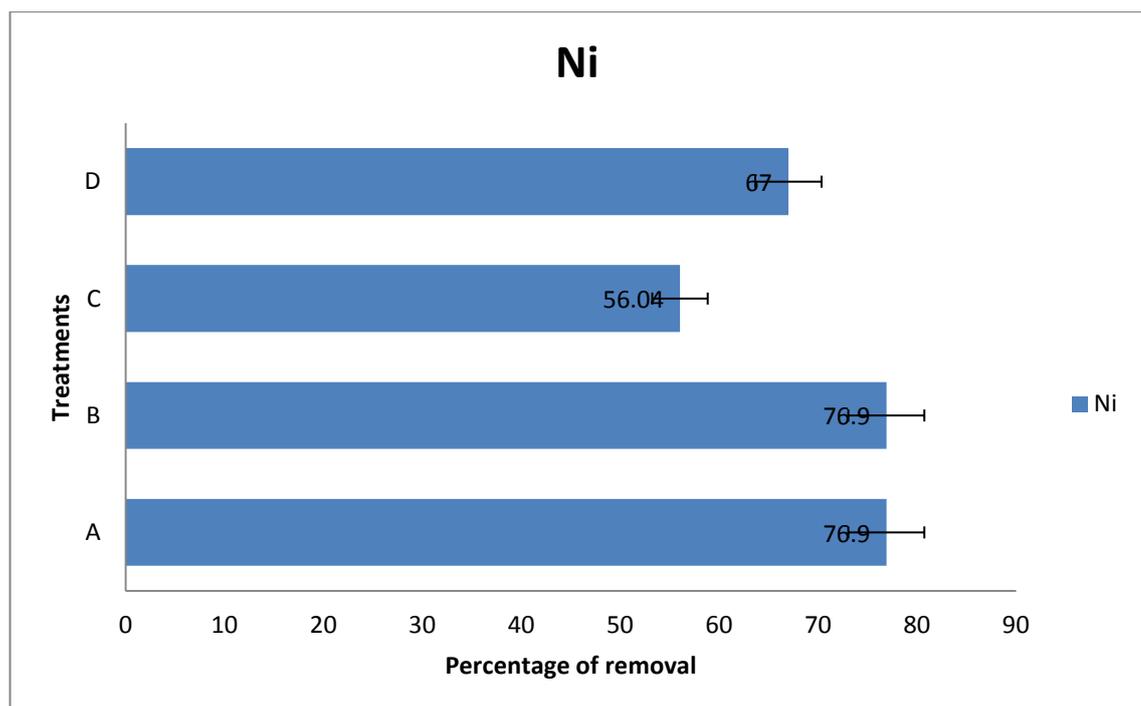


Figure 4.8 Percentage of Ni removed during bioremediation

Therefore, the edge associated to treatments A and B may be due to selective optimization of some of the microbes known to be implicated in previous studies for Ni remediation. It may concur with Choudhary and Sar (2009) where *Pseudomonas sp*

isolated from uranium site, enhanced metal uptake at maximum loading of 1048 nmol Ni<sup>2+</sup> mg<sup>-1</sup> dry weight. Similarly, while varying Ni concentrations, *B.thuringiensis* did not only exhibit metal tolerance but also showed degradation of Ni contaminated soil (Oves et al, 2013). Suspecting the role of *B.thuringiensis* in this study may be supported by the fact that the microbe removed 8% of 10 mgL<sup>-1</sup> Ni obtained in a mine tailing extract medium (Babu et al., 2013). However, considering that significance in this study was statistically directed at comparisons between treatments A & C (p = 0.013) and A & D (p = 0.047), the edge might be due to the role of *B.vesicularis*. This is because non-living biomass of *B.vesicularis* enhanced the removal of Ni(II) (Singh & Gadi, 2012). The microbe was absent in treatment B, but the reason why treatments A and C did not show similar result (since both contained *B.vesicularis*) may require further investigation as non-living biomass of the microbe must have been higher in a particular treatment than the other at a given time during the experiment.

### Chromium (Cr)

Results from Table 4.3 also revealed that Cr among other heavy metals was also reduced across the treatments. With treatment D showing the highest residual value of 0.1 mg/kg in the leachate contaminated soil, it means that more than 50% of the bioavailable Cr contents of the soil were reduced. ANOVA result showed this observation to be statistically significant (p < 0.05) between the starting period of the experiment and final day of monitoring across all the treatments. Hence, this is to imply that while inoculants' role in Cr removal was anticipated, natural removal by the pre-existing microbes in the soil cannot be ignored. In fact, comparisons between treatments showed significant differences except in situations of treatments A & B (p = 1.00) and C & D (p = 0.82).

