CHAPTER 1

INTRODUCTION

1.0 Background

The Asia-Pacific region which shelters more than 60% of the world's population is currently the most dynamic region in the world with regards to industrialization, commercial and economic activity. The increase in urban population in Malaysia from 6.05 million in 1988 to more than 28.3 million in 2010 (Department of Statistics Malaysia, 2010) resulted in the acceleration of waste generation in urban areas. The refuse generation rate has shown an uptrend from 241 kg/capita/year in 1988 to 438 kg/capita/year in 2007 (Agamuthu, 2010).

World Bank had identified improper waste management to be one of the three main contributors of environmental degradation in Asian countries (Agamuthu, 2011; World Bank, 2011). In Malaysia, urban waste generation increased 3% annually due to urban migration, affluence and rapid development (Agamuthu, 2001). The GNP per capita was RM 22,564 as of 2009 at a growth rate of 4.6% (Department of Statistics Malaysia, 2010). The increase in per capita income, as well as accelerated development of urban areas, the rural-urban migration and changes in consumption pattern (Agamuthu et. al., 2009a) caused the production of Municipal solid waste (MSW) at an alarming rate. The economic boom has also made way to the development and progress of urbanization and these countries faced challenges in terms of volume and complexity of the generated wastes. The scenario becomes more critical due to high population density at some of these countries, like Vietnam, Laos, Indonesia, Thailand, China and others.

Rapid industrialization and urbanization had brought in numerous revolutions in the management of solid waste generated. Some of the changes that have taken place due to developments are:

a) Changes in waste characteristics in accordance with the progressive development and standard of living
b) Increased volume of waste generated
c) Use of modern techniques like pyrolysis, gasification, dry distillation etc. in waste management in some countries
d) Increase in number of high-rise buildings, hypermarkets and industries which are correlated with rapid economic growth and concentration of large population in a particular area.

The quantity of waste produced has increased tremedously and this is creating great pressure on local governments. The local authorities are responsible in managing the wastes efficiently beginning from waste bins, to waste collection, transportation and lastly treatment and disposal. In the past, waste management practices were mostly aimed at short-term solutions with insufficient concern or awareness for long-term integrated waste management solutions. Integrated waste management strategy takes into consideration scarcity of land, financial limitations, effects on health and environment, suitable technology as well as protection and preservation of resources through 3R (reduce, re-use and recycle) programs. The strategies for waste management in developing countries involve many mutually related factors and the challenges faced by authorities are to transform these elements into adaptable solutions.

Malaysians generate approximately 31,000 tonnes of municipal waste daily (Agamuthu et. al., 2009a) and only 75% of these wastes are being collected by the authority, whereas 20% is dumped illegally, and 5% is recycled. Level of per capita solid waste generation changed accordingly with urbanization of more areas, as well as with the advancement of the people's quality of life in the country. The rate in the 1980's was 0.5kg/day and had increased to 1.3kg/day in year 2006 (Agamuthu et. al., 2009a). Current rate ranged at 1.5 - 2.0kg/day in most cities in Malaysia (Agamuthu, 2010). This accelerating trend could be possibly due to the changes in consumption habits which are subsequently influenced by the increasing affordability to consume more goods (Agamuthu et. al., 2009a). The economic status of individuals has a direct impact on the waste generation and waste characteristics. According to an article in local newspaper, The Star dated 10 June 2011, organic kitchen waste for instance leftover food made up nearly 50% of the 31,000 tonnes of wastes generated by Malaysian citizens. Approximately 95% of the collected waste volumes are sent to the landfills (Agamuthu et. al., 2009a) while 5% is recycled. Among these landfills, 90% of them are non-sanitary landfills lacking landfill liners and gas pipes.

Municipal solid waste is defined as solid, other than emission or effluent, and is regarded as inevitable, valueless-by product due to human activities, and is generated at a rate and discarded after use when no longer needed by the generator (Tchobanaglous et. al., 1993; Agamuthu 2010). Leachate is produced from the infiltration of precipitation and internal biological activities at landfills (Zalesny et. al., 2007b; Duggan, 2005). An estimated 2.1 x 10^7 m^3 of leachate is generated per day in at least 90% of the 260 dumps in Malaysia (Agamuthu et. al., 2009b) and without proper treatment, leachate is released into the environment.

Landfill leachate contains organic compound, inorganic ions and heavy metals which alter due to the biochemical processes that takes place during natural decomposition of the waste products (Kjeldsen et. al., 2002; Zalesny et. al., 2007b) and were found to increase environmental strain. Based on the material density, heavy metals are categorized as a group of elements with a density higher than 6g cm^{-3}, such as Cd, Cu, Ni, Zn, Pb and Cr (Mangkoedihardjo et. al., 2008; Jankaite and Vasarevisius, 2007). Among the metals, the presence of As, Fe, CN and Ammoniacal Nitrogen were significant in the raw/ untreated leachate from Jeram landfill, and hence these parameters were investigated in this study.

As is an ubiquitous element which poses a critical harm to human health. It is a naturally occurring pollutant where it gets released through geological weathering. As is given greater importance as it is generated by human activities due to its use as a wood preservative, pesticide, and as an artifact of valuable metal ores (Arthur et. al., 2003) and released through the environment. Reports show that soil arsenic concentrations above 40mg kg^{-1} likely cause toxicological risks, especially in children (Arthur et. al., 2003). As is a carcinogenic substance where high concentrations of As in human body cause endocrine disruption and also variety of other abnormalities (Nilsson, 2000).

Fe is a non biodegradable metal and the toxicity of the metal would make threats on aquatic ecosystem when the leachate was released into the river system. Therefore, removal of Fe by means of wastewater treatment is important and becomes a major concern for protecting aquatic life (Wittbrodt and Palmer, 1995).

Cyanide (CN) is a fast-acting, highly toxic substance but, at the same time, is deemed essential for sustaining life as well as our standard of living (Young, 2001). CN commonly occurs as industrial contaminants of soil. CN reaches toxic levels in the environment solely because of anthropogenic activities although it is produced in small quantities by many different organisms. CN is used in several industrial processes as it has the ability to form stable complexes with a range of metals (Johannes et. al., 1992).

One of the major challenges generally experienced by landfill operators is the concentration of Ammoniacal Nitrogen (NH_3-N) in landfill leachate. Slow leaching of wastes produces nitrogen and unavailability of notable methodology for conversion of NH_3-N in the landfills implies a high volume of NH_3-N in leachate after a long period of time (Hamidi et. al., 2004). There were limited studies in Malaysia on the aspect of removal of NH_3-N, especially in adsorption treatment because literatures reported that the treatment of Ammoniacal Nitrogen from leachate was not well documented (Hamidi et. al., 2004; Bashir et. al., 2010).

Leachate treatment is essential to prevent ground and surface water contamination although contaminant level generally decreased with landfill age (Wong and Leung, 1989; Zalesny et. al., 2007b). Measures to remediate metal polluted soil emphasized the physicochemical techniques such as soil removal and landfilling, stabilization or solidification, physicochemical extraction, soil washing, and flushing (Hosain et. al., 2005; Mangkoedihardjo et. al., 2008). Heavy metals cannot be degraded but can be phytoremediated through stabilization or extraction in harvestable plant parts (Ho et. al., 2008). The uses of phytotechnology in remediating heavy metal contaminated sites have been extensively studied as being reported by Duggan (2005), Jankaite and Vasarevisius (2007), and Arthur et. al., (2003). Hyperaccumulator plants often accumulate a specific element, performs agronomic characteristics and breeding potential (Ho et al, 2008; McIntyre, 2003). They have a potential of accumulating copper (Cu) > 1,000 mg kg^{-1}, lead (Pb) > 1,000 mg kg^{-1} and zinc (Zn) > 10,000 mg kg^{-1} in their shoot dry matter (Haque et. al., 2007). In that case, an ideal plant for reclamation would be a high biomass producing crop which can bioaccumulate, as well as, tolerate Fe, As, CN and NH_3-N and well-adapted to the harsh environment of leachate polluted site.

The two test plants selected for this study are Kenaf and Akasia. Kenaf (*Hibiscus cannabinus* L.), a potential paper pulp crop is an herbaceous plant. Numerous recent research and development works have demonstrated the suitability of Kenaf for use in building materials (particleboards), as adsorbents, for textiles and fibers in new and recycled plastics (Kalaycioglu and Nemli, 2006; Ho et. al., 2008), soil-less potting mixes and as packing material. In the previous studies, phytoremediation potential of

Kenaf was assessed mainly for Cd, Pb and Se uptake (Banuelos, et. al., 1997; Hiroyuki et. al., 2006). Kenaf was also extensively studied as a promising candidate for forage production.

Acacia mangium is a fast growing leguminous tree and very tolerant to disturbed well-drained acid soils (Norisada et. al., 2005). The timber *of Acacia mangium* is suitable for the manufacture of furniture, turnery, wall panelling, veneer and plywood, composite panel products, fibre for medium density fibreboard (MDF) manufacture, tool handles, interior finishing and other general utility purposes. It has been reported that the sawdust of Acacia provides a good medium for the production of shitake mushrooms (Lim et. al., 2003). Looking at the economical potential of the plant selected, this research aims to assess the effectiveness of Kenaf and Akasia for removal of As, Fe, Cn and NH_3-N from the soil treated with leachate.

Kenaf and Akasia were engaged for this study as both plants are aesthetically appealing to provide landscape-pleasing environment and they are potential phytoaccumulators of nutrients and metals (Choo et. al., 2006). Both Kenaf and Akasia have extensive root system and accomodate large surface areas for the formation of biofilms and hence intensify the microbial activities in the degradation of pollutants (Choo et. al., 2006). To date there have been very few investigations on the use of Kenaf and Akasia for wastewater treatment.

High costs associated with the treatment of heavy metal waste from contaminated soil have created an opening in the environmental remediation market for innovative and cost effective technologies. Phytoremediation systems based on utilizing the metal accumulating ability of certain plants for removal of heavy metals and radionuclides from soils and water, promised efficient and lower cost for clean-up of sites contaminated with low levels of metal. Phytoremediation gives advantages of non-intrusive treatment methods used to reduce the amount of hazardous material by clean up technology (Kamal et. al., 2004). Phytoremediation is exceptionally diverse, and can be employed anywhere for instance soil, water, from hazardous waste sites to low-level contaminated sites only in need of superficial treatment. It is also very productive when applied in conjunction with other mechanisms of remediation. Phytoremediation systems focused on heavy metal treatments which are classified into three processes, phytoextraction, phytostabilisation and rhizofiltration (Salt et. al., 1995). Phytoextraction and phytostabilisation refer to decontamination/ detoxification of contaminated soils using special metal accumulating plants. Rhizofiltration processes focus on using plant roots to purify contaminated waters. Terrestrial plants are hydroponically grown and supported so that their roots are immersed in the target water. The toxins in the water are removed by the roots via physical and active biological processes, resulting in a purified effluent and a small amount of hazardous biomass requiring disposal.

Wetlands treatment is defined as "A wastewater treatment system utilising the aquatic root system of cattails, reeds, and similar plants for the treatment of wastewater applied either above or below the soil surface" (Pankratz, 2001). Constructed wetlands are man-made system which associates altering the existing terrain to simulate wetlands conditions (Idris et. al., 2010). Constructed wetlands have been utilised for a variety of functions, from rehabilitation of areas to serving much specialised aim such as wastewater treatment (Liu et. al., 2007). Recently, there has been particular concern in the application of constructed wetlands for the uptake of toxic metals from contaminated water, soils, and sediments (Deng et. al., 2004). The various capacity and quality values of wetlands are widely recognized, particularly with regard to their abilities to improve water quality for reuse, also as a final polishing system (Fields, 1992; Hung et. al., 2010) for various types of polluted effluents before discharging to other receiving water systems. It has recently been proven to be one of the cost effective means of leachate and wastewater management (El-Gendy, 2003). Constructed wetland treatment systems offer an effective low cost, low technology alternative method for pollutants removal compared to conventional technologies. This biological treatment system only requires lower operation and maintenance costs and rely on renewable energy sources such as solar and kinetic energy (Idris et. al., 2010), as well as potential energy present in associated plant biomass and soils for continued operation. Wetland plants and microbes are the active agents in these pollutants removal processes (Idris et. al., 2010). In the case of this study, the terrestrial plants used in the "pot-culture" were tested hydroponically. Metal uptake efficiency of the test plants were compared between two (2) systems applied.

Currently, the availability of a decision support system for phytoremediation process in the market or free downloadable online softwares are next to nil. Therefore, development of a Decision Support System (DSS) entitled Phytoremediation Modeling System version 1.1 (e-PMS) is proposed to evaluate contaminant uptake ability by the test plants. This new and innovative artificial intelligence system will be developed using the platform of Visual Basic Edition 2005 programming language. The ability to build a decision support as an assessment tool of phytoremediation process, i.e. mathematical modeling of Bioconcentration Factor (BCF), Transocation Factor (TF) and deciding on phytoremediation mechanism is critical for the implementation of pilot or large scale systems based on these processes.

This study was carried out utilizing wastewater in the form of landfill leachate as a source of irrigation and fertilization for Kenaf in 'pot culture system' and also leachate as nutrient source in 'hydroponic system'. In 'pot-culture', leachate was prepared in different concentrations and applied to the soil to investigate the optimum concentration at which phytoremediation process works effectively. The effectiveness of phytoremediation process was assessed by three determining factors: 1)

Bioaccumulation factor (BCF); 2) Translocation factor (TF) and 3) Relative growth rate (RGR) and 4) First-order Kinetics.

Therefore, this research is proposed to determine the potential of Kenaf and Akasia plants in removing heavy metals: Fe and As; metal: CN and also macronutrient: Ammoniacal Nitrogen from leachate contaminated soil. A comparative study would involve the contaminant removal in hydroponic system. The study would also investigate the effects of different leachate treatments on the uptake ability of Kenaf and Akasia in removing toxic metals. Besides that, the mechanism of toxic metals' removal will also be determined.

1.1 Problem Statement

Landfill leachate is classified as problematic wastewater due to its toxicity and represents a dangerous source of pollution to the environment. Leachate purification is crucial, challenging and often insufficient; therefore the quality of the surface and underground waters are seriously endangered. Contaminated waters from leachate posed major environmental and human health problems. When untreated leachate is discharged, it will cause problem to the environment, especially aquatic life such as fishes and also affects drinking water quality. In addition, leachate contains numerous pathogenic microorganisms, toxic compounds, hormones, nutrients or other compound that may affect human health.

In unlined landfills, leachate is frequently discharged into groundwater or appears as a surface drainage around the base of the landfill. In modern lined landfills, leachate is collected from lined cells and routes to treatment units. Disposal of landfill leachate has become a serious issue in handling solids waste from municipalities due to recent stringent environmental regulations and a high potential for soil, surface and groundwater pollution. Therefore, landfills leachate treatment is needed as an significant element of solid waste management.

Various methods are being used in order to treat leachate. The most familiar system used for leachate treatment is conventional treatment processes that cooperates physical, chemical and biological methods (Wiszniowski et. al., 2006). However, numerous compounds generated from industrial wastes made leachate treatment difficult and costly to be treated using conventional and advanced leachate treatment processes. Besides that, great number of manpower and management are required.

Therefore, it is suggested that phytoremediation can be applied within the integrated system in order to treat landfill leachate. It is a plant based remediation that can remove organic, inorganic pollutants and heavy metal. These plants can remove pollutants because of their plant tissues and their capability to uptake contaminants to extract heavy metals from wastewater. For these reasons, phytoremediation was suggested for this study since the remediation technology may provide an economical solution, that cost less to build and operate, requires less energy and act as natural process for the remediation of both organic and inorganic pollutants present in leachate. Futhermore, phytoremediation techniques applied to landfill leachate is a relatively new field of research in tropical nations and that is why, this study was carried out.

1.2 Research Objectives

The objectives of this study:

1. To analyze the characteristics of Jeram Sanitary Landfill leachate
2. To study the removal rate of selected parameters: Fe, As, CN and NH_3-N from leachate using models, e.g. BCF, TF, RGR and First-order Kinetics

3. To study the effect of phytoremediation using Kenaf and Akasia on landfill leachate
4. To optimize the phytoremediation rate using two test plants: Kenaf and Akasia as well as, two different system: Pot-culture and Hydroponic system
5. Development of Decision Support System (DSS) software for the evaluation of the potential phytoremediator plants

1.3 Research Hypotheses

The overall objective of my research was to test the phytoremediation potential of *H. cannabinus* L. and *A. mangium* in order to make recommendations for similar studies and operational projects, where phytoremediation potential included successful establishment, growth, productivity, and nutrient/ chemical sequestering ability. I sought to identify terrestrial plants which are of economical value with elevated biomass accumulation and tissue concentration of identified elements by conducting *ex situ* experiments. My overarching null hypothesis tested in all experiments was that no differences for phytoremediation capability would be present under the different concentrations of leachate tested. Among other hypotheses are as listed below:

1. *Hibiscus cannabinus* L. and *Acacia mangium* are potential accumulator of Fe, As, CN and NH_3-N from landfill leachate. The contaminant uptake efficiency of both plants is equal. Removal rate of contaminants in both test plants are expected to be in the range of 70-90%.
2. Leachate treatments are superior to control and inorganically fertilized treatments in inducing plant growth and metal uptake efficiency. Leachate treatments (different N-content) which are below than 50% Treated Leachate offers optimum condition in the efficiency of contaminant uptake in this phytoremediation study.

3. Kenaf and Akasia plants grown in Pot Culture system will record better accumulation of Fe, As, CN and NH_3-N compared to test plants grown in Hydroponic-culture system.

This thesis and the published research paper from my work detail the establishment success, growth, productivity, and tissue composition of two different test plants under contrasting irrigation conditions (different concentrations of leachate, varied culture system: Pot-culture and hydroponic culture) resulting in phenotypic responses that will be useful for site managers and serves as a platform for future phytoremediation research and projects direction.

1.4 Dissertation Organization

A brief Introduction (Chapter 1) and subsequent Literature Review (Chapter 2) precedes the Materials and Method (Chapter 3). Results and Discussion (Chapter 4) highlights a detailed technical discussion and general discussion of my key research findings. A Conclusion (Chapter 5) includes my recommendations for future research.

One manuscript that has been published in *International Journal of Phytoremediation* is appended in Appendix E. A Compact Disc containing DSS software developed for the study which is published in .exe format is provided in Appendix D. Additional information that did not fit into any of the Chapters but are relevant to my project is provided in the Appendices.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition and Classification of Solid Waste

Solid wastes are defined as wastes generated from anthropogenic (human) and animal activities. They are commonly solid and unneeded. The solid waste is classified using various manners. Among the classifications are physical (solid, liquid, gaseous); waste materials (glass, paper, plastics, steel, oil); origin (domestic, commercial, industrial, agricultural, medical); original use (packaging waste); physical properties (combustible, compostable, biodegradable) and safety parameters (hazardous, inert, radioactive) (Agamuthu, 2001).

The four main groups of solid waste are:

1) Municipal solid waste (MSW)
2) Agricultural waste
3) Industrial waste
4) Hazardous waste

MSW is primarily the household waste including commercial waste and institutional waste. Composition of MSW depends on several factors i.e. geographical locations which include cultural habits of individuals, living standards, type of residence and seasons. MSW problems have been gaining importance in Malaysia due to the rapid increasing waste generation and the ineffectiveness of the existing mechanism to curb the problem holistically (Agamuthu and Fauziah, 2006).

Hazardous waste contains substances which cause hazards to humans and also the environment. This group of waste is defined by certain characteristics fixed by the individual country (UK, US, etc.). The hazardous effect could be on account of any or all of the following: ignitability, corrosivity, reactivity, toxicity and infectivity (Musee et. al., 2008). Animal waste and plant residue makes up the agricultural waste. Agricultural wastes such as fungicide, herbicide and pesticide containers are classified as hazardous. Industrial waste consists of wastes from industrial processes and some of these may also include hazardous waste.

2.2 Definition of MSW

MSW is an inevitable by-product of human activity (Agamuthu, 2011). MSW basically applies to all wastes produced, collected, transported and discarded of within the rights of a municipal authority. In most scenarios, it contents primarily food, waste and rubbish from housing areas, street sweepings, trade and institutional non-hazardous trash including construction and destruction waste in some countries (Agamuthu, 2011).

2.3 MSW generation in Malaysia

Malaysia, with a total land area of Malaysia is 329,847 km², had a population of approximately 28.96 million in 2010, with a per capita Gross National Income of USD 3,311.76 per person (Department of Statistics Malaysia, 2010). In Malaysia, urban waste generation increased 3% annually (Agamuthu, 2001) and the generation of MSW in Malaysia has increased more than 100% over the past 13 years. Generation of domestic and economic waste hit 8.0 million tonnes/ year, and one quarter of the total solid waste was produced in the Klang Valley by the year 2000 (Agamuthu et. al., 2009a; Agamuthu, 2001; Nasir et. al., 2000). Solid waste generation of Kuala Lumpur is escalating at an alarming rate. The amount of daily residential solid waste generation is reported to be close to 1.62 kg/capita; with the national average at 0.8–0.9 kg/capita. By year 2024, the MSW generation is expected to be rising linearly, reaching to 2.23 kg/capita (Saeed et. al., 2009). In year 2008, approximately 31,000 tonnes of waste were disposed off into 260 landfills in Malaysia (Agamuthu et. al., 2009a). An estimated 20% is burned or dumped into rivers or at illegal sites, while 5% is recycled (Agamuthu et. al., 2009a).This phenomenon is due to rapid development of urban areas, increase in per-capita income, rural-urban migration, which included the changes in consumption patterns which was influenced by the progress of nation (Agamuthu and Fauziah, 2006; Agamuthu and Khan, 1997).

Changes in MSW production rates are influenced by the demographic factors and infrastructures accomodated by the corresponding departments (Manaf et. al., 2009. Table 2.1 shows the relationship between growth of population and solid waste produced in different states in Peninsular Malaysia. Urban population which makes up 69.6% of the total population in Malaysia as at year 2007 (UNdata, 2010), is the main waste generator.

Table 2.1: Waste generation in Peninsular Malaysia

States	Population (2000)	Waste generated (2000)	Population (2001)	Waste generated (2001)	Population (2002)	Waste generated (2002)
Kuala Lumpur	1400,000	2520	1435,000	2635	1470,875	2755
Selangor	3325,261	2826	3408,393	2955	3493,602	3090
Johor	2252,882	1915	2309,204	2002	2366,934	2093
Kedah	1557,259	1324	1596,190	1384	1636,095	1447
Kelantan	1216,769	1034	1247,188	1081	1278,368	1131
Melaka	605,361	515	620,495	538	636,007	562
N.Sembilan	890,597	757	912,862	791	935,683	827
Pahang	1126,000	957	1154,150	1001	1183,004	1046
Perak	1126,000	1527	1841,489	1597	1887,527	1669
Perlis	230,000	196	235,750	204	241,644	214
Penang	1279,470	1088	1311,457	1137	1344,243	1189
Terengganu	1038,436	883	1064,397	923	1091,007	965

Source: Manaf et. al., 2009

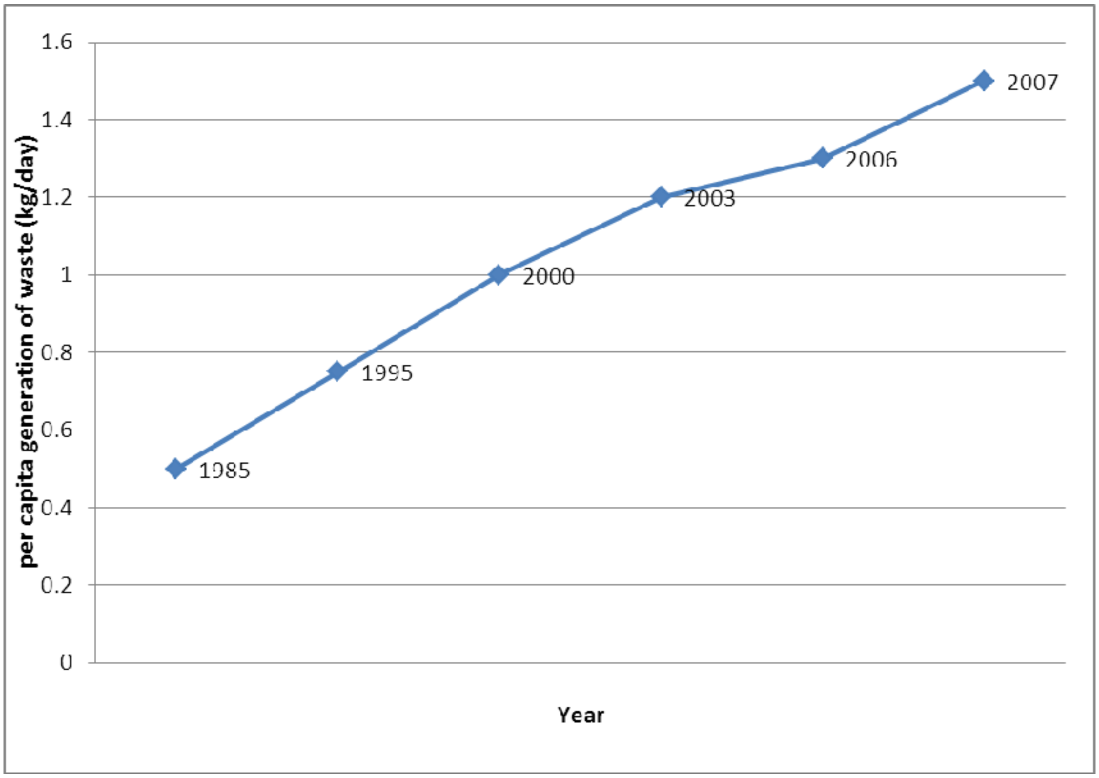
Table 2.2 below shows the trend of waste production in major urban areas in Peninsular Malaysia from 1970 to 2006. The generation volume is dominated by several factors such as family income level, type of housing, consumption pattern, season, education, waste collection system and frequency, and socio-economic practices (Agamuthu, 2011). The average quantity of municipal solid waste (MSW) produced in Malaysia was 0.5 - 0.8kg/person/day and it has rised to 1.7 kg/person/day in major cities (Kathirvale et. al., 2004) in year 2003. Waste quantities generated depended also on the source and the min activities that occurred in the area. The increasing trend per-capita generation of MSW is displayed in Figure 2.1.

Table 2.2: Generation of MSW in major urban areas in Peninsular Malaysia (1970 – 2006)

Urban centre	Solid waste generated (tones/ day)				
	1970	1980	1990	2002	2006*
Kuala Lumpur	98.9	310.5	586.8	2754.0	3100.0
Klang (Selangor)	18.0	65.0	122.8	478.0	538.0
Johor Bharu (Johor)	41.1	99.6	174.8	215.0	242.0
Ipoh (Perak)	22.5	82.7	162.2	208.0	234.0
Georgetown (P.Pinang)	53.4	83.0	137.2	221.0	249.0
Kuala Terengganu (Terengganu)	8.7	61.8	121.0	137.0	154.0
Kota Bharu (Kelantan)	9.1	56.5	102.9	129.5	146.0
Kuantan (Pahang)	7.1	45.2	85.3	174.0	196.0
Seremben (N.Sembilan)	13.4	45.1	85.2	165.0	186.0
Melaka	14.4	29.1	46.8	562.0	632.0

Source: Ministry of Housing and Local Government (2002)

*= estimated figure



Source: Agamuthu, 2001; Ministry of Housing and Local Government, 2002

Figure 2.1: Increasing trend in per-capita generation of municipal solid waste (MSW) from 1985 to 2007

2.4 Malaysian MSW Composition

MSW in Malaysia is highly heterogeneous. Composition of MSW is dynamic and changes with several factors such as status of income, lifestyle patterns, residential type , season, affluence and geographical location. The composition of solid waste from industries differed substantially among the local authorities. In areas where industrial activities are highly concentrated such as Kuala Lumpur city, the composition of wastes from industries was similar to those from the households. Malaysian MSW have capacity for high concentration of organic waste and results in high moisture content and bulk density in the range of 200 to 250 kg/m^3 (Manaf et. al., 2009) as shown in Table 2.3.

Table 2.3: Various data on the characteristic of Kuala Lumpur MSW

Proximate analysis (wet)	**Weight (%)**
Moisture content	55.01
Fixed carbon content	4.37
Volatile matter content	31.36
Ash content	9.26
Elemental analysis (dry)	
Carbon content	46.11
Oxygen content	28.12
Hydrogen content	6.86
Nitrogen content	1.26
Sulfur content	0.23
Heavy metals (dry)	ppm
Chlorine	8.840
Lead	26.27
Mercury	0.27
Cadmium	0.99
Chromium	14.41
Other parameters	
Bulk density (kg/m^3)	240
Net calorific value (MJ/kg)	9.13

Source: Manaf et. al., (2009)

A MSW characterization study generated by different sources in Kuala Lumpur (Table 2.4) observed that food, paper, and plastic were the main components of Malaysian waste which contributed to 80% of overall weight (Manaf et. al., 2009;

Kathirvale et. al., 2004). These characteristics addressed the habits, economic level and nature of the Malaysian population. Early management of solid waste involved little effort since waste was generated at a manageable level and it generally consists of organic materials such as food waste, paper, wood, and others (Fauziah et. al., 2007).

Table 2.4: Average composition (weight percentage) of components in MSW generated by various sources in Kuala Lumpur

Sources	High income residen-tial (%)	Medium income residential (%)	Low income resident-ial (%)	Commer-cial (%)	Institutional (%)
Food/organic	30.84	38.42	54.04	41.48	22.36
Newsprint	6.05	7.76	3.72	7.13	4.31
Mix paper	9.75	7.22	6.37	8.92	11.27
High grade paper	–	1.02	–	0.35	–
Corrugated paper	1.37	1.75	1.53	2.19	1.12
Plastic (rigid)	3.85	3.57	1.90	3.56	3.56
Plastic (film)	21.62	14.75	8.91	12.79	11.82
Plastic (foam)	0.74	1.72	0.85	0.83	4.12
Textile	1.43	3.55	5.47	1.91	4.65
Pampers	6.49	7.58	5.83	3.80	1.69
Rubber/leather	0.48	1.78	1.46	0.80	2.07
Wood	5.83	1.39	0.86	0.96	9.84
Yard	6.12	1.12	2.03	5.75	0.87
Glass (clear)	1.58	2.07	1.21	2.90	0.28
Glass (colored)	1.17	2.02	0.09	1.82	0.24
Ferrous	1.93	3.05	2.25	2.47	3.75
Non-ferrous	0.17	0.00	0.18	0.55	1.55
Aluminum	0.34	0.08	0.39	0.25	0.04
Batteries/hazards	0.22	0.18	–	0.29	0.06
Fine	–	0.71	2.66	0.00	0.39
Other organic	0.02	0.00	–	1.26	1.00
Other inorganic	–	0.27	0.25	–	8.05
Others	–	–	–	–	6.97
Total	100.00	100.00	100.00	100.00	100.00

Source: Manaf et. al., (2009)

The lifestyle modification, especially in the city areas, has persuaded to more chronic waste problems. The 9th Malaysia Plan projected that food waste will make up approximately 45% of the future waste, plastic will make up 24%, 7% consist of paper, and 6% of iron and glass, with the balance made up of other materials (Manaf et. al., 2009) as shown in Table 2.5 and Figure 2.2. The scenario is further depressible in the squatter areas and in slum areas with additional challenges of closely-packed housing and traffic, and water and air pollution (Manaf et. al., 2009).

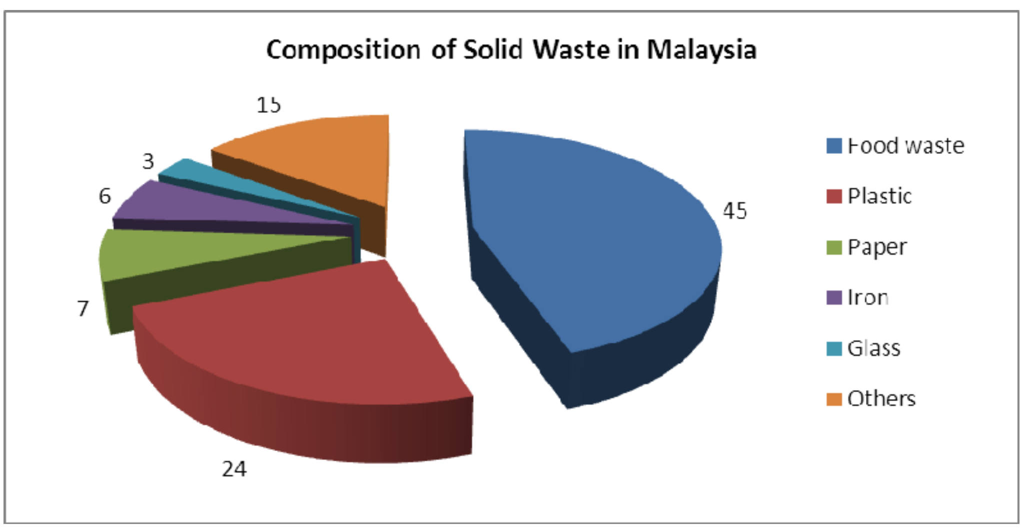
The waste characteristics and compositions in the country were observed to vary with the degree of affluence and urbanization of the served area. Wastes received at landfills in Malaysia are highly mixed with various refuse types, which made the extraction of recyclables almost impracticable. More packaging materials, in the form of plastics and paper are being generated with greater consumerism. Paper wastes include mixed paper, corrugated paper, white paper, newsprint, phonebook and magazines. Food waste was one of the major portions in organic component.

Table 2.5: The composition of solid waste in Malaysia in 2005 (RMK9)

COMPONENT	PERCENTAGE (%)
Food waste	45
Plastic	24
Paper	7
Iron	6
Glass	3
Others	15
Total	100

Source : Ministry of Housing and Local Government

(2010)



Source : Ministry of Housing and Local Government (2010)

Figure 2.2: Composition of solid waste in Malaysia (9th Malaysian Plan)

2.5 Solid Waste Disposal Technology

Treatment and disposal of solid waste varies among different countries depending on the composition of waste, infrastructure, land availability, economic aspects, labour, public awareness, recycling strategy, energy availability and demand, and environmental impact (Agamuthu, 2001). The treatment of solid waste prior to disposal includes recycling, compaction or pulverisation.

Landfilling is the main method used for the disposal of MSW in Malaysia at the present moment (Manaf et. al., 2009). Most of the landfill sites are open dumping areas, which pose critical environmental and social threats (Yunus and Kadir, 2003; Agamuthu and Fauziah, 2011). Landfilling is the cheapest means of eliminating solid urban waste, in terms of capital cost and exploitation (Agamuthu and Said, 2009). Landfilling offers lower cost of operation at only RM 35/tonne, as compared to RM 500/tonne for incineration and RM 216/tonne for composting (Agamuthu, 2001). Landfilling as a mean of wastes disposal is becoming uncompromising because existing landfill sites are filling up at a rapid rate. Nevertheless, construction of new landfill sites is also becoming more problematic because of land scarcity and the increase in land price and high demands, especially in urban areas due to the increase in population growth (Manaf et. al., 2009).

Sanitary landfill or controlled tipping is defined as a method of disposing refuse on land without causing hazard to public health and safety. This method utilizes the principles of engineering to compact the waste to the smallest area and volume and covers the waste with a layer of earth at the end of each day's operation or at more frequent period of time as may be required. Sanitary landfill comprises of depositing the waste in 1-2 m thick layers in low-lying lands or excavations, compacting it to the smallest volume and covering it with earth to a thickness of 15-25 cm daily. Lining materials at the bottom of the landfill prevents toxic elements and heavy metals in the leachate from leaching into the groundwater.

In Malaysia there are nearly 230 landfills identified authoritatively and an approximate of three times more illegal dumps. Most of the landfills are not equipped with facilities to collect and/or remediate the leachate and there is no infrastructure to exploit the landfill gas, therefore they are not classified as the sanitary landfill. There were 155 disposal sites under the responsibility of local authorities in Malaysia in year 2001 (Wan and Kadir, 2001) ranging in size from 8 to 60 ha, subjecting to the location and amount of waste disposed (Manaf et. al., 2009). Many of these sites were open dumpsites, and the ability to contain waste has been overloaded. The operation of these sites has been extended due to the inadequacy of suitable and economical solutions to remediate the waste (Manaf et. al., 2009). Table 2.6 illustrates the present waste management methods in practice since 2002 and it also illustrates the prospective technologies to be applied by 2020.

The impact of MSW landfill on the community is always negative, causing concern and fear not only about gas explosion and odour from such landfill but the pollution of water resources due to landfill leachate contamination (Agamuthu, 2001). Purity of ground water is threatened by the discharge of unmanaged or unmonitored leachate.

Several studies also suggest that of the total MSW collected currently, less than 4% is recycled and the remainder is disposed in landfill sites. Among the factors that contribute to the low rate of recycling is that more than half of the MSW contains food and other organic waste which has limited recycling potential (Agamuthu, 2007). In Malaysia, there are small incinerators in some municipalities but in most places incineration is not being carried out. Therefore, the majority of waste generated is land filled.

The Malaysian government recommended to gradually set up a couple of MSW incineration plants, consequently paying emphasize to the 3Rs (reduse, reuse and recycle) in near future, as conceptualised in Part X of the Solid Waste Bill. The Solid Waste and Public Cleansing Management (SWPCM) Bill 2007 is targetted to bring major transfrmations and challenges in waste management in Malaysia (Agamuthu et. al., 2009a).

Table 2.6: Methods of waste disposal practices in Malaysia

Treatment	Percentage of waste disposal		
	2002	2006	Target 2020
Recycling	5.0	5.5	22.0
Composting	0.0	1.0	8.0
Inert landfill	0.0	3.2	9.1
Incineration	0.0	0.0	16.8
Sanitary landfill	5.0	30.9	44.1
Other disposal sites	90.0	59.4	0.0
Total	100.0	100.0	100.0

Source: Agamuthu et. al., 2009a

2.6 Definition of landfill

Landfills are land disposal of waste where solid wastes are left to disintegrate and contaminants from the waste attenuates naturally. A landfill as shown in Plate 2.1 can be conceptualized as a biochemical reactor, with solid waste and water as the major input and landfill gases and leachate as the principal output (Hassan et. al., 1999). “Sanitary landfill” defined as “engineered facility for the disposal of municipal solid wastes designed and functioned to reduce public health and environmental impact” (Zaini, 2004). Landfilling – systematic operation by which residual solid waste is disposed in landfill, includes supervising the incoming waste stream, distribution and compaction of the waste, and set up of landfill environmental monitoring and manage facilities.

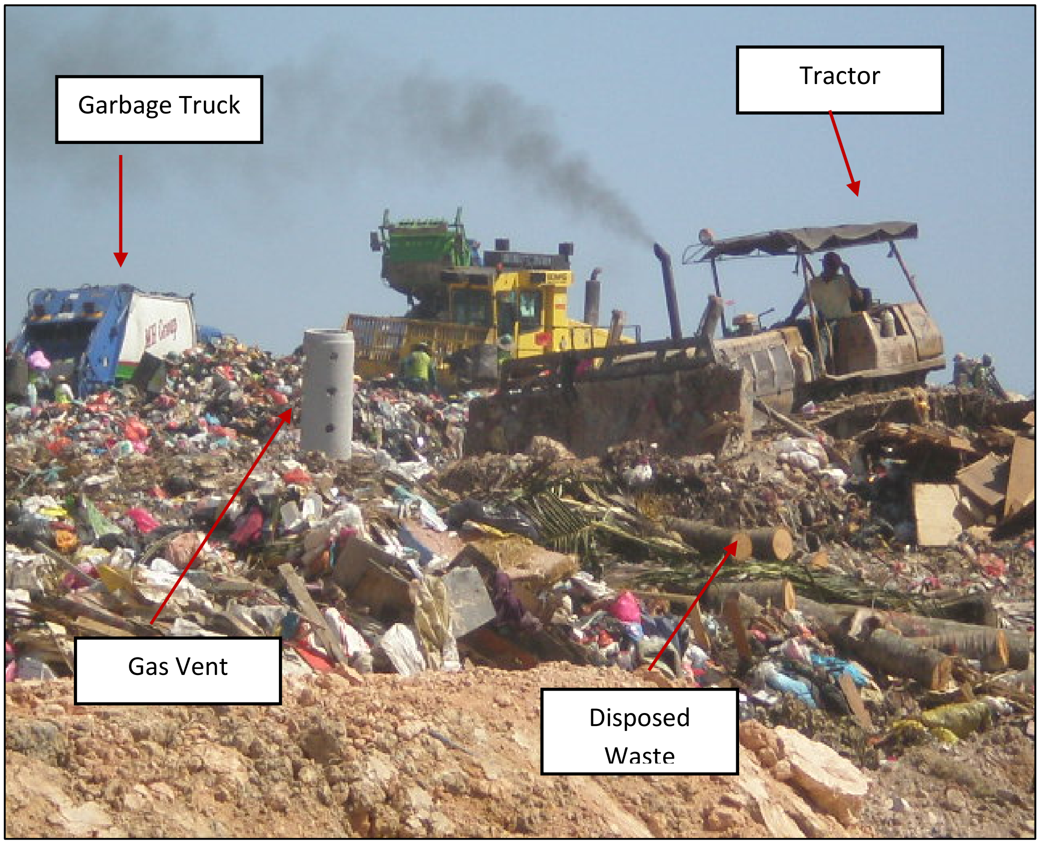


Plate 2.1: Typical view at a landfill

Table 2.7 and Table 2.8 show the landfill classification and landfilling methods in Malaysia (Zaini, 2004).

Table 2.7: Landfill classification

Class	**Type of Wastes**
I	Hazardous waste
II	Designated waste
III	Municipal solid waste

Source: Zaini, 2004

Table 2.8: Landfilling Methods

Methods	Types of wastes
Excavated cell	• Adequate depth of cover material is available • Far from water table
Area	• Where terrain is unsuitable for excavation of cells • High groundwater conditions
Canyon/ Depression	• Canyons, ravines, dry barrow pits and quarries • Depends on availability of adequate material to cover the individual lifts and final cover

Source: Zaini, 2004

Sanitary landfills are generally classsified into 5 levels (Department of Local Government, 2006b) and there are four levels of betterment, which are addressed (Idris et. al., 2004; Manaf et. al., 2009) as listed in Table 2.9. Higher level landfill will pose lower environmental impact and thus fewer countermeasures will be necessary for closure and subsequent post closure utilization. New facility should be designed to achieve at least Level 3 landfill. However, for existing landfills, rehabilitation and improvement targets must achieve Level 3 or higher (Department of Local Government, 2006a). An example of open dumpsite and sanitary landfill Level IV are illustrated in Plate 2.2 and Plate 2.3.

Table 2.9: Level of sanitary landfill system

Level	Description
Level O	Open dumpsite
Level I	Controlled tipping
Level II	Sanitary landfill with bund and daily cover
Level III	Sanitary landfill with leachate recirculation system
Level IV	Sanitary landfill with leachate treatment facilities and more

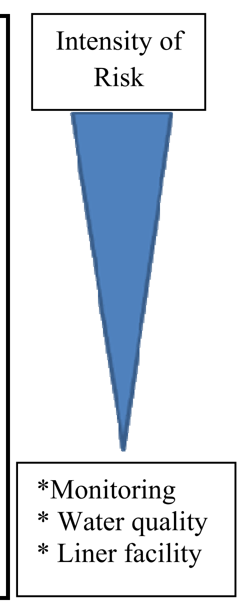


Plate 2.2: Open dump in Selangor (municipal solid waste) – Level 0 Landfill

Plate 2.3: Waste Management Centre in Bukit Nenas, Negeri Sembilan (Scheduled waste) - Level IV Landfill

In the year 2009, there are approximately 290 landfills in Malaysia, where 61% of the total was in operation and the rest have been closed as shown in Table 2.10. In Malaysia only 10% of the landfills are sanitary while the remaining 90% are either modified dumps or illegal dumping sites. Figure 2.3 displays the features of a typical sanitary landfill. Leachate treatment techniques vary with the level of landfill but in most cases it is still very rudimentary. In some landfills total integrated system is used, i.e. Bukit Tagar landfill where physical, chemical and biological treatments are in place. Additionally phytoremediation is employed to enhance the effluent quality to Malaysian standards (Agamuthu and Said, 2009).

Table 2.10: Number of landfills that were in operation or closed throughout Malaysia as at September 2009

STATES	NUMBER OF LANDFILLS IN OPERATION	NUMBER OF LANDFILLS THAT IS NOT IN OPERATION
Johor	13	21
Kedah	10	5
Kelantan	13	4
Melaka	2	5
Negeri Sembilan	8	10
Pahang	19	13
Perak	20	9
Perlis	1	1
Pulau Pinang	1	2
Sabah	21	1
Sarawak	51	12
Selangor	6	12
Terengganu	9	12
Wilayah Persekutuan KL	1	7
Wilayah Persekutuan Labuan	1	0
Total	176	114
Grand total	290	

Source: Ministry of Housing and Local Government, 2010

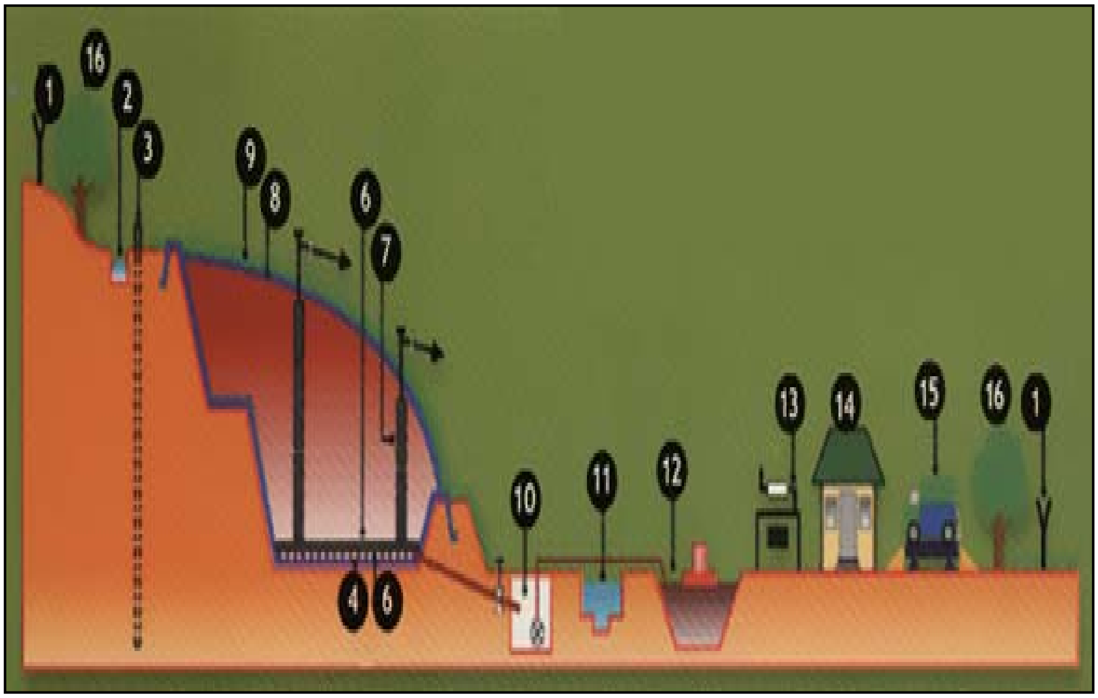


Figure 2.3: Features of a Sanitary Landfill

1- Perimeter fencing	9- Restored Surface
2- Surface Run-off Drainage	10- Leachate Pumping Sump
3- Groundwater Monitoring	11- Perimeter Drainage System
4- Lining System	12- Leachate Treatment Plant (LTP)
5- Leachate Collection System	13- Landfill Gas Power Generation Plant
6- Leachate Drainage Pipe	14- Operation Office
7- Gas Well for Landfill Gas	15- Weighing Bridge
8- Capping	16- Screening Tree

2.7 Jeram Sanitary Landfill

Jeram landfill is a young landfill of 4 years, commenced since 1 Jan 2007. Jeram landfill, a Class IV landfill is one of the 2 operating landfills managed by Worldwide Landfill Sdn Bhd. in the west part of Selangor state. WORLDWIDE Landfills Sdn. Bhd.

is a well known company engaged in waste management in Malaysian and committed to provide Selangor (the most developed state in Malaysia) with state-of-the-art waste management services. The landfill is located in Mukim Jeram, Selangor and used for the disposal of municipal solid waste (MSW) generated mainly from western part of Klang Valley. The site covers 64.7 hectares of Selangor state land near Mukim Jeram and located approximately 25km northwest of Shah Alam. Jeram landfill is situated within the Tuan Mee Oil Palm Estate. It is situated about 10 km southeast of Jeram town, between Sembilang River in the north and Tambak Jawa Besar River in the south. Figure 2.4 shows the Google map of Jeram landfill.

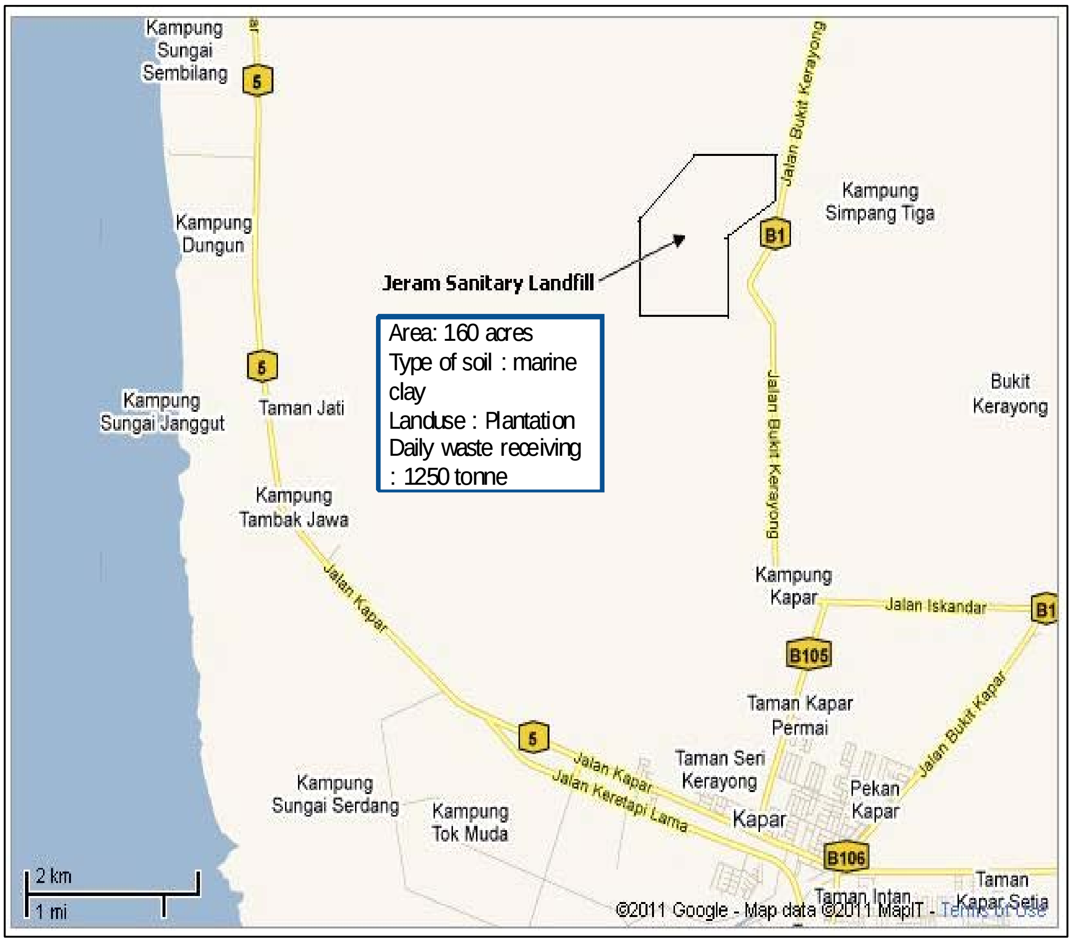


Figure 2.4: Google map of Jeram Sanitary Landfill

The site has been designed to receive about 1,000 to 1,500 tonnes of waste per day and expected to have an operation lifespan of approximately 10 years. Till August

2008, the capacity of the Jeram landfill has increased to 2,000 tonnes waste daily. The landfill is part of the Integrated Waste Management System (IWMS) for the State of Selangor and the Klang Valley, complementing other solid waste disposal sites in both regions, which have been identified by the Government of Malaysia. Table 2.11 summarises the characteristic of Jeram landfill.

Table 2.11: Summary of Physical Characteristic of Jeram sanitary landfill

Site Name	Jeram Sanitary Landfill
Area	64.7 ha.
Height of landfill	20 m
Site Location	• Ladang Tuan Mee, Mukim Jeram
Topography	• Flat with average ground level of RL 3.5 m. • Small hillock of elevation less than 100m
Geology and soils	• Overlain by thin layer of peat • Underlain by marine deposits of Quaternary age • Bedrock of Carboniferous age of phyllite and sandstone
Climate & Metrology	• Tropical climate with high rainfall and uniformly high humidity and temperature
Land use	• Oil palm plantation
Hydrology and drainage	• Near Sg. Sembilang river system
Groundwater & number of boreholes	• 4 boreholes available

In general, the area of Tuan Mee Oil Palm Estate is flat. The area is located approximately 8km from the Mean High Water Spring and therefore does not fall under the category of coastal area. Yearly trend for 5-year average of rainfall is between 2,640.6 and 2,822.6 mm, temperature is 27.5 to 28.1 ^{o}C, and relative humidity is 78.0 to 80.0%. The area is underlain by soft to medium stiff soil of low permeability. The soil is mainly composed of marine clay and sandy silt at a thickness of about 10 to 25 m. The upper layer of the soil (5 to 15 m of thickness) is softer and clayey as it was developed by the recently deposited marine alluvial clay. The soil is underlain by very hard soil which is mainly the slightly weathered shale. There is a buffer zone of 500m around the vicinity of the landfill site.

Jeram landfill operates based on Cell Method where the high density polyethylene membrane (HDPE) is used as the base liner (Plate 2.4) for the cell and GCL (synthetic clay liner) used in between geotextile. The landfill has 500 metres of buffer zone. Types of solid waste permitted in Jeram sanitary landfill are household/ domestic waste, market waste, street/public cleaning waste, commercial waste, light industrial waste, construction waste and also condemned food waste.

This landfill does not accept the disposal of toxic and hazardous clinical wastes. Features of Jeram Sanitary landfill includes: Lining system; leachate collection system (Plate 2.5); Gas Well for landfill gas (Plate 2.6); leachate treatment plant; Operation office; Weighing bridge (Plate 2.7); and compaction of waste (Plate 2.8).

Plate 2.4: HDPE liner for the cells at Jeram landfill

Plate 2.5: Equalisation Lagoon at Jeram landfill

Plate 2.6: Gas collection system at Jeram landfill

Plate 2.7: Weighing bridge

Plate 2.8: Compaction of waste

2.8 Composition of biomass in Jeram MSW

Biomass material, i.e. paper, food, and yard wastes, wood, leather and textiles constitutes 62% of the MSW (Table 2.12). The other components include inorganic materials such as metals, glass, gypsum/asbestos from construction and other minerals. The highest percentage of the waste disposed in Jeram sanitary landfill is organic waste, which includes kitchen waste (32%). This is typically similar to other developing and developed countries, as accounted by World Bank, 2011.

Table 2.12: Waste composition (based on on-site segregation) in Jeram Sanitary Landfill (2010)

Type of waste	Collected amount [tonne/day]	Waste composition (Percentage by wet weight basis, %)	Typical waste composition in developing countries (World Bank, 2011)	Typical waste composition in developed countries (World Bank, 2011)
Organic waste	52.30	32.38	58	50
Hard Paper	21.00	13.00	15*	20*
Soft Plastic	18.60	11.52	-	-
Hard Plastic	13.80	8.54	11**	9**
Soft Paper	11.60	7.18	-	-
Debris	10.00	6.19	-	-
Glass	9.70	6.00	2	3
Wood	9.00	5.57	2.9	-
Textile	6.01	3.72	1.3	-
Tin/ Alloy	5.00	3.12	-	-
Polystyrene	1.96	1.21	-	-
Aluminium cans	1.63	1.01	-	-
Electronics (wires)	0.50	0.31	-	-
Metal	0.40	0.25	3	5
Others (Miscellaneous)	-	-	-	-
TOTAL	161.5	100	-	-

* Hard paper and Soft paper are shown generally as Paper by World Bank, 2011

** Hard plastic and Soft plastic are shown generally as Plastic by World Bank, 2011

Source: World Bank, 2011

2.9 Leachate

The three main outputs from a sanitary landfill are gas, leachate and inert solid wastes. Leachate has the capability as contaminating liquor, which accumulates underneath a landfill site. Leachate is the outcome of the infiltration and percolation of rainfall, groundwater, runoff, or flood water into and through an operational or abandoned solid waste landfill site (Alkassasbeh et. al., 2009). Landfill leachate is generated when rainwater mixes with the waste in a landfill (Tatsi and Zouboulis, 2002). It is generated as a result of the degradation of organic materials and the quantity of leachate generated depends on many factors such as precipitation, surface runoff, evapotranspiration, final cover and moisture content (Mohammed and Agamuthu, 2008).

Leachate contains considerable amounts of dissolved organics (BOD and COD), Xenobiotic Organic Compound (XOCs), ammonia, heavy metals, inorganic salts and other toxicants as per reported by Alkassasbeh et. al. (2009), Pivato and Gaspari (2005) and Christensen et. al. (2001). The leachate produced is highly variable in quality (Table 2.13) depending on soil type, waste composition, rainfall, degree of compaction, evapotranspiration, landfill type and age (Agamuthu, 2001).

The average amount of leachate generated is 150L/tonne of waste. It is estimated that the total volume of leachate generated from landfills in Malaysia is about 3.0 million litres per day (Agamuthu and Said, 2009). Municipal landfill leachate have been identified to contain more than 200 organic compounds (Paxeus, 2000; Schwarzbauer et. al., 2002), with more than 35 compounds having the potential to bring harm to the human health and environment (Paxeus, 2000).

Landfill leachate can cause great environmental degradation if it gets into groundwater because it contains large concentration of organic matter (both biodegradable and non-biodegradable carbon), nitrogen (ammoniacal), suspended solids, heavy metals, inorganic salts, chlorinated organic and coloring matter. Usually the concentration of eluted matters in leachate will be the highest during the first 3-8 years (Table 2.14), when biological decomposition is most active but it is very much dependable on factors discussed above, especially the age of a particular landfill (Table 2.15). High level of ammonia is deadly to many living organisms in surface water and results in depletion of dissolved oxygen and eutrophication (Roadman et. al., 2003).

Table 2.13: Components of landfill leachate

Constituent	Value (mg/L) *	
	Range +	Typical
BOD_5	2,000-30,000	10,000
TOC (Total organic carbon)	1,500-20,000	6,000
COD	3,000-45,000	18,000
TSS	200-1,000	500
Organic nitrogen	10-600	200
Ammonia nitrogen	10-800	200
Nitrate	5-40	25
Total Phosphorus	1-70	30
Orthophosphorus	1-50	20
Alkalinity as CaCO3	1,000-10,000	3,000
pH	5.3-8.5	6
Total hardness as CaCO3	300-10,000	3,500
Calcium	200-3,000	1,000
Magnesium	50-1,500	250
Potassium	200-2,000	300
Sodium	200-2,000	500
Chloride	100-3,000	500
Sulphate	100-1,500	300
Total iron	50-600	60

Source: Tchobanoglous et. al. (1977)
*Except pH
+Representative range of values.

In Malaysia, there have been widespread of a great number of unmanaged landfills without suitable bottom liners and leachate collection systems. There are approximately 230 landfills with different ages and sizes identified officially and an estimated three times more illegal dumps. Most of the landfills are not classified as sanitary landfill due to absence of services to collect and/or treat the leachate and there are no facilities to exploit the landfill gas (Alkassasbeh et. al., 2009).

Table 2.14: Physical and chemical characteristics of sanitary landfill leachate from two Malaysian landfills compared with leachate from other countries.

Parameter#	[1]North Jinjang	[1]Kelana Jaya	[2]Canada	[3]Spain	[4]Norway	[4]USA
COD	184.32	619.84	860	113.65	110-9425	3800-38800
pH	8.18	8.15	6.8	8.4	5.9-7.0	5.4-6.4
Conductivity (mS)	27.5	5.04	-	18.52	-	-
N-total	19.36	20.25	-	-	16.6-254	56-630
P-total	25.33	8.25	-	-	0.1-7.7	5.9-11.3
Mg	327.49	32.99	350	-	13-19	-
Ca	919.98	94.98	180	318.5	99.400	-
K	5099.96	524.96	16	730	21.3-219	-
Na	2319.88	389.88	140	938	34.8-462	-
Cl	2979.8	439.42	190	-	68-680	-
F	2.75	3.25	-	-	-	-
Fe	115.5	5.4	5	148.5	11.5-234	-
Mn	8.88	0.63	5	10.52	-	-
Zn	11.65	0.65	2.5	2.16	0.055-2.65	5.3-155
Pb	5.2	2.5	1	0.52	0.001-0.015	0.1-1.4
Cu	0.79	0.79	1.7	0.34	0.008-0.085	0.18-1.3
Cd	0.59	0.19	-	0.23	0.0001-0.002	0.01-0.03

Sources: [1]Agamuthu (2001); [2]Warith and Yong (1991); [3]Corona et. al. (1988); [4]Johnsen and Carlsen (1976)

mg/L except pH (units) and conductivity (mS).

.

There are only several sanitary landfills with leachate treatment and gas exploitation facilities (Alkassasbeh et. al., 2009; Agamuthu, 2001). This leachate is released into waterways through migration away from landfill boundaries after full or partial treatment (Plate 2.9) and the release to the adjacent environment is a critical environmental concern and a danger to public health and safety. Treated leachate contains nutrients and minerals, and thus it is possible that it can be used for agro-irrigation with some pre-treatment.

Table 2.15: Characteristics of leachate from MSW landfills of different age

Component	[1] First 1.3 L/ft^3	[2] 6-9 months	[3] Air Hitam landfill 1 ½ yrs (as at March 1998)	[3] Beringin landfill 15 yrs (as at March 1998)
pH	6.0-6.5	5.6-5.7	7.6-7.9	8.07-8.50
Conductivity (mS)	-	-	17-25	12.6-34.6
Hardness as $CaCO_3$	890-7600	-	315-317	430-720
Alkalinity as $CaCO_3$	730-9500	-	8800-9000	4380-7580
Ca	240-2330	-	48-48	63-166
Mg	64-410	-	47-48	34-81
Na	85-1700	600-900	3040-5660	4200-5640
K	28-1700	600-900	720-1820	1660-1940
Fe, total	6.5-220	60-200	9-10	7-9
Ferrous ion	8.7-8.7	-	-	-
Cl	96-2350	900-1100	2000-2200	1450-2250
Sulphate	84-730	1700-1900	19-20	66-70
Phosphate	0.3-29	-	21-22	9-24
Total-N	-	-	131-140	104-630
Organic-N	2.4-465	-	-	-
NO_3-N	-	<0.1-0.1	-	-
NH_4-N	0.22-480	400-700	2-8	2-23
BOD	21700-30300	10000-20000	1560-1800	560-1520
COD		20000-40000	5370-7040	2050-5230
Total organic C		5000-10000	1614-1694	1380-2070
TSS			1090-1250	670-1050
Organic acid: Acetic Propionic n-Butyric i - Butyric		 2200-3700 1300-2500 2400-5100 200-300		

Source: [1]Mantell, 1975; [2]Wilson, 1981; [3]Agamuthu, 2001
Concentration range in mg/L except pH and conductivity (mS).

Plate 2.9: Leachate flowing into Sg. Kembong river system

Landfill leachate characteristic is influenced by the waste composition in the landfills as shown in Table 2.16. Besides that, leachate characteristics also depend on the volume of the downpour, stage of stabilization and others (Agamuthu and Fauziah, 2010).

Leachate analysis results indicate high amount of pollutants load were released into the adjacent rivers. Malaysian landfill leachate was reported to contain high COD of 1250 to 6660 mg/l and BOD readings of 120 to 1990 mg/l. Presence of high concentrations of K, Na and Ca was also indicate by studies conducted and pretreatment is required prior to biological treatment (Agamuthu and Fauziah, 2010).

Treatment of leachate is an important aspect in municipal waste management system. A number of options and technologies have been applied for the treatment of leachate. This falls into two basic types: physical/ chemical treatment and biological treatment. This is so because of the high ratio of COD/BOD and Ammonia-Nitrogen (NH_3-N), which poses major difficulties in biological treatment of leachate (Daekeun et. al., 2007).

Table 2.16: Characteristics of landfill leachate

Parameter	Kundang landfill (Urban)	Sungai Sedu landfill (Semiurban)	Panchang Bedena landfill (Rural)	EQA 1974 (Amendment 5, 2007)	
				Std A	Std B
BOD_5 (mg/l)	27.5 ± 0.66	22.27 ± 0.46	348.7 ± 134.2	20	50
COD (mg/l)	6232 ± 1824.3	169.3 ± 76.95	5056.7± 867.4	50	100
pH	7.43 ± 0.04	6.72 ± 0.02	8.1 ± 0.1	6-9	5.5-9
TSS (mg/l)	0.06 ± 0.01	0.09 ± 0.001	1.6 ± 0.4	50	100
Hardness ($CaCO_3$) (ppm)	429.3 ± 240.0	135573.3± 3144.9	30533.3± 57.3	-	-
Cd (ppm)	Not detected	0.002 ± 0.001	Not detected	0.01	0.02
Cr (ppm)	0.193 ± 0.02	0.006 ± 0.005	Not detected	-	0.05
Cu (ppm)	0.003 ± 0.002	0.005 ± 0.004	1.0 ± 0.3	0.2	1.0
Pb (ppm)	0.027 ± 0.012	0.147 ± 0.172	41.7 ± 39.2	0.1	0.5
Zn (ppm)	0.060 ± 0.044	0.153 ± 0.102	675.7 ± 548.7	0.2	1.0
Mg (ppm)	4.245 ± 0.420	7.480 ± 3.780	36533.3± 671	-	-

Source: Agamuthu , 2010

Biological treatments such as activated sludge process, attached-growth biomass system, sequencing batch reactor, trickling filters, anaerobic filter and fluidized bed reactor have been used for treatment of leachate. Treatment by biological methods is efficient for leachate of less than 5 years old (Rodriguez et. al., 2000). Physical/chemical treatment such as coagulation-flocculation, flotation, adsorption, chemical precipitation, chemical oxidation and air stripping are being applied successfully for treatment of leachate to remove color, suspended solids, colloidal particles, floating materials and toxic compounds (Renou et.al., 2008; Christensen et. al., 2001). The efficiency of leachate treatment processes depends on the composition of the leachate and concentration of pollutants.

Coagulation-flocculation process has been applied to landfill leachate for the removal of organic and inorganic substances in the form of colloids and suspended solids. Many studies were carried out using coagulation/flocculation process for treatment of landfill leachate (Tatsi et. al., 2003). In coagulation process, very small suspended particles or colloids attract one another when a coagulant such as aluminium sulphate or a polymer is added to the leachate to form larger particles (Bratby, 2006). The coagulant neutralizes the electrostatic charges on the particles, so that the particles can repel each other and permits the destabilized particles to attach together (Rivas et. al., 2003). The process of coagulation can be considered as three sequential steps: coagulate formation, particle destabilization and interparticle collisions. Flocculation is a slow mixing of the coagulant particles to enhance the formation of larger and heavier particles (floc) that can be easily settled by gravity (Tridib and Bhudeb, 2006).

2.10 Standards of Acceptable Conditions for Discharge of Leachate from Landfill according to Malaysian Legislation

In Malaysia, acceptable conditions for discharge of leachate are referred to Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 of Environmental Quality Act 1974. These Regulations applies to solid waste transfer stations and landfills which discharge or release leachate.

2.10.1 Acceptable conditions for discharge of leachate

According to the Regulation, no person shall discharge leachate which contains substances in concentrations greater than those specified as acceptable conditions as shown in the third column of the Second Schedule, onto or into any soil, or into any inland waters or Malaysian waters.

ENVIRONMENTAL QUALITY (CONTROL OF POLLUTION FROM SOLID WASTE TRANSFER STATION AND LANDFILL) REGULATIONS 2009

SECOND SCHEDULE
(Regulation 13)

ACCEPTABLE CONDITIONS FOR DISCHARGE OF LEACHATE

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

**ADMI – American Dye Manufacturers Institute*

Source: Department of Environment Malaysia, 2010

Here are some criterias listed in the Regulation extracted from Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 (Department of Environment Malaysia, 2010):

2.10.2 Monitoring of leachate discharge

An owner or occupier of a landfill that discharges leachate onto or into any soil, or into any inland waters or Malaysian waters shall, at his own expense—

(a) Monitor the concentration of ammoniacal nitrogen in the leachate discharged from the landfill on a continuous basis using the online instrumentation system which is linked to the Department of Environment;

(b) Monitor the concentration of other parameters as listed in the first column of the Second Schedule in the leachate discharged from the landfill; and

(c) Install flow-meters, monitoring equipment, sampling equipment and recording equipment for the purpose of monitoring leachate discharge.

2.10.3 Methods of Analysis of Leachate: THIRD SCHEDULE (Regulation 15)

(a) The 21st edition of "Standard Methods for the Examination of Water and Wastewater" published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Environment Federation of the United States of America

(b) "Code of Federal Regulations, Title 40, Chapter 1, Subchapter D, part 136" published by the Office of the Federal Register, National Archives and Records Administration, United States of America (USA)

2.10.4 Specification of Point of Disharge of Leachate: FOURTH SCHEDULE (Regulation 16)

(a) The discharge point is located within the boundary of the landfill, immediately after the

final unit operation or unit process of the leachate treatment system.

(b) The location of the discharge point is easily accessible and does not pose any safety hazards to personnel performing site inspection or leachate sampling.

(c) The leachate is discharged through a pipe, conduit or channel to facilitate leachate sampling.

(d) The discharge point is physically identified by installing a metal identification sign which reads “Final Discharge Point”.

(e) The discharge point and its surrounding are properly maintained to be free from any obstruction that may pose difficulty or hazards during site inspection or leachate sampling.

2.11 Heavy metals in landfills

Heavy metals in leachate are inorganic elements which have their own importance. Heavy metals are classified as transition metals or metalloids elements that have the higher atomic number than iron (Fe atomic number: 26) and high electronegativity that show toxicity effects on biological systems (Connel and Miller, 1984; Duffus, 2002). Heavy metals are hard to be removed from the environment and different from many other pollutants, cannot be biologically or chemically decomposed and are ultimately undestroyable. Mobility of heavy metals is strongly affected by pH, redox potential and the presence of complexing agents (Bozkurt et. al., 2000).

Generation of acids in aerobic conditions caused the concentration of heavy metals in leachate to be greater during the acidogenic phase of waste (Agamuthu and Fauziah, 2010; Suffian et. al., 2002). Besides that, the surrounding soil of a landfill gets heavily contaminated with metal and non metal elements. The metal contaminants could also exceed the Dutch Intervention Value. Among the examples in Table 2.17 and Table 2.18 illustrates the amount of pollutants released daily from a landfill in an urban area and also the result of surface and deep soil analysis respectively.

Rivers have always been associated with human civilization and often are considered as the life veins of a country. They not only provide many essentials and benefits to mankind such as source of water supply for domestic and industrial usage, irrigated agriculture, livestock rearing and mining activities but in facilitationg transportation and providing drainage needed to alleviate flooding. From an ecosystem viewpoint, rivers are essential for ecological balance in supporting wetlands, aquatic fauna and flora and also fisheries. Heavy metals if not removed, could cause serious consequences to human beings and the environment. Heavy metals can accumulate in the biological tissues of the body and cause serious diseases such as neurotoxic effects, renal failure (lead), genetic anomalies and cancer risk (cadmium, arsenic). This indicates clearly that need for pretreatment of leachate prior to biological treatment (Hamidi et. al., 2007).

Table 2.17: Impact on river pollution caused by leachate contamination

Parameter (g/day)	River adjacent to Kundang landfill
BOD_5	1 238
COD	280 440
TSS	2.7
Hardness (CaCO3)	19 320
Cd	Not detected
Cr	8.7
Cu	0.14
Pb	1.22
Zn	2.7
Mg	191

Source: Agamuthu, 2010

Table 2.18: Average concentration of metal and non-metal elements in surface and deep soil from an ex-landfill

Parameter	Unit	Surface soil (5cm from ground surface)	Deep soil (5cm below the ground surface)	Dutch Intervention Standard
Phosphate	mg/kg	2.5-5.5	0-13.6	-
Fluoride	mg/kg	2.4-7.0	0.5-0.9	-
Sulphate	mg/kg	30.2-946.3	4.2-10.2	-
pH	n.a.	5.8-9.9	7.3-8.2	-
Chloride	mg/kg	6.3-238.3	2.1-8.1	-
Nitrate	mg/kg	4.7-83.3	0.5-5.0	-
Nitrite	mg/kg	1.1-2.9	Not detected	-
Zn	mg/kg	7.7-129.8	Not detected	720
Sb	mg/kg	0-3.0	Not detected	15
Cd	mg/kg	0-0.6	Not detected	12
Cr	mg/kg	0.5-14.1	Not detected	380
Cu	mg/kg	2.3-17.3	Not detected	190
Pb	mg/kg	2.7-148.0	Not detected	530
Ni	mg/kg	0.3-5.0	0-9.0	210
Ag	mg/kg	0-1.2	Not detected	15
Tl	mg/kg	0-58.0	Not detected	15
As	mg/kg	8.8-64.5	0.3-2.7	55
Hg	mg/kg	0-1.4	8.5-11.5	10

Source: Agamuthu and Fauziah, 2010

Arsenic is of particular environmental interest due to its great extent of contamination and toxicity. The United States Environmental Protection Agency had considered Arsenic as carcinogenic in the A group (USEPA, 2003). Several countries are affected by the presence of the element over the maximum allowed limit of 0.05 mg/L (USEPA, 2003). It occurs predominantly in organic form as arsenate (AsV) and arsenite (AsIII). High Arsenic level in landfills is most probably resulted by the disposal of batteries and industrial waste. Hg could have originated from fluorescent bulbs or disposed batteries. As and Hg are of particular attention due to its highly toxic nature (Agamuthu and Fauziah, 2010).

Fe comes from the waste material and the cover soil. It is a vitally important substance discovered in all plant and animal tissues. Fe is essential for photosynthesis and enzyme production in plants and oxygen storage and transportation in animals (Ghaly et. al., 2008). However, an extreme amount of Fe is released in the liquid waste streams from many industries into natural ecosystems. Among the industries are: a) etch baths and spent pickle from plating shops and steel production industry (Park et. al., 2005) (b) acid mine streams from metal and coal mines (Sheoran and Sheoran, 2006) and (c) leachates from MSW landfills (Christensen et. al., 2001). Ferrous iron (Fe^{2+}) is an extremely soluble element which present in the aqueous phase and it is easily absorbed into biological tissues. Ferrous iron is identified to be the most severely harmful form of Fe (Ghaly et. al., 2008).

Cyanide is also highly toxic and reacts rapidly. CN evaporates at pH values lower than 11.5. Thus, cyanide solutions must be handled at a place with good ventilation, and skin contact with either solid or aqueous cyanide species must be refrained. Cyanide is present naturally in plant cells as a by-product in the last step of ethylene synthesis and is rapidly detoxified by reacting with cysteine to form asparagines (Ebel et. al., 2007). The enzyme β-cyanoalanine synthase (CAS) catalyses the conversion of cyanide and cysteine to β-cyanoalanine and sulfide. CAS is greatly apportioned in higher plants and plays a vital role in metabolism of Cyanide (Maruyama et. al., 2001; Ebel et. al., 2007).

The occurence of Ammoniacal Nitrogen ($N\text{-}NH_3$) in leachate is one of the difficulties generally faced by landfill operators. Slow leaching of wastes producing nitrogen (N_2) and insignificant mechanism for conversion of $N\text{-}NH_3$ in the landfills causes a high concentration of $N\text{-}NH_3$ in leachate over a long duration of time (Hamidi et. al., 2004). Past studies reviews that the reduction of ammoniacal nitrogen from leachate was not well documented and to date, there were limited studies in Malaysia on this aspect, particularly in adsorption treatment (Hamidi et. al., 2004). Among the major routes of ammonia removal in constructed wetlands were immobilizations into microbial cells and adsorption onto wetland media besides nitrification (Sun et. al., 2005). Figure 2.5 shows a conceptual model to highlight the possible routes of Ammoniacal Nitrogen removal.

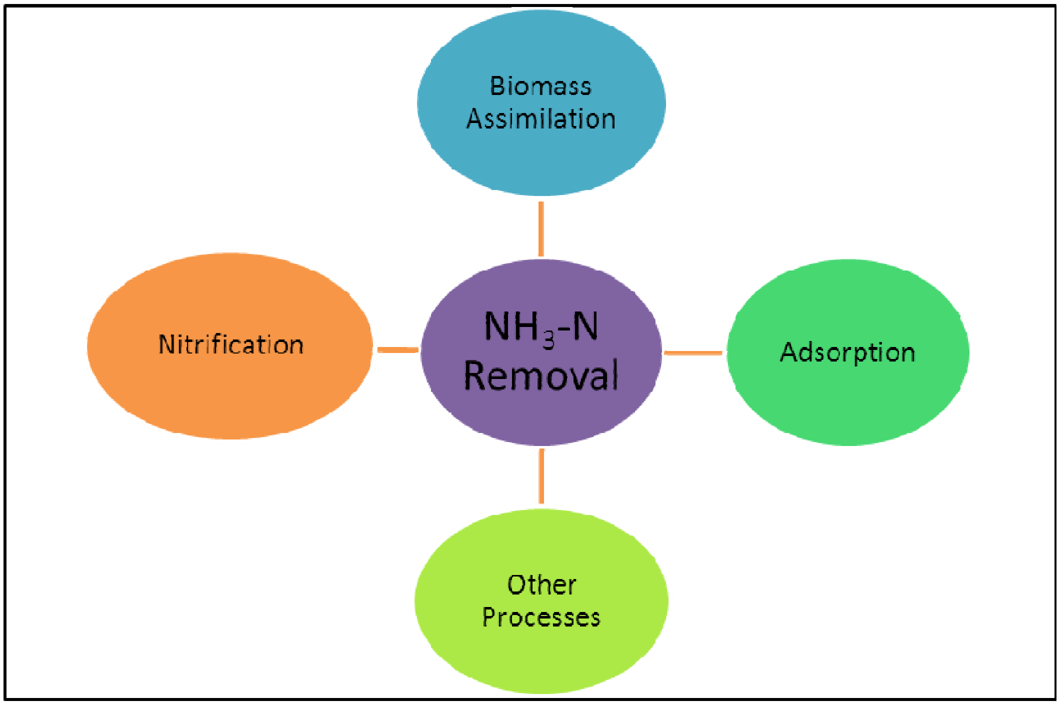


Figure 2.5: The route of NH_3–N removal

2.12 Phytoremediation

2.12.1 Definition of Phytoremediation

Phytoremediation is a technology which utilizes various plants to contain, degrade, extract, or immobilize contaminants from polluted soil or water (USEPA, 2000). The term phytoremediation can be simplified as 'phyto' which is known as plant while 'remediation' known as correct evil (USEPA, 2000). It is also known as a process whereby plants are used for the cleanup of pollutants contamination in soil and water. Phytoremediation takes advantage of the selective and remarkable uptake capabilities of plant root systems as well as the bioaccumulation, pollutants degradation potential, translocation of the entire plant (Tangahu et. al., 2011). Figure 2.6 shows processes of a phytoremediation system model.

Phytoremediation tied the science of plantation forestry with environmental clean- up strategies to attain the following important ecological advantages: 1) phytoremediation takes advantage of natural plant activities whereby the leachate could be biologically treated to remove many of the abundant nutrients and chemicals 2) depending on the contaminants, phytoremediation plants may be harvested in 8 to 10 years for energy or fiber, utilizing short rotation forestry to compensate demand and conserve natural forest stands (Zalesny et. al., 2007a) when plants extracts and sequester excess nutrients and contaminants present in the leachate, it controls the unwanted leaching of potentially toxic contaminants into nearby watersheds.

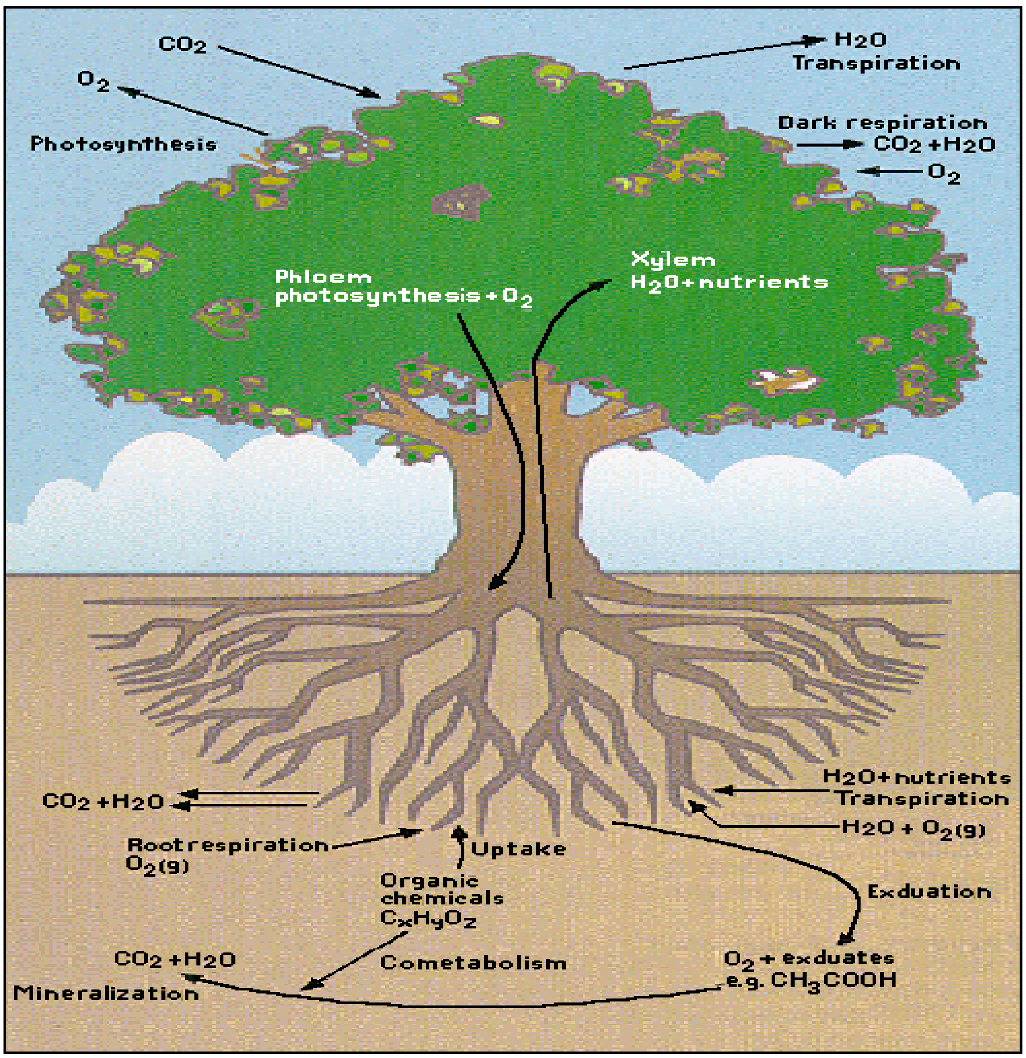


Figure 2.6: Model of Phytoremediation system

Field trials ranged from the removal of radionuclides from a contaminated pond at Chernobyl using sunflowers, to the treatment of lead contaminated soil in New Jersey using successive croppings of a cultivar of Indian mustard. While these experiments are indicative of the success of the phytoremediation technology on the experimental scale, the leap to a marketable, full-scale remediation system poses certain challenges in integrating engineering with biological knowledge.

Phytoremediation covers a diverse range of mechanisms such as phytodegradation, phytostabilisation, rhizofiltration, and phytoextraction. All these mechanisms depend on media (soil or water) and plants used. Current attempts now aimed on extending the phytoremediation methodologies to addresss pollute soils and air pollutants in an effort to preserve the biodiversity of soil and its biota (Markert, 1994). Some plants have the ability to accumulate metals thus called hyper-accumulators or metalloids and some has evolved to tolerate metals.

In achieving phytoremediation for decontaminating soils, plants used for the purpose must have the ability to accumulate pollutants in harvestable segment of the plant. Apart from that, plants suited for phytoextraction should grow at a fast rate and reach high biomass. Plants that produce high biomass and accumulate 1-3% metal, by dry weight are required in decontaminating sites in a reasonable number of harvests (Cunningham and Ow, 1996). To date there are approximately 400 species of hyper-accumulator belonging to 45 families have been identified (Reeves and Baker, 2000). However not all hyperaccumulators suit for phytoremediation if it's slow growing and produce low biomass, which is a waste of time and costly. Another limitation is the possibility of the food chain contamination if animals graze on the heavy metal contaminated crops. In addition, the disposal of harvested plant biomass remains unresolved (Khan et. al., 2000).

Indigenous microorganisms are defined for the microorganisms that are discovered to be already living at a chosen site. Proper/ optimum soil temperature, oxygen, and nutrient content may need to be provided to stimulate the growth of these indigenous microorganisms. Microorganisms from other locations, whereby their effectiveness has been tested, can be added to the soil at the contaminated site if the biological activity needed to degrade a particular contaminant is not present at the site. These additional microorganisms are called exogenous microorganisms. The soil condition at the new site is required to be adjusted to ensure that the exogenous microorganisms can develop vigorously (Prasad, 2007).

Properties of a good phytoremediator plants include high tolerance to the contaminants; rapid growth; intense biomass production; high accumulation of pollutants; economic/commercial value; able to compete with/ tolerant with other species; and large, deep root system.

2.12.2 Uses of Phytoremediation

The advantages of phytoremediation are as listed below:

- **Remediation of different substrates:** Air, Soil, Sediments, Groundwater,Wastewater streams from industrial, agricultural and municipal sources
- **Remediation of different pollutants:**

➢ **Inorganics:** Metals (Pb, Cd, Zn, Cr, Hg); Metalloids (Se, As); Nutrients (K, P,N, S); Radionuclides (Cs, U)

➢ **Organics:** PCBs; PAHs; TNT; Pesticides; Petroleum hydrocarbons

2.12.3 Advantages and limitations of phytoremediation

2.12.3.1 Advantages of phytoremediation

Phytoremediation offers various advantages to people and the environment. The most widely touted advantage of phytoremediation is its cost (USEPA, 2000). Phytoremediation gives lower operating costs compared with other treatment technologies like advanced wastewater treatment. Due to its performance, phytoremediation offers a great number of advantages. It can be permanent treatment solution and as in situ methods. It also has capability of remediation bioavailable fraction and mineralising organics. Other advantages are its applicability to a variety of contaminants and remediating a pollutant with plants is affirmed to be more aesthetically pleasing and possibly more acceptable by the public. This can be simplified as presented in Table 2.19.

Table 2.19: General advantages and limitations of phytoremediation (USEPA, 2000)

Advantages	**Disadvantages/Limitations**
Cost: Low capital and operating cost compared to other treatments	Time: Slower than some alternatives treatments
Performance: • Works on a variety of organic and inorganic compounds • Permanent treatment solution • Can be either in situ or ex situ • Easy to implement and maintain	Performance: • Biological methods does not have the capacity of 100% reduction • May not be applicable to all wastewater/ mixed wastes • High metal concentrations or other pollutants may be toxic
Others: Public acceptance: aesthetically pleasing and environmentally friendly Can be used with other methods	Others: Regulators may be unfamiliar with the technology and its capabilities

2.12.3.2 Limitations of phytoremediation

In spite of the advantages discussed, there are some drawbacks of using phytoremediation as an alternative of bioremediation. The fundamental limitations include the duration needed to achieve cleanup, the restricted depth of treatment, harmful effect on plants, the transfer of contamination across media, and regulatory acceptance. The time period needed for treatment can be a difficulty utilizing phytoremediation, as the process may require a longer time period than other remedial strategies to fulfill sufficient clean up levels. Phytoremediation is also restricted to the depths where the plant roots can reach. In addition, phytoremediation is limited to sites with low concentrations of contaminant by reason of concentrations that are too high can be deadly to plants. Nonetheless, the competency of phytoremediation process can be achieved, if the strategies are combined with other technologies, and further research is needed in the future.

2.12.4 Mechanisms of Phytoremediation

Below are discussions on the mechanisms of phytoremediation which include Phytoextraction, Phytostabilisation, Phytodegradation, Rhizofiltration/Phytofiltration, Phytovolatilisation and Phytostimulation.

2.12.4.1 Phytoextraction

Phytoextraction (Figure 2.7) is also known as phytoaccumulation. It involves a process where the uptake and concentration of contaminants (metals or organics) within the roots or aboveground portion of plants (Prasad, 2007) and the accumulation of contaminants into plant shoots and leaves. Element accumulating plants are harvested in order to remove the specific substances from the soil. Phytoextraction is usually applied to metal contaminated soil by transport and concentrate metals from the soil to roots and aboveground shoots such as leaves (USEPA, 2001).

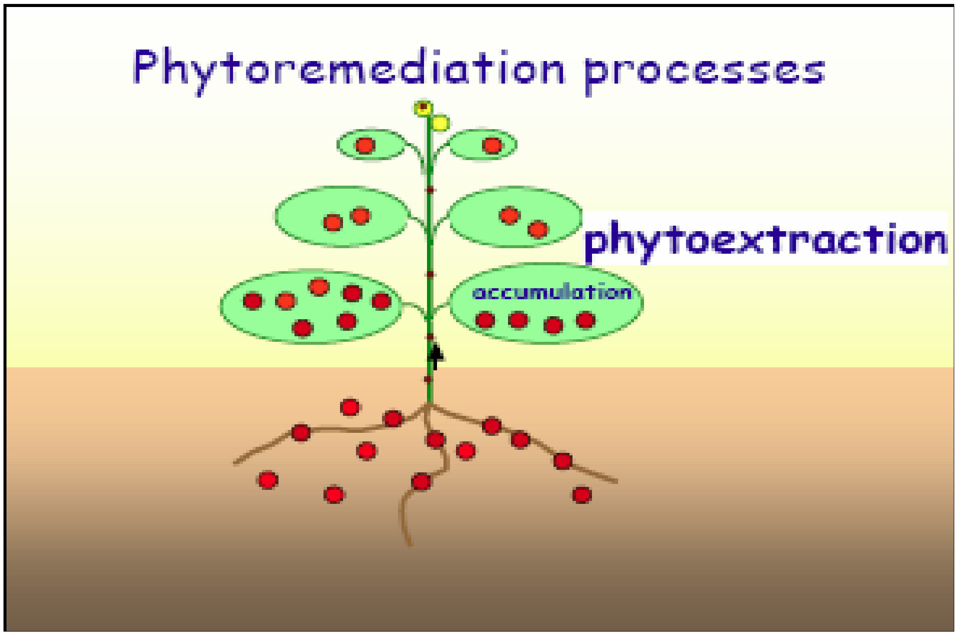


Figure 2.7: Phytoextraction

Blaylock and Huang (2000) suggested that the process of metal concentration outcomes in reduction of contaminated mass and together a transfer of the metal from an aluminosilicate-based matrix (soil or water) to a carbon-based matrix (plants). The carbon (C) in the plant biomass would be oxidized to carbon dioxide (CO_2); following that, the mass of material to be treated, disposed of or recycled will be further decreased and concentrated (Marques et. al., 2009). The phytoextraction process is dependent on the metal being first accumulated by plant roots and then translocated to harvestable plant tissues. The harvestable portions are generally regarded as the aboveground plant material, although roots of some crops may also be harvestable (Marques et. al., 2009).

The use of plants to extract toxic compounds from soil (phytoextraction) is being developed as a method for remediation of metal contaminated soil (Koopmans et. al., 2008). Plants that can accumulate and tolerate unusually high concentrations of heavy metals in their tissues were responsible for drawing attention to the possibility of using plants in this manner. Accumulation of Nickel (Ni) and Zinc (Zn), for example have been reported to contain as much as 5% of these metals on a dry-weight basis (Baker et. al., 1994). Phytoextraction coefficient is defined as the ratio of metal concentration in the plant (g metal/g dry weight tissue) to the initial soil concentration of the metal (g metal/g dry weight soil), for phytoextraction of metals (Prasad, 2007).

2.12.4.2 Phytostabilisation

Phytostabilisation (Figure 2.8) is the phenomenon of formulation of chemical compounds by plants to immobilize contaminants at the surface boundry of roots and soil (Kumpiene, 2005). Plants are capable of immobilizing contaminants in soil through absorption and accumulation by roots (USEPA, 2000).

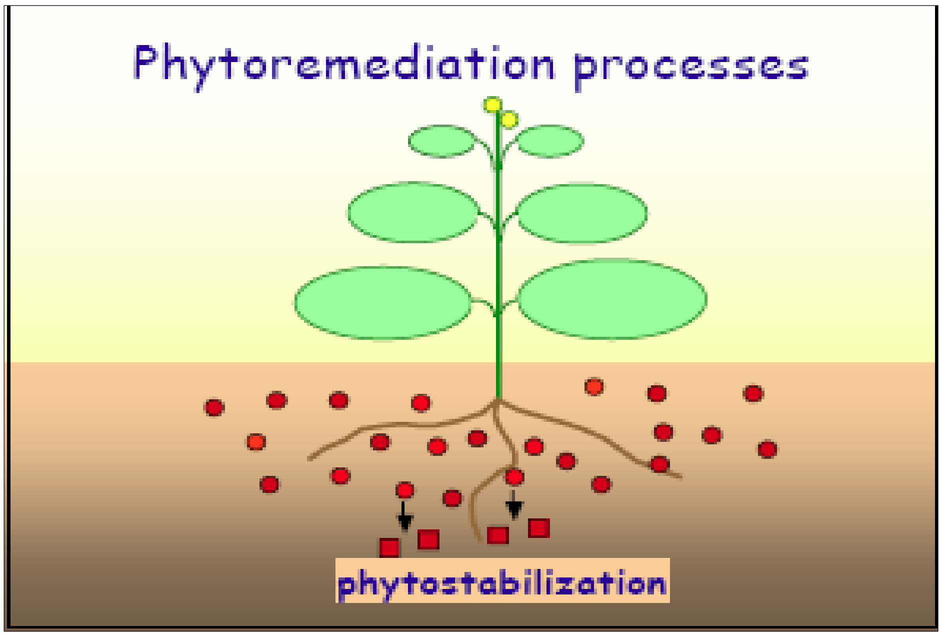


Figure 2.8: Phytostabilisation

2.12.4.3 Phytodegradation

Terry and Banuelos (2000) stated that phytodegradation (Figure 2.9) is the process where organic contaminants are metabolised within plant tissues. Enzymes, such as dehalogenase and oxygenase are produced by plants that help catalyse degradation. Here, the plant has taken up the organic contaminants, they are decomposed through metabolic pathways and incorporated into the plant tissues and used as mineral nutrients (USEPA, 2001).