Interpretation of the result was made clearer in Figure 4.9 where treatments A and B shared the same degree of Cr reduction (81%) as against 67% and 64% shown by treatments C and D, respectively. This implied that whereas natural removal of Cr from contaminated soil is possible, enhanced removal can be achieved by the introduction of the isolated microbes. Interestingly, reason for the similarity in the degree of removal between treatments A and B may be embedded in discrete organisms which have previous record of Cr removal from contaminated environment (soil or water).

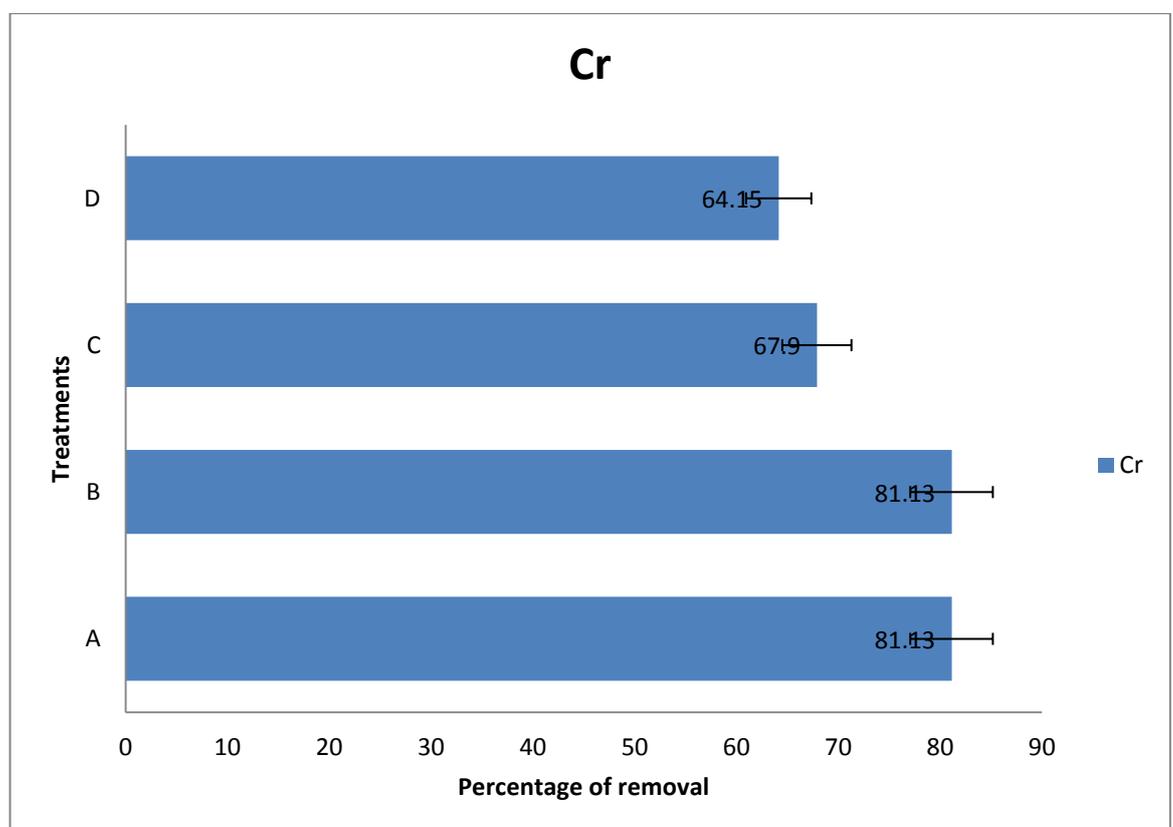


Figure 4.9 Percentage of Cr removed during bioremediation

One of the microbes with such potential is *L.sphaericus*. This is because another genus of Lysinibacillus, *L.fusiformis* ZC1 when isolated from wastewater highly exhibited an extremely rapid Cr(VI) reduction capability (He et al., 2011). Also the protein toxins produced by *L.sphaericus* may be exhibiting bioremediation potential since it tends not to have an adverse effect on other organisms except mosquito (Berry, 2012). Similarly,

*Bacillus sp* isolated from wastewater sample was not only resistant to Cr toxicity but its introduction resulted in the reduction of 83% of 10  $\mu\text{g Cr(VI) ml}^{-1}$  (Zahoor & Rehman, 2009). Oves et al (2013) also confirmed that *B.thuringiensis* potentially reduced Cr in polluted soil. Both *B.thuringiensis* and *Pseudomonas sp* enhanced bioremediation of Cu and Cr (Hassen et al., 1998). Hence, the combination of the microbes still require further investigation in order to type down the actual relationship existing between individual bacteria species and their discrete interactions that enhance metal removal from contaminated soil.