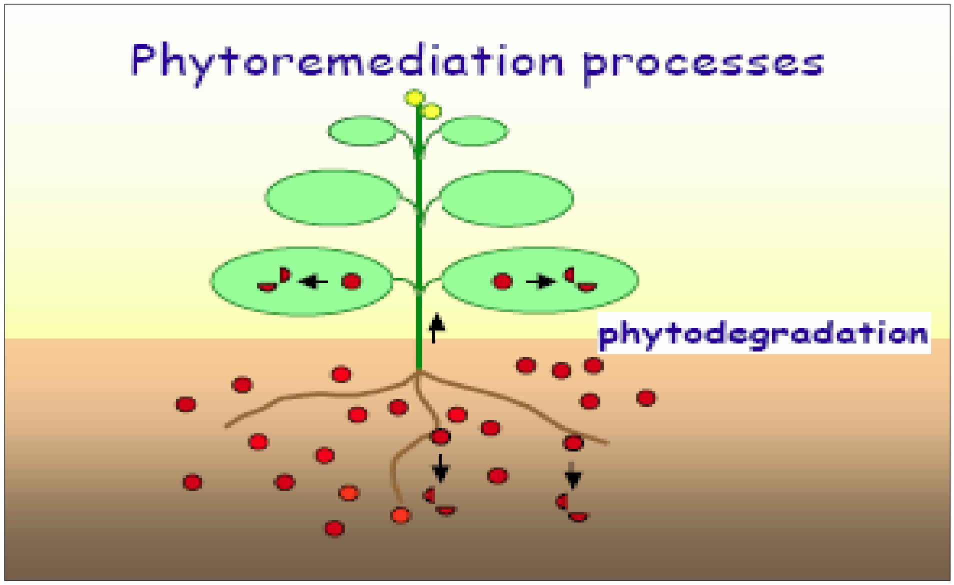


Figure 2.9: Phytodegradation

This process does not apply to inorganic contaminants such as metals, because metal cannot be degraded (Lasat, 2002; Beolchini et. al., 2011). Phytomining is the use of plants to extract inorganic substances from mine ore.

2.12.4.4 Rhizofiltration/ Phytofiltration

Rhizofiltration/ Phytofiltration (Figure 2.10) are the process that involves adsorption or precipitation onto plant roots that is in solution surrounding the root zone (USEPA, 2000). Plant uptake, bioconcentration, and translocation might occur, depending on contaminant. Pollutants removed from water by plant roots in hydroponics system. Exudates from plant roots might cause precipitation of some metals. Contaminants are then removed by physically harvesting the plant. Raychaudhuri et. al., (2008) stated that rhizofiltration act as "biofilter" or biocurtains where it involves biological activity and high-surface-area plant roots. Rhizofiltration is effective in absorbing pollutants from water. For rhizofiltration process, plant should be able to accumulate significant amounts of the contaminant of concern and to tolerate high levels of a toxic metal (at least temporarily). It should also have a high root, shoot ratio and grow safely in environment.

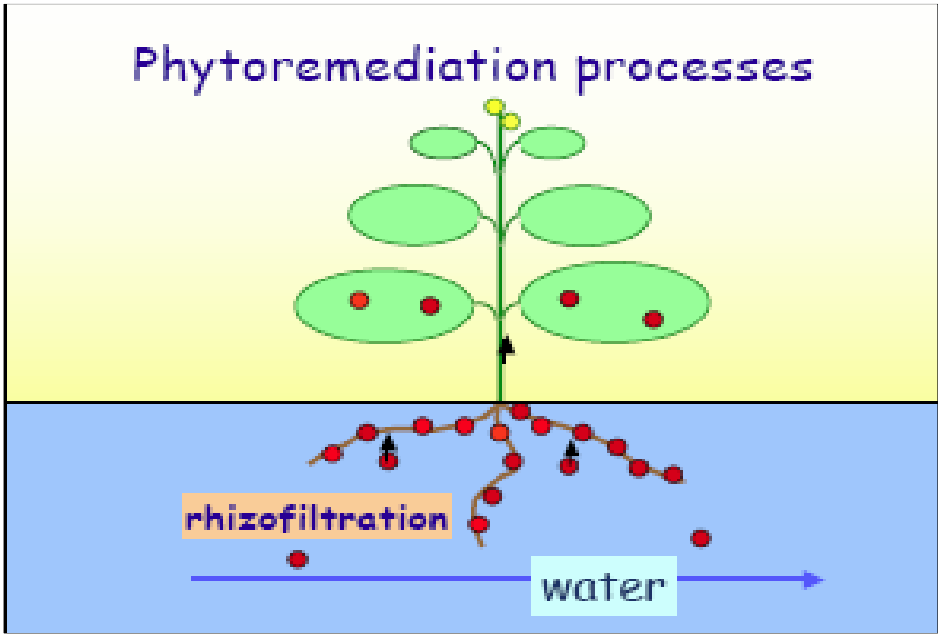


Figure 2.10: Rhizofiltration

2.12.4.5 Phytovolatilisation

Phytovolatilisation (Figure 2.11) includes the uptake and transpiration of a contaminant by a plant. It includes the release of the contaminant or a converted form of the contaminant to the atmosphere from the plant (Vanek et. al., 2010).

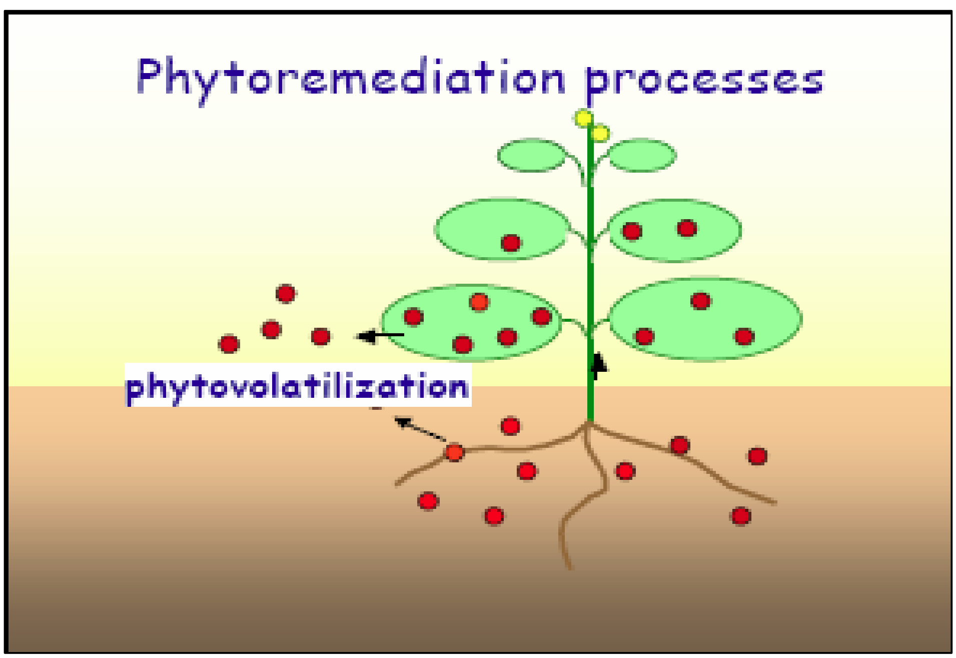


Figure 2.11: Phytovolatilisation

2.12.4.6 Phytostimulation

The mechanism of phytostimulation (Figure 2.12) is the process whereby plant roots stimulate degradation of pollutants by rhizosphere microbes.

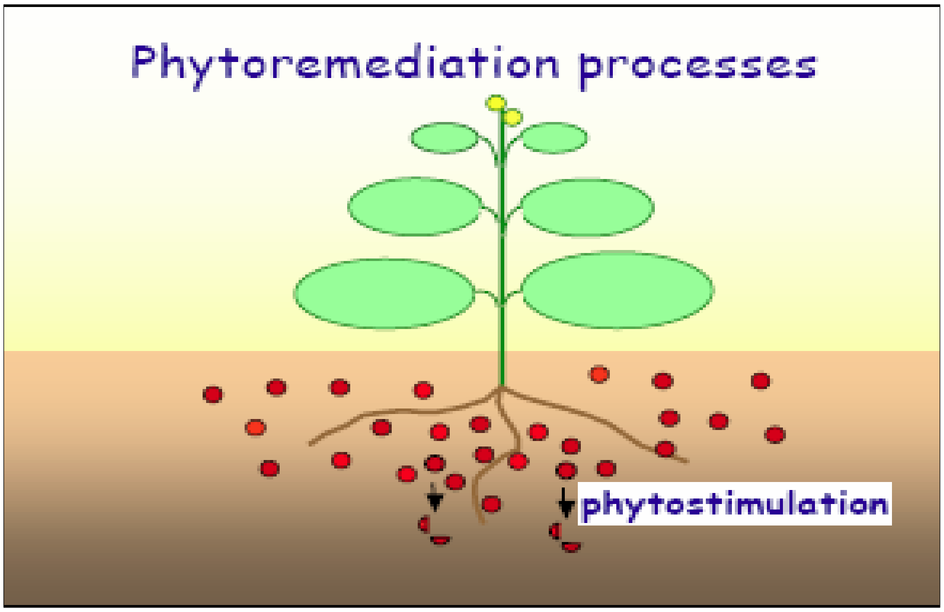


Figure 2.12: Phytostimulation

Table 2.20 shows the media, contaminants and typical plants with difference mechanisms of phytoremediation.

Table 2.20: Phytoremediation overview

Application	Media	Contaminants	Typical plants
Phytoextraction	Soil, Brownfields, sediments, sludges	Metals(Ag,Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn)	Indian mustard, Pennycress, Alyssum, Sunflowers, Hybrid poplars
Phytostabilisation	Soil, sediments, sludges	Metals(As,Cd, Cr, Cu, Hs, Pb, Zn) and Hydrophobics organics (PAH, DDT, PCB)	Indian mustard, Hybrid poplars, grasses
Phytodegradation	Soil, sediments, sludges, groundwater, surface water, landfill leachate, wastewater	Organic compounds, chlorinated solvents (TCE), phenols, herbicides, munitions	Algae, Stonewort, Hybrid poplar, Black willow, Bald cypress
Rhizofiltration	Groundwater, surface water	Metals, radionuclides	Sunflowers, Indian mustard, water hyacinth

Source: USEPA, 2000

2.13 Plant Responses to Pollutants

2.13.1 Plant responses to heavy metal toxicity

The final sink for heavy metal pollutants is the burial in soils and atmospheric deposition (Khan et. al., 2000). They often accumulate in the top layer therefore accessible for uptake by plant roots, which are the principal entry of heavy metal into a plant (Figure 2.13). Plants may take up an excess heavy metal which eventually affecting different physiological process. Stunted growth, leaf epinasty, necrosis, chlorosis (as a result of inhibition of chlorophyll synthesis) and discoloration of leaves are the characteristics symptoms visible to the naked eye in plants affected by severe pollutant toxicity due to altered processes at the cellular level (Diaz et. al., 2001).

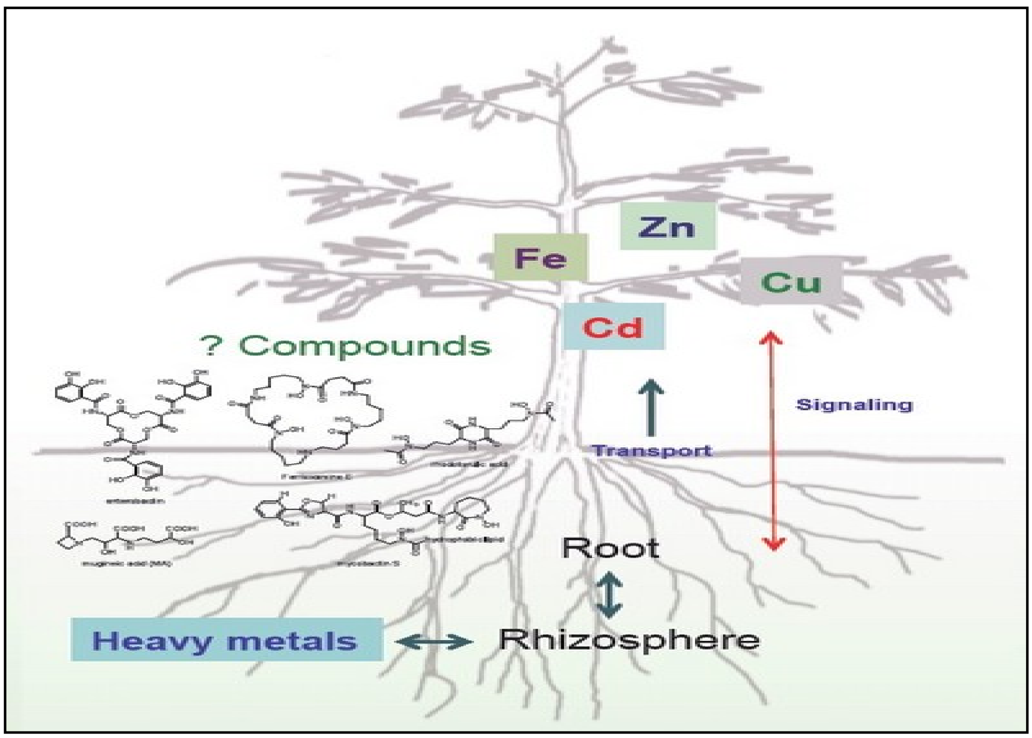


Figure 2.13: Interaction between plants and soil for metal ion acquisition and homeostasis

Depending on plant species, heavy metal tolerance or adaptations that enable them to grow in heavy metal contaminated soils are the result from two basic strategies, exclusion and accumulation. Exclusion strategy comprised of inhibition of uptake and restriction of transport to the shoot (Zhang et. al., 2002). Accumulation strategy involves physiological processes that require the cells to maintain the intracellular heavy metal ions in non-toxic forms (Cobbet, 2000) and storage of heavy metal ion complexes may be for removal by leaf fall (Zenk, 1996).

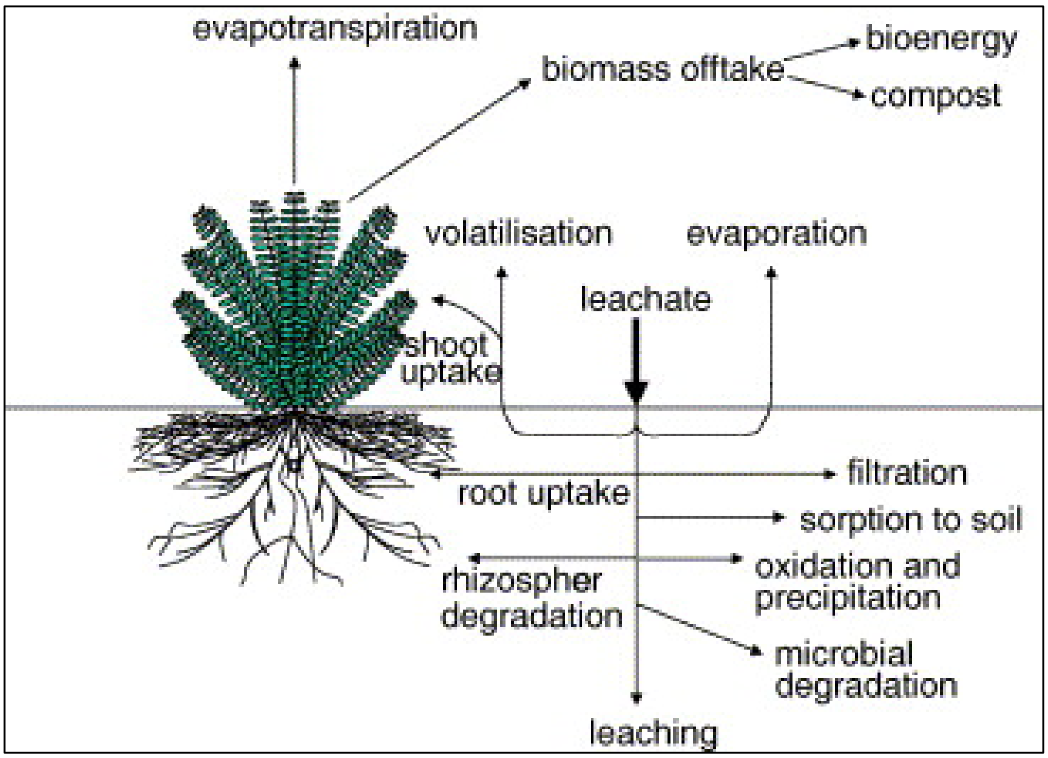
Water environment which is contaminated with heavy metal is a critical problem because it endangers not only the aquatic ecosystems but also human health. Heavy metals cannot be removed from water environment through decomposition by biological metabolism unlike organic pollutants (Liu et. al., 2007). In recent years, much attention had been also focused on constructed wetlands for treating toxic metals from wastewater. Plants play a significant role in metal remediation through filtration, adsorption, cation exchange, and root-induced chemical conversions in the rhizosphere zone (Wright and Otte, 1999). Plant species have diversified abilities in accumulating and degrading heavy metals. Some plant species such as duckweed (*Lemna minor*), salix, cattail (*Typha latifolia*) and common reed (*Phragmites australis*) can absorb much more heavy metals than others (Liu et. al., 2007).

The aquatic macrophytes remove metals and contaminants by three models (1) metals are bioaccumulated in the root, but translocation to the shoot is restricted (2) metals are constrained from entering the plant and attaches to the cell wall (3) hyperaccumulation whereby metals are bioconcentrated in the plant tissues. The hyper accumulative potential of the aquatic macrophytes are useful for the removal of heavy metals.

2.13.2 Plant responses to landfill leachate toxicity

A number of phytoremediation works utilized wastewater in the form of landfill leachate as a source of irrigation and fertilization for poplar trees (Zalesny et. al., 2007a; Erdman and Christensen, 2000). Leachate irrigation did not boost the tree growth and biomass accumulation for most genotypes of Populus species in the research carried out by Zalesny and associates (2007a). But nevertheless, significant reductions in productivity associated with the leachate were also not observed in the particular study.

Phytoremediation processes employ the capacity of the natural or effectively managed soil–plant integration to detoxify, degrade and inactivate potentially toxic elements in the leachate (Jones et. al., 2006). Figure 2.14 shows the main features of this bioremediation system. A principally phytoremediation system involves a combination of above- and below-ground processes.



Source: Jones et. al., 2006

Figure 2.14: Schematic model of the soil–plant bioreactor for the plant–soil based biological treatment of landfill leachate

Above-ground processes are inclusive of: (1) the foliar uptake of gaseous nutrients released from the applied leachate and their use in making new plant biomass for instance, ammonia (NH_3); (2) the foliar uptake of dissolved minerals and metals from the leachate applied and their use for growth/ biomass accumulation (such as nitrate (NO_3^-) and zinc) or their sequestration in the leaves (for example, lead); (3) the foliar uptake of volatile and dissolved organic elements within the applied leachate (such as chlorinated hydrocarbons) and their consecutive detoxification or sequestration; and (4) the increased evaporation of water from the leachate during and after irrigation, consequently reducing the volume of effluent (Jones et. al., 2006).

Below ground processes are inclusive of: (1) the uptake of water from the soil to enhance shoot transpiration, which begins by drawing the elements contained in the leachate towards the root, where they can be taken up by the plant, and secondly reduces volume of leachate and subsequently minimises migration of contaminants downward; this process, however, may also cause the intermittent accumulation of leachate constituents in soil (e.g., sodium); (2) the root uptake of inorganic nutrients (Potassium, K and Ammonium, NH_4^+) and other metals (e.g., Na, heavy metals) which can either be removed, used in biomass accumulation or transported to the shoots; (3) the uptake of organic compounds which can either be sequestered, degraded, used in growth or transported to the shoots; (4) the stimulation of rhizosphere microorganisms (including mycorrhizas) which reduce the BOD load of effluents, detoxify organic pollutants and sequester and provide some metals non-toxic (i.e., copper, Cu). This process is recognized as rhizoremediation. The roots also support the development of microsites which favour specific soil chemical transformations (e.g., denitrification, $NO_3^- \rightarrow N_2$); (5) the sorption, complexation and fixation/precipitation of metals onto the soil's solid phase; which includes immobilization onto both soil organic matter and mineral particles; (6) the sorption and degradation (biotic and abiotic) of organic compounds contained in leachate; and (7) the improvement of soil structure by plant roots, which enhances infiltration of leachate into the soil and reduces the risk of surface run-off. The enhancement of soil structure also promotes better soil aeration which stimulates a more productive biodegradation of organic compounds (Jones et. al., 2006).

There are a huge number of complex physical, biological and chemical interactions which takes place between plants and the soil when effluent is applied (Duggan, 2005). Cheng and Chu (2007) reported on both the positive and detrimental effects of landfill leachate on plant growth, depending on the plant species used and the concentration of the leachate. Therefore, identification and understanding of plant stress in the field would be of great importance in assessing the short-term negative response (Dimitriou et. al., 2006). However, Menser and collegues (1983) explained that irrigation with leachate could lead to yield reduction, eg. damage, premature senescence and poor plant survival. In contrast, Cheng and Chu (2007) proposed the use of landfill leachate as irrigation water in dry seasons to boost the growth, survival and stomatal conductance of *Acacia confuse*, *Leucaena leuocephala* and *Eucalyptusb torelliana*. Leachate application had significantly recorded higher growth in *Phalaris arundinacea*, *Salix babylonica* and *Populus nigra*. However, some phytotoxicity symptoms were observed in poplar leaves, such as brown leaves and necrotic spots, while chlorophyll degradation or complete chlorosis was found in willows (Cheng and Chu, 2007). Growth rates and biomass production are common indicators of imposed stress (Dimitrious et. al., 2006).

Research works have reported that there can be harmful result of applying leachate to willows as phytotoxic effects have been investigated (Stephens et. al., 2000). Several studies have reported detrimental effects on trees when spray irrigation has been used (Duggan, 2005). Photosynthetic rates and water use efficiency showed decline (34 and 70%, respectively) when spray irrigation of leachate was applied onto foliage of sugar maple although transpiration rates were largely unaffected (Duggan, 2005). In contrast, when spray irrigation on foliage with lower strength leachates were applied, no negative impact has been observed (Shrive et. al., 1994). Biomass production was also significantly greater for leachate irrigated plots than for water-fed controls according to

Tyrrel et. al. (2001). Landfill leachates with high (EC) in the range 0.2–0.4 S m^{-1} may cause osmotic or ionic stress when irrigated onto tree crops (Duggan, 2005). Soil salinity appears to be the main restricting factor for leachate amelioration (Bowman et. al., 2002). Electrical Conductivity has been proposed as a suitable indicator of toxicity (Alker et. al., 1998; Stephens et. al., 2000; Duggan, 2005). There can be an outstanding effect of leachate on leaf area. Trees receiving leachate had remarkable greater leaf area than water irrigated controls (Duggan, 2005).

Constructed wetlands, most regularly reed beds, have been applied successfully for a number of years as a polishing treatment in landfill leachate treatment. Reeds have demonstrated elevated levels of metals in roots proposing that they act as a filtering mechanism, limiting metals accumulating in the shoots or rhizomes (Duggan, 2005; Peverly et. al., 1995). However, it is strongly accepted that the reed beds are not capable of treating high strength leachates when faced with elevated levels of Ammoniacal Nitrogen (Robinson et. al., 1999). Due to the toxicity of the leachate to the reeds, therefore reed beds are basically considered inapplicable for primary treatment of landfill leachate, with the possible exception of leachate from older landfill sites. Nevertheless, after aerobic biological pre-treatment, they can be a very efficient polishing treatment (Robinson et. al. 1999).

2.14 Test Plants

There are several types of plants used to remediate water and soil, such as aquatic plants, woody plants and vegetable plants. Different plants have their own ability in absorbing or accumulate pollutants. This depends on the plants characteristics. In this study, there were two chosen plant species. They are *Hibiscus cannabinus* and *Acacia mangium*.

2.14.1 *Hibiscus cannabinus* (Local Name: Kenaf)

Kenaf [Etymology: Persian], ***Hibiscus cannabinus***, is a plant from the family of Malvaceae. Kenaf (Plate 2.10) is from the *Hibiscus* genus (Table 2.21) and is very likely native to southern Asia, although its exact natural origin is unidentified. The name also refers to the fibre produced by this plant. Kenaf is one of the associated fibres of jute and resembles similar characteristics (Kenaf, 2012).

Plate 2.10: *Hibiscus cannabinus* L. plant

Kenaf is an annual or biennial herbaceous plant (rarely a short-lived perennial). It grows to 1.5-3.5 m tall with a woody base and the stems are 1–2 cm diameter, often but not always branched (Kenaf, 2012). The flowers are 8–15 cm diameter, white, yellow, or purple; when white or yellow, the centre is still dark purple (Plate 2.11). The fruit is a capsule with a diameter of 2 cm and contains several seeds. The length of the leaves are 10–15 cm (Plate 2.12), variable in shape, with leaves near the top of the stem are shallowly lobed or unlobed lanceolate, while leaves near the base of the stems being deeply lobed with 3-7 lobes (Kenaf, 2012).

Table 2.21: Scientific Classification of Kenaf

Scientific Classification	
Kingdom	: Plantae
Division	: Magnoliophyta (angiosperms)
Class	: Magnoliopsida (dicots)
Order	: Malvales
Family	: Malvaceae
Genus	: *Hibiscus*
Species	: *H. cannabinus*
Binomial name	
Hibiscus cannabinus	

Plate 2.11: *Hibiscus cannabinus* flower

Plate 2.12: *Hibiscus cannabinus* leaves

Kenaf is cultivated in India, Bangladesh, United States of America, Indonesia, Malaysia, South Africa, Vietnam, Thailand, parts of Africa, and to a small extent in southeast Europe for its fibre. Kenaf was grown in Egypt over 3000 years ago. It matures in 100 to 200 days. The stems yield two types of fibre, a finer fibre in the core, and a coarser fibre in the outer layer (bast fibre). It is a potential paper pulp crop. Kenaf makes good animal forage with high crude protein in leaves (Ho et. al., 2008; Webber et. al., 2002). The Kenaf leaves were consumed by human and animal. The high quality fibre of Kenaf was used for the manufacture of bags, cordage, and the sails of Egyptian boats. This crop was only introduced into southern Europe in the early 1900s (Kenaf, 2012). Today, China and India is the principal farming areas and it is also cultivated in many other countries such as the USA, Mexico and Senegal. The main uses of Kenaf fibre have been rope, twine, coarse cloth (similar to that made from jute), and paper.

Wayback in 1992, 3,200 acres (13 km^2) of Kenaf were grown, most of which was used for animal bedding and feed in California, Texas, Louisiana and Mississippi.

Kenaf has been used as a cordage crop to produce twine, rope, and sackcloth for over six millennia (Dempsey, 1975). Other uses of Kenaf fibre include engineered wood, insulation, clothing-grade cloth, soil-less potting mixes, animal bedding, packing material, and material that absorbs oil and liquids. It is also useful as cut bast fibre for blending with resins for plastic composites, as a drilling fluid loss preventative for oil drilling muds, for a seeded hydromulch for erosion control. Kenaf can be processed into various types of environmental mats, such as seeded grass mats for instant lawns and moldable mats for manufactured parts and containers. A remarkable local use of Kenaf is the success of Panasonic setting up a plant in Malaysia to manufacture Kenaf fibre boards and exports them to Japan (Kenaf, 2012).

The utilization of Kenaf in paper production promotes many environmental benefits over producing paper from trees. In 1960, the USDA recognized Kenaf as the most promising source of "tree-free" newsprint after surveying more than 500 plants. In 1970, Kenaf newsprint produced in International Paper Company's mill in Pine Bluff, Arkansas, was successfully used by six U.S. newspapers. Since 1992, printing and writing paper made from the fibrous Kenaf plant has been offered in the United States. Again in 1987, a Canadian mill produced 13 rolls of Kenaf newsprint which were used by four U.S. newspapers to print experimental issues. They found that Kenaf newsprint made for stronger, brighter and cleaner pages than standard pine paper with less harm to the environment (Kenaf, 2012). Kenaf fibres are being naturally whiter than tree pulp therefore less bleaching is required to create a brighter sheet of paper. Hydrogen peroxide has been used with much success in the bleaching of Kenaf. It is an environmentally-safe bleaching agent that does not create dioxin.

Various studies reported that the energy requirements for producing pulp from Kenaf are about 20% less than those for wood pulp, most probably due to the lower lignin content of Kenaf. Kenaf have been adapted by many of the facilities that now process Southern pine for paper use. Kenaf is covered by the International Year of Natural Fibres 2009 as one of the world's important natural fibres,

2.14.2 *Acacia mangium* (Local Name: Akasia kuning)

The most valuable in agroforestry and reforestation is the ability of leguminous tree to fix atmospheric nitrogen into a plant usable form and Acacia mangium is the fastest to grow compared to other leguminous plants used in agroforestry purposes. *Acacia mangium* is in the family of Leguminosae and sub-family of Mimosoideae (Table 2.22).

Table 2.22: Scientific Classification of Akasia

Scientific Classification	
Kingdom	: Plantae
Division	: Magnoliophyta (angiosperms)
Class	: Magnoliopsida (dicots)
Order	: Fabales
Family	: Fabaceae
Subfamily	: Mimosoideae
Genus	: Acacia
Species	: *A. mangium*
Binomial name	
Acacia mangium	

It is a low-elevation species associated with rain forest margins and very tolerant to disturbed well-drained acidic soils (Norisada, et. al., 2005) and it is primarily found in the humid, tropical lowland climatic zone characterized by a short dry season and mean annual rainfall between 1446 and 2970 mm. *A. mangium* can withstand a minimum annual rainfall of 1000mm (Krisnawati et. al., 2011).

Acacia mangium (Plate 2.13), originally from Papua New Guinea, is a fast growing leguminous tree. One of its principle qualities is that it fixes nitrogen and recycles large quantities of leaves to the soil (adding organic matter), improving soil fertility. *A. mangium* is among the major rapid growing vegetation used in plantation forestry programs around Asia and the Pacific. *A. mangium* is playing a progressively significant role in efforts to sustain commercial supply of tree products and at the same time cuts down the pressure on natural forest ecosystem due to its fast growth and tolerance of very poor soils (Galiana, et. al., 1998).

Plate 2.13: *Acacia mangium* plant

Figures 2.14 and 2.15 shows the photographs of Akasia plant's flower and leaves respectively.

Plate 2.14: *Acacia mangium* flower

Plate 2.15: *Acacia mangium* leaves

Landfills hold back pollutants generated from waste from outbreak, thus it is very critical to reckon a treatment method to detoxify or attenuate heavy metals. Chemical treatments such as soil flushing, liming, electro kinetic, soil washing, pyrometallurgical extraction and many others are surely to be costly. Thus, using plants as a remediation technique has the advantages. By using the plants, less maintenance or control needed and it also helps to futher enhance the appearance of landfill. Futhermore, landfill will be landscaped after its ceased operation as for reclamation to be made possible. Phytoremediation will be most suitable to be adapted at shallow soils with low levels of contamination (2.5 – 100mg/kg) for polishing (Mulligan et. al., 2001).

2.15 Contaminant Uptake by Test Plants in Pilot Scale Constructed Wetland via Mechanism of Rhizofiltration

Developing cost efficient and eco-friendly technologies for the treatment of wastewaters polluted with toxic substances has gained attention globally. The potential of metal- accumulating plants in wetland remediation has been recently valued (Liao and Chang, 2004). Several physical, chemical and biological processes are incorporated in the conversion and consumption of organic matter and plant nutrients within the wetland. Liao and Chang (2004) defined rhizofiltration as the use of plant roots to absorb heavy metals from polluted effluents.

Constructed wetlands are self-sustaining, cheaper systems that have been used to treat various types of toxic metals contaminated wastewaters. They are used for the removal of heavy metals from municipal sewage, landfill leachate, stormwater runoff, agricultural runoff, mining effluent and industrial wastewater besides in the degradation of organic substances and nutrients (Liu et. al, 2007). In constructed wetlands, substrate interactions remove most metals. The permanent or temporarily anoxic condition in wetland soil assists in creating a condusive environment for immobilization of heavy metals in the highly reduced sulfite or metallic form. Processes that play an important role for remediation of heavy metal contaminated wastewaters in wetlands include: sedimentation and filtration of solids, precipitation as sulphides and oxyhydroxides, ion exchange with the sediments and plant uptake (Ghaly et. al., 2008; Kadlec and Wallace, 2009).

There are several literatures reporting on the wetland plants ability to accumulate heavy metals in their tissues, such as duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), salix (Stoltz and Greger, 2002), cattail (*Typha latifolia*) and common reed (*Phragmites australis*) (Deng et. al., 2004). Cattail and common reed have been extensively used for phytoremediation of Pb/ Zn mine tailings under waterlogged conditions (Ye et. al., 2001). Duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata* R. Br) have been studied to accumulate metals like Fe and Cu by up to 78 times the concentrations in the wastewater (Liao and Chang, 2004; Jain et. al., 1989). Water hyacinth is a popular plant used in wastewater treatment systems to upgrade the water quality by reducing the concentrations of organic and inorganic constituents (Delgado et. al., 1995; Liao and Chang, 2004). According to Falbo and Weaks (1990), the plant potentially reduces the level of heavy metals in acid-mine drainage water. There also literatures indicating that some spesies can accumulate specific heavy metals, such as the Spirodela polyrhiza for Zn (Liu et. al., 2007; Markert, 1993). Nevertheless, heavy metals cause phytotoxic effects on plants which results in the inhibition of chlorophyll synthesis and necrosis (Cervantes et. al., 2001).

Trace elements removal by wetland vegetation can be greatly enhanced by selection of suitable wetland plant species. The selection is carried out on the basis of the types of elements to be remediated, the geographic location, microclimate, hydrologic conditions, soil properties, and known accumulation capacities of the species. Bioavailability of metals to aquatic plants is also related to many factors including ambient metal concentrations, pH of soil or water, concentration of ligands, competition with other metals for binding sites, and mode of exposure (Miretzky et. al., 2004; Van Leeuwen et. al., 2005). Besides the factors mentioned above, metal uptake by wetland plants is also influenced by variations in species of plant, the maturity phase of the plants, and element characteristics which control absorption, accumulation, and translocation of metals. In addition, toxic metal accumulation by sequestration of metals in the roots is controlled by physiological adaptations (Deng et. al., 2004).

The placement, immobilization and accumulation of metals in the root structures of the test plants could be possibly due to the process of rhizofiltration, which is commonly observed in aquatic plants (Choo et. al., 2006). Root exudates in the rhizosphere may also cause the metals to precipitate onto the root surfaces (Ma et. al., 2011). Metal ions can be absorbed actively into the root cells via plasmalemma, and adsorbed on the cell walls via passive diffusion or moved acropetally in the roots of aquatic macrophytes (Choo et. al., 2006). The differential in localization of metals within the plant cells is also essential in deciding how well the metals may be bound and released on senescence of the plants. Past studies indicate that cell wall-bound metals are released slowly due to the slow breakdown of the cell walls. As a result, The metals may be exported from a wetland to a lesser extent than those metal bound intracellularly (Malik, 2007). Nevertheless, both plants and microbes have particular restrictions pertaining to their individual abilities to metabolise the pollutants. Interactive actions by both rhizosphere microorganisms and plants could possibly solve some of the limitations and thus offer a beneficial basis for enhancing remediation of contaminated environment (Chaudhry et. al., 2005). Although the role of the plants in supplying oxygen and mineral nutrients to the microbes in rhizosphere via fine roots and of the advantageous effect of microorganisms on plant root growth had been explored, very few studies identified the degradative abilities of plant-microbe relationship (Malik, 2007; Ramos et. al, 2005).

In Malaysia, various studies of phytoremediation have been conducted using aquatic plants. Water hyacinth (*Eichhornia crassipes*), are among the local aquatic plants that have been extensively used. Besides that, some other plant species including Water lettuce (*Pistia stratiotes*) (Plate 2.16), *Lepironia articulata*, *Typha angustifolia*, *Phragmites karka*, duckweed (*Lemna minor)*, and others were used to evaluate their effectiveness and potential in improving water quality. It was discovered that the local aquatic plants tested show no evidence of senescence or reduced growth.

Plate 2.16: Water lettuce

The major characteristics of water hyacinth (Plate 2.17) are their extensive root system and rapid growth rate that make them an attractive biological support media for bacteria. Water hyacinth in a natural wetland system serves as “nature’s kidneys” for efficient effluent treatment in order to preserve and conserve the earth’s precious water resources from contamination. It has been used extensively for removal of inorganic nutrients, toxic metals and persistent organic pollutants (Malik, 2007). Water Lily (Plate 2.18) has an extensive root system with rapid growth rates, but is sensitive to cold temperature; therefore it is an ideal plant for water treatment in warm climates. Duckweed (*Lemma* spp.) as shown in Plate 2.19 has greater cold tolerance and a good capacity for nutrient absorption.

Plate 2.17: Water hyacinth

Plate 2.18: Water lily

Plate 2.19: Duckweed

In general, literature reviews stated that in hydroponic systems, floating aquatic or submerged plants with short or medium root system are some of the successful plants used. Therefore, this study is among one of a novel research carried out using terrestrial plants grown hydroponically to provide a comparison study to 'pot-culture' system of contaminant uptake efficiency.

2.16 Development of a Decision Support System for Phytoremediation

A Decision Support System (DSS) is an Information Technology (IT) system used to simulate different combinations of a decision options with the purpose of selecting the best set of options in finding solution to a given problem. The DSS may have a complex simulation model as its foundation or a simple economic comparison model (Burckhard et. al., 1999). . The types of variables needed as input to the DSS depends on the type of problem that is being solved. Output from the system is dependable on the possible solution types that are simulated. The output for a landfarming DSS may include the depth to place the contaminated soil, the recommended intervals for aerating the soil during treatment, and the possible length of time required for treatment (Burckhard et. al., 1999).

There are several DSS being developed by researchers in the field of phytoremediation but nevertheless, readily available softwares in the market or online are next to nil. Among some the available developed systems include DESYRE, RGS, and Graphical User Inter-face DSS for Vegetated Treatment System etc. as discussed below.

The proposed DSS system developed for this project is e-PMS, a Graphical User Interphase. The DSS system assists decision makers in determining potential phytoremediator plants to be used for in situ or ex situ bioremediation of contaminated soil or wastewater. There is a useful database with models involved in determination of potential bioaccumulation of pollutants from contaminated sites. Among the existing systems that are available for comparison are GIS based system, systems that operate on fuzzy logic, socioeconomic analysis and others. The e-PMS proposed was developed on Visual Basic programming language and the proposal incorporated a user-friendly interphase with displays of test plants. There were various models proposed to determine the performance of phytoremediator plants. There are also additional features like glossary, Q & A and scientific classification of test plant display. In addition, all data inputs can be saved in the form of .txt files.

2.16.1 Decision Support System for the Requalification of Contaminated Sites (DESYRE)

DESYRE is a GIS-based decision support system (DSS) designed specially to address the integrated management and remediation of contaminated megasites (i.e., large contaminated areas or impacted areas characterized by multiple site owners and multiple stakeholders) (Carlon et. al., 2007). The main aspects associated to a remediation process— site characterization, analysis of social and economic benefits and constrains, risk assessment, selection of best available technologies, creation of sets of technologies to be applied, analysis of the residual risk, and comparison of different remediation scenarios were included in the development and conceptual design of DESYRE (Carlon et. al., 2007).

The DESYRE DSS is GIS-based software composed of 6 interconnected modules (Carlon et. al., 2007). In the characterization module, chemical and hydrogeological data are structured in a relational database and geostatistic tools are used to map contaminants' distributions. The socioeconomic module focuses on the socioeconomic limitations though a fuzzy logic analysis for the selection of best land use. The risk assessment module is separated into 2 topics. In the preremediation topic, an original procedure takes into consideration assessing and representing the spatial distribution of risks posed by contaminants in soil and groundwater, supplying a risk-based zoning of the site. In the technology assessment module, options of suitable technologies and creation of different technology sets, considering both technical requirements and site-specific features are available. This module is performed by experts supported by multicriteria decision analysis tools. A simulation of applied technologies offers residual risk maps with related uncertainty maps in the postremediation risk assessment. Lastly, in the decision module, alternative remediation scenarios are described by a set of indices. These indices can be used to compare and rank by interested stakeholders using multicriteria decision analysis methodologies. The paper addresses original procedural steps and functionalities of DESYRE and analyzes its main points of strength and potentialities, including its limits (Carlon et. al., 2007).

2.16.2 Rhizofiltration Greenhouse System (RGS)

A paper on "Computer Model for Full-Scale Phytoremediation Systems using Rhizofiltration Processes" stated that Rhizofiltration Greenhouse System version 1.1 (RGS) is an example of DSS software developed by Fleisher and associates (1997). This system provided decision support information for design and operation of a rhizofiltration system. A Michaelis - Menton based model was developed and incorporated into a series of algorithms which process information relevant to the rhizofiltration system design. The system incorporated an engineering economic analysis tool within the software for analysis of impact of critical design variables on hydroponic system efficiency.

2.16.3 Design of a Graphical User Inter-face (GUI) Decision Support System for a Vegetated Treatment System

The use of plantation in remediating contaminated soils and sediments has been researched for a number of years (Riser-Robert, 1998). Positive laboratory results have resulted in the use of vegetation at field sites. The design process engaging field sites and the related decision processes have been developed. As part of this progress, a computer-based graphical user interface (GUI) decision support system was outlinedfor use by practicing environmental professionals. The stages involved in development of the GUI were combination of the pollutant degradation model and the designing of the decision support system for a vegetated treatment system for vehicle wash pit waste (Burckhard et. al., 1999). Many parameters were incorporated with simulation models in the vegetated treatment system,

The contaminant degradation model used for this DSS is a 1-D solute transport model, associating root growth, water movement, contaminant movement, contaminant degradation, and the impact that test plants has on these (Burckhard et. al., 1999).

Required inputs to this are soil texture, climate data, plant type, contaminant type, and contaminant level. A list of the typical parameters is shown in Table 2.23.

The expected output from the DSS is as explained in Table 2.24. A flowchart of the GUI DSS operation was generated (Figure 2.15) by comparing the list of readily available input parameters and the desired outputs, along with a description of the required simulation model for the simulation of fate and transport of contaminant under the influence of a vegetated treatment system.

Table 2.23: Typical parameters related to the simulation of a vegetated remediation treatment system

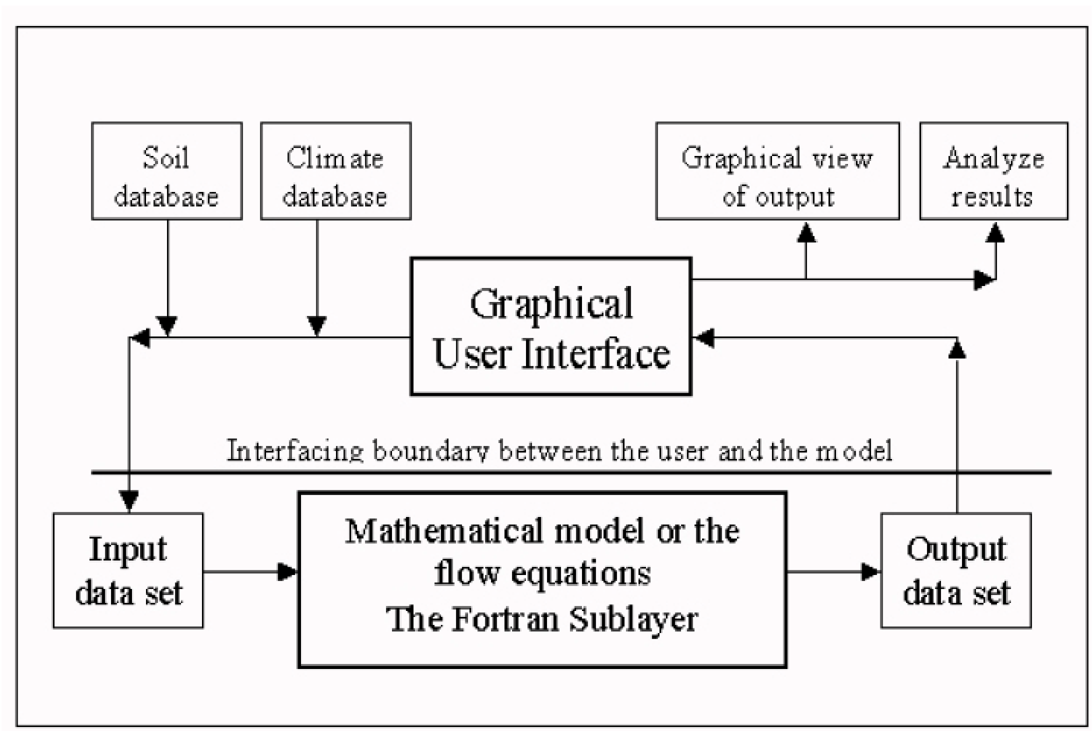
Parameter type	Examples of required information
Soil	Texture, specific storage, specific retention, saturated and unsaturated hydraulic conductivity, specific yield
Vegetation	Plant type, leaf area index, root hydraulic conductivity, root permeability, root death rate, root proliferation rate, root elongation rate, biomass yield, permanent wilting point
Contaminant	Henry's law coefficient, solubility, adsorption coefficient, degradability, diffusion coefficient, dispersion coefficient, root concentration factor, transpiration stream concentration factor
Simulation Controls	Number of nodes in problem; maximum allowed number of plants, soils, contaminants; output variables, time step for calculations
Initial conditions	Soil, plant, contaminant, water content

Source: Burckhard et. al., 1999

Table 2.24: Input and output parameters of GUI

Readily available input parameters	Desired output parameters
Soil texture, plant type, climate TPH contaminant level, target contaminant treatment level, volume of soil requiring treatment	Depth-to-layer contaminated soil for treatment, timeto- reach-target contaminant level, management needs of treatment system

Source: Burckhard et. al., 1999



Source: Burckhard et. al., 1999

Figure 2.15: Flowchart describing the interactions between the simulation model and the graphical user interface

From the brief description on various softwares developed in relation to the field of phytoremediation, it was found that e-PMS and DSS for a Vegetated Treatment System had similarity in terms of Graphical User Interphases. The idea of point and click applications made it possible for mor people to use computer systems without extensive training (Burckhard et. al., 1999). Visual Basic programming language and also mathematical modeling was chosen for the development of Rhizofiltration Green house System (RGS) and the proposed e-PMS. Nevertheless, e-PMS system has integrated few models, namely BCF, TF, RGR and First-Order Kinetics in the decision making process of potential phytoremediators.

Both the e-PMS and RGS are user-interactive program which combines user inputs and phytoremediation process model into an integrated series of 'windows'. RGS was developed given particular attention to the design and operation of a simulated full scale facility of a greenhouse. Greenhouse dimensions, numbers, interior layouts, trough dimensions, system performance, operation and an engineering economic analysis tool were included in the software. But on the other hand, e-PMS was developed with an emphasis on the plants performance in the phytoextraction and rhizofiltration system.

The DESYRE software system applied 6 comparative macrocriteria and evaluation matrix for the comparison of remediation technologies of contaminated sites. The selected technologies are ranked according to a multicriteria decision analysis (MCDA) approach (Critto et. al., 2006). e-PMS software application does not apply ranking or evaluation (scoring) approach. This system applied user input data set on plant type, test plant (leaves, stem, root, and total plant dry biomass) growth, concentration of contaminants in soil/wastewater and plant root, stem and leaves, time period of study which would generate a desired output data set comprising Relative Growth Rate (RGR); Bioconcentration Factor (BCF); Translocation Factor (TF); Bioaccumulation rate constant (k) and half-life of contaminant (t) through First Order Kinetics. Results are analysed and a series of Questions are provided to enable users to determine the efficiency and mechanism of contaminant removal by selected test plants.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Description of Study Site

This study was conducted in open environment at the nursery of Herbal Garden, National Hydraulics Research Institute of Malaysia which is located at 03° 00.058’ N and 101° 41.083’ E, town of Seri Kembangan, Selangor, Malaysia. The experiment trials were conducted under a netted plant shelter with dimensions of 3.3 m x 1.2 m x 1.2 m to protect the test plants from direct rain and sunlight. Temperature at the nursery site ranged from 21-32 ^{o}C and annual precipitation was 2,452 mm. Plate 3.1 and Plate 3.2 show the seedlings tray preparation and nursery experimental setup.

Plate 3.1: Trays containing black soil prepared for seedlings planting

Plate 3.2: Nursery setup

The bags were placed on raised planks inside a "shed" covered all around with nylon netting to avoid leakage of leachate to neighbouring poly bags.

3.2 Preparation of Test Plants

Kenaf seeds (variety V36 LTN) as shown in Plate 3.3 were collected from Malaysian Tobacco Board (LTN) while the Akasia seeds (Plate 3.4) were collected from Forest Research Institute of Malaysia (FRIM).The seeds were sown in trays containing 2 kg of black soil. The plants were adapted under *ex situ* nursery environmental conditions to avoid transplant shock. At 7 days and 30 days after germination for Kenaf and Akasia, healthy and uniform seedlings with a height of 10 – 12 cm (2 weeks old for Kenaf and 1 month old for Akasia) each were selected for the study (Plates 3.5 and 3.6). About two to three seedlings were transplanted into freedraining poly bags measuring 12” by 12” containing 5 kg of black soil per poly bag as shown in Plate 3.7.

Plate 3.3: Kenaf seeds

The seedlings were transplanted at a rate of two to three plants per bag, spaced 5 cm apart to reduce inter-plant competition for nutrients. All plants were well-watered daily until leachate application began on the 30th day after seedling transplantation. At this time, the Kenaf plants (Plate 3.8) had on average 2 mm expanded leaf length, 2 mm leaf width, and 15 mm root length by destructive sampling and 30 mm stem height. The Akasia plants (Plate 3.9) had on average 30 mm expanded leaf length, 15 mm leaf width, and 10 mm root length by destructive sampling and 30 mm stem height.

Plate 3.4: Akasia seeds

Plate 3.5: Kenaf seedlings of 10 – 12 cm tall (7 days of growth)

Plate 3.6: Akasia seedlings (2 weeks old)

Plate 3.7: Poly bag containing 5 kg of black soil

Plate 3.8: Kenaf seedlings after transplanted into polybag

Plate 3.9: Akasia seedlings before being transplanted measuring 10 cm (after 1 month of germination)

3.3 Test Media

Three replicates of raw/ untreated landfill leachate for irrigation of test plants were sampled from Jeram Sanitary Landfill (JSL), Selangor, Malaysia (Plate 3.10). The landfill is located at 3^{o} 11’ 27’’ N and 101^{o} 22’ 3’’ E. The leachate was collected in high-density polyethylene bottles at the outlet of the leachate pipe, before its entry into the leachate treatment plant. The bottles contained 1mL of concentrated Nitric acid to prevent sorption of the metals. The sample bottles were labeled and transported to the laboratory. The samples collected were kept in containers at 4 ^{O}C in cool boxes. The sample bottles were then placed in cold room at 4 ^{O}C prior to analysis. Samples were taken over a period of 3 months from June to August 2009 and was analysed to establish the mean characteristics of the leachate. Since Malaysia does not have distinct seasonal variations, the sampling period represents the drier months as well as the monsoon season.

Plate 3.10: Leachate pond at Jeram Sanitary Landfill, Selangor

Leachate treatment in this landfill included a pumping chamber to the Equalization Lagoons (Plate 3.11). Leachate then goes through physical, biological and chemical treatment before being released into the nearby river. The leachate was dark brownish in color as shown in Plate 3.12 and had a putrid odor.

Plate 3.11: Equalization Lagoon

Plate 3.12: Leachate sampling

Jeram Sanitary Landfill leachate characteristics were determined based on American Public Health Association, APHA (APHA, 1998) and Hach method (Hach, 2002). Leachate analysis included pH and conductivity, measured using a pH and conductivity probe (HANNA Model, No.8033). Total suspended solid (TSS) was determined using a spectrophotometer, HACH Model (DR/4000). BOD_5, COD and Total N were analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998) while heavy metals were determined using acid digested leachate by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Chloride, Sulphide, Phenol and Phosphorus were analysed according to the Standard Methods of APHA (APHA, 1998) and Hach (Hach, 2002).

- **Biological Oxygen Demand (BOD_5):** BOD was determined using BOD bottles incubated for 5 days at 20^{O}C. The dissolved oxygen (DO) before and after incubation was measured using DO meter YSI Model 57 and the BOD was computed from the difference in DO values.

- **Chemical Oxygen Demand (COD):** COD was obtained using dichromate reflux method using potassium dichromate, silver nitrate and sulphuric acid in a TECATOR COD digestion unit. The excess dichromate was titrated with 0.1M ferrous ammonium sulphate. COD was calculated based on the difference in dichromate levels.

- **Total – N:** Total-N was determined using Kjeldahl method.

Chemical treatment of leachate was done via a Jar Test (a six-paddle Flocculater SW6 from Stuart Scientific (Flocculator SW6), United Kingdom equipped with 6 beakers of 500 mL each. Ferric (III) Chloride concentration of 0.4% w/v or 2g/500mL (Agamuthu and Said, 2009) in solid state was used as effective coagulant dosage for 500 mL of raw leachate at pH 7 (Said, 2008) for the preparation of treated leachate. The mixing speed of flocculator was set at 100 rpm for 10 minutes (Agamuthu and Said, 2009). Rapid mixing ensured the total mixing of the coagulant in the solution whereas slow mixing causes the agglomeration of the flocs produced during the rapid mixing (Nor Asikir and Agamuthu, 2007). After mixing, the samples were allowed to settle for 60 minutes. During the settlement process, liquid (supernatant) was siphoned out and used as test media for irrigation of test plants.

Leachate was used as a source of Fe, As, CN and NH3-N for this study. The test media was organized for 9 compositions; i.e. Zero–N Control; 100 % raw leachate; treated leachate (TL) at 100%; 75%; 50%; 25%; 12.5%. The experimental design is shown in Table 3.1.

Inorganic fertilizer preparation at the same N-equivalent as the leachate at 100 % IF and 50% IF + 50% treated leachate were included. The 100% TL treatments had total Nitrogen content of 0.96% N, while the raw leachate treatment had 0.98% N-content. The 50% TL and 100% IF treatments were standardized at 0.48% N. Both the 75% TL and 50% TL + 50% IF treatments had the same N-content (0.72% N). The N-content of 25% TL and 12.5% TL treatments were 0.24% N and 0.12% N respectively. This standardization was important because N is the most limiting factor in plant growth system, and N addition is a proven method for increasing overall productivity of the trees (Coyle and Coleman, 2005; Brown and van den Driessche, 2002).

Control treatments consisting of bags of Kenaf plants without leachate treatment were also prepared. The Control treatments were irrigated with same volume of distilled water. Soil control treatments were also prepared where the soil was applied with all 9 treatments as shown in Table 3.1 but without plants. Amendment of inorganic fertilizer was considered to improve nitrogen sources for plant growth and as comparison study of contaminant uptake ability of plants with unamended soil.

Table 3.1: Experimental Design of Test Media

Treatments	Details of treatment	
Control	Distilled water + Soil + Plants	0
100% IF	Inorganic Fertiliser N:P:K = 1:1:1 + Soil + Plants	0.48
12.5% TL	12.5% Treated Leachate + 87.5 % distilled water + Soil + Plants	0.12
25% TL	25% Treated Leachate TL + 75 % distilled water + Soil + Plants	0.24
50% TL	50% Treated Leachate + 50% distilled water + Soil + Plants	0.48
50% TL + 50% IF	50% Treated Leachate + 50% Inorganic Fertiliser + Soil + Plants	0.72
75% TL	75% Treated Leachate + 25 % distilled water + Soil + Plants	0.72
100% TL	100% Treated Leachate + Soil + Plants	0.96
100% RL	100% Raw Leachate + Soil + Plants	0.98

3.4 Test Operations and Sampling

Each of the test plant was grown in the pots containing 5 kg of black soil. The soil samples were collected from a nursery in Sungai Buloh, Selangor, Malaysia. Experiments were carried out with randomized blocks of 9 treatments replicated six times (54 experimental units per harvest). Deionised water was applied to all seedlings for an establishment period of 30 days. Following establishment, trees were hand irrigated with leachate at a rate of 200 ml/day (twice daily) before 9 AM and after 5 PM.

Fertigation was increased to 250mL (twice a day) when the plants were more expanded because of higher evapo-transpiration (Alaribe and Agamuthu, 2010). Total leachate application was performed for 3 months. No pesticide was applied to all the test plants till the end of the experiment. Hand weeding was performed weekly to prevent infestations of weeds because they are difficult to manage once they have invaded and to ensure maximum plants' survival.

Kenaf and Akasia plants were harvested by uprooting every 8^{th}, 12^{th} and 16^{th} week of growth. At each harvest, plants were sectioned into parts of leaf, stem and root (Plate 3.13) after rinsing thoroughly in tap water. The leaf length (LL) and stem height (SH) were measured prior to harvesting while the root length (RL) (Plate 3.14) was measured after harvest. Total plant height measurements were carried out by placing the harvested plants on polystyrene (Figure 3.15).

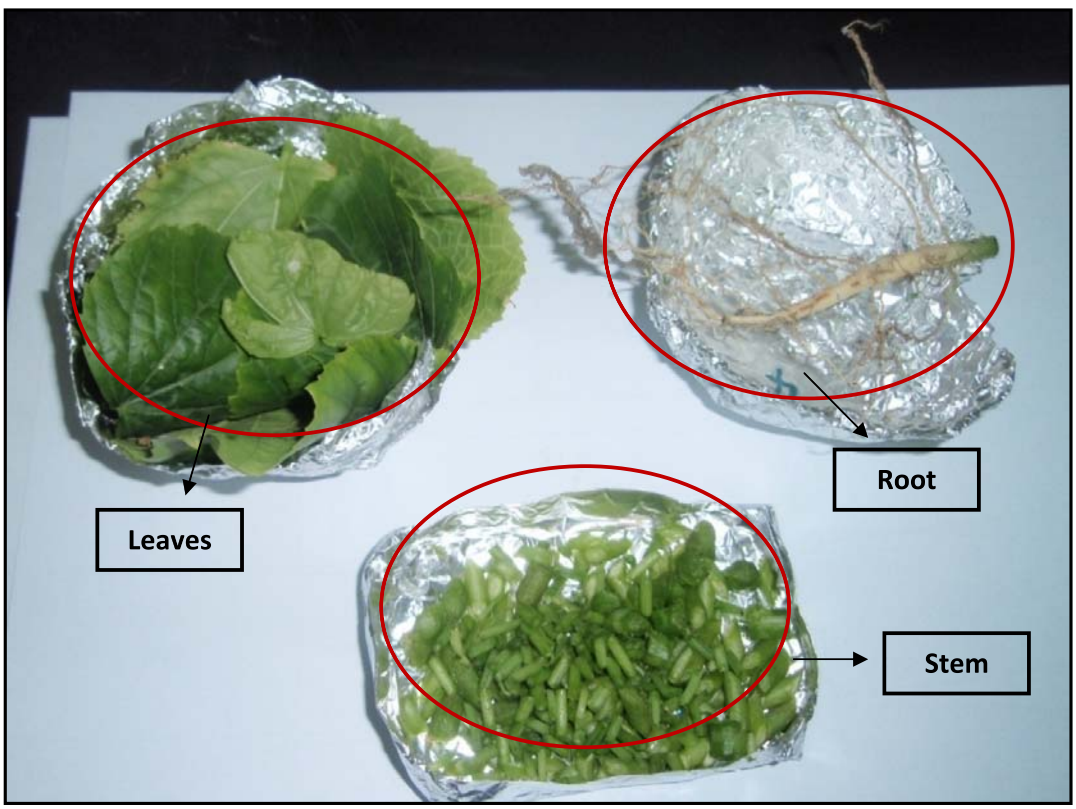


Plate 3.13: Harvested plants separated into leaves, stem and root

Plate 3.14: Akasia plant root measurement

Plate 3.15: Harvested Akasia placed on polystyrene for total plant height measurement

Fresh weights of the separated leaves, stem, roots and soil sample were determined. Height was measured from ground level to the base of the apical bud on the terminal shoot (Zalesny et. al., 2007a). Dry matter yield was obtained for roots, stem, leaves and soil sample after drying in a forced draft oven (GO-251) at 80 ^{O}C for 3 days until constant weight (Mangkoedihardjo et. al., 2008). The samples were then ground to a fine powder using a silica pestle and mortar and acid digested. Fe, As, CN and NH_3-N concentrations in plant and soil samples were analysed using Atomic Absorption Spectrometry, Method APHA 3114B, APHA 3111B, USEPA SW 84 9014 and MS417: Part3: 1994, respectively.

Topsoil samples (< 15 cm) were also collected within the root zone of Kenaf and Akasia, approximately 0 to 5 cm from the surface by scrapping with a metal scoop and packed in aluminium foil at every harvest. Soil samples were also dried in the oven at temperature at 80 ^{O}C for 3 days or constant weight. Dried soil samples were then crushed with pestle and mortar before digestion. Soils samples were also measured for Fe, As, CN and NH_3-N using ICP-OES.

Pollution impacts based on the heavy metal content in the soil and in the test plants, *Acacia mangium* and *Hibiscus cannabinus* were compared with The Ministry of Agriculture, Fisheries and Forest (MAFF) United Kingdom and FAO/WHO permissible concentration standard.

3.5 Sample Preparation for Heavy Metal Analysis: Acid Digestion

After drying, the dry weights (DW) of plants and soil were recorded to the nearest gram, following that the samples were individually ground to pass a 2-mm screen in a laboratory mill (Serial no. 39017, Christy and Norris LTD, Chelmsford, England). Approximately 0.5 to 1.0 g of dried samples of leaves, stem, root and soil sample were first weighed and then placed in clean 500 ml volumetric flasks respectively. Then a mixture of concentrated nitric acid, HNO_3 and perchloric acid, $HClO_4$ with mixed ratio of 4:1 was then added to digest the samples. The digestion first started with temperature at 40^oC for about an hour by mounting the flask on the digestion heater (EAM 9203 heating mantle) and then the temperature was increased to 140^oC for another 2 to 3 hours until digestion process completed. Digestion was complete when the sample solution was clear or pale yellow and no more brownish fume released.

For the soil samples, 3 mL (30%) H_2O_2 and 10 mL HCL were added while refluxing for 15 min. The resulting solution was cooled, filtered and diluted to 50 mL using deionized water, and was analyzed for Fe, As, CN.

After the solution has cooled down, the samples were then diluted to make 40mL solution and filtered through filter paper no.42 into pillboxes. Blanks were carried out the same way as samples. These solutions were then analysed with Perkin Elmer Inductively Coupled Plasma-Optical Emission Spectrometer (*ICP-OES*), for three different elements: Fe, As, CN. No further treatment was given for water samples after being filtered and acidified with nitric acid in the field. Water samples were directly analysed with AAS and the concentration ontained from AAS was based on 1 L of water samples.

Prior to analysis in the laboratory, glassware or containers for the samples have been acid washed by soaking the glasswares overnight in 5% acid solution (HNO_3 or HCl) and then rinsed thoroughly with distilled water. Deionised water was used in diluting the sample solutions to minimize metal contamination (Van Loon, 1985).

3.6 Sample Preparation for Macronutrients (NH_3-N) analysis

For analysis of macronutrients, different reagents for digestion were employed. Approximately 0.1 to 0.5g of dried Kenaf and Akasia plant samples; i.e. leaves, stem and root were weighed and inserted into digestion tubes. Then Kjeldahl catalyst tablets (1.0g sodium sulfate anhydrous and 0.05g selenium) was inserted in each tubes and 5mL concentrated sulfuric acids, H_2SO_4, were added. Following that, the samples were heated up at a temperature of 60 ^{o}C for about an hour before the temperature was increased gradually to 300 ^{o}C for another 3 to 5 hours until the fume subside and the solution become pale yellow. The solution was then let to cool and deionised water added to make 100mL. The solution was then filtered through filter paper no. 42 into pillboxes. Blanks were carried out the same way as samples (Allen et. al., 1974).

3. 7 Data Analysis

All data obtained from the ICP-OES were in mg/L unit. The readings had been multiplied with dilution factor (total volume) and divided with sample weight to obtain the actual concentration of heavy metals per kilogram samples (mg/kg unit).

$$\text{Heavy metals concentration in a kg sample} = \frac{C \times D.F}{W}$$

C = Concentration in mg/L obtained from AAS

D.F = Dilution factor (total volume of sample solution)

W = Sample weight (kg)

The Bioconcentration Factor (BCF) and Translocation Factor (TF) were calculated according to Majid and associates (2012) and Yoon et. al. (2006) as following:

Eqn. (1)

$$\text{Bioconcentration Factor (BCF)} = \frac{C_{root}}{C_{soil}}$$

where, C_{root} is the concentration of contaminants in root and C_{soil} is the concentration of total metal in soil.

Eqn. (2)

$$\text{Translocation Factor (TF)} = \frac{C_{aerial}}{C_{root}}$$

where, C_{aerial} is the concentration of contaminant in aerial plant parts.

3.8 Plant response/ growth

The concept of Relative Growth rate (RGR) was applied for shoot, leaf, root and total plant biomass in order to compensate for the marked differences in initial plant development (Dimitriou et. al., 2006). The RGR from initial to final harvest was calculated based on a model introduced by Dimitriou and associates (2)06). Plant response assessment on relative growth rate (RGR) was addressed for aerial plant parts consisting of leaf length (L), stem height(S), root length(R) and total plant dry biomass (TP). RGR was defined as follows:

Eqn. (3)

$$\boxed{\text{RGR} = \{(\text{Ppt} - \text{Ppi}) / \text{Ppi}\} / dt}$$

where; Ppt is plant parameter such as leaf area at time of measurement, Ppi is plant parameter initially measured, *dt* is time of exposure (Mangkoedihardjo et. al., 2008).

Statistical analysis of the RGR data was carried out using STATISTICA Version 9. The data were analysed by ANOVA (analysis of variance), considering the treatments as independent variables. Correlation test with significance level reported ($p<0.05$) was based on Pearson's coefficients.

3.9 Determination of Biaoccumulation rate constant and Half-life

The approach used in this study was based on a first order kinetic model and depends on the heavy metal accumulation in the plant biomass. First Order Kinetics model of Yeung et. al. (1997) was used to determine the rate of biodegradation of used oil in the various treatments. Data for the sampling periods were combined before this model could be used (Abioye et. al., 2010). Final and initial metal concentration was fitted to first-order kinetics model of Yeung et. al. (1997).

Eqn. (4)

$$\boxed{y = a.e^{-kt}}$$

Where y is the final metal concentration in plant (mg kg^{-1}), a is the initial metal concentration in plant (mg kg^{-1}), k is the bioaccumulation rate constant (day^{-1}), and t is time (day). The model estimated the bioaccumulation rate and half-life of metals in plant relative to treatment applied. Half-life ($t_{½}$) was then calculated from the model of Yeung et. al. (1997) as following:

Eqn. (5)

$$\boxed{\text{Half life } (t_{½}) = \ln(2)/k}$$

This model was based on the assumption that the bioaccumulation rate of metals positively correlated with the metal pool size in soil (Yeung et. al., 1997).

3.10 A Suspended Net-Pot, Non-Circulating Hydroponic Method System for Assessment of Contaminant Uptake by Test Plants

The ability of *Acacia mangium* and *Hibiscus cannabinus* L. to absorb and translocate Fe, As, CN and NH_3-N was studied in hydroponics system based on "Artificial Constructed Wetland Treatment System" at a laboratory scale. Figure 3.1 shows the flow diagram of activities carried out for this study.

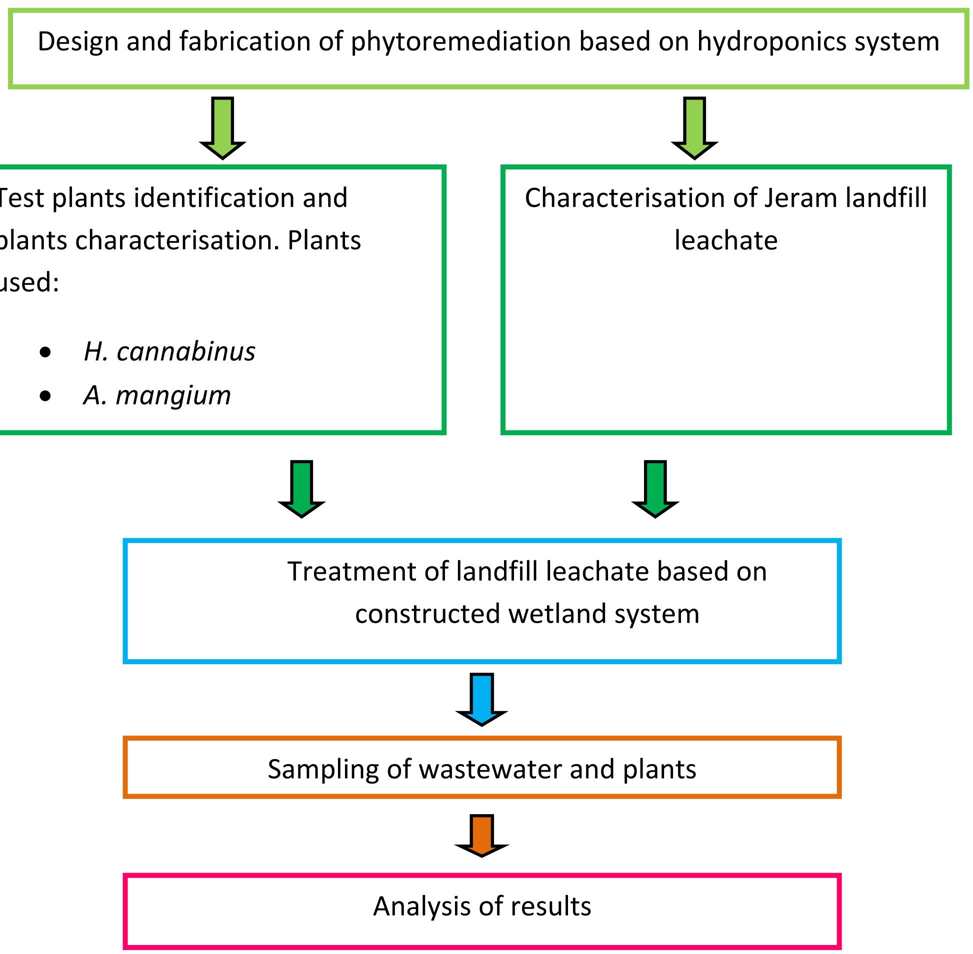
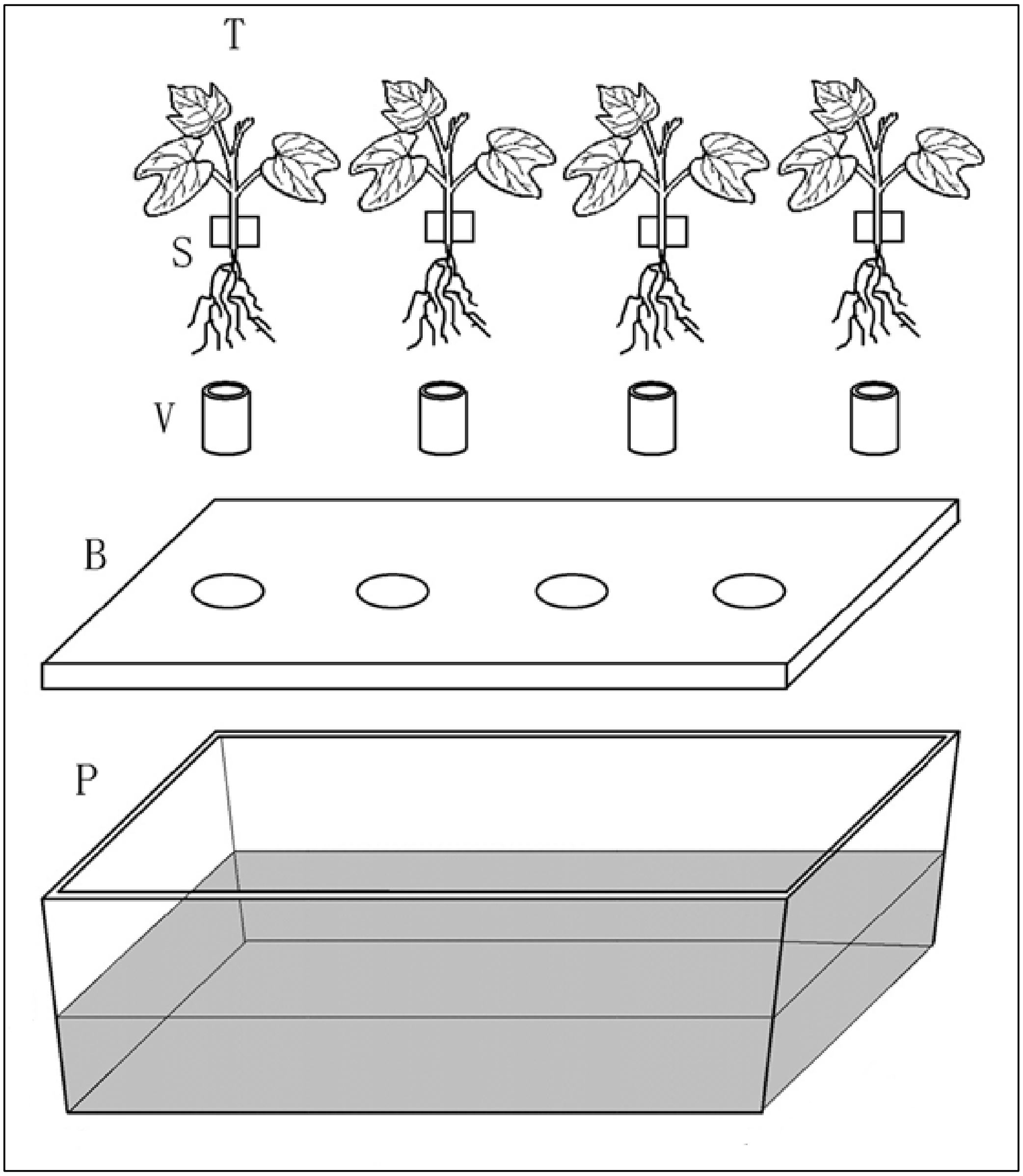


Figure 3.1: Flow diagram of activities to be carried out for the hydroponics system studied in phytoremediation

3.10.1 Experimental set-up



T: Plants	B: Polistyrene
S: Sponge	P: Holding tank
V: Net-Pot	

Figure 3.2: Schematic diagram of hydroponic system

A wetland system was constructed in tanks in laboratory scale to study the metal and macronutrient removal by the two terrestrial plants tested for 'pot culture'. The study was conducted for a period of 5 weeks from 10 January 2011 to 14 February 2011. The plants were grown vegetatively in nutrient solution in indoor under controlled environmental conditions with a 12 h light period, a 25 ^{O}C light/ 20 ^{0}C dark regimes and 60% relative humidity. About 80 plant seedlings for each species were placed in 9 tanks (approximately 9 plants per each species per tank). Each tank was resembled by high-quality polyethylene tank (55 cm length x 41 cm width x 8 cm depth), laid at the ground level, and filled with 20 L of stagnant nutrient solution. Figure 3.2 above shows a schematic diagram of experimental setup of a hydroponic system.

3.10.2 Design and fabrication of system

Plate 3.16: Apparatus used for the hydroponics system set up

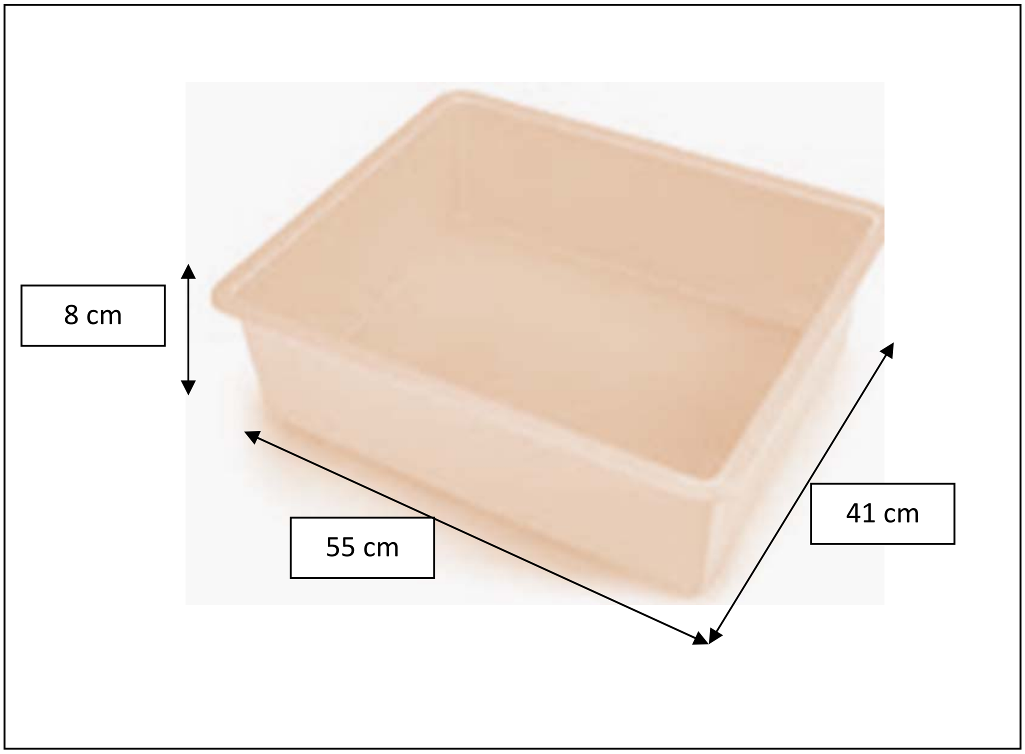


Plate 3.17: Diagram of hydroponic tank used for phytoremediation study

Plate 3.18: Net-pot

Apparatus used for setting up the hydroponics system is as shown in Plate 3.16. During 10 days of acclimatization, the Kenaf and Akasia plants were planted in nutrient stock solution in the holding tank (Plate 3.9.17). The plant seeds (4 - 5 seeds per pot) were sown on the media thread placed in net-pot as shown in Plate 3.18. After acclimatization period, the nutrient solution of each 9 tanks was prepared for 9 treatments, respectively, by adding appropriate amounts of leachate (Refer to Section 3.9.5). The control tank was not treated with leachate and thus filled up with deionised water. The adopted leachate concentrations were similar to those used in pot culture studies. The experiment was carried out in three replicates.

The total volume of water and the pH in each tank were monitored daily and maintained constant throughout the duration of the experiments (Sanita diToppi et. al., 2007). Every 3 days, the water in the 9 tanks was replaced with new treatments amended with nutrient solution specific for hydroponic cultures (Gibeaut's Nutrient Solution). pH of the nutrient solution were held constant at pH 6.0 throughout the 5 week of plants growth period. Every day, the water in every tank was stirred thoroughly (Sanita diToppi et. al., 2007) to agitate the water to ensure good aeration of the test plants' root system.

3.10.3 Test plant material for hydroponic culture

Healthy and uniformly aged seeds of *A. mangium* and *H. cannabinus* L. plants provided by FRIM and Malaysian Tobacco Board were utilized for the experiments. Terrestrial plants used for 'pot-culture' system were hydroponically grown and supported so that their roots were immersed in the target water. The toxins in the water were removed by the roots via physical and active biological processes, resulting in a purified effluent. About 4 -5 seeds of Akasia and Kenaf were planted in each pot respectively (Plate 3.19 and Plate 3.20).

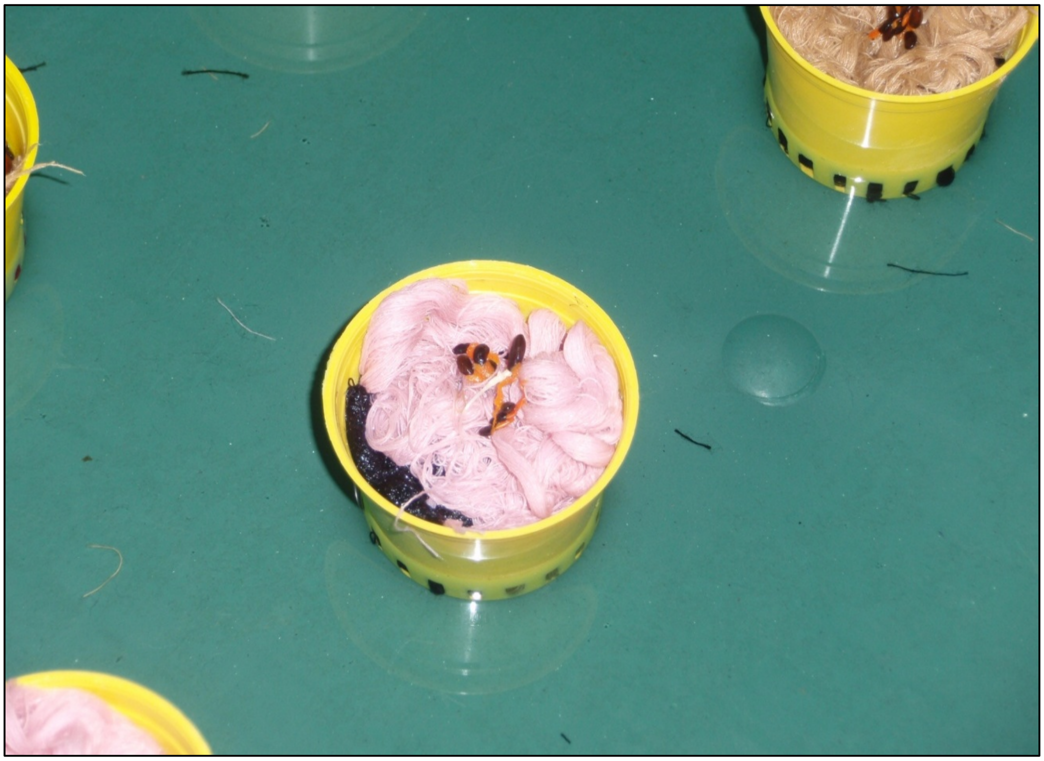


Plate 3.19: Akasia seeds sown into thread media in a net-pot

Plate 3.20: Kenaf seeds planted hydroponically (4-5 seeds per pot)

3.10.4 Preparation of stock solution

Nutrient stock solution, used during the acclimatization period of the test plant seedlings was prepared based on Gibeaut's nutrient stock solution (Gibeaut et. al., 1997). Solutions were made from macronutrient and micronutrient as listed in Table 3.2. The macronutrient stock solutions were stored in different containers. The Fe stock solution was contained in aluminum-foil-covered or dark-brown bottle in order to prevent light degradation. The micronutrient stock solution was a combination of all micronutrients mixed together in a single container. All solutions were prepared with distilled-deionized water. After making the complete Gibeaut's solution, the pH was quite high and was adjusted to nearly pH 6.0 with 10 M HNO_3. The key point was to change the solution when it goes above pH 7.0 or add HNO_3 to reduce pH back to pH 6.0.

Table 3.2: Preparation of Gibeaut's Nutrient Solution

Nutrients	**Stock**	**Stock Solution (g/L)**	**mL Stock Solution/ 1L**	**Molecular weight**
Macronutrients				
$Ca(NO_3)_2 \times 4H_2O$	1M	236.15	1.50	236.15
KNO_3	1M	101.11	1.25	101.11
$Mg(SO_4) \times 7H_2O$	1M	246.48	0.75	246.48
KH_2PO_4	1M	136.09	0.50	136.09
$Na_2O_3Si \times 9H_2O$	0.1M	28.420	1.00	284.20
Fe (Sprint 330 iron chelate)	0.072 M	40.0	1.00	10% (w/w)
Micronutrients				
KCl	50mM	3.728	1.00	74.56
$MnSO_4 \times H_2O$	10mM	1.690	1.00	169.01
$CuSO_4 \times 5H_2O$	1.5mM	0.375	1.00	249.68
$ZnSO_4 \times 7H_2O$	2mM	0.575	1.00	287.54
H_3BO_3	50mM	3.092	1.00	61.83
$(NH_4)_6Mo_7O_{24}$	0.075mM	0.093	1.00	1235.9

Source: Gibeaut et. al., 1997

Even though Silicon (Si) is not categorised as an essential nutrient for plants, Si was included in this study because it naturally occur in the plant cell wall and in the soil solution. Si is believed to play role in mechanical strengthening, improving nutritional balance, and pathogen resistance (Gibeaut et. al., 1997).

3.10.5 Leachate as Growth Media

Leachate solution was used as a source of nutrient solution after 10 days of acclimatization of the test plants. The leachate solution was prepared for 20 L for each and every tank. Fe, As, CN and NH3-N uptake by the Kenaf and Akasia plants were assessed for the purpose of this study. The test media was organized for 9 compositions; i.e. Zero–N Control; 100 % raw leachate; treated leachate (TL) at 100%; 75%; 50%; 25%; 12.5%. The experimental design is shown in Table 3.3.

Table 3.3: Experimental Design of Leachate Nutrient Solution

Treatments	Details of treatment	N-content (%)
Control	Distilled water	0
100% IF	Inorganic Fertiliser N:P:K = 1:1:1	0.48
12.5% TL	12.5% Treated Leachate + 87.5 % distilled water	0.12
25% TL	25% Treated Leachate TL + 75 % distilled water	0.24
50% TL	50% Treated Leachate + 50% distilled water	0.48
50%TL+50% IF	50% Treated Leachate + 50% Inorganic Fertiliser	0.72
75% TL	75% Treated Leachate + 25 % distilled water	0.72
100% TL	100% Treated Leachate	0.96
100% RL	100% Raw Leachate	0.98

3.10.6 Sampling Operations

Plant material were sampled and analysed at the end of the 5th week of experiment. Water samples were collected from each tank at a depth of 1 cm from the mouth of tanks. The Kenaf and Akasia plants were harvested, divided into shoots, leaves and roots and the length and weight determined. During sampling, test plants species which were homogeneous in their health state and size were randomly collected for each treatment and control and carefully rinsed with distilled water. Root, shoot and leaf dry matter were measured after oven drying at 70 ^{O}C (Dimitriou et. al., 2006) for 3 days.

The effect of leachate concentration on plant growth was also studied. Fe, As, CN, NH_3-N content in root, stem and leaves of *A. mangium* and *H. cannabinus* L. were measured by ICP- OES. Leachate treatments were also measured for contaminant concentration in wastewater after plant uptake at the final harvest. Plate 3.21 shows the wastewater sampled in graduated square bottles of 30 mL covered with aluminium foil to prevent from light degradation.

Plate 3.21: Leachate treatment sampled in graduated square bottles

3.10.7 Plant cultivation in hydroponic culture

Each of the test plant was grown in the net-pots containing sponge which acts as wick. Experiments were carried out with randomized blocks of 9 treatments replicated eight times (72 experimental units). Deionised water was applied to all seedlings for an establishment period of 10 days. Following establishment, seedlings were treated with leachate or fertilized water. Total leachate treatment application was performed till plants showed signs of retarded growth. Test media was agitated at daily basis. No pesticide was applied to all the test plants till the end of the experiment.

Kenaf and Akasia plants were harvested by uprooting at 5th week of growth. During harvest, plants were sectioned into parts of leaf, stem and root after rinsing thoroughly in tap water. The leaf length (LL) and stem height (SH) (Plate 3.22) were measured prior to harvesting while the root length (RL) was measured after harvest (Plate 3.23). Total plant height measurements were carried out by placing the harvested plants on polystyrene. Fresh weights of the separated leaves, stem, roots and sail sample were determined. Refer to Materials and Methodology: Section 3.4 for processes carried out after harvest.

Wastewater samples were also collected within the root zone of Kenaf and Akasia, approximately 0 to 5 cm from the surface by scooping the water samples with a graduated square bottle and packed in aluminium foil. Wastewater samples were also measured for Fe, As, CN and NH_3-N using ICP-OES.

Plate 3.22: Stem height measurement of harvested hydroponic plants

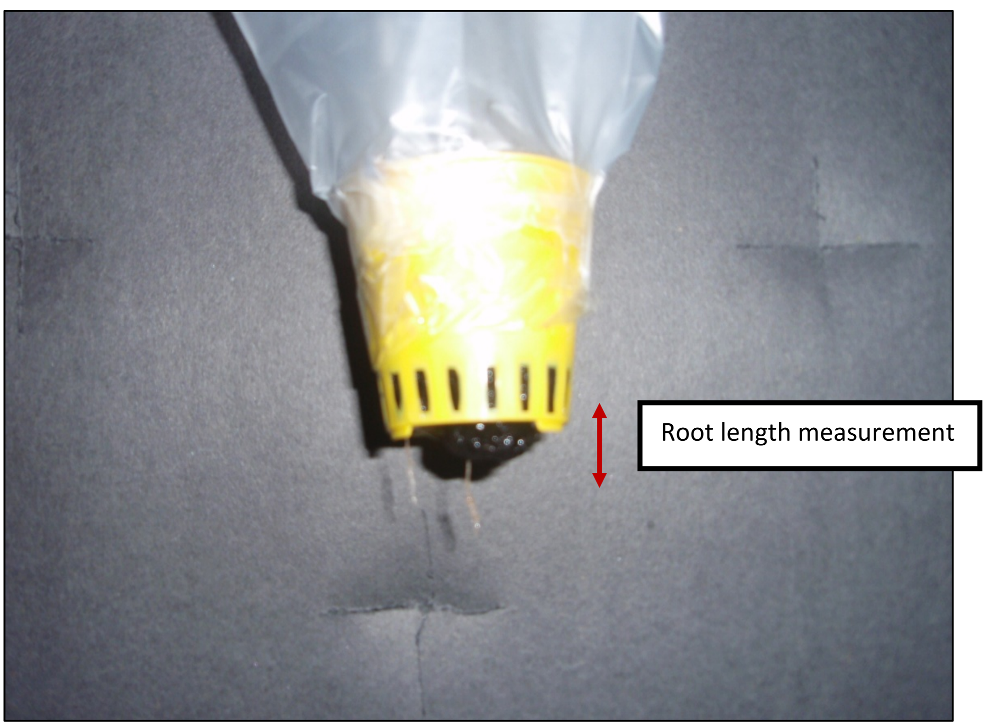


Plate 3.23: Root length measurement of harvested hydroponic plants

3.10.8 Toxicity experiments and metal removal

Statistical analysis was carried out for the metal removal data collected. The Bioconcentration Factor (BCF) and Translocation Factor (TF) were calculated according to Yoon (Yoon et. al., 2006) as shown by Equation 1 and 2 in Section 3.7.

3.11 Decision Support Software for Phytoremediation Systems

Microcomputer software was developed to provide decision support information on phytoremediation efficiency based on proposed models for selected plant species. A mathematical modeling based on Kinetics Model, Relative Growth Rate (RGR), Bioaccumulation Factor (BCF) and Translocation Factor (TF) was developed to quantitate the ability of the selected test plants in removal of contaminants within the phytoextraction and phytostabilisation system, a phytoremediation based technology referring to decontamination/ detoxification of contaminated soils using special metal accumulating plants.

A First Order kinetics based model was developed and incorporated into the system which process information relevant to the system design of phytoremediation process. Physical components of the phytoremediation system – plant scientific classification, photographs of plants, phytoremediation efficiency model are coupled with engineering and biological aspects of the process.

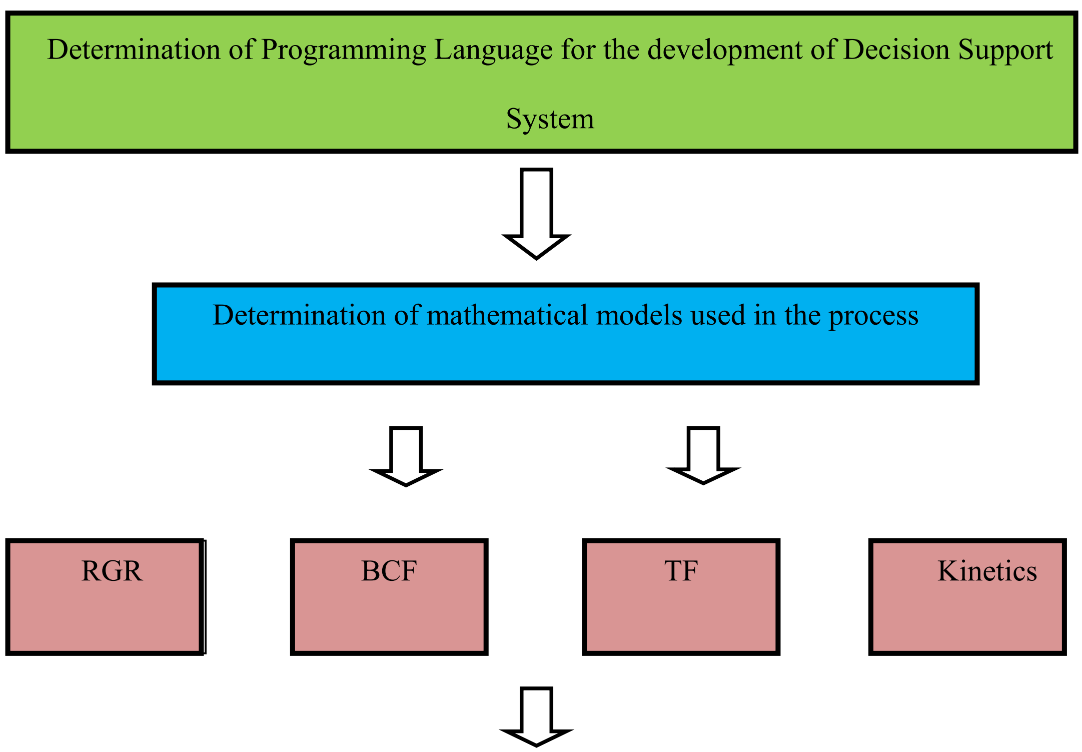
The ability to develop artificial intelligence system for operation and evalution of phytoremediation efficiency is critical for the implementation of *in situ* pilot studies based on these processes.

3.11.1 Process and Systems Model Integration

Microsoft's Visual Basic version 2005 Express Edition programming language was used to develop a computerized artificial intelligence tool, e-PMS, for evaluation of phytoremediation efficiency of selected test plant species. e-PMS, short for Phytoremediation Modeling System, is a user-interactive program which combines user inputs and the phytoremediation models into an integrated series of 'windows', each with specific information about the selected test plants, photographs, glossary, quizzes and mathematical models pertaining to phytoremediation system.

Experimental results for the calculation model were supplied from the experiment conducted at the nursery of National Hydraulics Research Institute of Malaysia. Modeling efforts focused on evaluating the value of Bioaccumulation rate constant, half-life of metal in plants, RGR, BCF and TF.

The following flow diagram (Figure 3.3) shows series of activities and procedures carried out for the development of e-PMS application.



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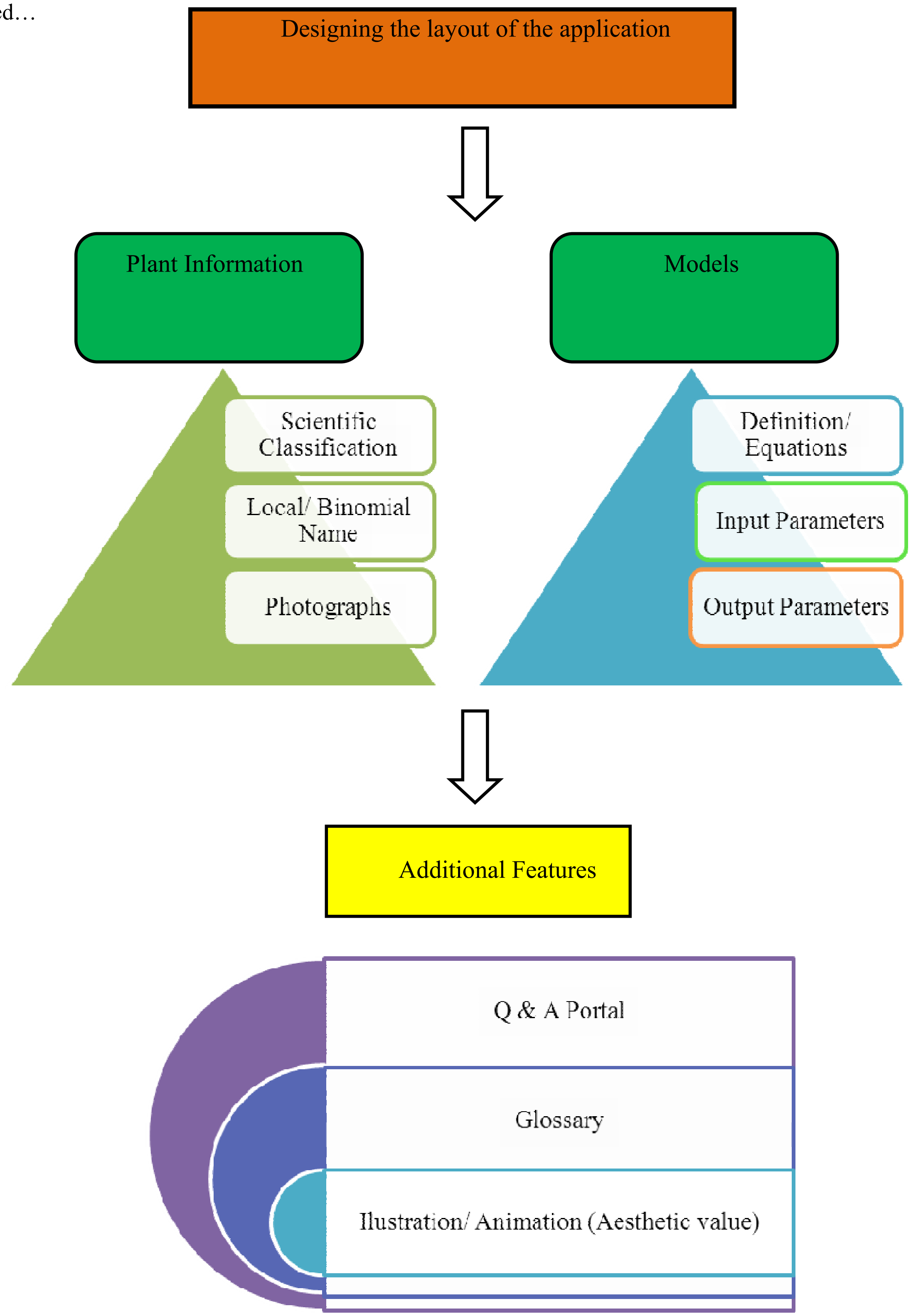


Figure 3.3: Flow Chart of Development of e-PMS Decision Support System

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physicochemical properties of leachate waste used for phytoremediation

The physicochemical properties of raw leachate from Jeram Sanitary Landfill are shown in Table 4.1. The untreated leachate had high BOD and COD, approximately 15,000mg/L and 53,000 mg/L, respectively and these high readings indicate that there are organic materials in the leachate which are highly biodegradable.