#### **4.4.3 The rate constant of heavy metals removal**

The study further generated the removal rate constants of the heavy metals (Pb, Cd, Al, Mn, Cu, Zn, Fe, Ni, Cr) per day in the different microcosms using the first order kinetic model. This was to show estimated daily removal ability of the microbial combinations as it pertains each heavy metal and in comparison to the natural remediation potential as exhibited in the control experiment.

Table 4.7 revealed that removal rate constant of Cu in leachate contaminated soil amended with the three microbes (*B.thuringiensis*, *L.sphaericus* & *R.wratislaviensis*) within 100 days of the study was highest among all the heavy metals than in other treatments studied. This is to imply that optimal removal rate for the studied heavy metals were achieved with treatment B. Reason for the foregoing observation may be linked to an interaction that is very specific but complex. This is because treatment A which contained all the microbes may be expected to show the best rate of removal but it might be that in the presence of some organisms, some degree of antagonistic reaction

take place which invariably impair the optimal performance of the discrete organisms especially on those that individually possess remediation capabilities. Further explanation on this can be seen in the treatment B wherein *L.sphaericus* was present and may have availed optimal performance of *B.thuringiensis* that have a bioremediation potential (Luo et al., 2011; Babu et al., 2013) and *R.wratislaviensis* which have record of degrading mainly nitroaromatic compounds and monocyclic hydrocarbons (Navratilova et al., 2005; Warhurst & Fewson, 1994). Therefore, considering the fact that *L.sphaericus* possessed a hex-histidine tag (His<sub>6</sub> – tag) at the C-terminus of its S-layer protein SbpA, it is possible that the metal binding property of His<sub>6</sub> – tag was better expressed when in association with *B.thuringiensis* and *R.wratislaviensis* alone, hence providing the bioremediation edge for treatment B.

Table 4.7 Removal rate constant (k) of heavy metals across treatments

	Removal per day (day <sup>-1</sup> )			
	Treatment A	Treatment B	Treatment C	Treatment D
<b>Pb</b>	0.0097	0.0124	0.0089	0.0053
<b>Cd</b>	0.0053	0.0053	0.0053	0.0053
<b>Al</b>	0.0151	0.0127	0.0127	0.0089
<b>Mn</b>	0.0099	0.0105	0.0099	0.0027
<b>Cu</b>	0.0124	0.0212	0.0099	0.0078
<b>Zn</b>	0.0092	0.0131	0.0084	0.0065
<b>Fe</b>	0.0089	0.0111	0.0082	0.0065
<b>Ni</b>	0.0151	0.0151	0.0082	0.0114
<b>Cr</b>	0.0167	0.0167	0.0108	0.0097

Furthermore, the removal rate result showed a flat reading for Cd across all the treatments (0.0053 day<sup>-1</sup>). This might be an evidence of natural attenuation/remediation, wherein the localized microbes were able to biodegrade the Cd content regardless of the amendments in other microcosms. Hence, it is a proof that nature can readjust in situations of pollution through a slow manner. Results from treatment D may be justified with the observation on Cd that some metals may undergo natural remediation

especially when a polluted soil is left undisturbed, whereas the removal rates for the other heavy metals which showed low rates when compared to treatments A, B, C is an evidence of restricted ability of localized/pre-existing microbes to be self sufficient in bioremediation of contaminated soil.

In general, it is evident that the amended soils showed more bioremediation ability than the control experiment (not amended). However, the amendments differently prioritized heavy metals removed. Treatment A prioritized Cr ( $0.0167 \text{ day}^{-1}$ ) removal over Ni ( $0.151 \text{ day}^{-1}$ ) while treatment B prioritized Cu ( $0.212 \text{ day}^{-1}$ ) as against the  $0.0127 \text{ day}^{-1}$  for Al recorded in treatment C. Therefore, this gave an insight that more complex interactions existed within the microcosms. Hence blending the microbes gives an optimal removal as observed in treatment B.

#### **4.4.4 Bacterial Count**

Population count of the bacteria during the bioremediation revealed a fluctuating distribution across the treatments and biomonitoring days. This is in concordance with another bioremediation study (Lin et al., 2010). Figure 4.10 showed a comparison of the initial bacterial count (start of the experiment) and the first 20 days ranged within  $6.5 \times 10^{10} - 4.3 \times 10^{11} \text{ CFU/g}$ . Basically, the result is expected since 300 ml of inocula were introduced into the treatments (A – C); hence concentrations of bacteria must have increased. However, it slightly contradicted when treatment A and D are compared. The former showed lower count than the later, whereas it is also a surprise that treatment B which contained only three microbes showed the highest count as against C that was comprised of nine bacterial species. Generally, this might be that the organisms

regardless of the treatments were still adjusting to the toxic nature of soil due to leachate contamination.

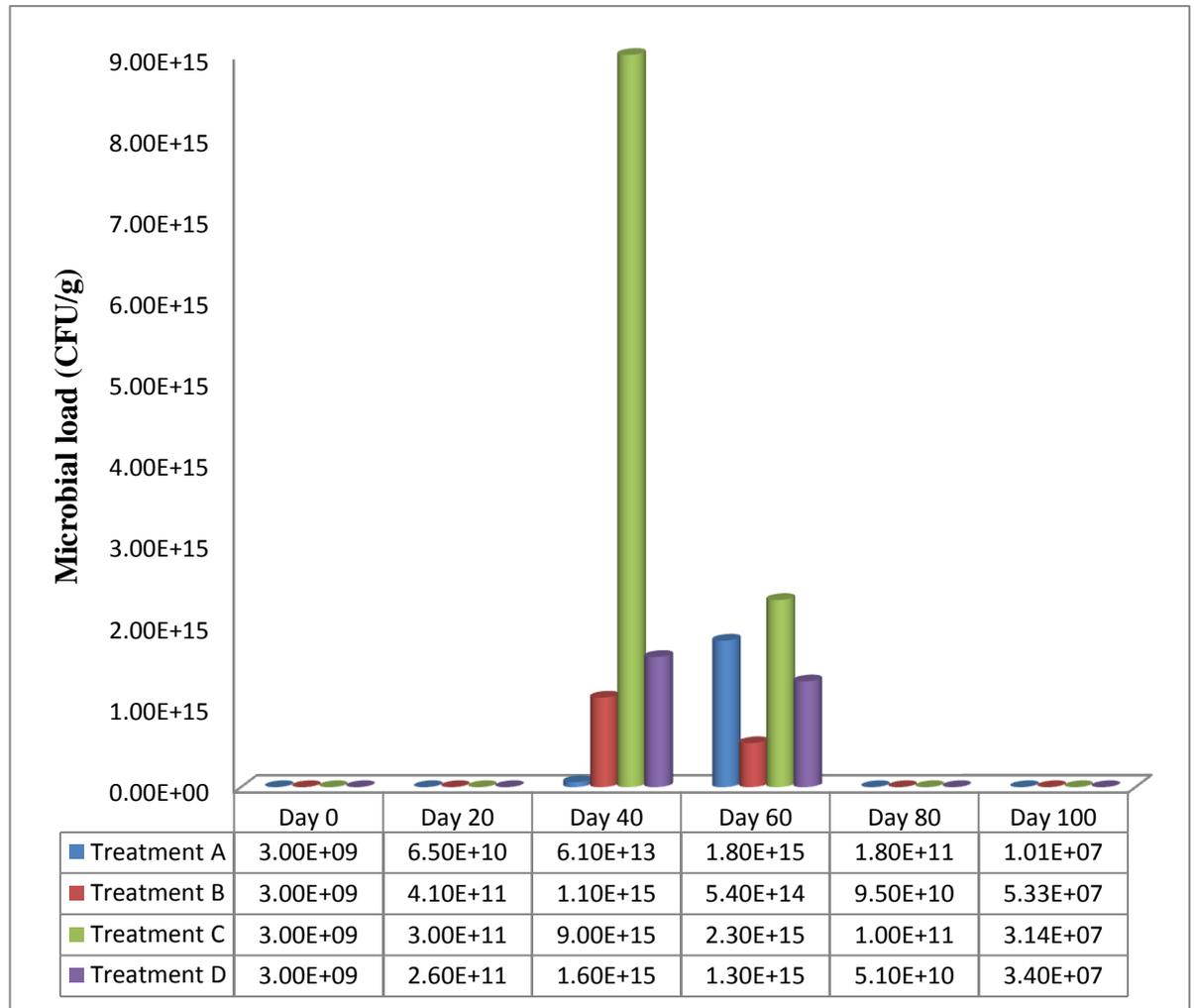


Figure 4.10 Bacterial counts from the start of experiment to 100 days of biomonitoring

The result however revealed higher bacterial growth on 40<sup>th</sup> day of biomonitoring in all treatments. Treatment A still showed the least count ( $6.1 \times 10^{13}$  CFU/g) as against  $9 \times 10^{15}$  CFU/g recorded in treatment C which turned out to be the highest. Considering the number of bacterial species in treatment C, the increased population was expected and this might also be due to availability of other nutrients that abound both in the soil and leachate (though not evaluated).

Bacterial count continued to increase even after 60 days but was not rapid as recorded between 20<sup>th</sup> and 40<sup>th</sup> days of biomonitoring. This might be that some cells were no longer duplicating as before due to stress associated to the biodegradation process, just as the available nutrient may be depleting as well. However, treatment A now recorded higher count ( $1.8 \times 10^{15}$  CFU/g) than treatments B ( $5.4 \times 10^{14}$  CFU/g) and D ( $1.3 \times 10^{15}$  CFU/g), whereas treatment C ( $2.3 \times 10^{15}$  CFU/g) maintained the highest count. This increase in treatment A's bacterial count may be due to optimized interaction among organisms introduced into the microcosm which seemingly took longer time. Yet, the fact that there was continued increase in treatment D may imply that though leachate environment is toxic, it still avail microbes nutrients necessary for selective survival and eventual degradation of the heavy metals.