Table 4.1: Characteristics of untreated Jeram landfill leachate compared with Acceptable conditions for Discharge of Leachate Regulations 2009, Environmental Quality Act (EQA 1974), MALAYSIA

Parameter	Unit	Raw Leachate (RL)	Standard	Method
pH at 25^{0}C		3.6±0.9	6.0-9.0	APHA 4500-H^+B
Temperature	^{O}C	35.5±1.5	40.0	APHA 2550B
BOD at 20 ^{0}C	mg L^{-1}	14549.0±12.3	20.0	APHA 5210B
COD	mg L^{-1}	53165.0±22.4	400.0	APHA 5220C
Suspended Solids	mg L^{-1}	24450.0±7.7	50.0	APHA 2540D
Oil and Grease	mg L^{-1}	411.0±4.1	5.0	APHA 5520B
Dissolved Oxygen	mg L^{-1}	1.9±0.2	NA	APHA 4500-O G
Chloride	mg L^{-1}	2675.0±6.3	NA	Hach 8113
Mercury as Hg	mg L^{-1}	ND	0.005	APHA 3112B
Cadmium as Cd	mg L^{-1}	ND	0.01	APHA 3120B
Chromium Hexavalent as Cr^{6+}	mg L^{-1}	ND	0.05	APHA 3500-CrB
Arsenic as As	mg L^{-1}	0.5±0.1	0.05	APHA 3120B

* **Table 4.1** continued

Parameter	**Unit**	**Raw Leachate (RL)**	**Standard**	**Method**
Cyanide as CN	mg L^{-1}	0.4±0.1	0.05	Hach 8027
Lead as Pb	mg L^{-1}	0.3±0.0	0.10	APHA 3120B
Chromium Trivalent as Cr^{3+}	mg L^{-1}	0.4±0.0	0.20	APHA 3120B & 3500-CrB
Copper	mg L^{-1}	2.4±0.4	0.20	APHA 3120B
Manganese, Mn	mg L^{-1}	6.4±0.6	0.20	APHA 3120B
Nickel, Ni	mg L^{-1}	0.3±0.0	0.20	APHA 3120B
Zinc, Zn	mg L^{-1}	4.7±1.1	2.0	APHA 3120B
Boron, B	mg L^{-1}	2.6±0.0	1.0	APHA 3120B
Iron, Fe	mg L^{-1}	223.6±10.3	5.0	APHA 3120B
Phenol	mg L^{-1}	0.3±0.1	0.001	APHA 5530 B&C
Magnesium, Mg	mg L^{-1}	119.7±1.6	NA	APHA 3120B
Calsium, Ca	mg L^{-1}	757.5±26.5	NA	APHA 3120B
Potassium, K	mg L^{-1}	1039.7±14.2	NA	APHA 3120B
Ammoniacal Nitrogen	mg L^{-1}	14.0±2.6	5.0	Hach 8155
Total Nitrogen	mg L^{-1}	958.0±6.4	NA	APHA 4500-N
Sulphide	mg L^{-1}	6.0±1.0	0.50	Hach 8131
Phosphorus	mg L^{-1}	62.0±0.7	NA	Hach 8048
Turbidity	NTU	50.0±2.5	NA	APHA 2130B
Salinity	ppt	3.0±0.6	NA	Hach 10073

The ratio of COD/BOD_5 of raw leachate was about 3.6 and this value shows that the organic material in the leachate is easily biodegradable (Mizanur et. al., 1999).The values of BOD_5 and COD were considered very high compared to Environmental

Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 of Acceptable Conditions For Discharge Of Leachate (Second Schedule of Environmental Quality Act 1974) (Department of Environment Malaysia, 2010). All parameters which do not comply with the Standard are marked in red font.

The pH of the leachate sample was 3.60 and this indicates that the landfill is in an acidic phase. The acidic pH is caused by high production of volatile fatty acids and the high partial pressure of CO_2 (Robinson, 1989). The acidic pH of the leachate is also possibly due to the availability of easily biodegradable organic matter and thus the microorganisms produce significant concentrations of H^+ ion (Tchobanoglous et. al., 1993). Leachate contains high concentrations of suspended solid (24,450 mg L^{-1}) and ammonia (14 mg L^{-1}) due to the decomposition of nitrogenous substances of waste mass (Aziz et. al., 2007), which also indicates that the landfill is in acidic phase (Robinson, 1989). The leachate sample also recorded high turbidity (50 NTU) and suspended solids as a consequence of organic and inorganic solids present. The presence of high salt concentrations in the leachate are due to the large amount of garbage (food waste) disposed in to the landfill. Sulphide was also recorded high at 6 mg/L compared to Acceptable Conditions for Discharge of Leachate Regulations 2009, Environmental Quality Act of Malaysia (Department of Environment Malaysia, 2010).

Leachate from Jeram Sanitary Landfill also contained high concentration of heavy metals such as, As (0.5 mg/L), Pb (0.3 mg/L) and Fe (224 mg/L) and metals like CN (0.4 mg/L), Cu (2.4 mg/ L) and Mn (6.4 mg/L) and results obtained were in accordance to characteristic of a young methanogenic leachate as reported in DoE (DoE, 1995). All parameters measured exceeded the regulatory standard of limit of EQA 1974 Discharge of Leachate -Regulations 2009 according to Department of Environment Malaysia (2010). Potassium is released during refuse decomposition and the main source of potassium is plant material and discarded food (Robinson, 1989), which is the main component of Malaysian Municipal Solid Waste (MSW).

The presence of heavy metals in the leachate is due to industrial waste and household non-hazardous waste (Accot Technologies, 2008). Alaribe and Agamuthu (2010), had also reported high heavy metal composition of Ampar Tenang landfill leachate but Jeram landfill leachate had higher readings of BOD and COD in comparison to Ampar Tenang landfill, which exhibited typical characteristics of an ageing landfill.

The physicochemical properties of Jeram Sanitary Landfill leachate treated with Ferric (III) Chloride (w/v: 5 g L^{-1}) are shown in Table 4.2. Treated leachate presented a lower BOD, COD and Suspended solids concentration compared to untreated leachate at approximately 4500, 7600 and 1050 mg L^{-1}, respectively. Some metals, for instance As, Mn, Zn, Mg and Ca showed reduction in concentration after coagulation (Table 4.2). Reduction of Fe, As and NH_3-N was 0.8%, 20% and 17.1%, respectively. No reduction noticed in CN concentration.

Sulphide, Phosphorus and Turbidity concentrations also showed improvement with the application of $FeCl_3$. Coagulation is the procedure of chemically stimulating primary treatment to minimise suspended solids and organic loads from primary clarifiers (IWA Water Wiki, 2010). When metal coagulants (aluminum sulfate, aluminum chloride, sodium aluminate, ferric sulfate, ferrous sulfate, ferric chloride and ferric chloride sulfate) are added to water, the metal ions (Al and Fe) hydrolyze rapidly but in a unregulated manner. A series of metal hydrolysis species is formed. The efficiency of rapid mixing, the pH, and the coagulant dosage decides which hydrolysis species is efficient for treatment (IWA Water Wiki, 2010).

Table 4.2: Characteristics of $FeCl_3$ treated leachate of Jeram landfill compared with Acceptable conditions for Discharge of Leachate Regulations 2009, Environmental Quality Act (EQA 1974), MALAYSIA.

Parameter	Unit	Untreated Leachate (RL)	Treated Leachate (TL)	Standard	Method
pH at 25^{0}C		3.6±0.9	8.1±0.8	6.0-9.0	APHA 4500-H^+B
Temperature	^{O}C	35.5±1.5	34.0±1.8	40.0	APHA 2550B
BOD at 20 ^{0}C	mg L^{-1}	14549.0±12.3	4493.0±11.5	20.0	APHA 5210B
COD	mg L^{-1}	53165.0±22.4	7613.0±24.1	400.0	APHA 5220C
Suspended Solids	mg L^{-1}	24450.0±7.7	1050.0±6.7	50.0	APHA 2540D
Oil and Grease	mg L^{-1}	411.0±4.1	10.1±2.2	5.0	APHA 5520B
Dissolved Oxygen	mg L^{-1}	1.9±0.2	1.9±0.3	NA	APHA 4500-O G
Chloride	mg L^{-1}	2675.0±6.3	2678.0±4.7	NA	Hach 8113
Mercury as Hg	mg L^{-1}	ND	ND	0.005	APHA 3112B
Cadmium as Cd	mg L^{-1}	ND	ND	0.01	APHA 3120B
Chromium Hexavalent as Cr^{6+}	mg L^{-1}	ND	ND	0.05	APHA 3500-CrB
Arsenic as As	mg L^{-1}	0.5±0.1	0.4±0.1	0.05	APHA 3120B
Cyanide as CN	mg L^{-1}	0.4±0.1	0.4±0.0	0.05	Hach 8027
Lead as Pb	mg L^{-1}	0.3±0.0	0.3±0.1	0.10	APHA 3120B
Chromium Trivalent as Cr^{3+}	mg L^{-1}	0.4±0.0	0.4±0.0	0.20	APHA 3120B & 3500-CrB
Copper	mg L^{-1}	2.4±0.4	2.4±0.1	0.20	APHA 3120B
Manganese, Mn	mg L^{-1}	6.4±0.6	2.6±0.0	0.20	APHA 3120B
Nickel, Ni	mg L^{-1}	0.3±0.0	0.3±0.1	0.20	APHA 3120B
Zinc, Zn	mg L^{-1}	4.7±1.1	4.4±0.5	2.0	APHA 3120B

* **Table 4.2** continued

Parameter	Unit	Untreated Leachate (RL)	Treated Leachate (TL)	Standard	Method
Boron, B	mg L^{-1}	2.6±0.0	2.5±0.2	1.0	APHA 3120B
Iron, Fe	mg L^{-1}	223.6±10.3	221.8±4.3	5.0	APHA 3120B
Phenol	mg L^{-1}	0.3±0.1	0.2±0.0	0.001	APHA 5530B & C
Magnesium, Mg	mg L^{-1}	119.7±1.6	107.7±3.4	NA	APHA 3120B
Calsium, Ca	mg L^{-1}	757.5±26.5	309.5±14.5	NA	APHA 3120B
Potassium, K	mg L^{-1}	1039.7±14.2	1095.4±10.6	NA	APHA 3120B
Ammoniacal Nitrogen	mg L^{-1}	14.0±2.6	11.6±1.7	5.0	Hach 8155
Total Nitrogen	mg L^{-1}	958.0±6.4	903.0±3.8	NA	APHA 4500-N
Sulphide	mg L^{-1}	6.0±1.0	4.0±0.4	0.50	Hach 8131
Phosphorus	mg L^{-1}	62.0±0.7	48.0±1.2	NA	Hach 8048
Turbidity	NTU	50.0±2.5	20.0±1.1	NA	APHA 2130B
Salinity	ppt	3.0±0.6	0.3±0.1	NA	Hach 10073

4.2 Response of Kenaf plants to the leachate treatments and Biomass of Kenaf

The appearance and response of plants to various concentration of leachate was monitored during the 120 days of the study (Agamuthu et. al., 2010). No plant death was observed. Plates 4.1 to 4.3 show Kenaf plants uprooted at every 8th, 12th and 16th week of growth.

Plate 4.1: Harvested Kenaf at week 8 of growth

Plate 4.2: Kenaf plants at 12th week of growth

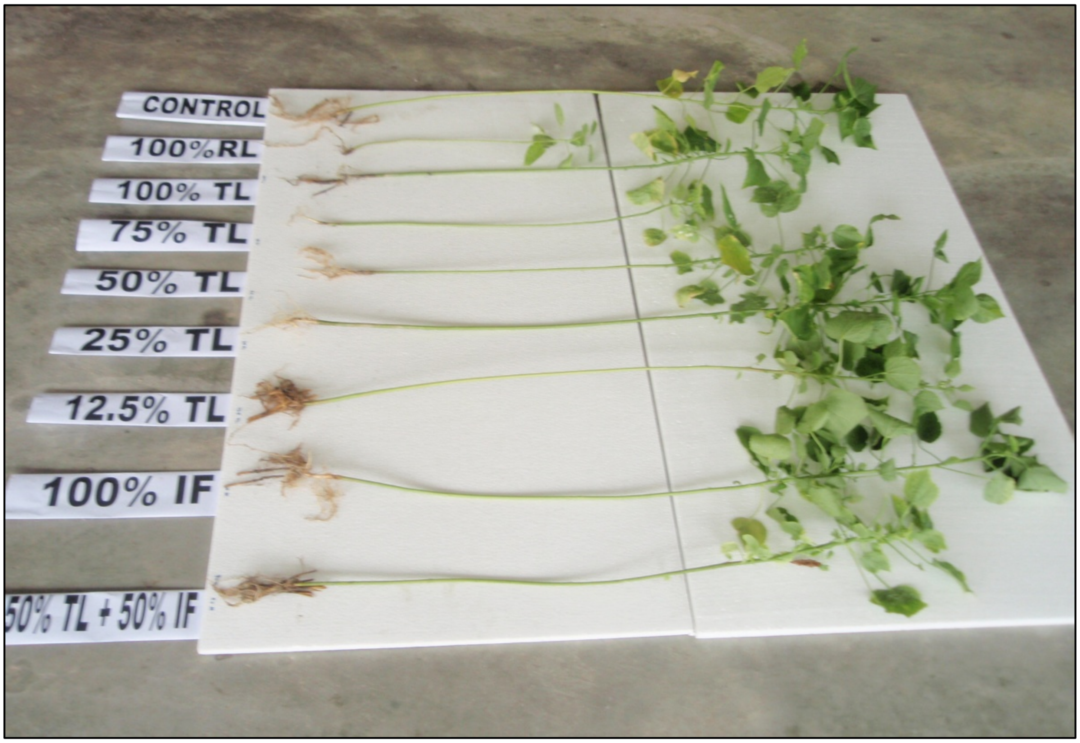


Plate 4.3: Harvested Kenaf plant at week 16 of growth

Relative growth rate of leaf, stem and root dry mass of the *Hibiscus cannabinus* L. plant for every treatment was calculated at the end of 3rd cycle (16th week) as shown in Table 4.3. The trend of RGR is illustrated in Figure 4.1. Factorial analysis revealed that the interaction between leachate treatments was significant in affecting the relative growth rate of Kenaf biomass ($p<0.01$).

The distribution of plant dry matter of Kenaf at final harvest was 32.3% leaves, 43.6% stems and 24.1 % roots, respectively. Inhibition of biomass accumulation was negligible in this study which indicated that As, Fe, CN and NH_3-N did not cause adverse effects on Kenaf growth. This is in sharp contrast with findings of Dimitriou et. al. (2006), who recorded 30 – 35% reduction in relative growth rate of aerial Kenaf plant parts treated with leachate compared to control. The results are similar to the records of Ho et. al. (2008), where significantly greater total biomass of Kenaf was found in metal-contaminated soil.

Table 4.3: Relative Growth Rate of *H. cannabinus* L. treated with different concentrations of leachate

TREATMENTS	RGR_L (cm/ week)	RGR_S (cm/ week)	RGR_R (cm/ week)	RGR_{TP} (cm/ week)
CONTROL	0.60±0.23	0.78±0.25	0.61±0.12	0.55±0.17
12.5 % TL *	0.87±0.21	1.02±0.38	0.89±0.24	0.94±0.19
25% TL *	2.20±0.22	2.19±1.04	1.52±0.41	1.76±0.06
50 % TL *	1.44±0.23	1.84±0.07	1.38±0.25	1.67±0.18
100 % IF *	1.21±0.24	1.73±0.11	1.08±0.20	1.47±0.19
50% TL+50%IF*	1.12±0.11	1.58±0.05	1.02±0.56	1.40±0.21
75% TL	1.01±0.30	1.34±0.21	0.96±0.49	1.33±0.19
100% TL	0.94±0.21	1.04±0.18	0.91±0.22	1.11±0.21
100% RL	0.67±0.19	0.82±0.04	0.70±0.20	0.61±0.15

* Significantly different RGR of leaves, stem, root and total PDM compared to control

RGR_L: Relative Growth Rate of leaves

RGR_{TP}: Relative Growth Rate of total plant dry biomass

RGR_S: Relative Growth Rate of stem

RGR_R: Relative Growth Rate of root

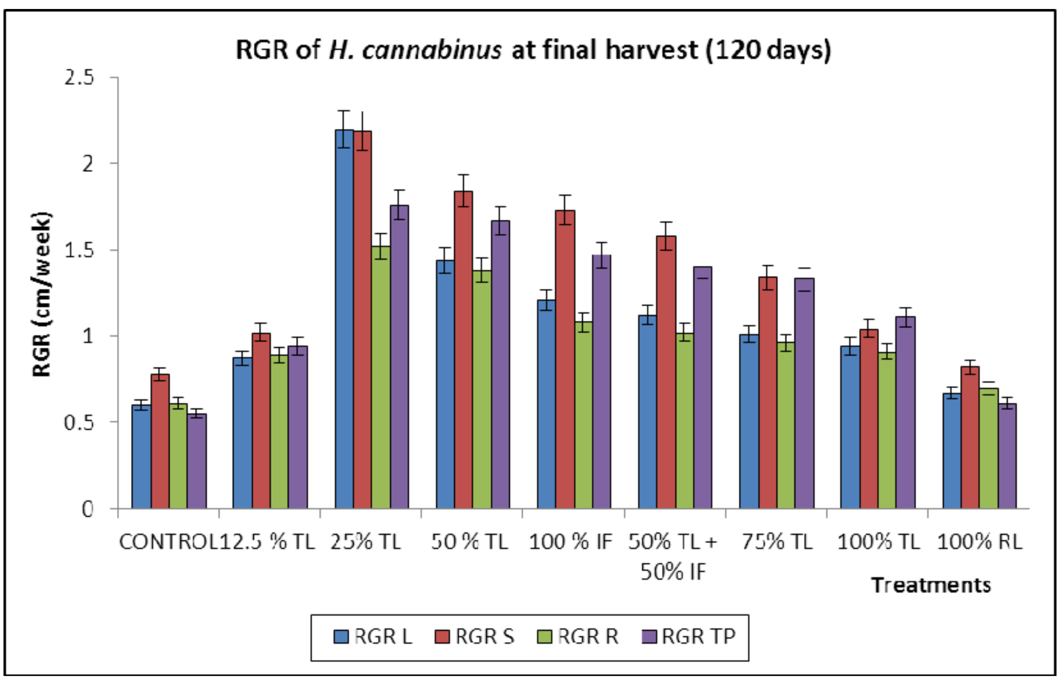


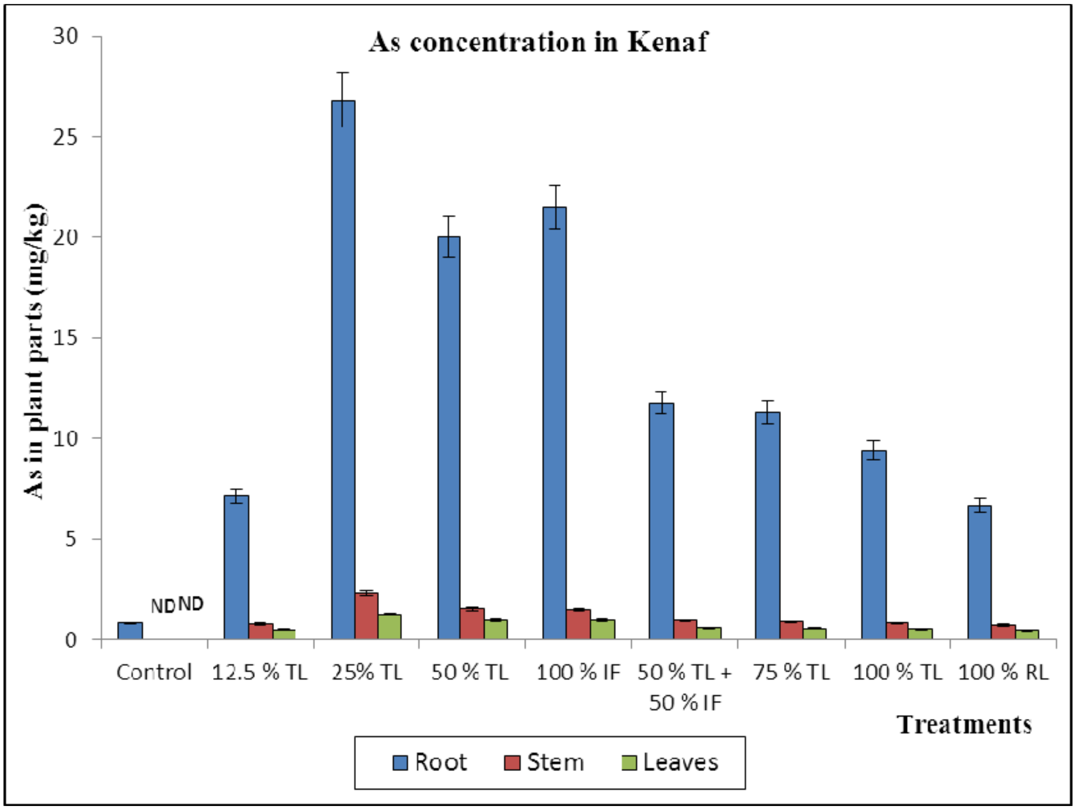
Figure 4.1: RGR of root (R), stem (S), leaves (L) and total Kenaf dry biomass (TP)

Pearson's correlation coefficient (r =0.84, r =0.93 and r =0.82, $p<0.05$) was obtained between RGR_L, RGR_S, RGR_R, respectively with total plant dry biomass (RGR_{TP}) indicating a strong correlation between these variables. The mean of RGR values was also significantly different among RGR_L, RGR_S, RGR_R, and RGR_{TP} ($p<0.05$). Kenaf irrigated with 25% TL (0.24% N-content) recorded highest significant RGR_L, RGR_S, RGR_R and RGR_{TP}. Treatment concentrations were significant at 12.5% TL, 25% TL, 50% TL and 50% TL + 50% IF compared to the control treatment.

4.3 Bioaccumulation of Fe, As, CN and NH_3-N in Kenaf planted in soil contaminated with different treatments of leachate

The bioaccumulation of Fe, As, CN and NH_3-N in plant parts, soil sample and control soil (without plants) at different concentrations of leachate contaminated soil at week 16 of harvest are appended in Appendix A (Table A.1, Table A.2, Table A.3 and Table A.4). Fe, As, CN and NH_3-N were detected in all plant parts irrigated with leachate treatments in the nursery pot experiment, with insignificant bioavailable metals present in leaves and stem. There was no insoluble Arsenic, Cyanide or Iron species noted on the roots of Kenaf upon harvest.

Figure 4.2 illustrates As uptake by Kenaf plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16^{th} of growth.



*ND: Non- Detectable

Figure 4.2: As concentration in root, stem and leaves of Kenaf under different leachate treatments

At final harvest, Kenaf plants irrigated with 25% TL recorded optimum As uptake in Kenaf root which recorded 26.82 mg As kg^{-1}. The Kenaf plant root sequestered 0.09 - 0.64 mg As/ g plant dry weight {Calculated as: eg: min/max As in root = (26.82/1000 mg/g * 24.00 {Total PDM for 25TL}). As concentrations in the Kenaf stem and leaves were 0.46 – 1.28 mg kg^{-1} and 0.72 – 2.34 mg kg^{-1}, respectively at the different leachate treatments during final harvest.

The high tolerance of As in Kenaf could be attributed to the detoxification of As by metal detoxifying enzymes called phytochelatins in plants (Schmoger et. al., 2000). As detoxification mechanisms involves reduction of As (V) to As (III) (Tu et. al., 2004; Tu and Ma, 2002). In addition to stimulating As reduction in the plant, As also enhances the production of phytochelatins (PCs) in both As-sensitive plants and As-tolerant plants (Tu et. al., 2004). Formation of As–PC complexes is expected to be a key mechanism of As detoxification in many plants (Schmoger et al., 2000).

Figure 4.3 illustrates Fe uptake by Kenaf plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

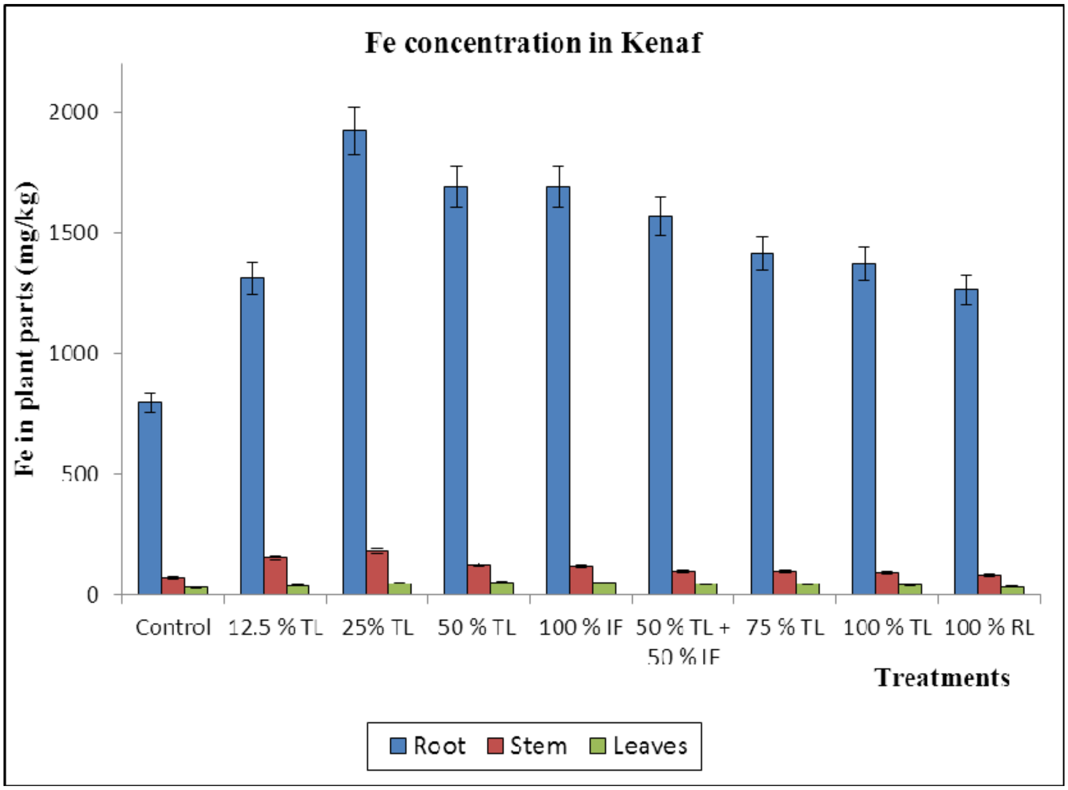


Figure 4.3: Fe concentrations in root, stem and leaves of Kenaf under different leachate treatments

At final harvest, highest concentration of Fe bioaccumulation was recorded by plants grown in 25% TL treatment. The Kenaf root sequestered 16.94– 46.19 mg Fe/ g plant dry weight. The results showed that Fe concentrations in stem and leaves of Kenaf 73.20 – 182.46 mg kg^{-1} and 30.62 – 52.46 mg kg^{-1}, respectively. Fe^{3+} is relatively insoluble in alkaline soils and two alternative mechanisms for Fe acquisition have evolved in plants. Nongraminaceous monocots and dicits, the ‘strategy I plants’, acidify the rhizosphere (presumably via an H+-ATP ase) to increase Fe solubility and use a ferric-reductase to reduce Fe^{3+} to Fe^{2+} which is transported into roots via an Fe^{2+}

transporter (Haydon and Cobbett, 2007). Graminaceous species utilize 'strategy II' whereby a metal-binding ligand, Mugineic acid, is synthesized enzymatically from three molecules of S-adenosyl methionine and is secreted from roots to bind Fe^{3+} in the rhizosphere. The Fe (III)-MA then enters the roots via a specific transporter (Haydon and Cobbett, 2007; Curie and Briat, 2003).

Figure 4.4 illustrates CN uptake by Kenaf plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

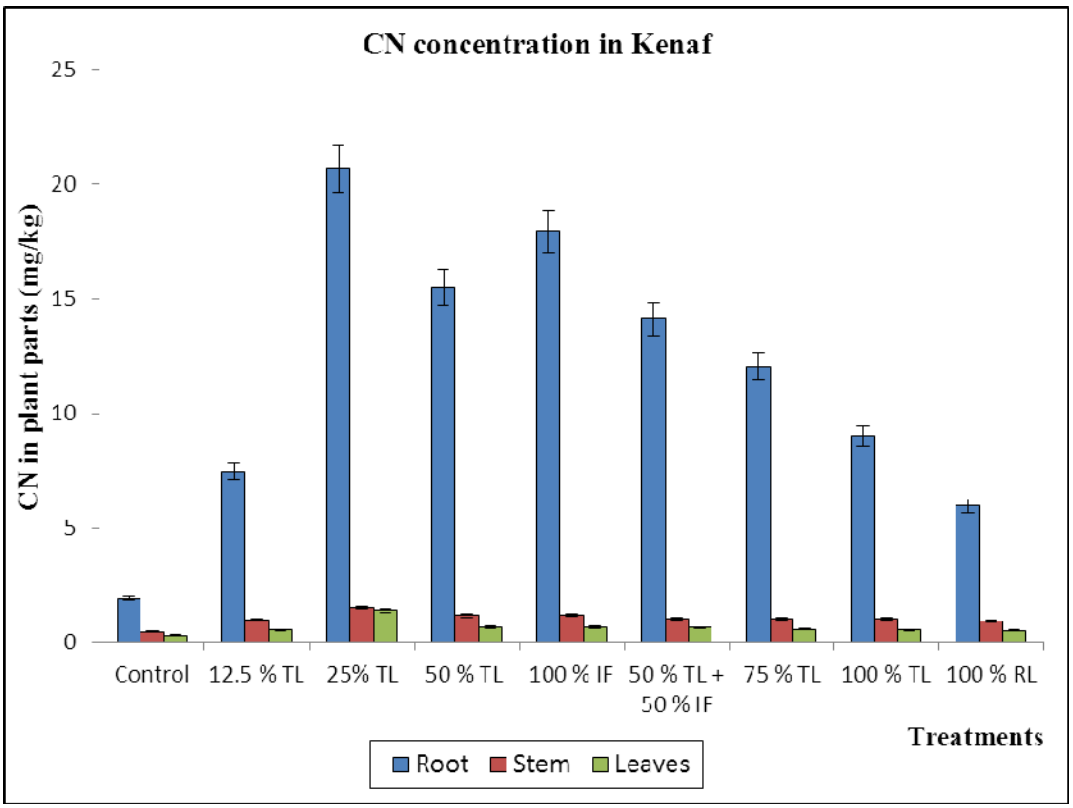


Figure 4.4: CN concentrations in root, stem and leaves of Kenaf under different leachate treatments

Approximately 21 mg CN kg^{-1} were recorded for roots of Kenaf planted in 25% TL contaminated soil. The Kenaf root sequestered 0.08 – 0.50 mg CN/ g plant dry weight at week 16th of plant growth. CN concentrations in stem and leaves of Kenaf were in the range of 0.47 – 1.51 mg kg^{-1} and 0.32 – 1.38 mg kg^{-1}, respectively. Cyanide metabolism is strongly related to ethylene production in plant tissues. In higher plants, the key enzyme to detoxify CN is β-cyanoalanine synthase which catalyzes the conversion of L-cysteine to β-cyanoalanine. β-Cyanoalanine synthase is widely distributed in higher plants (Ebel et. al., 2007). β-Cyanoalanine thus formed is further metabolized to asparagines or to γ-glutamyl-β-cyanoalanine (Pirrung, 1985).

Figure 4.5 illustrates NH_3-N uptake by Kenaf plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

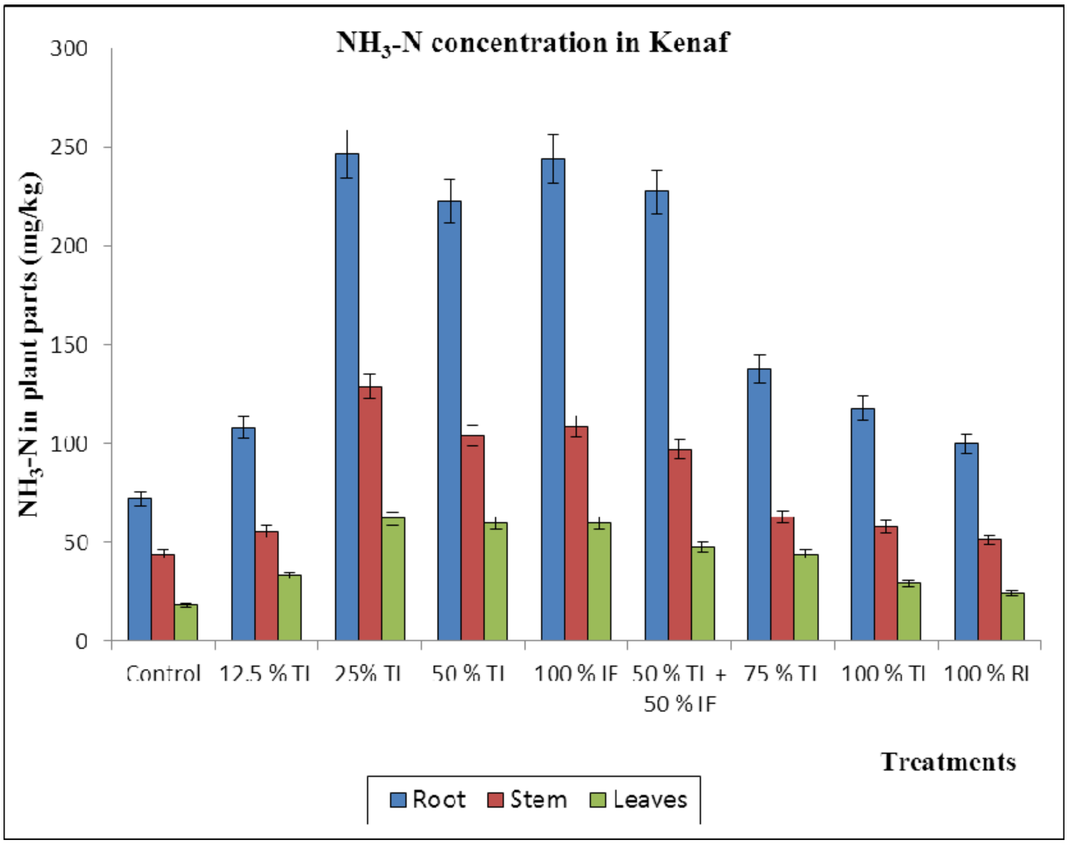


Figure 4.5: NH_3-N concentrations in root, stem and leaves of Kenaf under different leachate treatments

Bioremediation of landfill leachate at 25 % treated leachate recorded optimum efficiency for the removal of NH_3-N after 120 days of growth. The Kenaf root sequestered and 1.34 – 5.92 mg NH_3-N/ g plant dry weight. NH_3-N concentrations in the stem and leaves were 51.58 – 129.09 mg kg^{-1} and 24.45 – 62.09 mg kg^{-1} for leachate treatments, respectively. Plants have the capacity to absorb Ammoniacal Nitrogen via foliar stomata and cellular assimilation into plant cells through the glutamine synthetase and glutamate synthase pathways (Patterson et. al., 2008). At the right concentrations, NH_y (NH_3 + NH_4^+) would favour plant growth, but at a critical threshold it would cause tissue necrosis, reduced growth, and greater frost sensitivity (Van deer Eerden et. al., 1998).

Fe concentrations in roots of Kenaf (796.24 – 1923.81 mg/kg) were significantly higher than As, CN and NH_3-N concentrations in root at 0.82 – 26.82 mg/kg, 1.95 – 20.69 mg/kg and 72.42 – 246.47 mg/kg, respectively. This is line with study conducted by Mench and Martin (1991), where solubility of Fe and Mn was much higher than of the four other metals tested (Cd, Cu, Ni, Zn) in the presence or absence of root exudates of *Nicotiana* spp. and *Zea mays.* Mass of soil sample and plant dry matter (PDM) according to leaves, stems, roots and total PDM was recorded in Table 4.4.

Kenaf plant roots sequestered higher concentration of contaminant in their plant roots because of the direct contact and uptake from the soil; this is in congruence to the observation of Ho (Ho et. al., 2008), who recorded a maximum of 4,200 mg kg^{-1} lead concentrations in Kenaf roots compared to aerial plant parts such as shoot, leaves and bud.

As, Fe, CN and NH_3-N absorption efficiency in Kenaf plants decreased tremendously from root to stem and subsequently to leaves. These results are in contrast to studies done with hyperaccumulator plants (Xue et. al., 2004) where maximum accumulation of contaminants takes place at stem or shoots. The concentrations of As, Fe, CN and NH_3-N in roots, stem and leaves increased progressively with the increase of external leachate concentrations applied but decreased when it was above 100 % IF.

Table 4.4: *H. cannabinus* Plant Dry Matter (PDM) and Dried Soil Sample Mass at final harvest (week 16)

Treatments	PDM (g) and Dried Soil Sample Mass (g)				
	Roots	Stems	Leaves	Total PDM	Control Soil
Control(dH_2O)	1.61± 1.07	4.02 ± 1.99	3.56 ± 0.78	9.19 ± 1.41	4.06 ± 0.76
12.5 % TL	2.86 ± 0.26	6.97 ± 0.26	6.07 ± 0.27	15.90 ± 0.78	3.13 ± 0.95
25% TL	6.98 ± 0.49	9.24 ± 0.30	7.79 ± 1.06	24.01± 1.79	3.42 ± 0.49
50 % TL	6.28 ± 0.34	7.83 ± 0.49	7.62 ± 0.02	21.73 ± 0.83	3.90 ± 0.68
100 % IF	6.30 ± 0.13	7.80 ± 0.13	7.64 ± 0.18	21.74 ± 0.40	2.90 ± 0.78
50% TL+50% IF	5.34 ± 0.39	7.24 ± 0.38	7.39 ± 0.39	19.97 ± 1.17	3.01 ± 0.27
75 % TL	4.82 ± 0.44	7.06 ± 1.11	7.34 ± 0.75	19.22 ± 1.82	3.75 ± 1.29
100 % TL	2.94 ± 0.25	7.01 ± 0.94	6.87± 0.27	16.82 ± 1.27	3.21± 1.00
100 % RL	1.75 ± 0.21	5.63 ± 0.51	6.02 ± 0.67	13.40 ± 0.82	3.19 ± 0.83

The results also indicated the impacts of leachate concentrations towards Kenaf growth. Therefore, there is a possibility of having encountered a situation with two factors counteracting with each other. Application of leachate as strong as above 50% TL might have affected the plants negatively considering the high ionic strength of the leachate (Dimitriou et. al., 2006). Leachate concentrations below 25% TL resulted in lower nutrient additions, which in turn resulted in lower biomass produced as well as lower As, Fe, CN and NH_3-N concentrations; this is in agreement with Dimitrou et. al. (2006) who observed nutrient insufficiency in willows irrigated with high dilution of leachate. Essential metals like Fe, Zn and Cu are required for healthy plant growth but can be toxic when present in excess. Metal homeostasis mechanisms in plants allow uptake and distribution of metals to tissues. Metals are also maintained within plant cells or subcellular compartments below levels that can cause toxic symptoms (Haydon and Cobbett, 2007; Clemens, 2001). Some of the negative impacts of irrigation with landfill leachate are leaf damage, premature leaf senescence and reduced biomass production (Wong and Leung, 1989).

Despite high concentrations of As, Fe, CN and NH3-N in the roots, no visual symptoms of metal toxicity were observed indicating that Kenaf is a As, Fe, CN and NH_3-N tolerant species. According to Ho et. al. (2008), Kenaf is also a good Pb-tolerant species. Very little cellular metal ion content is expected to exist as free ions. According to Haydon and Cobbett (2007), metal ions that are not hold to sites in proteins are expected to be bound to low molecular-weight metal ligands. Metal ligands can have intracellular roles as chelators for sequestering metal ions in the cytosol or in subcellular compartments. Metals with low solubility such as Fe may be used either for mobilization from the soil or for translocation within the plant. Transport mechanisms are required for the secretion or uptake of metal ion ligands by cells or for the movement of ligands between subcellular compartments in order to fulfil these roles (Haydon and Cobbett, 2007).

All heavy metals above a concentration of 0.1% in the soil with the exception of Ferum become toxic to plants and subsequently change the community structure of plants in a polluted habitat (Bothe, 2011). Nevertheless, each plant species has a specific threshold value for every heavy metal where it triggers toxicity.

Relationship of As, Fe, CN and NH_3-N concentrations between the root and stem, root and leaf, and stem and leaf in *Hibiscus cannabinus* according to the paired Student T-test were as presented in tables below.

Table 4.5: Significant difference (P) of As bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student T-test (t)

As		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	4.0	<0.01
Root and leaf	3.9	
Stem and leaf	-3.9	

As bioaccumulation relationship between root and stem, root and leaf and stem and leaf were significantly different at confidence level of 99% according to paired Student t-test. Table 4.6 records the significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student t test (t).

Table 4.6: Significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student T-test (t)

Fe		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	18.5	<0.01
Root and leaf	16.9	
Stem and leaf	-6.4	

Fe bioaccumulation relationship between root and stem, root and leaf and stem and leaf were significantly different at confidence level of 99% according to paired Student T-test. Table 4.7 records the significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student T- test (t).

Table 4.7: Significant difference (P) of CN bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student T-test (t)

CN		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	4.1	<0.01
Root and leaf	3.9	
Stem and leaf	-2.8	<0.05

CN bioaccumulation relationship between root and stem and root and leaf were significantly different at confidence level of 99% according to paired Student t-test. Accumulation of CN in stem and leaf was also significant at $p<0.05$. Table 4.8 records the significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student t test (t).

Table 4.8: Significant difference (P) of NH_3-N bioaccumulation between the root and stem, root and leaf, and stem and leaf in *H. cannabinus* according to the paired Student T-test (t)

NH_3-N		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	5.3	<0.01
Root and leaf	4.8	
Stem and leaf	-0.2	*ns

*ns: non-significant

The t-test performed for mean of paired samples was recorded significantly different for all the pairs in the treatment of NH_3-N ($p<0.01$) except NH_3-N concentration in stem and leaf which was recorded insignificant.

Statistically strong association was observed between Fe in soil and Fe in Kenaf root ($r=0.92$, $p<0.05$) but low Pearson's coefficient correlation was found in soil As and root As ($r=0.50$, $p<0.05$) and also soil NH_3-N and root NH_3-N ($r=0.60$, $p<0.05$). This result gives an implication that the bioavailability of Fe in soil has a remarkable influence on the uptake of Fe by Kenaf root. CN concentration in soil and CN concentration in plant root also reported statistically strong association ($r=0.89$, $p<0.05$). Stoltz and Greger (2002) and also Palmroth et. al. (2002) have reported that different characteristics in root activities and exudates of different plants as well as microbial activities have been shown to affect significantly the phytoavailability of metals in soil. Bioconcentration factor of Ferum, Arsenic, Cyanide and Ammoniacal Nitrogen accumulation in leachate treated plants is significantly different ($p<0.01$) compared to control respectively.

Cumulative accumulation percentage of total plant As, Fe, CN and NH_3-N at each harvest cycle (week 8, 12 and 16th of Kenaf growth) is presented in the graphical form as shown below. Figure 4.6 shows the total bioconcentration of As (%) in Kenaf plant throughout week 8, 12 and 16 of growth.

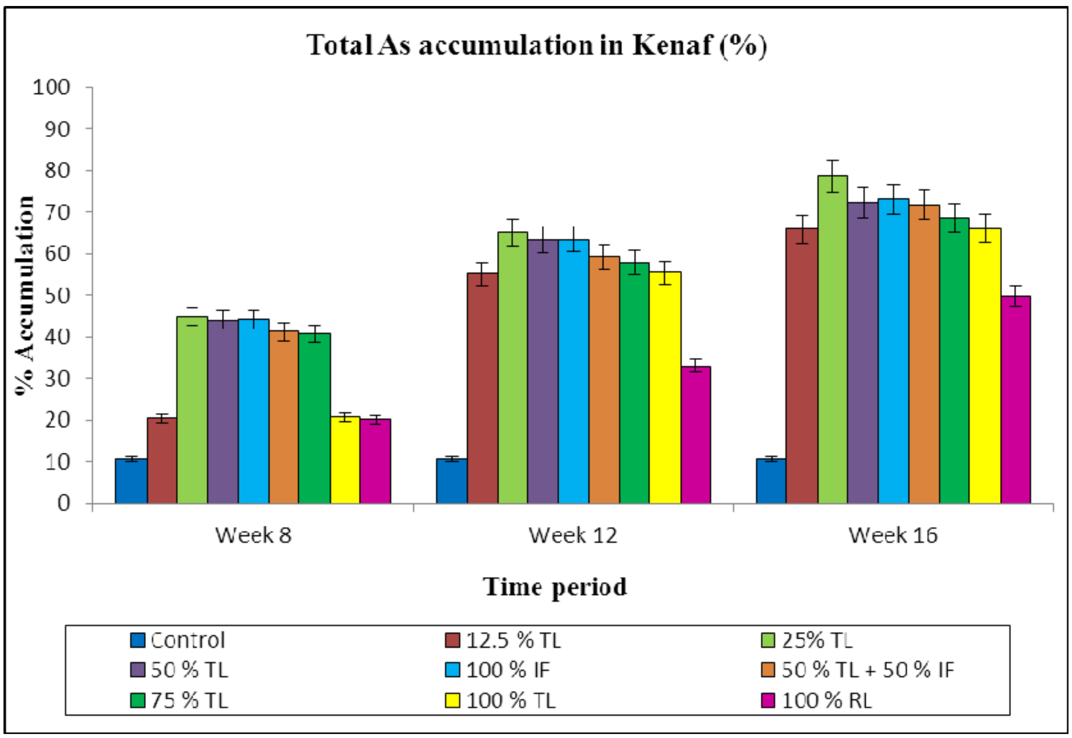


Figure 4.6: As accumulation (%) in Kenaf at week 8, 12 and 16 of growth

Total As accumulation in Kenaf plants grown under different leachate concentrations was 49.8 – 78.6 % at final harvest with highest accumulation was recorded by plants in 25% TL treatment. A constant accumulation of As was observed in control plants throughout 16 weeks of study. An average accumulation of 34.6%, 56.6% and 68.3% of As bioconcentration was observed in all leachate treated plants at week 8, 12 and 16, respectively excluding control treatment.

Figure 4.7 shows the total bioconcentration of Fe (%) in Kenaf plant throughout week 8, 12 and 16 of growth. Total Fe accumulation in Kenaf plants grown under different leachate concentrations recorded at 71.2 – 90.5 % at final harvest. Bioremediation of 25% treated leachate was recorded with significantly high bioaccumulation of total Fe in plant parts. Fe accumulation in control plants was insignificant during the 120 days of experiment.

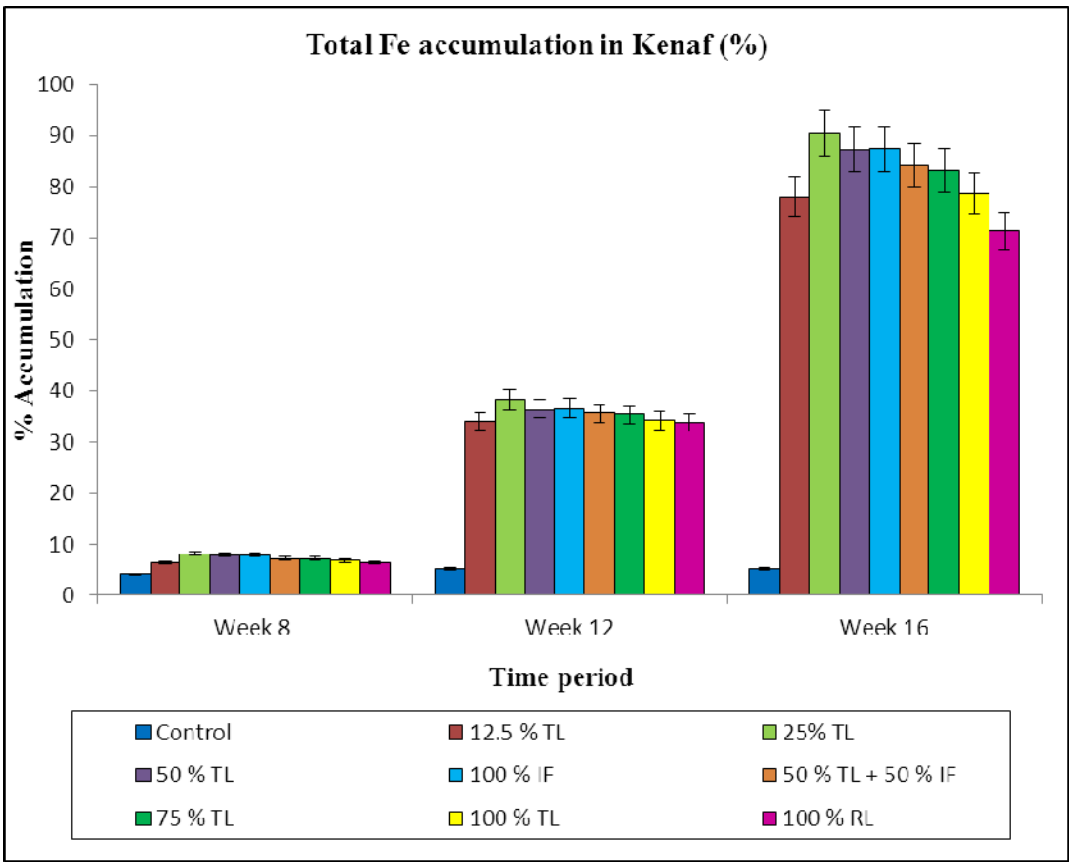


Figure 4.7: Fe accumulation (%) in Kenaf at week 8, 12 and 16 of growth

Figure 4.8 shows the total bioconcentration of CN (%) in Kenaf plant throughout week 8, 12 and 16 of growth. Total CN accumulation in Kenaf plants grown under different leachate concentrations was 60.6 – 79.6 % at final harvest with highest accumulation was recorded by *H. cannabinus L.* in 25% TL treatment. A constant accumulation of CN at 8.3% was observed in control plants throughout 16 weeks of study. An average accumulation of 40.7% and 73.2% of CN bioconcentration was observed in all leachate treated plants at week 12 and 16, respectively excluding control plants.

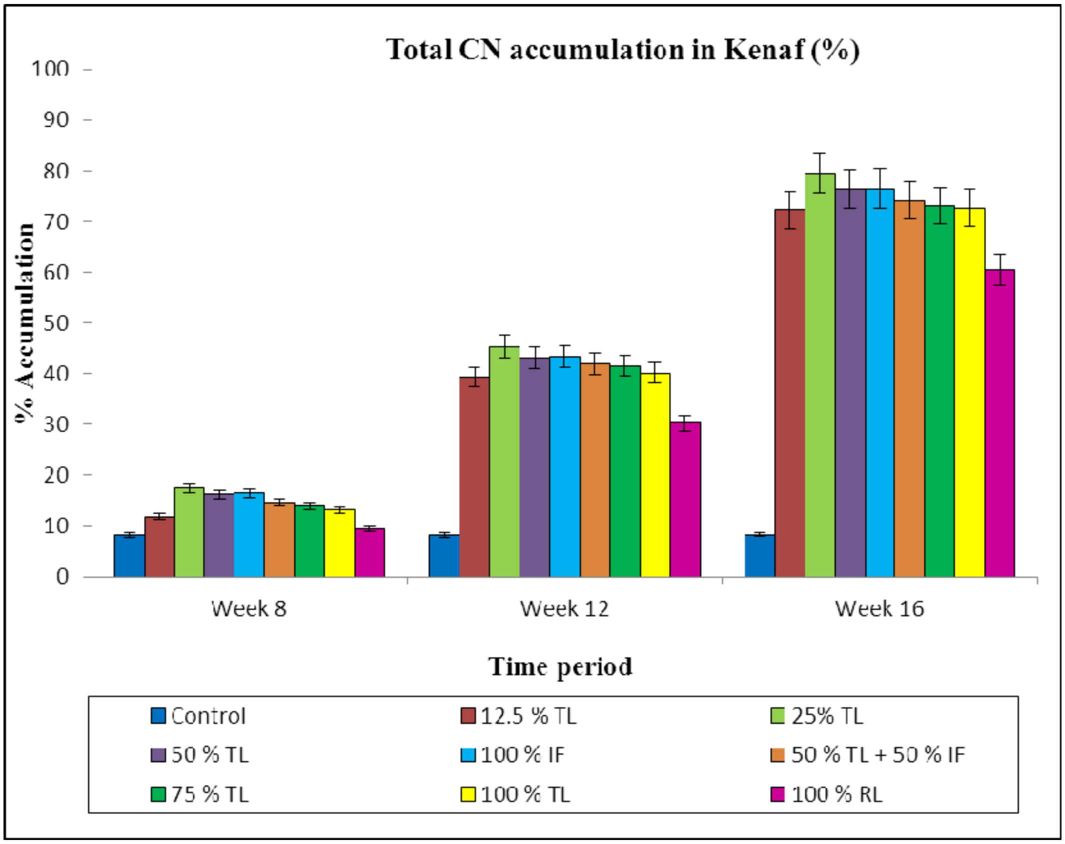


Figure 4.8: CN accumulation (%) in Kenaf at week 8, 12 and 16 of growth

Figure 4.9 shows the total bioconcentration of NH_3-N (%) in Kenaf plant throughout week 8, 12 and 16 of growth. Total NH_3-N removal from soil contaminated with different concentrations of Jeram landfill leachate was 75.4 – 90.3 % at final harvest with soil contaminated with 25% TL gave an optimum bioremediation rate. Among the eight leachate treatments applied in this experiment, 100% RL was observed to be the least optimum treatment for the pollutants removal by Kenaf plants from contaminated soil.

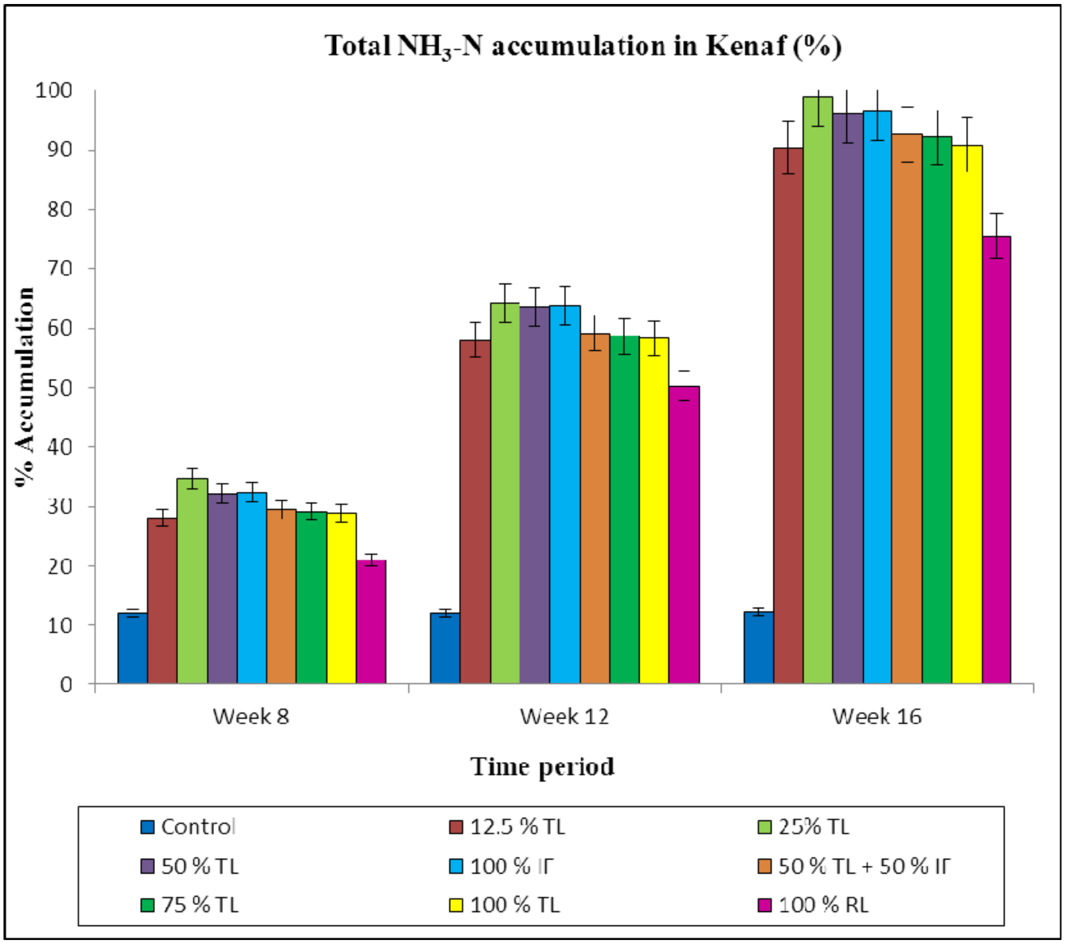
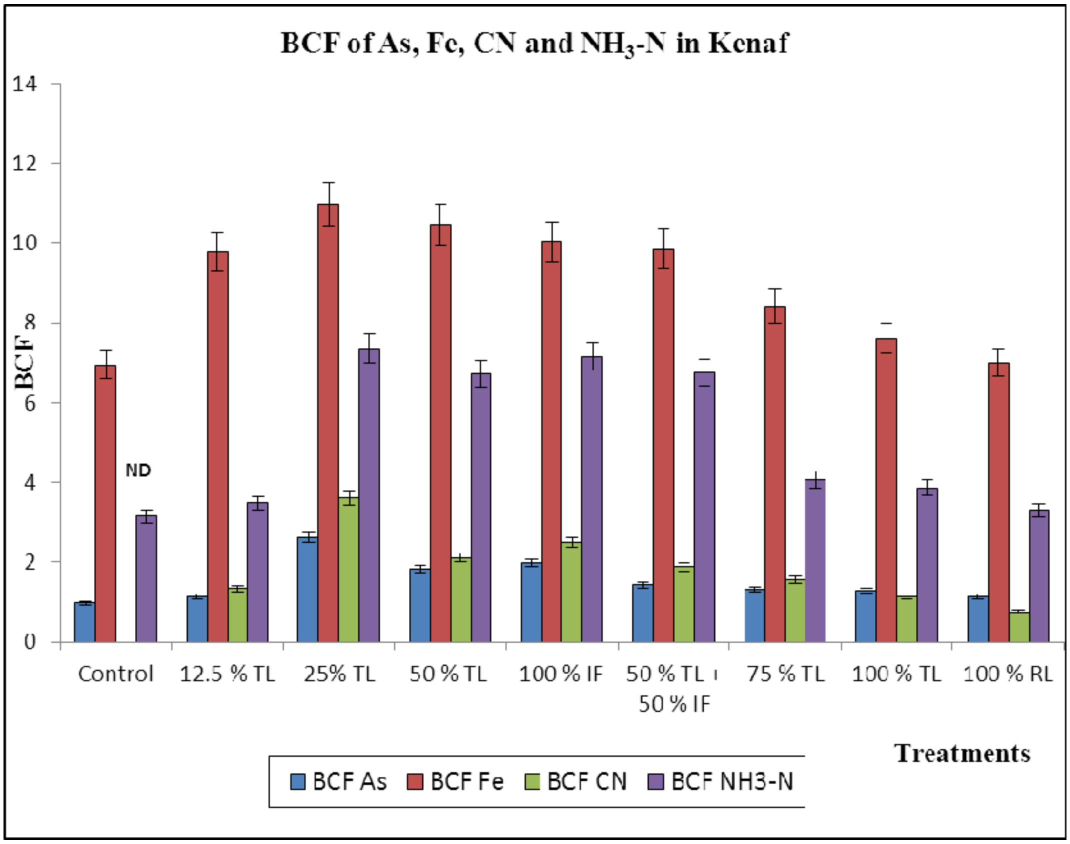


Figure 4.9: NH_3-N accumulation (%) in Kenaf at week 8, 12 and 16 of growth

4.4 Uptake of heavy metals by Kenaf

Accumulation of As, Fe, CN and NH_3-N in Kenaf root, stem, leaf and soil sample was significant based on ANOVA ($p<0.01$). Potential of a plant for phytoremediation process can be assessed through BCF value (heavy metal/ contaminant concentration ratio of plant roots to soil) and TF value (ratio of contaminants in aerial plant parts to root) (Yoon et. al., 2006).



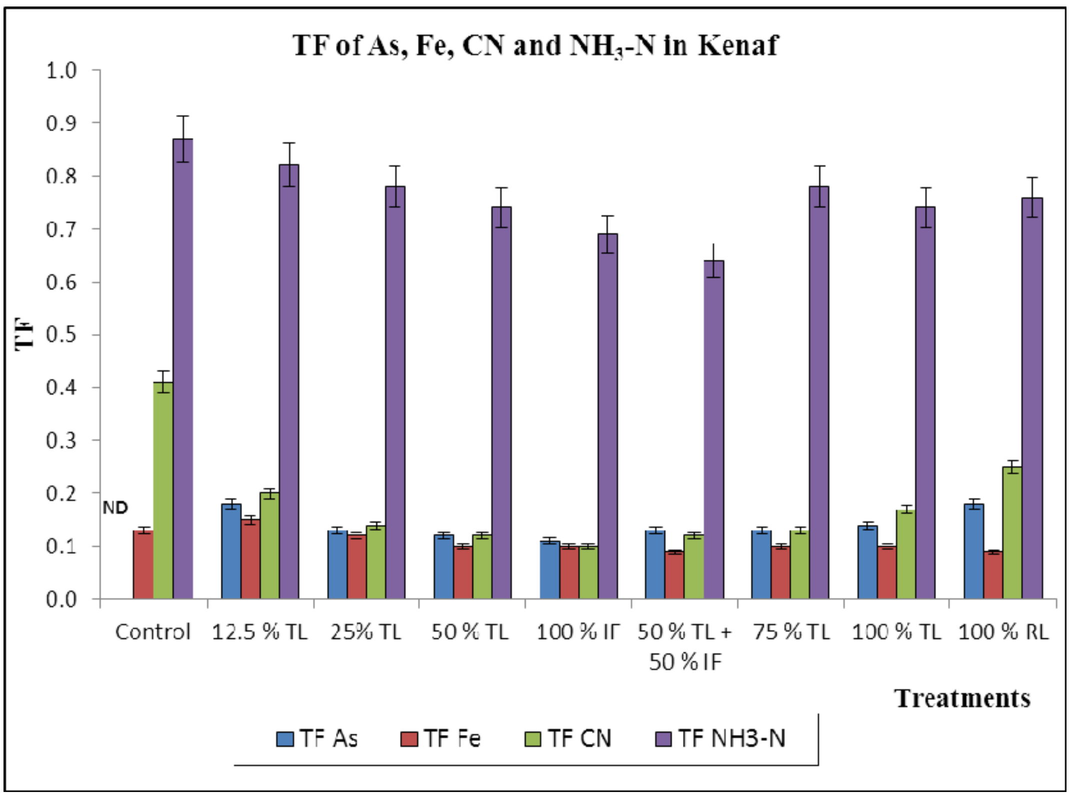
*ND: Non- Detectable

Figure 4.10: Bioconcentration Factor (BCF) of As, Fe, CN and NH_3-N in Kenaf under different leachate treatment. Bar indicates standard error (n=4)

In bioremediation of As, Fe, Cn and NH_3-N, Kenaf showed significantly higher BCF ($p<0.05$) in 25% TL (Figure 4.10). The BCF value in As, Fe, CN and NH_3-N removal showed reduction after the treated leachate concentration of 50% and 100% Inorganic fertilizer, which may be due to restriction in soil-root transfer at higher leachate concentrations (Ho et. al., 2008). Kenaf plants irrigated with 100% IF or 50% IF + 50% TL showed improved bioaccumulation of As, Fe, CN and NH_3-N in root with significantly higher BCF value compared to leachate treatment by 32.2% in average; in line with the findings of Wei et. al. (2010), who discovered the increase of Cd phytoremediation with fertilizer amendments. However, further confirmations needed to prove this phenomenon because many studies were carried out with organic fertilizers and urea; however inorganic fertilizer was used for the purpose of this study.

The range of BCF in As and CN accumulation in Kenaf grown in leachate contaminated soil in this study was 1.14 -2.63 and 0.76 – 3.62, respectively in comparison to NH_3-N accumulation which recorded higher BCF value in the range of 3.30 – 7.35. Fe accumulation in Kenaf reported highest bioconcentration factors in the range of 7.01 - 10.98. These results were relatively higher than Pb-treated Kenaf study done by Ho et. al. (2008), who recorded a BCF range of 1.92-3.21. The differences may be due to characteristics of the experimental soils or differences in microbial ecology (Stoltz and Greger, 2002).

Kenaf roots have proven to be effective in taking up large amounts of As, Fe, CN and NH_3-N but restricted its translocation to other plant parts at the same time. The translocation of As, Fe, CN and NH_3-N from root to aerial Kenaf parts was limited to TF values below one (TF <1) as shown in Figure 4.11.



*ND: Non- Detectable

Figure 4.11: Translocation Factor of Fe, As, CN and NH_3-N in Kenaf under different leachate concentrations. Bar indicates standard error (n=4)

According to Baker (1981), metal accumulator plants have TF values above one (TF >1) and metal excluder plants shows TF values lower than one (TF <1). The findings of this study is in agreement with a study done by Eduardo (2010), who discovered that As is quickly reduced to As (III) once inside root cells and plants cope with As via detoxification mechanisms, including complexation, compartmentalization and cell wall retention.

The TF values of As in the current study were significantly reduced in plants treated with different leachate concentrations (Figure 4.10). The translocation of As to aerial plant parts was restricted at leachate concentrations above 25% TL (TF in the range of 0.13 to 0.18). The transfer of Fe to aerial plant parts were optimum at 12.5% TL but leachate concentrations above that had strictly retained Fe in roots with TF range of 0.09 to 0.15. Translocation of Fe, CN and NH_3-N was highly efficient for control treatment. Kenaf plants performed a defense mechanism against As, Fe, CN and NH_3-N phytotoxicity through the retention of heavy metals in root cell walls and restriction from aerial plant parts (Ho et. al., 2008; Schmoger et. al., 2000). The translocation of CN was recorded in the range of 0.10 to 0.41 with optimum TF at 12.5% TL. The transfer of NH_3-N to aerial plant parts were optimum at 12.5% TL too but leachate concentrations above that had strictly retained NH_3-N in roots of Kenaf.

Kenaf demonstrated high BCF and low TF at different leachate concentrations in the study. The results suggest that the mechanism of Fe, As, CN and NH_3-N removal by the Kenaf plants may be via phytoextraction and phytostablisation in contrary to the role of hyperaccumulators. Therefore, *H. cannabinus* is a potential phytoremediator of landfill leachate to bioremediate the leaching of contaminants from landfill site to the river ecosystem.

Metallophytes extracts contaminant into roots, which is later translocated to the aerial plants parts i.e. shoot, leaves and buds. On the other hand, non-accumulators or excluders (Ho et. al., 2008) accumulates metals from soil and immobilizes them in the plant roots via exclusion mechanism. Translocation factor of Fe, As, CN and NH_3-N accumulation in leachate treated plants is insignificantly different compared to control in their respective treatments. Translocation factor of As was intimately related ($r=0.77$, $p<0.05$) to CN transport according to Pearson correlation statistical function , indicating probability that a small portion of As may have been co-translocated with CN from roots to shoots.

4.5 Response of Akasia plant to the leachate treatments and Biomass of Akasia

The relative growth rate of Akasia plants in response to different concentrations of leachate contaminant was monitored throughout the 120 days of the experiment. There was no plant deaths recorded for Akasia. Akasia plants were harvested by uprooting every 8th, 12th and 16th week of growth (Plates 4.4 and 4.5). Plate 4.6 shows harvested Akasia placed on polystyrene for plant height measurement.

Plate 4.4: Akasia at week 8th of growth

Plate 4.5: Akasia at week 12th of growth

Plate 4.6: Harvested Akasia at week 16th of growth

Relative growth rate of leaf, stem and root dry mass of the Akasia plant in each treatment was determined at the end of 16th week as shown in Table 4.9. Factorial analysis revealed that the interaction between leachate treatments was significant in affecting the relative growth rate of Akasia biomass ($p<0.05$). The RGR trend is illustrated in Figure 4.12.

Table 4.9: Relative Growth Rate of *A. mangium* treated with different concentrations of leachate

TREATMENTS	RGR_L (cm/ week)	RGR_S (cm/ week)	RGR_R (cm/ week)	RGR_{TP} (cm/ week)
CONTROL	0.71±0.19	0.82±0.14	0.78±0.22	0.89±0.10
***12.5 % TL**	1.04±0.14	1.04±0.06	1.09±0.16	1.30±0.07
***25% TL**	2.52±0.12	2.20±0.04	1.71±0.27	1.88±0.07
***50 % TL**	1.49±0.10	1.93±0.10	1.58±0.18	1.74±0.07
***100 % IF**	1.51±0.11	1.79±0.05	1.18±0.10	1.75±0.16
***50% TL+ 50%IF**	1.15±0.16	1.66±0.09	1.15±0.16	1.48±0.40
***75% TL**	1.12±0.10	1.44±0.03	1.14±0.05	1.45±0.31
100% TL	1.06±0.11	1.10±0.18	1.12±0.05	1.33±0.09
100% RL	0.88±0.07	0.96±0.11	0.86±0.05	0.94±0.07

* Significantly different RGR of leaves, stem, root and total PDM compared to control

RGR_L : Relative Growth Rate of leaves

RGR_S : Relative Growth Rate of stem

RGR_R : Relative Growth Rate of root

RGR_{TP} : Relative Growth Rate of total plant dry biomass

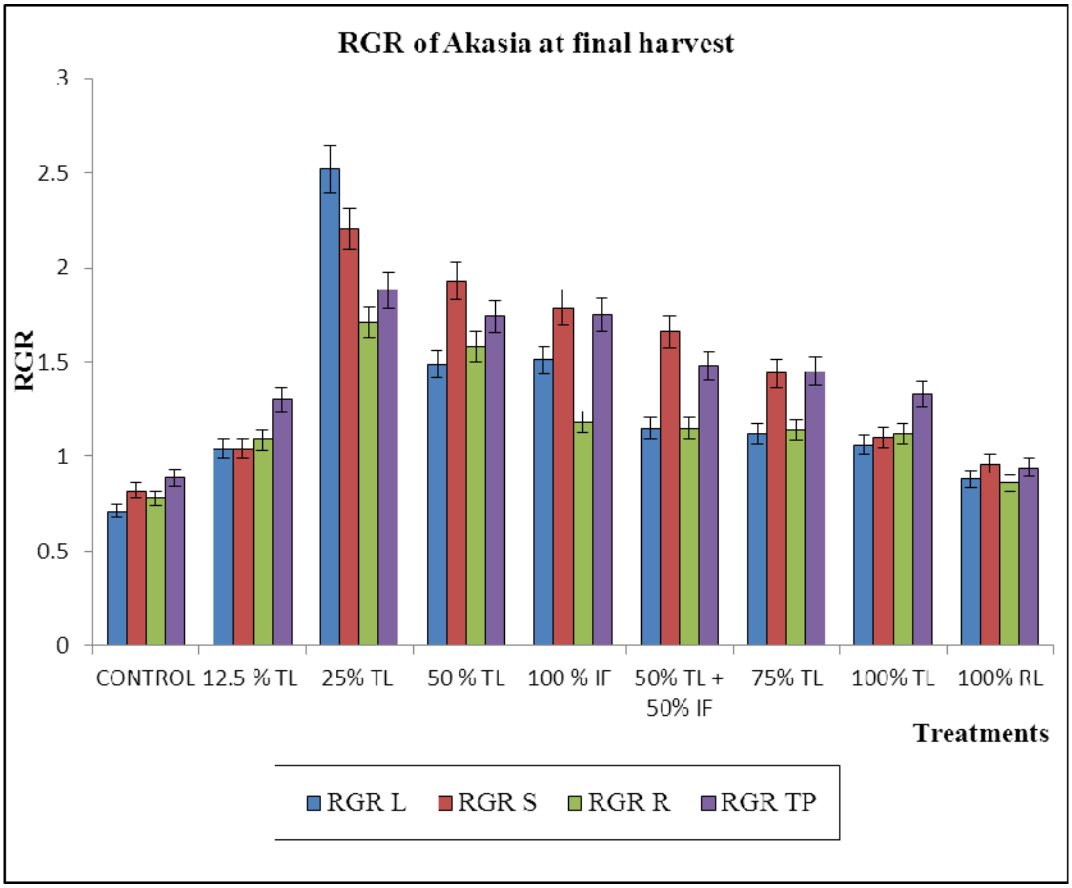


Figure 4.12: RGR of root, stem, leaves and total Akasia dry biomass

The distribution of plant dry biomass according to leaves, stems and roots were 31.5%, 29.3% and 39.2%, respectively at final harvest. Inhibition of biomass accumulation was not observed in this study which indicated that As, Fe, CN and NH_3-N did not cause adverse effect on Akasia growth. Pearson's correlation coefficient ($r=0.93$, $r=0.82$ and $r=0.88$, $p<0.05$) was observed between $RGR_{L,}$, RGR_S and RGR_R respectively with total plant biomass (RGR_{TP}) indicating a strong association between these variables.

The mean of RGR values were also significantly different among RGR_L, RGR_S, RGR_R, and RGR_{TP} ($p<0.05$) of Akasia. Akasia plants irrigated with 25% TL (0.24% N-content), recorded highly significant RGR_L, RGR_S, RGR_R and RGR_{TP}. Treatment concentrations were significant at 12.5% TL, 25% TL, 50% TL, 100% IF, 50% TL +

50% IF and 75% TL compared to the control treatment. According to Dimitriou et. al. (2006), the irrigation with landfill leachate resulted in reduced RGR in willow clones but there weren't any clear differences between the different treatments. The control plants of all clones had higher RGR compared to plants irrigated with landfill leachate which is in contrast with the results of this current study. The RGR results of plant dry weight (PDM) of leachate treated willows was in the range of 0.358 – 0.537. The highest RGR of PDM was 0.537 week^{-1} for control without leachate treatment and the optimum RGR of PDM was given by diluted leachate concentrations at 33% (0.381 week^{-1}) as reported by Dimitriou et. al. (2006).

4.6 Bioaccumulation of Fe, As, CN and NH_3-N in Akasia from soil contaminated with different treatments of leachate

The bioaccumulation of Fe, As, CN and NH_3-N in plant parts, soil sample and control soil (without plants) at different concentrations of leachate contaminated soil at week 16 of harvest are appended in Appendix A (Table A.5, Table A.6, Table A.7 and Table A.8). Fe, As, CN and NH_3-N was detected in all plant parts irrigated with leachate treatments in the nursery pot experiment, with insignificant bioavailable Fe, As and CN present in leaves and stem. NH_3-N was detected in all Akasia plant parts. There was no insoluble Arsenic, Cyanide or Iron species noted on the roots of Kenaf upon harvest.

Figure 4.13 illustrates As uptake by Akasia plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

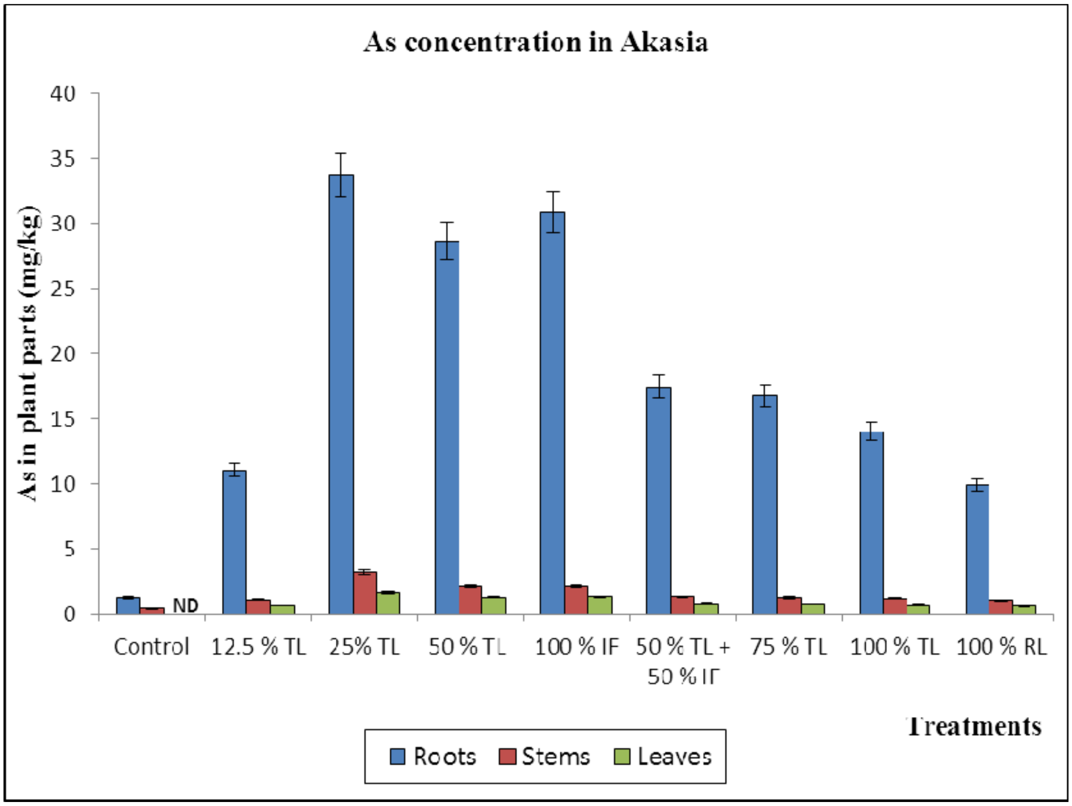


Figure 4.13: As concentration in root, stem and leave of Akasia under different leachate treatments

At final harvest, Akasia plants irrigated with 25% TL recorded optimum As uptake in Akasia root which recorded 33.69 mg As kg^{-1}. The Akasia plant root, stem and leaves sequestered 0.16 - 1.02 mg, 0.02 - 0.10 mg and 0.01 - 0.05 mg As per g plant dry weight, respectively. As concentrations in the Akasia stem and leaves were in the range of 0.97 – 3.21 mg kg^{-1} and 0.59 – 1.68 mg kg^{-1}, respectively at the different leachate treatments during final harvest. Figure 4.14 illustrates Fe uptake by Akasia plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16^{th} of growth. Figure 4.14 illustrates Fe uptake by Akasia plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16^{th} of growth.

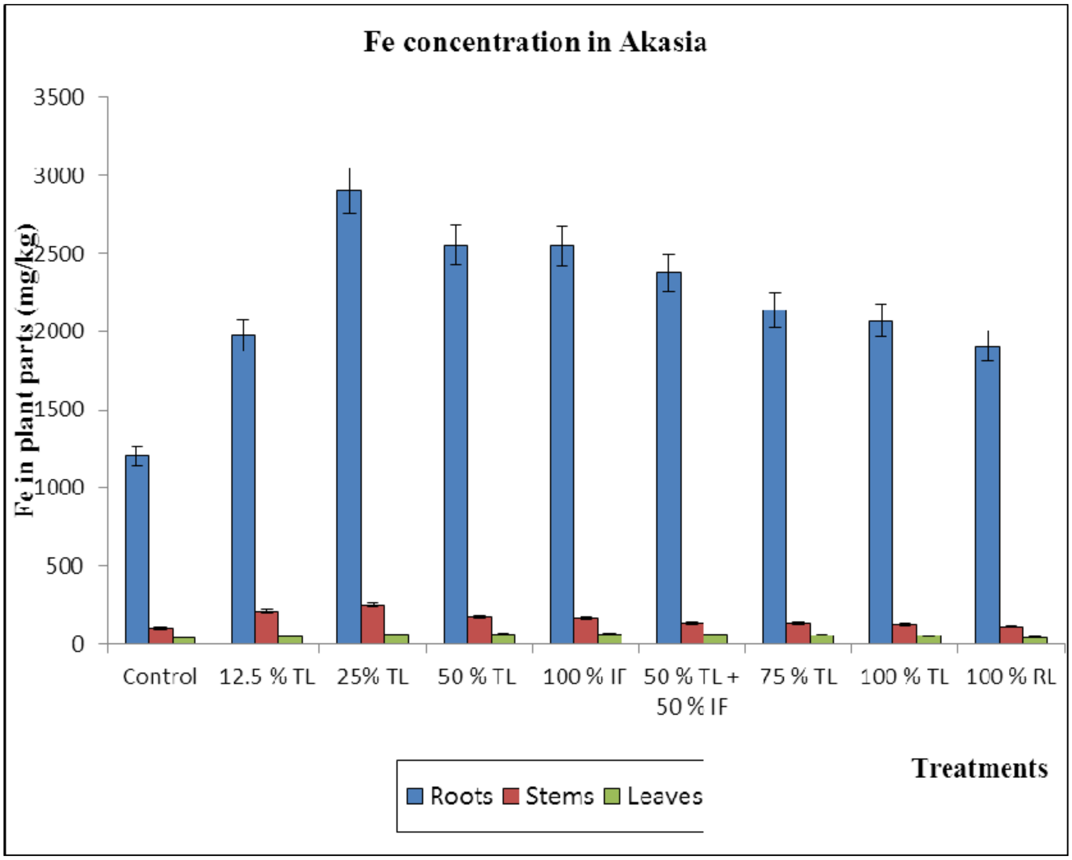


Figure 4.14: Fe concentration in root, stem and leave of Akasia under different leachate treatments

At final harvest, highest concentration of Fe bioaccumulation was recorded by Akasia grown in 25 % TL treatment. From a scientific standpoint, the possible causes of inhibition in plant growth with increasing Fe supply may include one or both of the following: (i) high concentrations of Fe result in iron toxicity within the plants and (ii) elevated Fe concentrations retard the uptake of other nutrients by the plants, therefore causes nutrient deficiency (Batty and Younger, 2002). The Akasia root, stem and leaves sequestered 31.70– 87.72 mg, 1.79 -7.50 mg and 0.67 – 1.76 mg Fe per g plant dry weight, respectively. The results showed that Fe concentrations in stem and leaves of Akasia grown in different leachate treatments during final harvest were in the range of 110.23 – 248.18 mg kg^{-1} and 41.13 – 64.51 mg kg^{-1}, respectively. Figure 4.15 illustrates

CN uptake by Akasia plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

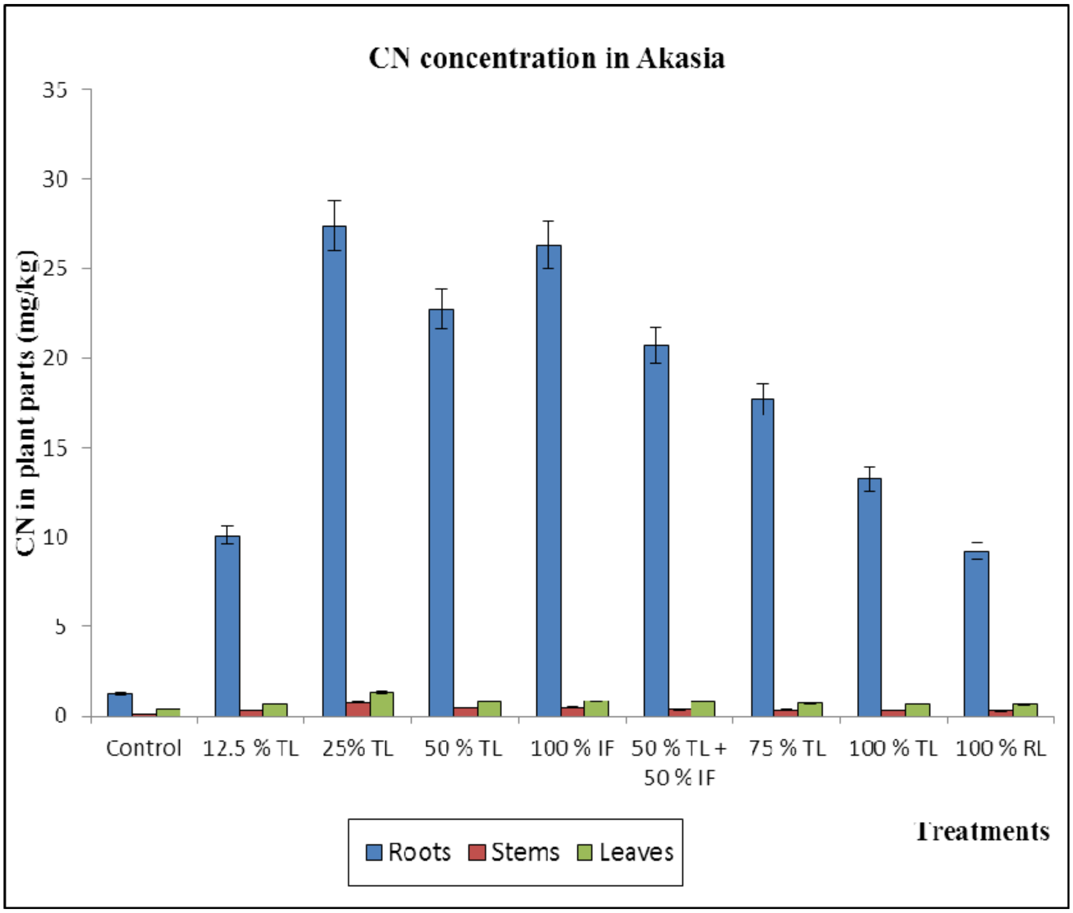


Figure 4.15: CN concentration in root, stem and leave of Akasia under different leachate treatments

Approximately 27 mg CN kg^{-1} were recorded for roots of Akasia planted in 25% TL contaminated soil. The Akasia root sequestered 0.15 – 0.82 mg CN/ g plant dry weight at week 16th of plant growth. CN concentrations in stem and leaves of Akasia were in the range of 0.29 – 0.73 mg kg^{-1} and 0.63 – 1.34 mg kg^{-1}, respectively. Figure 4.16 illustrates NH_3-N uptake by Akasia plants and concentrations in root, stem and leaves of the test plant under different leachate treatments at final harvest of week 16th of growth.

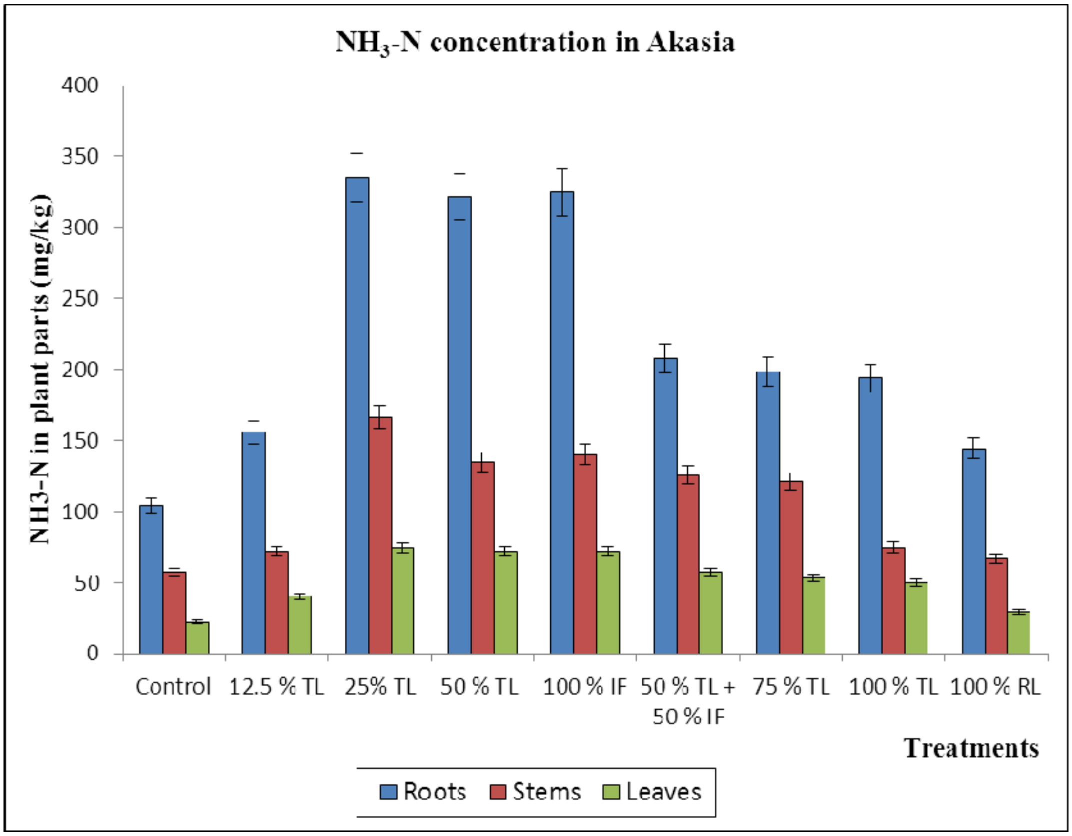


Figure 4.16: NH_3-N concentration in root, stem and leaves of Akasia under different leachate treatments

Bioremediation of landfill leachate at 25% treated leachate recorded optimum efficiency for the removal of NH_3-N after 120 days of growth. The Akasia root sequestered 0.15 – 0.83 mg NH_3-N/ g plant dry weight. Stem and leaves of Akasia sequestered 0.004 – 0.02 mg and 0.01 – 0.04 mg NH_3-N per g plant dry weight, respectively at the final harvest. The trend of NH_3-N accumulation in Akasia is best explained by N_2 fixation which was slightly reduced as soil N increased. High soil N fertility causes inhibition of nodulation and N_2 fixation (Turpin et. al., 2002).

Fe concentrations in roots of Akasia (1202.34 – 2556.21 mg/kg) were significantly higher than As, CN and NH_3-N concentrations in root at 1.19 – 33.69 mg/kg, 1.24 – 27.41 mg/kg and 104.28 – 334.91 mg/kg, respectively. Mass of soil sample and plant dry matter (PDM) according to leaves, stems, roots and total PDM was recorded in Table 4.10.

Table 4.10: *A. mangium* Plant Dry Matter (PDM in g) and Dried Soil Sample Mass (g) at final harvest (week 16)

Treatments	PDM (g) and Dried Soil Sample Mass (g)				
	Roots	Stems	Leaves	Total PDM	Soil Sample
Control(dH_2O)	2.13± 0.12	5.11 ± 2.04	4.24 ± 0.09	11.47 ± 3.41	4.01 ± 0.88
12.5 % TL	3.78 ± 0.13	8.85 ± 0.83	7.22 ± 0.26	19.85 ± 0.74	3.12 ± 0.06
25% TL	9.21 ± 1.06	11.73 ± 0.11	9.27 ± 0.33	30.22 ± 0.63	3.52 ± 0.74
50 % TL	8.29 ± 0.16	9.91 ± 0.56	9.07± 0.87	27.27± 2.56	3.34 ± 0.55
100 % IF	8.32 ± 0.18	9.94 ± 0.37	9.09 ± 1.44	27.35 ± 0.06	3.85 ± 0.07
50% TL+ 50% IF	7.05 ± 1.02	8.97 ± 0.68	8.79 ± 1.35	25.04 ± 0.52	3.44 ± 0.89
75 % TL	6.36 ± 0.08	8.90 ± 0.97	8.73 ± 1.03	24.06 ± 0.56	3.56 ± 0.23
100 % TL	3.88 ± 0.37	7.15± 1.45	8.18 ± 0.07	20.96 ± 2.52	3.56± 0.82
100 % RL	2.31 ± 0.06	3.01 ± 0.43	7.16 ± 0.26	16.62 ± 1.09	3.26 ± 0.76

Akasia plant roots sequestered higher concentration of contaminant in their plant root compared to aerial plant parts because of the direct contact and uptake from the soil. This is in congruence to study conducted by Ho et. al. (2008) where Pb was most pronounced in the roots of fertilized and unfertilized treatments in the Kenaf except in leaf. As, Fe, NH_3-N absorption efficiency in Akasia plants decreased tremendously from root to stem and subsequently to leaves. Phytoremediation of CN from leachate polluted soil with Akasia recorded an uptake efficiency in the ratio of root> leaves> stem. The concentrations of As, Fe, CN and NH_3-N in roots, stem and leaves increased progressively with the increase of external leachate concentrations applied but decreased when it was above 100 % IF.

The results also indicated the impacts of leachate concentrations towards Akasia growth. Application of leachate as strong as above 50% TL might have affected the Akasia's growth negatively due to the high ionic strength of the leachate. Leachate concentrations below 25% TL resulted in lower nutrient additions, which in turn resulted in lower biomass produced as well as lower As, Fe, CN and NH_3-N concentrations. Despite high concentrations of As, Fe, CN and NH_3-N in the roots, no visual symptoms of metal toxicity were observed indicating that Akasia too is a As, Fe, CN and NH_3-N tolerant species.

The plant's capacity to yield big quantities of root exudates (to solubilize soil arsenic), to produce high biomass of root, to effectively translocate Arsenic to the aerial plant parts, and to reduce arsenic from arsenate- AsO_4^{3-} to arsenite-AsO_2^- in the plant parts have all lead to its ability to hyperaccumulate arsenic (Peer et. al., 2006). The high tolerance of CN could have been due CN-resistant respiration which has been reported in tissues from a large number of plants, including a variety of angiosperms (Siedow and Berthold, 1986). Relationship of As, Fe, CN and NH_3-N concentrations between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test are presented in Table 4.11 to Table 4.14 below. The t-test performed for mean of paired samples was recorded significantly different for all the pairs except CN concentration in stem and leaf and NH_3-N concentration in stem and leaf which were recorded insignificant.

Table 4.11: Significant difference (P) of As bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test (t)

As		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	26.2	<0.01
Root and leaf	22.6	
Stem and leaf	4.2	<0.05

As bioaccumulation relationship between root and stem, root and leaf and stem and leaf were significantly different at confidence level of 95% according to paired

Student t-test. Table 4.12 records the significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test.

Table 4.12: Significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test (t)

Fe		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	5.8	<0.01
Root and leaf	5.8	
Stem and leaf	-3.3	

Fe bioaccumulation relationship between root and stem, root and leaf and stem and leaf were significantly different at confidence level of 99% according to paired Student t-test. Table 4.13 records the significant difference (P) of Fe bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student t test.