However, the 80<sup>th</sup> day biomonitoring witnessed drop in bacterial count across all treatments. This time treatment D recorded the least count ( $5.1 \times 10^{10}$  CFU/g) while treatment A took the lead at  $1.8 \times 10^{11}$  CFU/g. This may imply depletion in available nutrient required for bacterial survival as confirmed by Lin et al (2010).

Further degree of reduction in the bacteria count was observed after 100 days. The reduction was very drastic and even below the starting bacteria count. Whereas treatment A recorded the least ( $1.01 \times 10^7$  CFU/g) this time, treatment B showed highest count ( $5.3 \times 10^7$  CFU/g). This reduction is most likely due to highly reduced nutrient level. Also the bacterial species may be much stressed due to metabolic processes required for the heavy metals removal; hence mortality or inhibition of cell duplication took place. However, the higher count found in treatment B may be a reflection of higher resistance by the combined microbes which might have added to the inocula

ability to bioremediate higher percentage of the heavy metals and even at high removal rates.

In general, the bacterial count and associated variations along the different days of monitoring can be linked to the concentration changes in the heavy metals. This can be substantiated by the ANOVA evaluation of the changes in the heavy metals concentrations wherein significant differences ( $p < 0.05$ ) were recorded for all treatments when levels “0” and “5” were compared across the treatments, it may depict the peculiar degree at which the existing microbes (both inocula and natural ones) bio-remove the heavy metals. Hence, the trend of bacterial count in treatment B gave optimal removal of heavy metals from the leachate contaminated soil.

## CHAPTER FIVE

### GENERAL SUMMARY

#### 5.1 Introduction

Previously, wastes were managed via open-air burning, ocean dumping, open-pit dumping and some other alternative methods until the negative effects on such methods became evident. Hence landfilling came into existence as a way to give protection to the environment (Boni et al., 2006). However, the advantageous use of landfill had become an issue of discussion upon the realization of leachate formation among other problems associated to this waste disposal method.

Currently, landfilling has metamorphosed to a stage where a lot of landfill practices are standardized and upgrading of landfill status has given birth to classifications. Yet, generation of leachate and the required treatments remained the common factor among all landfill classes. Therefore, while Europe and the US have advanced much in technology in terms of alternate options to landfills over or even landfilling with utmost adherence to standards, developing countries are yet to catch up with the advanced trend, hence landfilling of generated waste is the ultimate disposal option. In most cases, old methods of landfill practice can still be seen in most Asian countries, which include Malaysia.

Sanitary landfills are still not enough in Malaysia, just as only a few out of the 306 officially recognized waste disposal sites have sanitary status, whereas the majority are non-sanitary or open dumpsites (Agamuthu, 2001; Jaffar et al., 2009; Agamuthu et al., 2011). With leachate treatment plants existing in few landfills, only some forms of

localized system of leachate treatment exist at the non-sanitary landfills, and even in some cases no treatment of any sort is observed. Therefore, leachate discharge into nearby watercourse leading to water pollution and seepage into soil causing soil contamination are issues that require critical attention, else environmental deterioration will be inevitable.

This is the reason the present study took into account, the characterization of leachate from active and inactive landfills with the sanitary and non-sanitary nature of the landfills as an in-depth classification that will represent the Malaysian scenario in terms of waste landfilling. Putting into consideration that chemical or physico-chemical characterizations of leachate may not give a correct idea of its potential effect in the environment, toxicity testing became an evaluation tool design to provide an in-depth information on interaction that exist between leachate as pollutant and the aquatic life. Since soil/Landfill often serve as the barrier/medium of exchange between terrestrial pollutants and aquatic pollution, the study adopted the approach of trying to remove some pollutants (heavy metals) from the soil before it gets into the surfacewater via bioremediation.

## **5.2 Landfill types and the leachate studies**

Based on standard landfills classifications (Fauziah & Agamuthu, 2003), Jeram landfill (JSL) and Air Hitam (AHL) have sanitary status as against non-sanitary nature of Bukit Beruntong landfill (BBL) and Taman Beringin landfill (TBL). MSW are still being sent to JSL and BBL, which accords them an active status whereas none waste disposal situation at AHL and TBL makes them non-active landfills. The study deduced that

these landfill conditions reflected in the varied concentrations of leachate components after characterization.

The study was able to establish that the landfills under review were at the methanogenic stage because the pH range was 6.8 – 8.2 (Lagerkvist, 1994) though such may not be enough evidence to determine methanogenic or acidogenic stages of most landfills. Therefore, the degree of organic composition of the leachate confirmed the conditions of the landfills. Assessment of the BOD/COD gave 0.26 – 0.53 which is typical of methanogenic phase of landfill (Christensen, 2001; Fan et al., 2006). This implied that regardless of the age differences of these landfills, they were currently undergoing the same phase. JSL which is the youngest of the four landfills recorded highest BOD and COD values, 27,000 mgL<sup>-1</sup> and 51,200 mgL<sup>-1</sup> which reflected the MSW nature in Malaysia to be highly composed of organic contents as confirmed by Agamuthu et al (2009) that more than 50% of Malaysian waste is organic. Such organic loading will make leachate discharge a huge subject of concern especially when not treated in any form.

Ammonical nitrogen (NH<sub>3</sub>-N) which is a known major aquatic pollutant was found high across the landfills with the highest value associated to AHL (880 mg L<sup>-1</sup>). With such NH<sub>3</sub>-N distribution across the landfills, then it may imply that concentration of nitrogenous compounds are high within Malaysia landfills and the subsequent biodegradation will continue to accord them the generation of high NH<sub>3</sub>-N. These pollutants most often find way to watercourses; hence a potential threat to the aquatic life. The potential threat of NH<sub>3</sub>-N to aquatic life in Malaysia had been described in Jaffar et al (2009) and Emenike et al (2012a), wherein they confirmed the toxic impact

of  $\text{NH}_3\text{-N}$  on fish species. Similarly, all other anionic compounds ranging from chloride to sulphate, as found in all leachate types exceed the discharge limits of Environmental Quality Act, in Malaysia.

A different variation was observed in metallic and monocyclic aromatic hydrocarbon contents of the leachate samples. Metal concentrations were found to be higher for JSL whereas it was devoid of monocyclic aromatics like benzene, toluene and ethyl benzene that were easily detected in AHL, TBL and BBL. Such characteristics most probably reflected the waste deposition. It may go on to infer the past and present waste disposal orientation of people and also the degree of present day waste segregation (though still low) that had directed monoaromatic hydrocarbons associated sources away from the waste pool in JSL.

However, the presence of volatile fatty acids was only found in the propanoic content of AHL, which makes it negligible in the study but is known to be a toxicant. The below detection limit of organophosphorus pesticides, organochlorine pesticides, alcohols and other chlorinated hydrocarbons may depict the degree of waste handling in the landfills which might have kept the sources off the landfill. Such will save the nearby terrestrial and aquatic environment of the associated contamination. In general, the concentrations of the analyzed leachate components were found to be higher in JSL than other leachate types. However to ascertain whether the totality of high concentrations in measured parameters accords any landfill leachate the highest toxic impact or if toxicity will be dependent on concentration of discrete toxicant, was better viewed through bioassay tests on fish species that are most resident in Malaysian or Southeast Asian waters.

Toxicology also looks at impact and fate of pollutants especially on living systems. Raw leachate samples from the landfills upon exposure on the ten fish species, demonstrated acute toxicity at different levels. *Clarias batrachus* which is known to be resilient to aquatic changes was found to show 50% mortality upon exposure to 5.99 – 20.1% v/v of the raw leachate samples. In fact, *Mystus nemurus* was the most vulnerable to the raw leachate exposure with 50% mortality at 1, 1.24, 3.18 and 5.18% v/v for each of AHL, JSL, TBL and BBL, respectively.

Exposure showed that acute mortality for AHL was recorded within discrete fish species exposure to range of 1 – 5.99% v/v whereas for JSL, TBL and BBL it was 1.24 – 7.99% v/v, 3.0 – 19.86% v/v and 5.18 – 20.01% v/v, respectively. Hence such ranges infer that AHL was the most toxic of all tested raw leachate samples.

To buttress the toxic effect and then prioritize the leachate samples, FLLTI was developed as modelled after Pesticide Toxicity Index (PTI) (Munn & Gillion, 2001). It was evident that mortality distribution across all the used fish species implied that raw leachate of Malaysian origin was toxic to both edible and non-edible fish species, since *Clarias batrachus*, *Pangasius sutchi*, *Leptobarbus hoeveni*, *Mystus nemurus* and *Oreochromis mossambicus* were edible while *Xiphophorus maculatus*, *Poecilia sphenops*, *Hyphessobrycon eques*, *Devario aequipinnatus* and *Danio rerio* were considered non-edible or ornamental fish species. FLLTI values generated was in the range of 0.4 – 2.31. Hence weight classification was assigned though more bioassays on additional fish species are required to generate a comprehensive weightage. However, high tolerance (Ht), mild tolerance (Mt) and low tolerance (Lt) were accorded to the fish species which were based on the fact that the higher an FLLTI value is, the more

susceptible the fish species can be to landfill leachate. The classification pitched *C.batrachus* and *P.sutchi* together since their FLLTI values were less than 1. Therefore the proximity values found among *D.rerio*, *O.mossambicus* and *D.aequipinnatus* are of significant importance. This is because *D.rerio* is utilized in most bioassays and if an alternative is found then it will avail ease in bioassay research especially in Southeast Asia where *D.rerio* may be scarce sometimes owing to the fact that it is more predominant in Europe. But considering the edible nature of *O.mossambicus*, *D.aequipinnatus* may be a preferred alternative.