Table 4.13: Significant difference (P) of CN bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test (t)

CN		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	2.3	<0.05
Root and leaf	3.9	<0.01
Stem and leaf	-2.8	*ns

*ns: non significant

CN bioaccumulation relationship between root and stem and root and leaf were significantly different at confidence level of 95% according to paired Student t-test. Accumulation of CN in stem and leaf was insignificant at $p<0.05$. Table 4.14 records the significant difference (P) of NH_3-N bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student t test (t).

Table 4.14: Significant difference (P) of NH_3-N bioaccumulation between the root and stem, root and leaf, and stem and leaf in *A. mangium* according to the paired Student T-test (t)

NH_3-N		
Bioaccumulation relationship	**t-value**	**p**
Root and stem	4.0	<0.01
Root and leaf	6.0	
Stem and leaf	0.5	*ns

*ns: non-significant

The t-test performed for mean of paired samples was recorded significantly different for all the pairs in the treatment of NH_3-N ($p<0.01$) except NH_3-N concentration in stem and leaf which was recorded insignificant.

Statistically strong association was observed between As in stem and As in leaves with As in Akasia root ($r=0.73$ and $r=0.74$, $p<0.05$) but very low correlation was found between Fe in stem with Fe in root ($r=0.20$, $p<0.05$). In the case of CN, a strong Pearson's correlation was observed in CN in stem, leaves and Akasia planted soil with CN in root ($r = 0.89$, $r=0.88$ and $r= 0.90$, $p<0.05$ respectively). In the case of NH_3-N, a strong Pearson's correlation was observed in NH_3-N in stem and NH_3-N in leaves with NH_3-N in root ($r = 0.75$, $r=0.89$, $p<0.05$ respectively). Bioconcentration factor of CN and NH_3-N accumulation in leachate treated Akasia plants is significantly different ($p<0.01$) compared to control.

Cumulative accumulation percentage of total plant As, Fe, CN and NH_3-N at each harvest cycle (week 8, 12 and 16th of Akasia growth) is presented in graphs below. Figure 4.17 shows the total bioconcentration of As (%) in Akasia plant throughout week 8, 12 and 16 of growth.

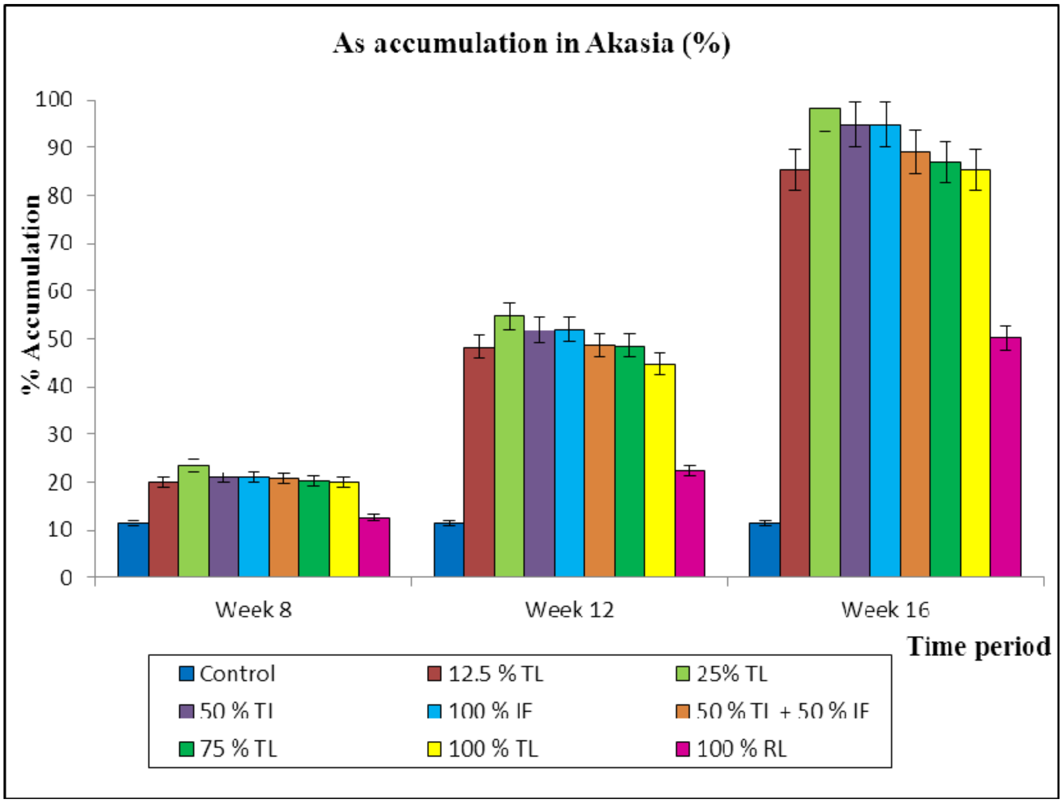


Figure 4.17: As accumulation (%) in Akasia at week 8, 12 and 16 of growth

Total As accumulation in Akasia plants grown under different leachate concentrations was 50.2 – 98.3 % at final harvest with highest accumulation was recorded by plants in 25% TL treatment. A constant accumulation of As was observed in control plants throughout 16 weeks of study. An average accumulation of 19.9%, 46.4% and 85.6% of As bioconcentration was observed in all leachate treated plants at week 8, 12 and 16, respectively excluding control treatment. Figure 4.18 shows the total bioconcentration of Fe (%) in Akasia plant throughout week 8, 12 and 16 of growth. Total Fe accumulation in Akasia plants grown under different leachate concentrations recorded at 75.3 – 94.6 % at final harvest. Bioremediation of 25% treated leachate was recorded with significantly high bioaccumulation of total Fe in plant parts. Fe accumulation in control plants was insignificant during the 120 days of experiment.

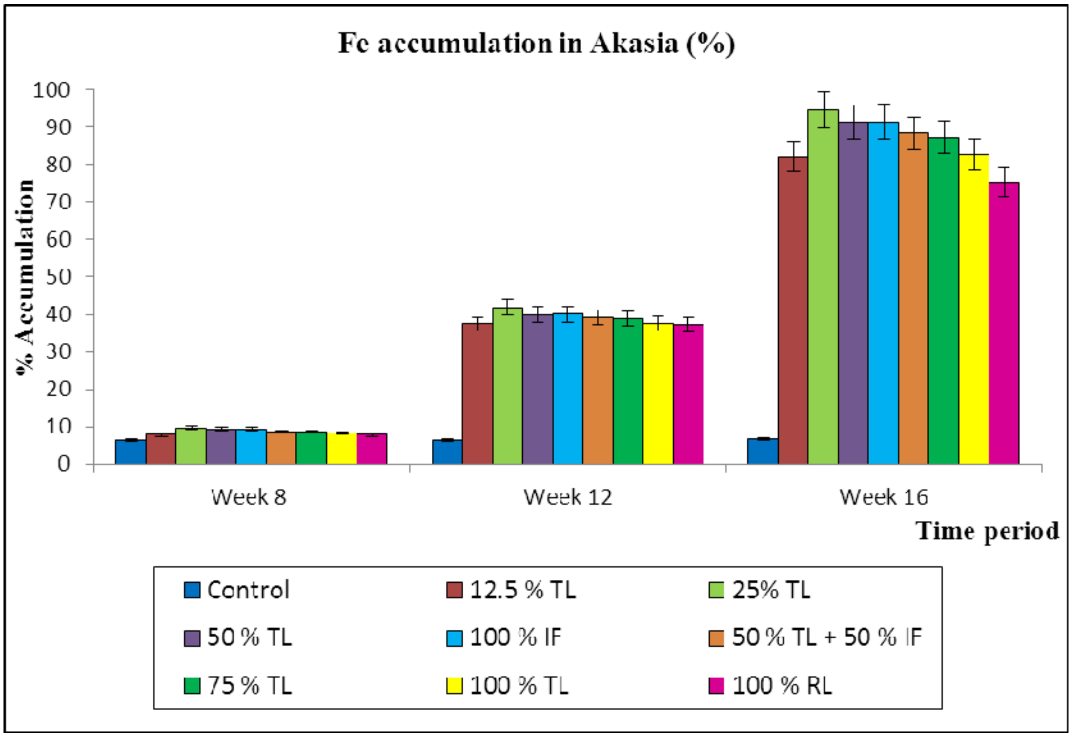


Figure 4.18: Fe accumulation (%) in Akasia at week 8, 12 and 16 of growth

Figure 4.19 shows the total bioconcentration of CN (%) in Akasia plant throughout week 8, 12 and 16 of growth.

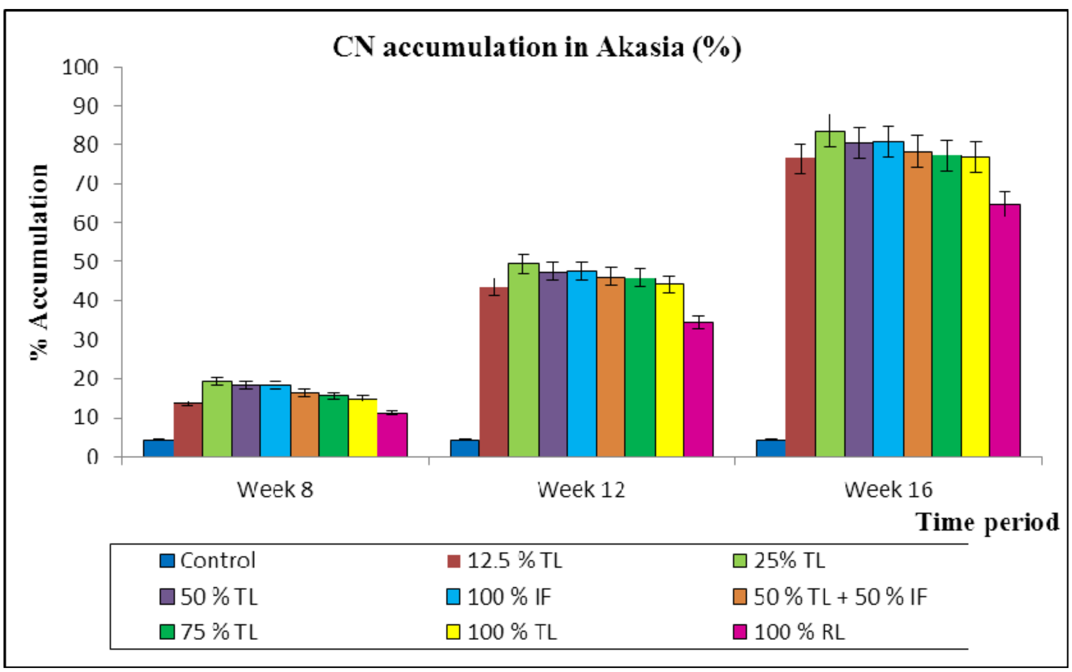


Figure 4.19: CN accumulation (%) in Akasia at week 8, 12 and 16 of growth

Total CN accumulation in Akasia plants grown under different leachate concentrations was 64.7 – 83.7 % at final harvest with highest accumulation was recorded by *A. mangium* in 25% TL treatment. A constant accumulation of CN at 4.4% was observed in control plants throughout 16 weeks of study. An average accumulation of 14.7%, 40.4% and 69.2% of CN bioconcentration was observed in all leachate treated plants at week 8, 12 and 16, respectively excluding control plants. Figure 4.20 shows the total bioconcentration of NH_3-N (%) in Akasia plant throughout week 8, 12 and 16 of growth.

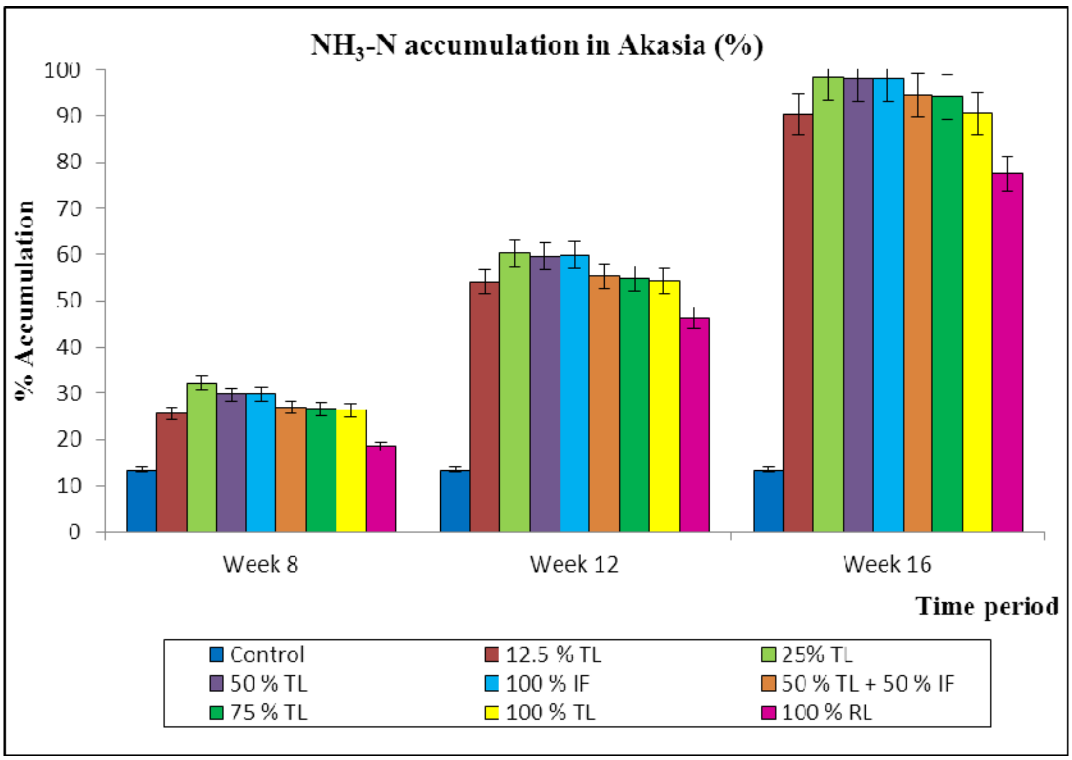


Figure 4.20: NH_3-N accumulation (%) in Akasia at week 8, 12 and 16 of growth

Total NH_3-N removal from soil contaminated with different concentrations of landfill leachate was 77.5 – 98.5 % at final harvest with soil contaminated with 25% TL gave an optimum bioremediation rate. Among the eight leachate treatments applied in this experiment, 100% RL was observed to be the least optimum treatment for the pollutants removal by Akasia plants from contaminated soil.

4.7 Uptake of heavy metals by Akasia

Akasia showed significantly higher BCF (BCF: 2.89, $p<0.05$) in 25% TL in remediation of Arsenic. As for Fe and CN, significantly higher BCFs were recorded by Akasia plants grown also in 25% TL (BCF: 14.29 and 4.27, respectively) as shown in Figure 4.21. Akasia grown in 25% TL, 50% TL and 100% IF showed high bioaccumulation of NH_3-N in their plant cells. The BCF value in As, Fe, CN and NH_3-N treatments showed reduction after the treated leachate concentration of 50% TL and 100% IF, which may be due to restriction in soil-root transfer at higher leachate concentrations (Ho et. al., 2008).

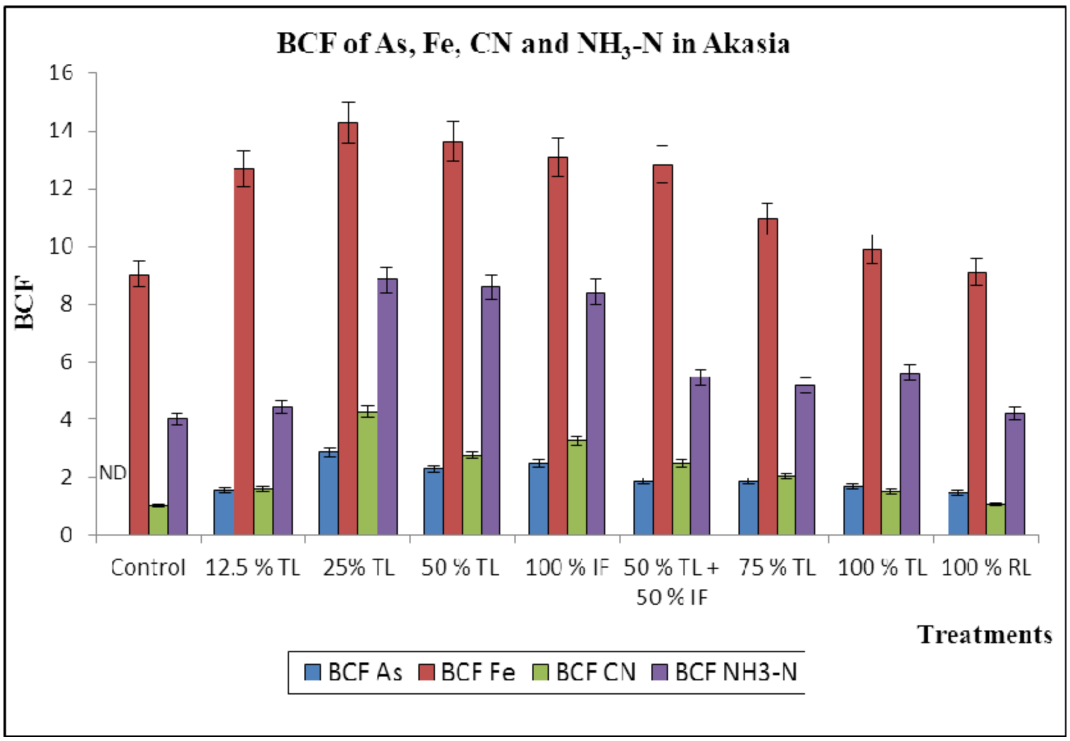


Figure 4.21: Bioconcentration Factor of As, Fe, CN and NH3-N in Akasia under different leachate treatment. Bar indicates standard error (n=4)

Akasia plants irrigated with 100% IF and 50% IF + 50% TL showed improved bioconcentration of Fe, As, CN and NH_3-N with significantly higher BCF value compared to leachate treatment by 55.7%, 60.6%, 65.95 and 93.12% respectively.

The range of BCF in removal of CN in Akasia in this study was 1.02 – 4.27 which is equivalent to As removal by Akasia with BCF readings ranging 1.48 – 2.89. The BCF range for NH_3-N removal was in the range of 4.02 – 8.84. Fe bioconcentration in plant cells was significantly high ranging from 9.04 to 14.29. The trend of heavy metal and macro nutrient accumulation from leachate polluted soil by *A. mangium* in decreasing order based on Bioconcentration Factor (BCF) were as listed below:

$$Fe > NH_3\text{-}N > CN > As$$

Akasia roots have proven to be effective in taking up large amounts of metals but restricted its translocation to other plant parts as the same mechanism which worked for Kenaf. Similar results was also reported by a study on Cd, Pb, Cr and Cu uptake by *A. mangium* from soil contaminated with sewage sludge where the roots showed the highest concentrations followed by stems and leaves (Islam et. al., 2011; Majid et. al., 2011). The translocation of As and Fe from root to aerial Akasia plant parts was limited to TF values below one (TF< 1) as illustrated in Figure 4.20. On the other hand, translocation of NH_3-N was very significant in Akasia where TF values above one (TF> 1) for all the 9 treatments prepared. The CN also showed a poor translocation of pollutant to aerial plant parts where Translocation Factor was in the range of 0.05 – 0.40. The findings of this study is in agreement with a survey which had been carried out on the presence and distribution of three enzymes that breaks down cyanide in a various higher plants in both cyanogenic and non-cyanogenic species (Miller and Conn, 1980). β-cyanoalanine synthase, rhodanese and formamide hydrolyase were the three enzymes investigated. β-cyanoalanine synthase was discovered to be available in each higher plant tested but rhodanese was discovered to be present far less commonly in plants (Miller and Conn, 1980).

In a study conducted by Islam et. al. (2011), Cadmium and lead were highly concentrated in the stems while chromium and copper in the roots of A. mangium grown in sewage sludge contaminated soil. *A. mangium* seems to have a high potential to absorb much amounts of Cd, Pb, Cr and Cu in the leaves, stems and roots. *A. mangium* was able to tolerate and accumulate high concentrations if heavy metal. The TF values of As, Fe and CN in the current study were significantly reduced in plants treated with different concentrations of leachate (Figure 4.22). The translocation of As to aerial plant parts was restricted at leachate concentrations above 50% TL (TF in the range of 0.06 – 0.16). The transfer of Fe to aerial plant parts were optimum at 12.5% TL but leachate concentrations above that had strictly retained Fe in roots with TF range of 0.08 to 0.13.

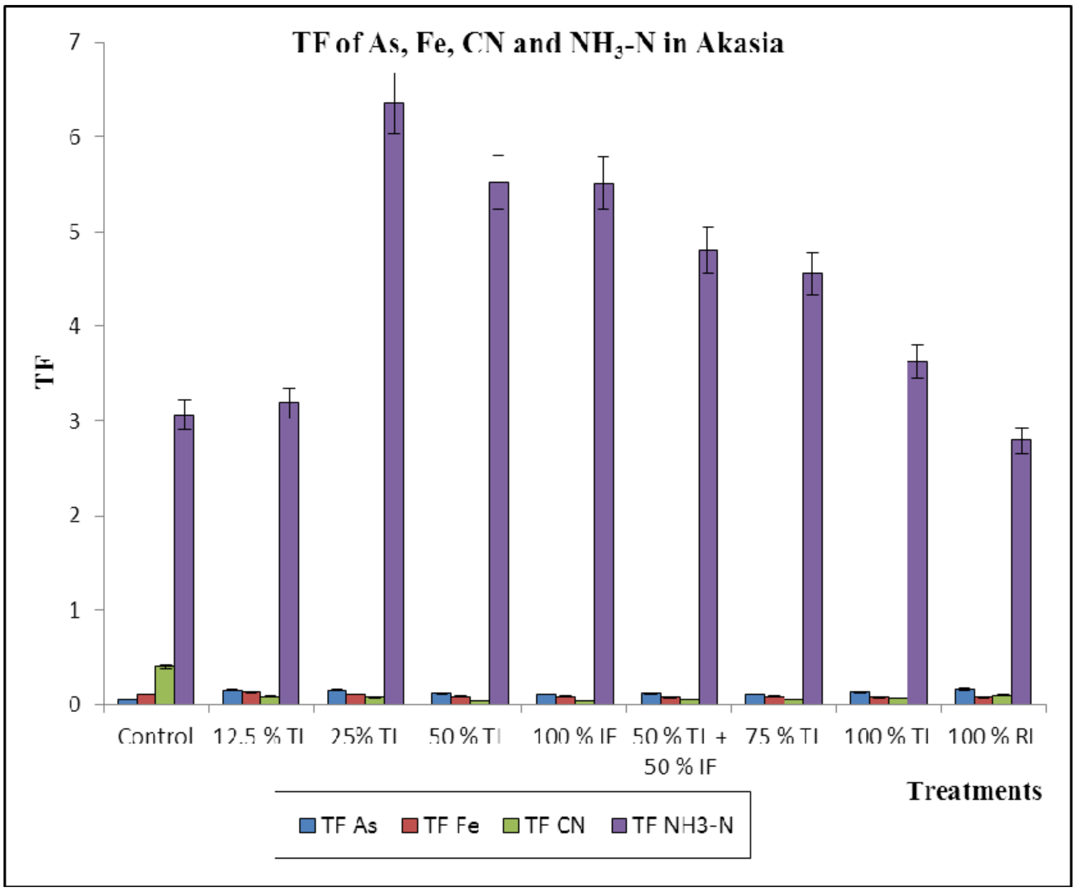


Figure 4.22: Translocation Factor of As, Fe, CN and NH_3-N in Akasia under different leachate treatment. Bar indicates standard error (n=4)

Translocation of Fe was high for control, 12.5% TL and 25% TL treatments. Translocation of CN and Fe in Akasia was highly efficient for control treatment. TF of

CN was optimum at Akasia irrigated with distilled water (TF value at 0.40) and decreased gradually at concentrations above that. In the case of NH_3-N, Akasia proved to be a good accumulator of Nitrogen with TF values above 1 for all the treatments (TF value ranging from 2.79 to 6.36).

Akasia demonstrated high BCF and lower TF at different leachate concentrations in the study. The results also suggest that the mechanism of Fe, As, CN and NH_3-N removal by the Akasia plants may be via phytoextraction and phytostablisation in contrary to the role of hyperaccumulators. Therefore, *A. mangium* is also an efficient phytoremediator of landfill leachate polluted soil.

Translocation factor of As and NH_3-N accumulation in leachate treated Akasia plants is insignificantly different compared to control in their respective treatments. Translocation of Fe and CN was significantly different compared to control in their respective treatments. Translocation factor of Fe and CN was associated ($r=0.70$ and $r=0.69$, $p<0.05$ respectively) to NH_3-N transport, indicating probability that a small portion of Fe and CN may have been co-translocated with NH_3-N from roots to the Akasia shoots (Cobbet, 2000).

According to Tyrrel et. al. (2002) and Sun et. al. (2005), several research works have observed that the increase in nitrite- and nitrate-nitrogen concentrations does not balance the reduction of Ammoniacal-Nitrogen in wastewater. Although the 'removal' of ammonia can be possibly due to plant uptake and volatilization, it is investigated that these processes only make a minor contribution. The removal of inorganic nutrients in wetlands is rarely discussed and associated with the process of immobilization into microbial cells, for example biomass assimilation (Sun et. al., 2005). Biomass assimilation process may have played an important role in the removal of Ammoniacal-nitrogen, taking into consideration that a major part of biomass is constituted by nitrogen. This is because a large quantity of organic substance is removed by the growth and respiration of microorganisms during the experiments (Cannon et. al., 2000). It is therefore a rational fact that together with nitrification, immobilizations into microbial cells and adsorption onto wetland/soil media are among the major routes of ammonia reduction (Sun et. al., 2005) or ammonia metabolism in phytoremediation process.

4.8 Comparative Study of RGR and Contaminant Bioaccumulation based on N-content of Treatments

For both Kenaf and Akasia, highest RGR was recorded by treatments containing 0.24% N treatment which recorded highest RGR. Fe, As, CN and NH_3-N accumulation in Kenaf and Akasia plants increased with the increase in N-content but after the concentration of 0.48% N-content, the bioaccumulation of contaminants or metals decreased. Bioconcentration of As, Fe, CN and NH_3-N showed highest in 0.24% N-content in Kenaf. In Akasia, highest bioconcentration of As, Fe, CN and NH_3-N was also recorded by 0.24% N-content.

Comparison among 100% IF and 50% TL treatments applied to Kenaf and Akasia, both containing 0.48% N-content, the test plants proved a higher Relative Growth Rate of leaves, stem, root and total plant dry biomass for 100% IF. Better accumulation of As, CN and NH_3-N was observed in 100% IF treatment compared to 50% TL treatment. Fe concentration in both Kenaf and Akasia was equivalent for both the treatments. The bioconcentration of contaminants in Akasia also recorded higher BCF values for plants treated with 100% IF compared to plants treated with 50% TL. Rate of metal degradation in soil amended with fertilizer is better probably due to its low pH along the 120 days of investigation because low pH condition affects the growth and metabolic activities of microbes in soil (Abioye et. al., 2010).

Among 50% TL + 50% IF and 75% TL treatments where by both contained 0.72% N-content, RGR of leaves, stems, roots and plant dry matter showed a better results with application of 50% TL + 50% IF treatment. The bioaccumulation of Fe, CN and NH_3-N also proved a higher concentration in Kenaf plants at 50% TL + 50% IF. The bioaccumulation of As was nearly similar in both the 0.72% N treatments. Cumulative accumulation of As, CN, and NH_3-N at final harvest recorded higher percentage for Kenaf plants treated with the above mentioned combination of fertilizer and leachate preparation. As for Akasia, 50% TL + 50% IF recorded a better growth rate of plant parts compared to 75% TL. Higher concentration of Fe, CN, and NH_3-N was found in the 50% TL + 50% IF treatment though As accumulation was equivalent in both the treatments. Removal percentages of Fe, As, CN and NH_3-N from contaminated soil also was recorded lower in 75% TL comparable to 50% TL + 50% IF. BCF of all 4 parameters tested was significantly higher in 50% TL + 50% IF.

Comparison analysis was also carried out for 100% TL (0.96% N-content) and 100% RL (0.98% N-content) treatments. RGR of both Kenaf and Akasia plants proved to show better results with 100% TL. Same goes for Bioconcentration Factor of Fe, As, CN and NH_3-N in the two test plants which recorded a higher accumulation in plants treated with 100% TL.

Soil N-content was obmitted in all the treatments applied in this study. Control treatment (Zero N-content) with recorded a lower Kenaf and Akasia growth rate compared to 100% IF (0.48% N-content) and also 100% RL. Bioconcentration values of Fe, As, CN and NH_3-N was shown to be higher in 100% IF and 100% RL for both the test plants compared to Control. Cumulative percentage of parameters tested at week 8, 12 and 16th of Akasia growth proved that plants treated with fertilizer amendment showed better accumulation percentage. This phenomenon proved the hypothesis of better metal accumulation in plants from fertilizer amended soil. The Control treatments gave an insignificant constant bioconcentration of contaminants in test plant cells throughout the study period.

4.9 Comparison Study of Control Soil and Soil Standard

Soil control was a preparation of nine pots of soil applied with nine treatments of leachate without plants for a period of 120 days. Pollution impacts based on the heavy metal content in the soil and in the test plants, *A. mangium* and *H. Cannabinus L.* were compared with maximum permitted concentration (MPC) of The Dutch Intervention Standard. The Dutch Intervention Standard was used as a reference because there are fewer references on the soil standard in Malaysia. The Dutch Intervention Standard for surface and deep soil analysis is appended in Appendix B (Table B.1).

According to Table 4.15, among the elements compared, As concentration in Control Soil applied with the nine treatments after 120 days of experiment, was within the Dutch Intervention Standard.

Table 4.15: Concentration of contaminants in control soil at 120 days of treatments

Treatments	**Metal/ Contaminants (mg/kg)**			
	As	**Fe**	**CN**	**NH_3-N**
Dutch Intervention Standard	**55**	**NA**	**NA**	**NA**
Control	1.27±0.02	122.01±5.50	2.96±0.21	40.84±1.02
100 IF	8.25±0.02	142.21±11.23	7.46±1.02	42.15±15.87
12.5% TL	11.32±0.02	201.41±8.40	7.70±0.88	47.84±15.86
25% TL	15.41±0.01	211.45±2.30	7.81±2.10	50.11±2.15
50% TL	3.41±0.10	130.08±17..75	4.10±0.25	50.45±10.66
50% TL+ 50% IF	12.56±0.04	215.47± 11.22	7.71±3.01	50.96±1.45
75% TL	17.53±0.04	227.46±10.05	7.95±1.45	53.21±16.52
100% TL	18.87±0.01	231.44±20.04	7.95±2.09	56.14±12.98
100% RL	18.96±0.50	233.06±11.10	7.97±1.21	56.15±10.74

NA = Not available

Arsenic is of particular concern besides Hg because of its highly toxic nature. Furthermore, polluted soil surface may result in contamination of surface and groundwater via run-off. The Dutch Intervention Standard does not provide a maximum permitted concentration for other parameters used in this study such as Fe, CN and NH_3-N.

The Soil control results were also compared to regulatory standards set by the United States Environmental Protection Agency (USEPA) as shown in Table 4.18. According to the table below, maximum concentration of As permitted in industrial waste or sludge must be within 75 mg/kg. Fe, CN and NH_3-N concentrations in contaminated soil is less discussed in the international soil standards available. The maximum As concentration in control soil as per reported by 100% RL was 18.96 mg/kg which is still within the regulatory limits of USEPA as shown by the Table 4.16 below.

Table 4.16: USEPA Regulatory limits on heavy metals applied to soils

Heavy metal	**Maximum concentration in sludge (mg/kg or ppm)**	**Annual pollutant loading rates**		**Cumulative pollutant loading rates**	
		kg/ha/yr	lb/A/yr	kg/ha	lb/A
Arsenic	75	2	1.8	41	36.6
Cadmium	85	1.9	1.7	39	34.8
Chromium	3000	150	134	3000	2679
Copper	4300	75	67	1500	1340
Lead	420	21	14	420	375
Mercury	840	15	13.4	300	268
Molybdenum	57	0.85	0.80	17	15
Nickel	75	0.90	0.80	18	16
Selenium	100	5	4	100	89
Zinc	7500	140	125	2800	2500

Source: Adapted from USEPA, 1993

The pollutant loading rate for the selected contaminants of As, Fe, CN and NH_3-N of this study was 12.99 kg/ha/application, 159.63 kg/ha/application, 5.46 kg/ha/application and 38.46 kg/ha/application, respectively.

4.10 Kinetics of metal uptake: Biaccumulation Rate Constant (k) and Half-Life ($t_{1/2}$)

Kinetic model is among one of the approach used in this study and it is influenced by the concentration of heavy metal in the plants' biomass. Evaluation of the specific metal uptake rate and the maximum specific content of the metal in the plant is enabled using this approach.

First order kinetics model of Yeung et. al. (1997) was applied to determine the rate of bioaccumulation of metal in the various treatments (Abioye et. al., 2010). The uptake of metal, i.e Iron by the test plant at given conditions (pH and temperature) can be represented as a function of the maximum concentration of metal that can be biaccumulated in the plant cells and the specific uptake rate applying the first-order kinetic model (Ghaly et. al., 2008).

$$\frac{d(y)}{dt} = k.(a) \qquad (1)$$

Where:

y = Maximum metal concentration in test plant at a given time (mg/kg)

a = Initial metal concentration in test plant (mg/kg)

t = Time (day)

k = Metal bioaccumulation rate constant (day-^{1})

Equation 1 represents that the higher the k-value the faster the metal accumulation by the plants. Equation 1 can be rearranged for integration using the limits $0 \rightarrow y$ and $0 \rightarrow t$ as follows:

$$\int_0^y \frac{d(y)}{a} = \int_0^t k.\,dt \qquad (2)$$

On integration, Equation 2 can be written as follows:

$$\left[\ln \frac{y}{a}\right] = k.t \qquad (3)$$

Equation 3 can also be written in a logarithmic form as follows:

$$2.3 \log \frac{y}{a} = k.t \qquad (4)$$

First order kinetic model can be also interpreted as below:

$$y = ae^{-kt} \qquad (5)$$

Or Equation 5 can be integrated as follows for the calculation of bioaccumulation rate constant (k):

$$\ln (y) = \ln (a) - kt \qquad (6)$$

Following that, Half- life ($t_{1/2}$) is calculated according to Equation 7 as shown below:

$$\text{Half life } (t_{1/2}) = \ln (2)/ k \qquad (7)$$

Table 4.17 and Table 4.18 show the bioaccumulation rate constant (*k*) and half-life ($t_{1/2}$) of parameters tested for the different treatments within the 120 days of study period in Kenaf. Soil treated with 25% treated leachate shows the highest bioaccumulation rate of 0.03/day and half-life 21.9 days for Fe; 0.02/day and half-life 36.3 days for As, 0.02/ day and half-life 34.2 days for CN and 0.02/day and half-life 51.4 days for NH_3-N respectively. The bioaccumulation rate of Control treatment was the least in As, Fe, CN and NH_3-N uptake in plant cells, 0.019/day and half-life of 46.3

days for Fe, 0.004/ day and half-life 81.3 days for As, 0.008/ day and half-life 58.4 days for CN and 0.009/ day and half-life 75.5 days for NH_3-N.

All readings of bioaccumulation rate constant and half-life of Fe, As, CN and NH_3-N in Kenaf were significantly different at confidence level of 99% ($p<0.01$). Summary of Descriptive statistics resulted that Fe has a mean k value of 0.0202 per day and mean half life of 35.7 days ($p<0.01$); As has a mean k value of 0.0072 per day and mean half life of 58.8 days ($p<0.01$); CN has a mean k value of 0.0143 per day and mean half life of 46.6 days ($p<0.01$); NH_3-N has a mean k value of 0.0127 per day and mean half life of 60.0 days ($p<0.01$).

Table 4.17: Bioaccumulation rate constant and half-life of Fe and As accumulation from leachate polluted soil planted with Kenaf

Treatments	**Fe**		**As**	
	Bioaccumulation constant (k) day $^{-1}$	**Half-life ($t_{1/2}$)**	**Bioaccumulation constant (k) day $^{-1}$**	**Half-life ($t_{1/2}$)**
Control	0.0110	46.32	0.0040	81.25
12.5% TL	0.0158	43.90	0.0055	65.77
25% TL	0.0327	21.85	0.0185	36.33
50% TL	0.0253	28.04	0.0064	51.94
100% IF	0.0250	29.12	0.0071	49.51
50% TL + 50% IF	0.0224	34.39	0.0062	51.97
75% TL	0.0180	36.33	0.0064	51.61
100% TL	0.0183	35.40	0.0058	65.68
100% RL	0.0138	46.27	0.0049	74.86

Table 4.18: Bioaccumulation rate constant and half-life of CN and NH_3-N accumulation from leachate polluted soil planted with Kenaf

Treatments	CN		NH_3-N	
	Bioaccumulation constant (k) day $^{-1}$	**Half-life ($t_{1/2}$)**	**Bioaccumulation constant (k) day $^{-1}$**	**Half-life ($t_{1/2}$)**
Control	0.0078	58.40	0.0087	75.48
12.5% TL	0.0138	48.52	0.0122	63.01
25% TL	0.0197	34.19	0.0152	51.39
50% TL	0.0168	40.86	0.0137	55.64
100% IF	0.0170	42.07	0.0137	49.55
50% TL + 50% IF	0.0152	45.25	0.0133	59.72
75% TL	0.0144	47.18	0.0129	59.91
100% TL	0.0129	50.32	0.0125	62.83
100% RL	0.0111	53.08	0.0125	62.84

Table 4.19 and Table 4.20 show the bioaccumulation rate constant (k) and half-life ($t_{1/2}$) of parameters tested for the different treatments within the 120 days of study period in Akasia. Soil contaminated with 25% treated leachate shows the highest bioaccumulation rate of 0.04/day and half-life 22.7 days for Fe, 0.02/day and half-life 32.1 days for As, 0.03/day and half-life 25.20 days for CN and 0.03/ day and half-life 50.4 days for NH_3-N accumulation in Akasia plants, respectively. The bioaccumulation rate of Control treatment was the least, 0.027/day and half-life of 30.9 days for Fe, 0.011/day and half-life of 62.7 days for As, 0.010/day and half-life of 61.3 days for CN and 0.018/day and half-life of 80.4 days for NH_3-N, respectively.

Table 4.19: Bioaccumulation rate constant and half-life of Fe and As accumulation from leachate polluted soil planted with Akasia

Treatments	**Fe**		**As**	
	Bioaccumulation constant (k) day $^{-1}$	**Half-life ($t_{1/2}$)**	**Bioaccumulation constant (k) day $^{-1}$**	**Half-life ($t_{1/2}$)**
Control	0.0268	30.87	0.0112	62.71
12.5% TL	0.0289	25.12	0.0150	54.05
25% TL	0.0406	22.65	0.0244	32.06
50% TL	0.0295	23.25	0.0215	42.25
100% IF	0.0298	23.23	0.0215	42.30
50% TL + 50% IF	0.0294	23.45	0.0203	46.46
75% TL	0.0290	24.94	0.0194	48.59
100% TL	0.0290	25.03	0.0177	51.63
100% RL	0.0283	27.01	0.0128	55.08

All readings of bioaccumulation rate constant and half-life of Fe, As, CN and NH_3-N in Akasia were significantly different at confidence level of 99% ($p<0.01$). Summary of Descriptive statistics resulted that Fe has a mean k value of 0.0301 per day and mean half-life of 25.1 days ($p<0.01$); As has a mean k value of 0.0182 per day and mean half-life of 48.3 days ($p<0.01$); CN has a mean k value of 0.0223 per day and mean half-life of 41.3 days ($p<0.05$); NH_3-N has a mean k value of 0.0243 per day and mean half-life of 68.8 days ($p<0.01$).

The kinetics parameters investigated in this study showed that the rate of uptake of metal in soil contaminated with leachate was relatively higher than control treatments; this is possibly due to high percentage of nitrogen present in leachate contaminated soil compared to other inorganic wastes and bioavailability of nutrients in leachate to microbe species in the leachate contaminated soil (Abioye et. al., 2010).

Table 4.20: Bioaccumulation rate constant and half-life of CN and NH_3-N accumulation from leachate polluted soil planted with Akasia

Treatments	CN		NH_3-N	
	Bioaccumulation constant (k) day $^{-1}$	**Half-life ($t_{1/2}$)**	**Bioaccumulation constant (k) day $^{-1}$**	**Half-life ($t_{1/2}$)**
Control	0.0104	61.26	0.0184	80.36
12.5% TL	0.0217	49.22	0.0226	73.60
25% TL	0.0342	25.20	0.0318	50.38
50% TL	0.0232	31.91	0.0268	64.83
100% IF	0.0240	31.50	0.0279	61.31
50% TL + 50% IF	0.0228	38.23	0.0251	67.77
75% TL	0.0224	38.92	0.0233	71.10
100% TL	0.0224	40.67	0.0226	73.84
100% RL	0.0200	54.74	0.0206	76.27

Treatments above 50% TL + 50% IF showed signs of toxicity as the k value of the metal and macronutrient accumulation in test plants/ treatment decreased with the increase in leachate concentrations in polluted soil. The results showed that the specific metal uptake rate and the maximum concentration of metal that can be accumulated in each species were influenced by the initial metal concentration in the soil and the type of plant. The bioaccumulation rate for plant species increased as the initial metal concentration in the soil increased (Ghaly et. al., 2008). However, the rate of metal bioaccumulation in soil treated with untreated leachate was lower than those treated with treated leachate and fertilizer amended soil; this may be due to its low pH condition during 120 days of experiment because low pH condition affects the proliferation and bioaccumulation activities of metal-utilizing-bacteria in soil (Abioye et. al., 2010).

4.11 Comparative Study of Metal and Macronutrient Accumulation in Test Plants via Hydroponic System

4.11.1 Bioaccumulation of Fe, As, CN and NH_3-N in hydroponically grown Kenaf from water contaminated with different concentrations of leachate

The Kenaf plants grown hydroponically were able to survive for a time period of 5 weeks. Plates 4.7 to 4.9 show the Kenaf growth in Hydroponic system.

Plate 4.7: Kenaf seedlings grown hydroponically after 1 week of growth

Plate 4.8: Kenaf plants at 4th week of growth in hydroponic culture

Plate 4.9: Kenaf plants ready to be harvested at week 5 of growth in hydroponic culture

Table 4.21 shows the growth measurements of leaf, stem and root of hydroponically grown Kenaf plants.

Table 4.21: Leaf length, Stem height and Root length of hydroponically grown Kenaf at week 5 of growth

Treatments	Growth (in cm) of hydroponically grown Kenaf		
	Root Length	Stem Height	Leaf Length
Control(dH_2O)	1.5±0.03	4.9±1.21	1.1±0.21
12.5 % TL	3.4±1.10	11.4±0.67	1.7±0.23
25% TL	4.3±0.82	13.5±0.86	2.1±0.87
50 % TL	3.2±0.61	11.0±1.54	1.5±0.67
100 % IF	3.4±0.70	10.9±0.23	1.7±0.22
50 % TL + 50 % IF	3.0±0.12	9.3±1.20	1.4±0.54
75 % TL	2.7±0.09	8.0±0.58	1.5±0.23
100 % TL	2.8±0.56	7.2±0.06	1.3±0.14
100 % RL	1.2±0.41	6.4±1.05	1.1±0.11

Kenaf plants grown in hydroponic culture with 25% TL treatment showed significantly the best growth record of root length, stem height and leaf length. The control and 100% RL treatments gave an insignificant reading of plant growth in hydroponic culture. Figure 4.23 explains the growth of hydroponically grown Kenaf graphically.

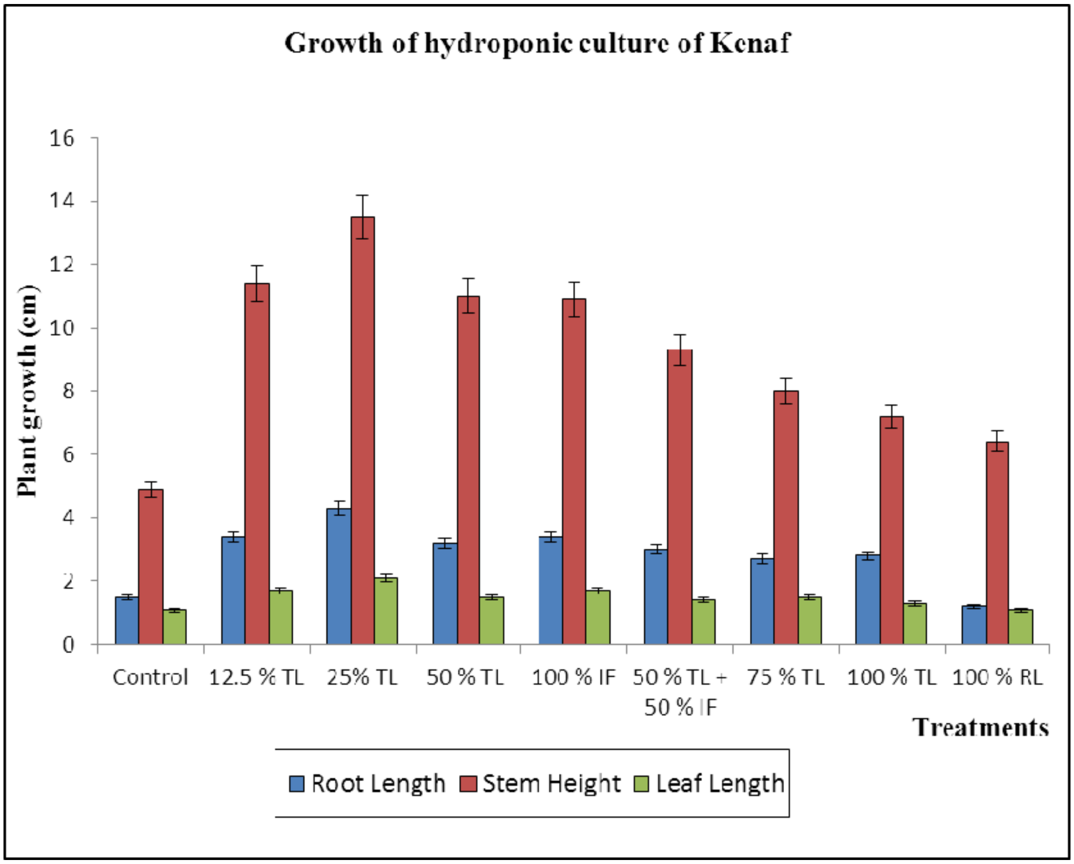


Figure 4.23: Growth of hydroponic culture of Kenaf in the treatment of leachate wastewater at week 7

Fe, As, CN and NH_3-N concentrations in root, stem and leaves of harvested Kenaf plants grown hydroponically under different leachate treatments at final harvest (week 5) is displayed graphically and discussed further. The tables of Fe, As, CN and NH3-N concentrations in Kenaf in hydroponics treatment are appended in Appendix C (Table C.1, Table C.2, Table C.3 and Table C.4). Figure 4.24 shows the As accumulation in root, stem and leaves of Kenaf grown hydroponically for the treatment of landfill leachate contaminated wastewater.