Also the tissue study on the exposed fish species showed impairment of the liver and gill cells. The use of H&E stain identified the impact of leachate exposure on the cellular components of the aforementioned tissues which concurs with Emenike et al (2012a). Poor stains of the nucleic and cytoplasmic compartments of the examined tissues gave an insight on the potential effect on continued exposure of fish species to leachate pollution.

Furthermore, the tissue disruption evidence enhanced further investigation into the bioaccumulation potentials of the fish in face of leachate pollution. Result showed that bioaccumulation of heavy metals which included Zn, Fe, Cr among others. The concentration of Zn found in the fish was considered lethal (Fauziah et al, 2013; Tuzen, 2003). Similarly, Fe has such toxic potential as well especially as it affects fish gills (Svobdova et al., 1993).

Therefore, having established that fish usually have the ability to bioaccumulate heavy metals in their muscles (Rahman et al., 2012) and considering that leachate may not

only flow directly into watercourses but may as well seep through soil compartments, then it became necessary to find a possible means of removing deposited heavy metals in the soil due to leachate seepage in order to avoid eventual wash off into surface waters. Hence, bioremediation of heavy metals from leachate contaminated soil was done.

Bioremediation carried out in the study was to remedy soil of one of the pollutants that have the potential to bioaccumulate in aquatic life in general and fish species in particular. Remediation using microbes was preferred in order to ensure a complete green approach which is geared towards environmental reclamation in a sustainable manner. Therefore, investigation into the microbial distribution of a leachate soaked site showed that some bacterial species can be isolated. This implied that such microbes were able to survive the pollution; hence capable of metabolizing in such environment.

*Bacillus thuringiensis*, *Lysinibacillus sphaericus*, *Microbaterium maritypicum* and *Rhodococcus wratislaviensis* were the gram-positive bacteria species isolated, whereas *Pseudomonas putida* biotype B, *Stenotrophomonas maltophila*, *Flavomonas oryzihabitans*, *Acinetobacter schindleri* and *Brevundomonas vesicularis* were the gram-negative group. Some of the microbes have clinical relevance and insecticidal properties but are commonly found in soil. However, some have bioremediation potentials. Hence to assess the potentials of the microbes to remediate heavy metals in leachate polluted environment, the three amendments (A, B, C) were prepared to make a comparison with a control microcosm which operated on the principle of natural remediation/attenuation.

Changes in heavy metals concentrations across the 20 day intervals of biomonitoring were found to be significant with all treatments ( $p < 0.05$ ). Removal of Cd was found to be the same in terms of percentage removed and removal rate in all treatments, including the control experiment. This may be associated to natural remediation which regardless of the introduced inocula still took place at the same rate.

However, the pool of the nine microbes used as microcosm C did not show the best heavy metal removal when compared with treatments A and B. When the removal rate of Pb was  $0.0089 \text{ day}^{-1}$  in treatment C,  $0.0097 \text{ day}^{-1}$  and  $0.0124 \text{ day}^{-1}$  were recorded in A and B, respectively. Similar situation was observed with every other heavy metal analyzed for in the study, except in the case of Al where same removal rate was maintained in treatments B and C ( $0.0127 \text{ day}^{-1}$ ) whereas faster rate  $0.0151 \text{ day}^{-1}$  was obtained in A. Cu removal rate ( $0.0212 \text{ day}^{-1}$ ) was the highest of the entire heavy metals and was obtained with treatment B.

Therefore with optimal heavy metal removal obtained in treatment B, it came as a surprise considering the fact that not only were just three bacteria species used to generate the inoculum, the genus of the bacteria were known to be either bioinsecticidal as with *B.thuringiensis* (Feitelson, 1993; Damgaard et al., 1996) and *L.sphaericus* (Berry, 2012) or just widely distributed in soil as with *R.wratislaviensis* (Warhurst & Fewson, 1994). However, the bioremediation edge of the inoculum in this study may be associated with the fact that *B.thuringiensis* actively thrive in heavy metals polluted environment (Luo et al., 2011) and among its other degradative ability have been found to degrade the metabolites of fipronil (Mandal et al., 2013). Also bioaccumulation of Cd was discovered in Bacillus strain H9 from metal-contaminated soil. Active effect of

*R.wratislaviensis* may be implicated as well considering the fact that it is a known biodegrader of organic pollutants, especially when it breaks down nitroaromatic compounds into 4-nitrocatechol (4-NC), 5-nitroguaiacol (5-NG) and 3-nitrophenol (3-NP) as reported by Navratilova et al (2005). Yet, it appeared the ability of the two aforementioned bacterial species had special degree of synergistic interaction with *L.sphaericus* to produce a better remediation effect which was not same when the entire nine microbes were used together. Most probably, the His<sub>6</sub> – tag at the C-terminus of its S-layer protein optimized its metal binding effect (Tang et al., 2009) in the presence of *B.thuringiensis* and *R.wratislaviensis*, just as it may be the ability that also enabled *L.sphaericus* DMT-7 to desulfurize 60% of dibenzothiophene into 2-hydroxybiphenyl in 15 days after it was isolated from diesel contaminated site (Bahuguna et al., 2011).

Though treatment A did not show equal removal with treatment B, it showed significant removal and *P.putida biotype B* may be highly implicated considering its track record in remediation studies (Farrell & Quilty, 2002; Newton et al., 2005; Valls et al., 2000; Choudhary & Sar, 2009; Plociniczak et al., 2013; Xu et al., 2012). But it may not have performed in isolation since *F.oryzihabitans*, though without report on its ability to degrade heavy metals but its ability to exhibit chemotactic effect towards oil-gas and hexadecane (Lanfranconi et al., 2003; Parales & Haddock, 2004) may imply its potential to remediate some other components which include heavy metals.

Therefore, bioremediating leachate contaminated soil of its heavy metals content is possible with the use of indigenous microbes. However, combination of the microbes affects the optimal removal of the heavy metals, hence selection and further trials become paramount.

## CHAPTER SIX

### CONCLUSION

The study concludes that leachate contained toxic components which include ammonia, dissolved organic matters, some semivolatile organic carbon compounds and monocyclic aromatic hydrocarbons. Comparison of the physico-chemical properties of raw leachate from the studied landfills proved that leachate regardless of the landfill status (active/inactive or sanitary/non-sanitary) still contains toxic compounds especially in combined state.

A whole effluent toxicity test gave the indirect information on the toxic effect of discrete chemical components when existing as a mixture like leachate. Tests have shown that even at low concentrations leachate can be toxic to aquatic life. Bioassay variation has shown a remarkable difference between impact of leachate from active and non-active landfills. It can also be concluded that ammonia toxicity might be a major concern when dealing with leachate from non-active landfill. Hence there is a strong relationship between the operational status of landfill and its toxicological or environmental impact.

This study herein proposed the FLLTI as a toxicity tool in Malaysia which can be used for leachate impact evaluation. It therefore concludes that FLLTI value above 1 should be considered too toxic. Such can be indicator for the need of adequate preventive measures necessary to block such leachate from finding its way into the aquatic environment.

It is established that leachate not only harbour pollutants like monocyclic hydrocarbons and organopesticides but heavy metals. It not only causes tissue/cellular disruption but

also can also initiate heavy metals bioaccumulation. Therefore, this study concludes that the concentration of heavy metals in *P.sutchi* exposed to the landfills leachate were high and may pose greater risk via bio-uptake in other organisms.

Tissue examination in this study confirmed that leachate exposure does not only allow for bioaccumulation of toxicants in the fish species, but it actually disrupts cellular arrangement and function. The study also concludes that the poor staining of the gills and liver implies that the nucleus and cytoplasm of the fish cells are lysed upon exposure to leachate.

Though landfill leachate is toxic, yet microbes especially bacteria still survive the leachate polluted soil. Distribution of microbes on the contaminated soil includes both gram-positive and –negative bacteria. Microbes found are those with either clinical relevance or known bioremediation potential, whereas a few are just known to be widely distributed in soil. Therefore diverse genera of bacteria are obtained in leachate contaminated soil.

Leachate-site persistent microbes can remedy heavy metals in leachate polluted soil. However, the study confirmed that selective use of the microbes especially the gram-positive bacteria give optimal heavy metal removal from the soil. Also wide spectrum of heavy metals is removed with Cu as the optimally removed in the presence of *B.thuringiensis*, *L.sphaericus* and *R.wratislaviensis*. The study therefore proposed the use of first order kinetic model for generation of the heavy metals removal rate for bioremediation leachate pollution in soil.

Finally concludes that the varied impact of leachate is a reflection of the variation in the heterogeneous nature of leachate across different landfills rather than differences in exposed fish species/types. Also bioaugmentation is sustainable and capable of removing heavy metals from leachate contaminated soil.

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