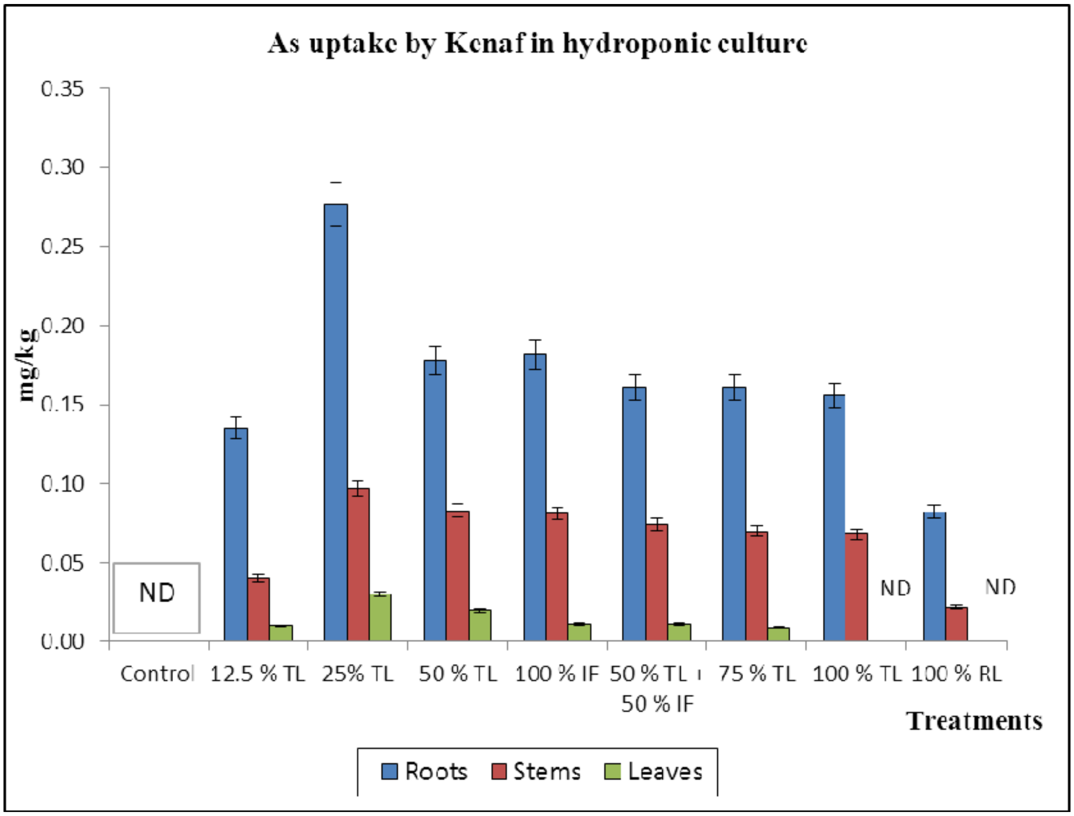


Figure 4.24: As concentration in root, stem and leaves of Kenaf grown in hydroponic culture under different leachate treatments

As concentration was not detected in Kenaf plants grown in Control treatment. As uptake was significantly greater in plant root (68.1% of As accumulation in Kenaf root) compared to plant stems and leaves which was observed 27.3% and 4.6% of As uptake, respectively. The treatment which contains 25% TL was the optimum concentration of treatment for high concentration of As accumulation in Kenaf cells.

Figure 4.25 shows the Fe accumulation in root, stem and leaves of Kenaf grown hydroponically for the treatment of landfill leachate contaminated wastewater. Fe uptake in the root, stem and leaves of hydroponically cultured Kenaf was in the range of 1.97 – 2.48 mg/kg, 0.41 – 0.85 mg/kg and 0.32 – 0.50 mg/kg, respectively with the highest Fe uptake took place in the 25% TL and the least concentration recorded by plants in 100% RL excluding control treatment.

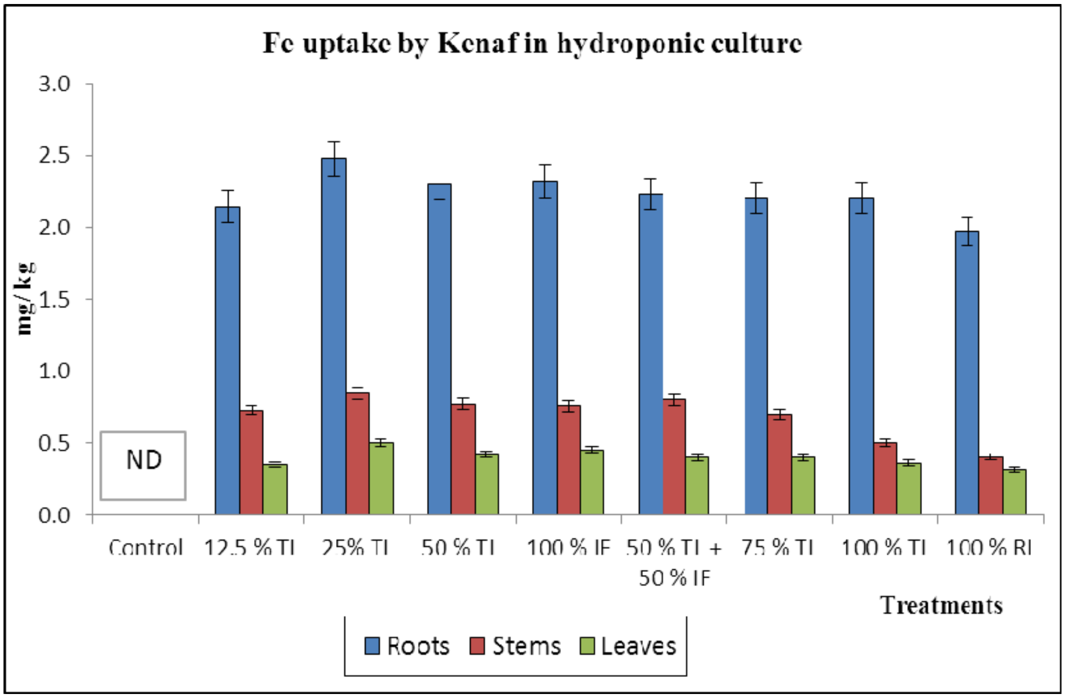


Figure 4.25: Fe concentration in root, stem and leaves of Kenaf grown in hydroponic culture under different leachate treatments

Figure 4.26 shows the CN accumulation in root, stem and leaves of Kenaf grown hydroponically for the treatment of landfill leachate contaminated wastewater.

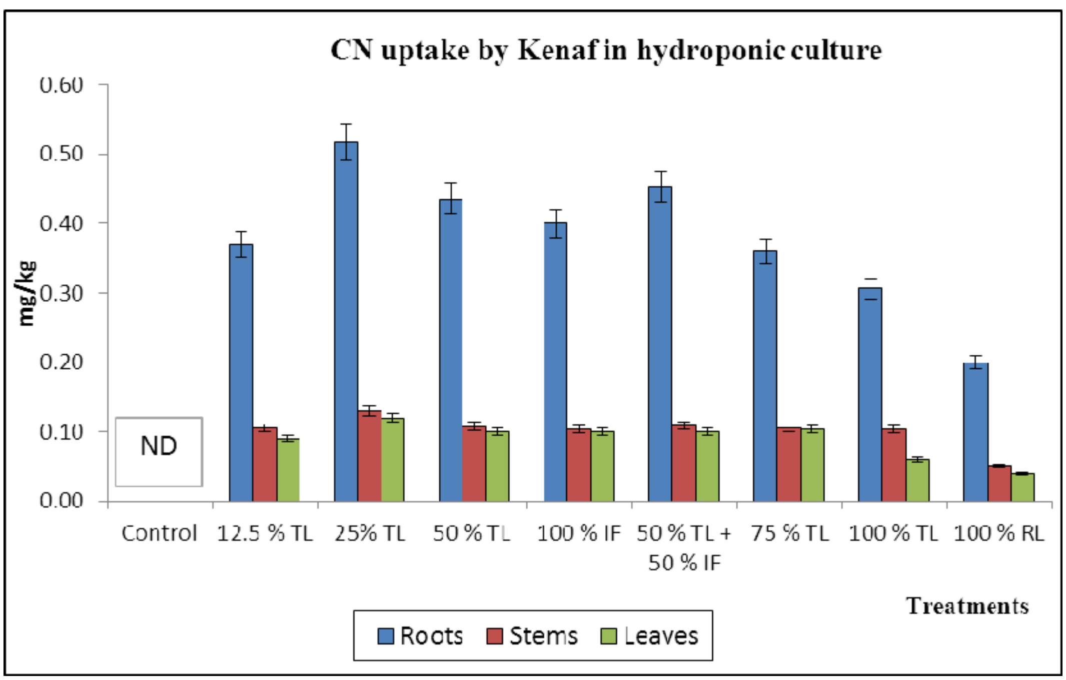


Figure 4.26: CN concentration in root, stem and leaves of Kenaf grown hydroponically under different leachate treatments

CN concentration was not detected in Kenaf plants grown in Control treatment. CN uptake was significantly greater in plant root (66.6% of As accumulation in Kenaf root) compared to plant stems and leaves which was observed 17.8% and 15.6% of CN uptake, respectively. The treatment which contains 25% TL was the optimum concentration of treatment for high concentration of CN accumulation in Kenaf. Figure 4.27 shows the NH_3-N accumulation in root, stem and leaves of Kenaf grown hydroponically for the treatment of landfill leachate contaminated wastewater.

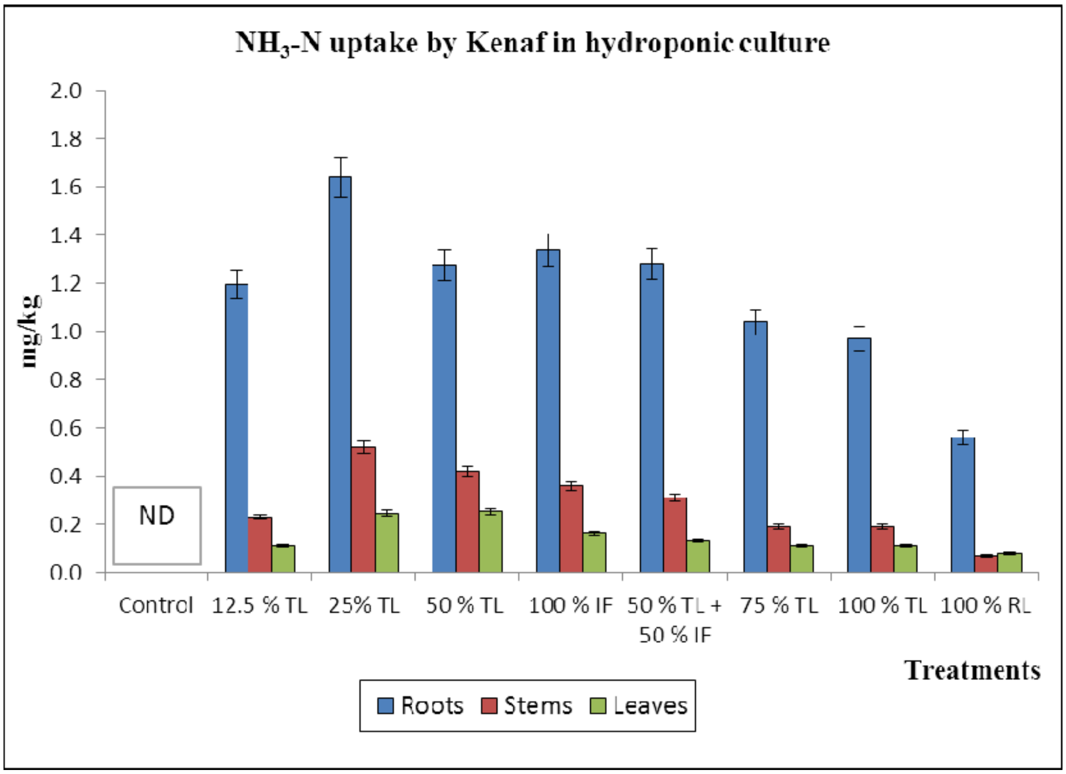


Figure 4.27: NH_3-N concentration in root, stem and leaves of Kenaf grown hydroponically under different leachate treatments

NH_3-N uptake in the root, stem and leaves of hydroponically cultured Kenaf was in the range of 0.56 – 1.64 mg/kg, 0.07 – 0.52 mg/kg and 0.08 – 0.25 mg/kg, respectively with the highest NH_3-N uptake took place in the 25% TL and the least concentration recorded by plants in 100% RL. NH_3-N concentration was not detected in Kenaf plants grown in Control treatment.

The results showed that NH_3-N concentrations in stem and leaves of Kenaf (0.07 – 0.52 mg/kg and 0.08 – 0.25 mg/kg respectively) were significantly higher than As concentrations in stems and leaves (0.02 – 0.10 mg/kg and 0.01 – 0.03 mg/kg respectively) and CN concentrations (0.05 – 0.13 mg/kg and 0.04 – 0.12 mg/kg respectively) in the stem and leaves respectively at the different leachate treatments. Fe was reported highest concentration in hydroponic culture of Kenaf at a range of 0.41 – 0.85 mg/kg in stems and 0.32 – 0.50 in leaves. The trend of heavy metal and macro nutrient accumulation from leachate wastewater by *H. cannabinus* L. in hydroponic system in decreasing order are as listed below:

$$Fe > NH_3\text{-N} > CN > As$$

Kenaf plants grown via hydroponics system in 25% TL recorded significant accumulation of As, Fe, CN and NH_3-N. Application of leachate as strong as above 50% TL had also affected the Kenaf plants growth negatively. Visual symptoms of metal toxicity were observed in plants treated with high concentrations of the heavy metals. All Kenaf plants dried up after 7 weeks of growth (Plate 4.10). The Kenaf leaves in hydroponic culture started turning yellow around week 5 of growth as shown in Plate 4.11. This is supported by study conducted by Batty and Younger (2002) whereby the seedlings of *P. australis* exposed to 2 mg/L Fe and above displayed diversified changes in morphology which includes stunted shoot growth, browning, and/or die-back of the leaves, brown patches on the leaves, dwarfing of root growth, reduced branching of roots, and root flaccidity.

Plate 4.10: Kenaf plants dried up after 5 weeks of growth

Plate 4.11: Kenaf dried up – leaves turn yellow

Statistically strong association was observed between As in stem and As in root and between As in stem with As in leaves ($r=0.77$ and $r=0.88$, $p<0.05$), respectively. Low correlation was found between uptake of Fe in stem with Fe in root ($r=0.39$, $p<0.01$) and Fe in leaves with Fe in root ($r=0.53$, $p<0.05$). In the case of CN, a strong Pearson's correlation was observed in CN in stem and leaves and with CN in root ($r = 0.95$ and $r=0.74$, $p<0.05$ respectively). As for NH_3-N uptake, a strong Pearson's correlation was observed in NH_3-N in stem with NH_3-N in root ($r = 0.71$, $p<0.05$).

According to Chen et. al. (2005), Iron oxides are basically reported to have great adsorption capacity for inorganic anions, especially for arsenate and phosphate. It also has a possible capacity to oxidize arsenite to arsenate (Chen et. al., 2005) and it is a common phenomenon in rice roots. On the other hand, As mobility and availability in soil also increases due to the presence of Fe and manganese oxides (Peralta-Videa et. al., 2009; Zavala and Duxbury, 2008). The transport and availability of As in soil is strongly inter-related with the soil pH. At high pH values (pH 6–8), As is mostly bound to calcium whereas at low pH values (pH 4), As is found complexed with iron (Peralta-Videa et. al., 2009).

4.11.2 Uptake of heavy metals by hydroponically grown Kenaf

In the process of Fe reduction, Kenaf showed significantly higher BCF ($p<0.05$) in 25% TL whereas in As accumulation, significantly higher BCFs were recorded by Kenaf plants grown in 25% TL, 50% TL and 100% IF (Figure 4.28). The BCF value in As treatment, however, showed reduction after the treated leachate concentration of 50%, which may be due to restriction in soil-root transfer at higher leachate concentrations (Ho et. al., 2008). Kenaf plants irrigated with 100% IF showed improved bioaccumulation of Fe in root with significantly higher BCF value compared to leachate treatment by 1.8%. Cyanide bioaccumulation in *H. cannabinus* L. showed significantly higher BCF ($p<0.05$) in 25% TL. The BCF value subsequently reduced after the treated leachate concentration of 50%. In NH_3-N accumulation, overall BCF values were below than one (BCF <1). Plants treated with 25% TL, 50% TL and 100% IF recorded high BCF compared to other leachate treatments.

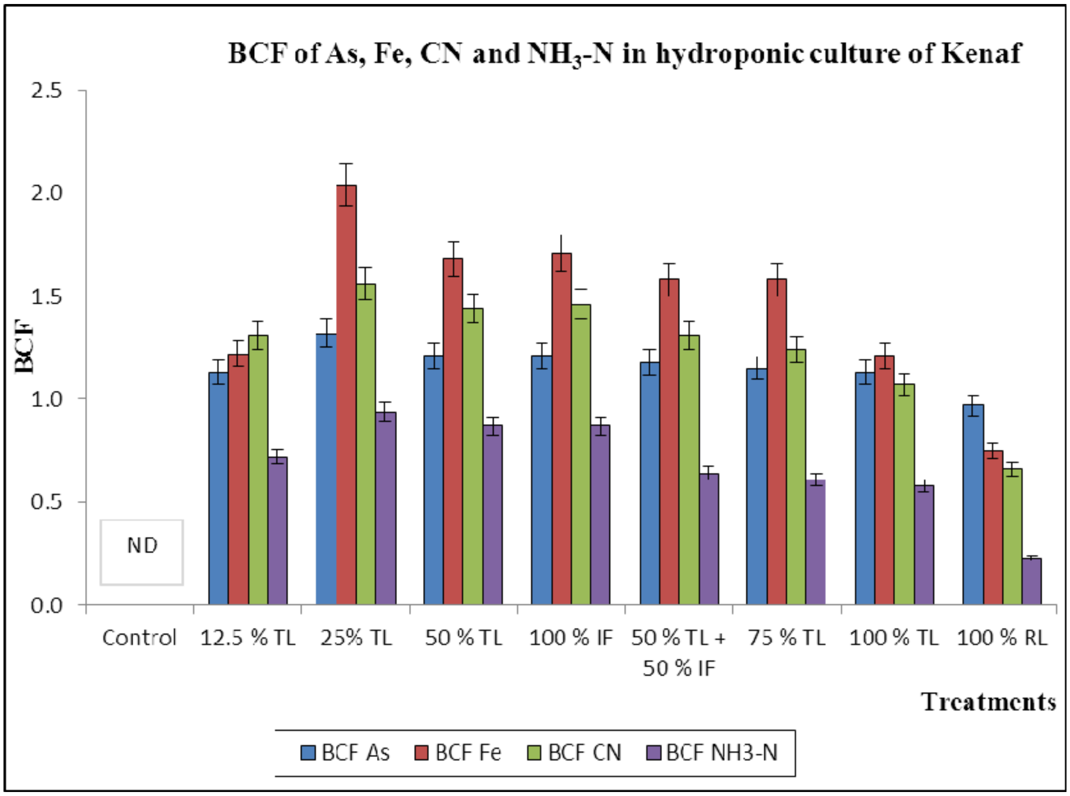


Figure 4.28: Bioconcentration factor (BCF) of As, Fe, CN and NH_3-N in hydroponically grown Kenaf under different leachate treatment. Bar indicates standard error (n=4)

The range of BCF in Fe and CN accumulation in hydroponic culture of Kenaf in this study was 0.75 - 2.04 and 0.66 – 1.56 respectively in comparison to As-accumulation which recorded higher BCF value in the range of 0.97 – 1.32. NH_3-N accumulation in Kenaf grown hydroponically for the treatment of landfill leachate contaminated wastewater reported bioconcentration factors in the range of 0.23 – 0.94. The ion selectivity by the Kenaf plants in hydroponic culture based on BCF were Fe> CN> > As > NH_3-N. This is line with a study conducted on constructed marsh receiving landfill leachate by Weis and Weis (2004). They found that most metals (Cu, Pb, Cd and Fe) accumulated only within roots, although Zn did accumulate in shoots. They also reported that the roots were significantly efficient at restricting flow into vascular tissue, thus limiting upward transport of the metals.

Kenaf roots in hydroponic culture were proven to be effective in taking up large amounts of As, Fe, CN but restricted its translocation to other plant parts at the same time. The translocation of As, Fe, CN and NH_3-N from root to aerial Kenaf parts was limited to TF values below one as shown in Figure 4.29 below.

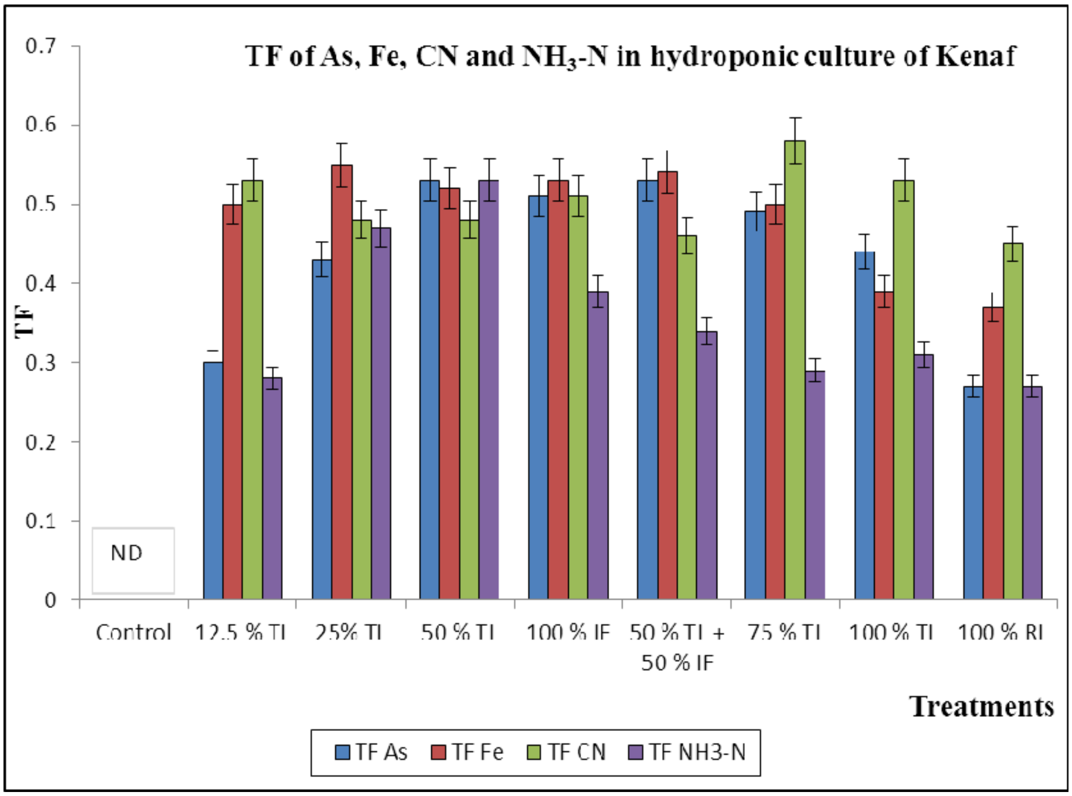


Figure 4.29: Translocation factor (TF) of As, Fe, CN and NH_3-N in hydroponically grown Kenaf under different leachate treatment. Bar indicates standard error (n=4)

Translocation ability was defined as the quantity of metal translocated in the plant's tissues, and was expressed as a root/aerial plant parts ratio (Liao and Chang, 2004). The ratio of translocation ability in the decreasing order among the four selected parameters were in the order of CN > Fe> As > NH_3-N in hydroponic culture of Kenaf. Quantity of elements that can be accumulated by Kenaf plants grown hydroponically has been shown to correlate well with concentration of heavy metals in the water according to Pearson's correlation test. As in root and As in solution recorded r= 0.60, p<0.05; Fe in root and Fe in wastewater solution showed r= 0.71, p<0.05; and CN in root and CN in leachate solution showed r = 0.95, p<0.05.

The results showed that the growth rate of leachate treated wetland plants was significantly higher than those in the control tanks. Total harvested biomass from treatment tanks was also much higher than from control tanks (Table 4.22). The nutrient uptake efficiency of Kenaf plant samples for the bioremediation of leachate is in the sequence of root > stem> leaves.

Table 4.22: *H. cannabinus* Plant Dry Matter (PDM in g) at final harvest (week 5)

Treatments	PDM (g) of hydroponically grown Kenaf		
	Roots	Stems	Leaves
Control(dH_2O)	0.12±0.03	0.06±0.01	0.29±0.09
12.5 % TL	0.16±0.01	0.14±0.01	0.40±0.11
25% TL	0.22±0.05	0.18±0.06	0.57±0.20
50 % TL	0.17±0.01	0.16±0.02	0.47±0.04
100 % IF	0.18±0.04	0.15±0.06	0.48±0.14
50 % TL + 50 % IF	0.17±0.02	0.15±0.0	0.43±0.03
75 % TL	0.14±0.03	0.12±0.04	0.37±0.07
100 % TL	0.12±0.01	0.11±0.01	0.33±0.10
100 % RL	0.10±0.04	0.02±0.0	0.21±0.06

Kenaf plants in hydroponic culture for the remediation of contaminants in landfill leachate wastewater had greater biomass of leave dry matter compared to root or stem dry biomass. The distribution of leaves, stem and root biomass were 59.0%, 18.1%

and 22.9%, respectively. At a higher concentration of leachate treatment, dry weights of Kenaf plants were significantly affected; in line with Batty and Younger (2002) who reported seedlings of *P. australis* grown in mine water wetlands at 5 mg/L Fe and above had lower shoot dry weights than those grown in 1 mg/L Fe and below. Table 4.23 displays the Pearson's correlation test between the root and stem, root and leaf, and stem and leaf of As, Fe, CN and NH_3-N uptake in *H. cannabinus.*

Table 4.23: Significant difference (P) of correlation between the root and stem, root and leaf, and stem and leaf of As, Fe, CN and NH_3-N in *H. cannabinus* according to the Pearson correlation test (r)

<table>
<tr><th rowspan="2">Pearson correlation test in contaminant uptake in plant parts</th><th colspan="2">As</th><th colspan="2">Fe</th><th colspan="2">CN</th><th colspan="2">NH3-N</th></tr>
<tr><th>r</th><th>p</th><th>r</th><th>p</th><th>r</th><th>p</th><th>r</th><th>p</th></tr>
<tr><td>Root and stem</td><td>0.77</td><td><0.05</td><td>0.39</td><td rowspan="2"><0.01</td><td>0.95</td><td rowspan="2"><0.05</td><td>0.71</td><td><0.05</td></tr>
<tr><td>Root and leaves</td><td>0.66</td><td><0.01</td><td>0.53</td><td>0.74</td><td>0.60</td><td rowspan="2"><0.01</td></tr>
<tr><td>Stem and leaves</td><td>0.88</td><td><0.05</td><td>0.71</td><td><0.05</td><td>0.66</td><td><0.01</td><td>0.26</td></tr>
</table>

* r= Correlation; p= Probability

In this present research, the removal efficiency of Fe, As, CN and NH_3-N from the wastewater by Kenaf plants were more than 50%. The 25% TL treatment marked a significant removal efficiency of 60.0%, 72.4%, 64.0% and 46.1% for the reduction of As, Fe, CN and NH_3-N from wastewater, respectively compared to other leachate treatments applied in this study.

4.11.3 Bioaccumulation of Fe, As, CN and NH_3-N in hydroponically grown Akasia in different treatments of leachate

Akasia growth in non-circulating constructed wetland system is shown in Plate 4.12 to Plate 4.15 below.

Plate 4.12: Akasia seedlings on growth bed in hydroponic culture

Plate 4.13: Akasia seedlings after 2 weeks of growth in hydroponic culture

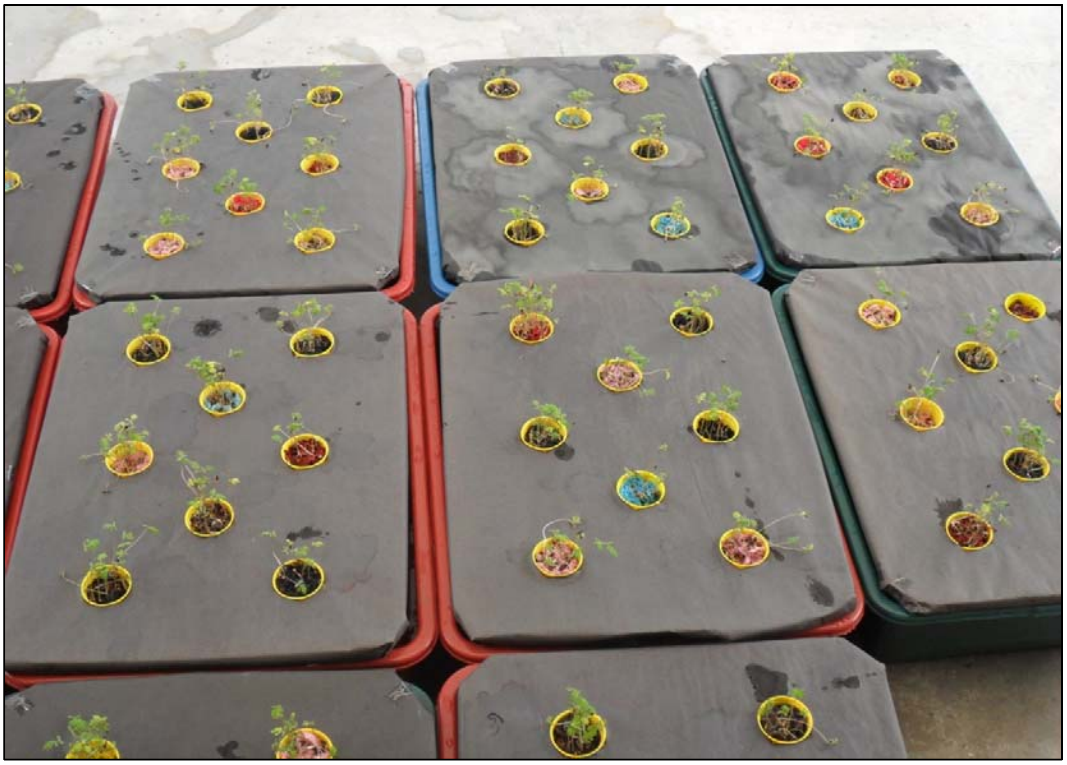


Plate 4.14: Akasia seedlings after 3 weeks of growth in hydroponic culture

Plate 4.15: Harvested Akasia plants at week 5 of growth in hydroponic culture

Table 4.24 shows the growth measurements of leaf, stem and root of hydroponically grown Akasia plants.

Table 4.24: Leaf length, Stem height and Root length growth measurement of hydroponically grown Akasia at week 5 of growth

Treatments	Growth (in cm) of hydroponically grown Akasia		
	Root Length	Stem Height	Leaf Length
Control(dH_2O)	3.5±1.02	6.6±0.15	1.1±0.21
12.5 % TL	4.6±0.56	10.2±1.24	1.3±0.11
25% TL	6.6±0.74	14.6±1.32	1.7±0.16
50 % TL	5.4±0.25	12.5±0.87	1.4±0.05
100 % IF	5.7±0.66	13.2±1.24	1.4±0.09
50 % TL + 50 % IF	5.2±0.34	12.4±0.88	1.4±0.41
75 % TL	4.8±1.05	10.2±0.75	1.3±0.21
100 % TL	4.5±0.92	9.5±1.40	1.2±0.11
100 % RL	2.4±0.11	4.9±0.36	1.0±0.21

Akasia plants grown in hydroponic culture with 25% TL treatment showed significantly the best growth record of root length, stem height and leaf length. The control and 100% RL treatments gave an insignificant reading of plant growth in hydroponic culture. Figure 4.30 explains the growth of hydroponically grown Akasia graphically. A reduction in growth was observed in plants treating wastewater with a concentration above 50% treated leachate and inorganic fertilizer.

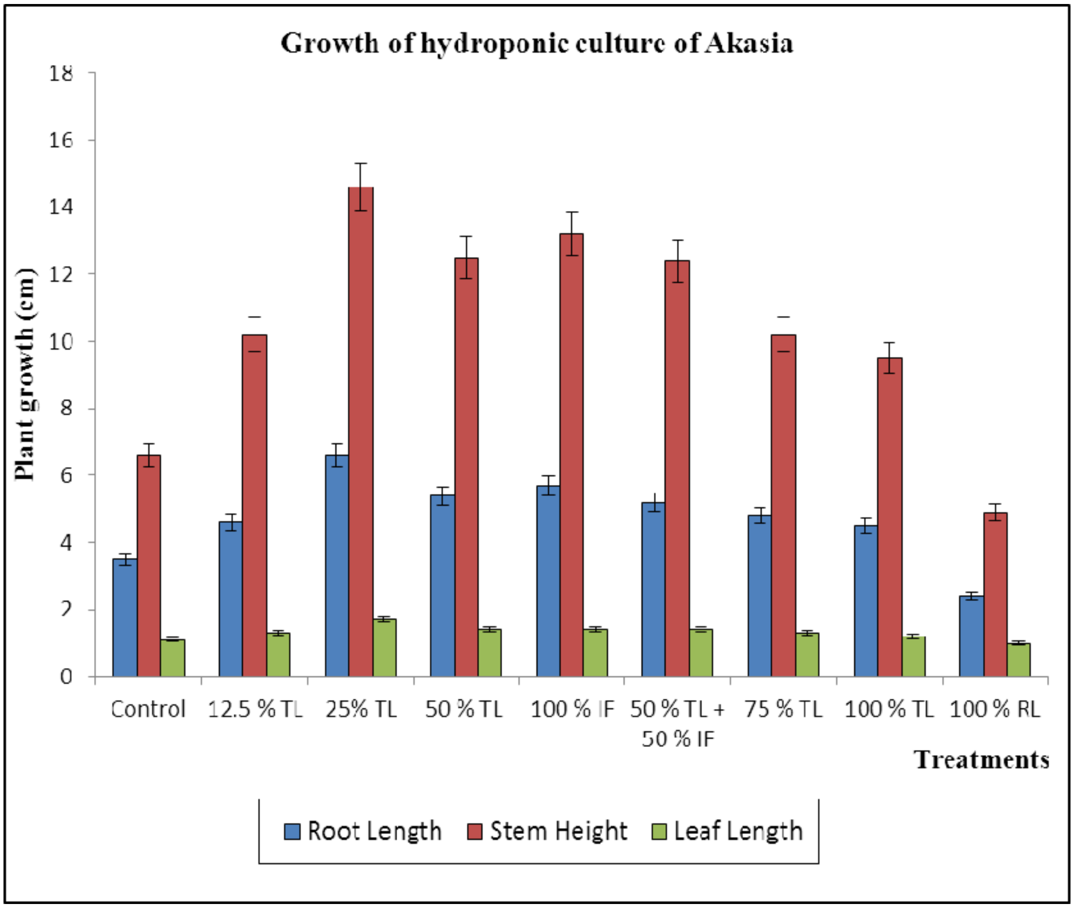


Figure 4.30: Growth of hydroponic culture of Akasia in the treatment of leachate wastewater at week 5

Fe, As, CN and NH_3-N concentrations in root, stem and leaves of harvested Akasia plants grown hydroponically under different leachate treatments at final harvest (week 5) is displayed graphically and discussed further. The tables of Fe, As, CN and NH_3-N concentrations in Akasia in hydroponics treatment are appended in Appendix C (Table C.5, Table C.6, Table C.7 and Table C.8). Figure 4.31 shows the As accumulation in root, stem and leaves of Akasia grown hydroponically for the treatment of landfill leachate contaminated wastewater.

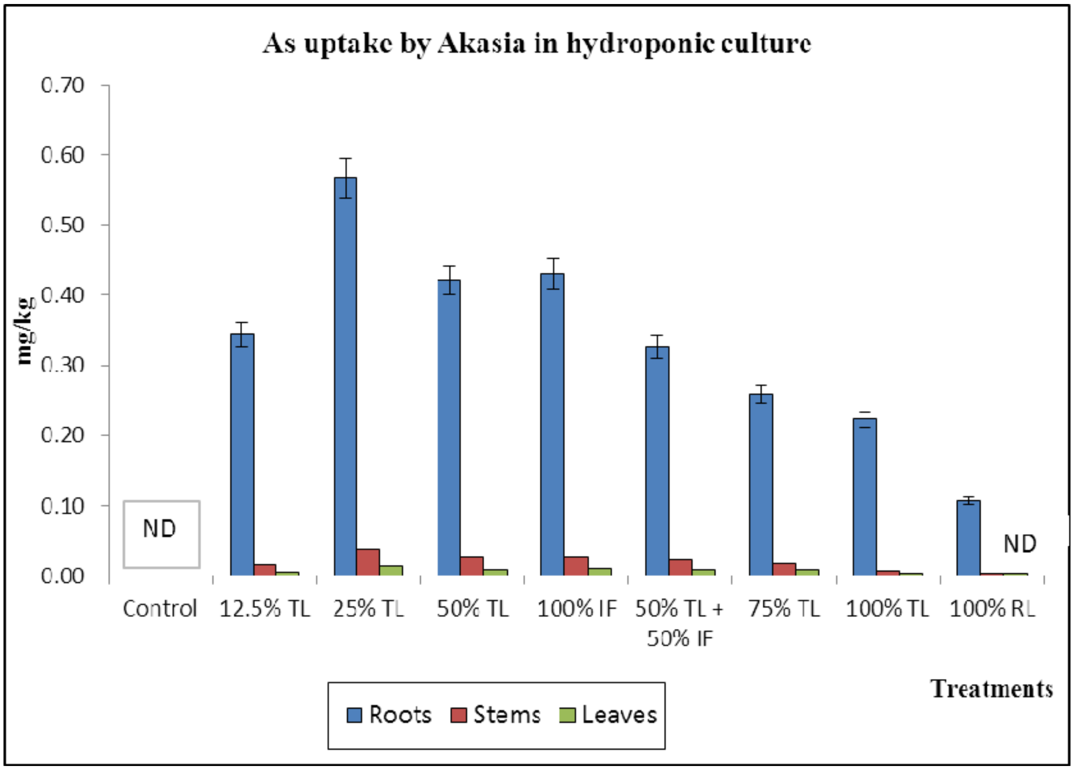


Figure 4.31: As concentration in root, stem and leaves of Akasia grown hydroponically under different leachate treatments (mean ± s.e.)

As concentration was not detected in Akasia plants grown in Control treatment of hydroponic culture. As uptake was significantly greater in plant root (92.5% of As accumulation in Akasia root) compared to plant stems and leaves which was observed 5.4% and 2.1% of As uptake, respectively. The treatment which contained 25% TL was the optimum concentration of treatment for high concentration of As accumulation in Akasia cells. Weis and Weis (2004) found a similar pattern in a study conducted in hydroponic conditions, but also observed that both root and shoot concentrations significantly increased with increased levels of As in the medium regardless of the chemical form of arsenic.

Figure 4.32 shows the Fe accumulation in root, stem and leaves of Akasia grown hydroponically for the treatment of landfill leachate contaminated wastewater. Fe uptake in the root, stem and leaves of hydroponically cultured Akasia was in the range of 1.75 – 3.08 mg/kg, 0.29 – 0.78 mg/kg and 0.20– 0.55 mg/kg, respectively with the highest Fe uptake took place in the 25% TL and the least concentration recorded by plants in 100% RL excluding control treatment.

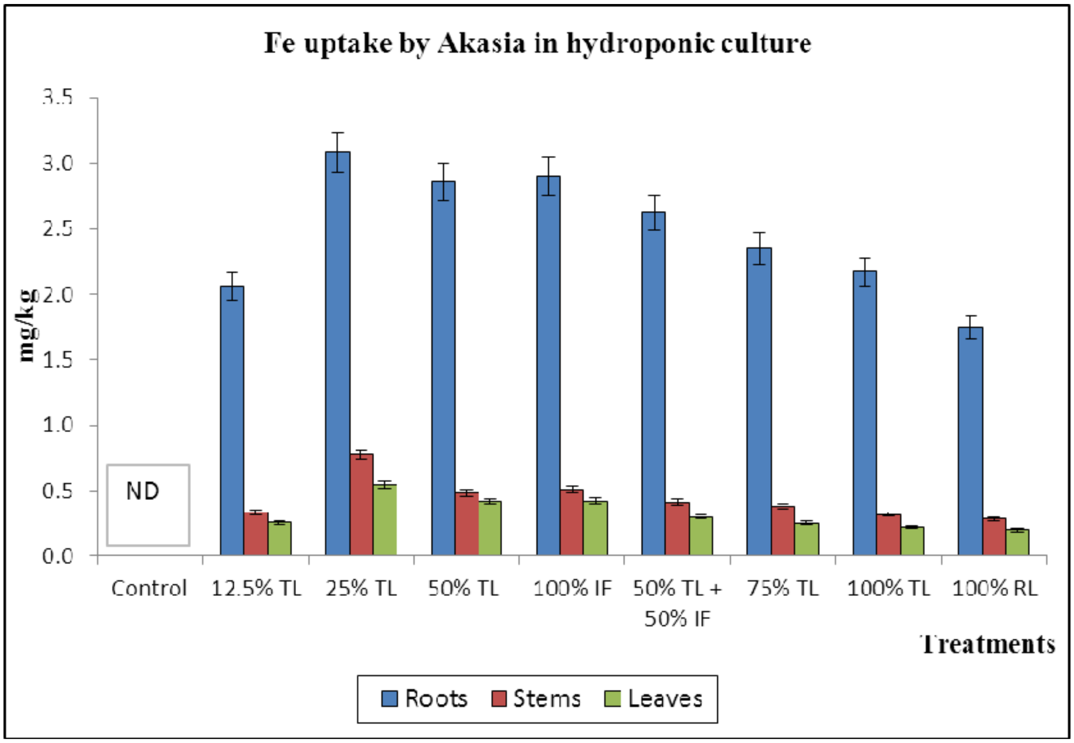


Figure 4.32: Fe concentration in root, stem and leaves of Akasia grown hydroponically under different leachate treatments (mean ± s.e.)

Figure 4.33 shows the CN accumulation in root, stem and leaves of Akasia grown hydroponically for the treatment of landfill leachate contaminated wastewater. CN concentration was not detected in Akasia plants grown in Control treatment. CN uptake was significantly greater in plant root (more than 75% of As accumulation in Akasia root) compared to plant stems and leaves which was observed 15.2% and 7.6% of CN uptake, respectively. The treatment which contains 25% TL was the optimum concentration of treatment for high concentration of CN accumulation in Akasia. Figure 4.34 shows the NH_3-N accumulation in root, stem and leaves of Akasia grown hydroponically for the treatment of landfill leachate contaminated wastewater.

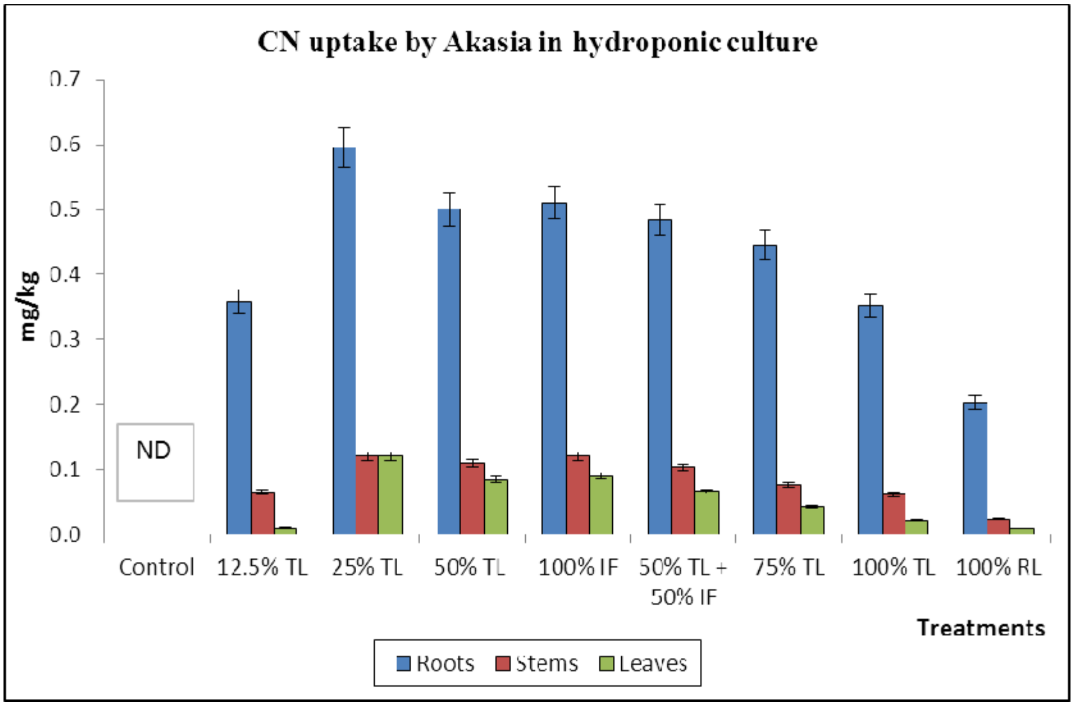


Figure 4.33: CN concentration in root, stem and leaves of Akasia grown hydroponically under different leachate treatments (mean ± s.e.)

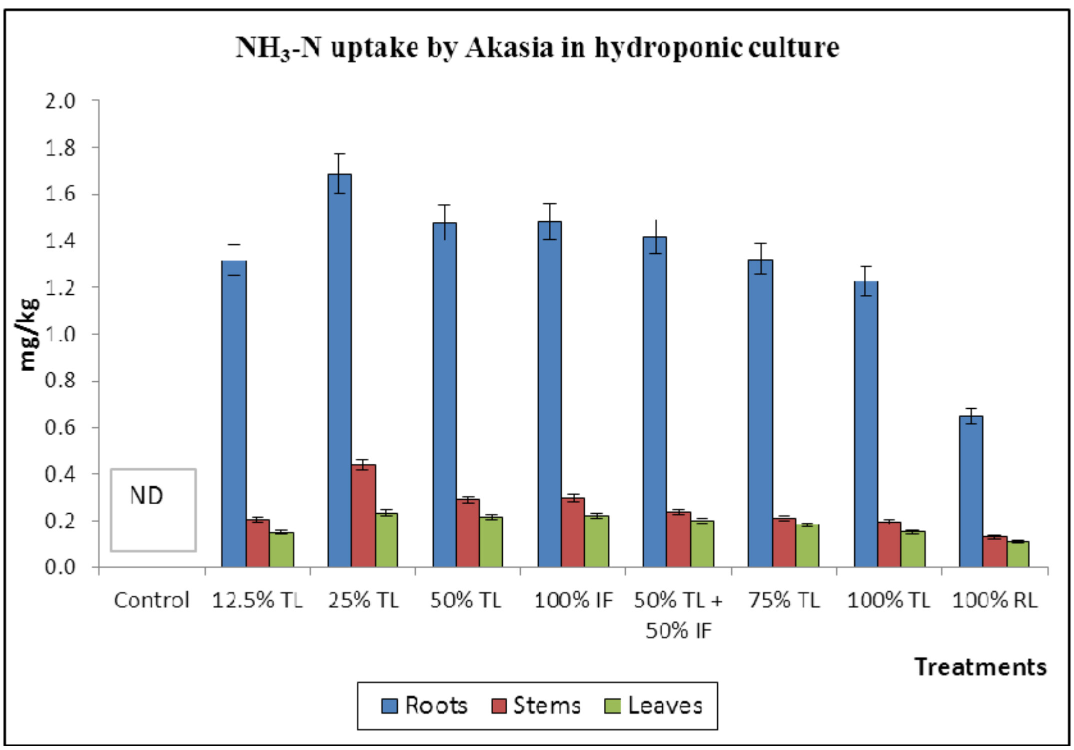


Figure 4.34: NH_3-N concentration in root, stem and leaves of Akasia grown hydroponically under different leachate treatments (mean ± s.e.)

NH_3-N uptake in the root, stem and leaves of hydroponically cultured Akasia was in the range of 0.65 – 1.69 mg/kg, 0.13 – 0.44 mg/kg and 0.11 – 0.23 mg/kg, respectively with the highest NH3-N uptake took place in the 25% TL and the least concentration recorded by plants in 100% RL. NH_3-N concentration was not detected in Akasia plants grown in Control treatment. The results showed that NH_3-N concentrations in root, stem and leaves of hydroponics Akasia (0.65 – 1.69 mg/kg; 0.13 – 0.44 mg/kg and 0.11 – 0.23 mg/kg respectively) were significantly higher than CN concentrations in root, stem and leaves (0.20 – 0.60 mg/kg; 0.02 – 0.12 mg/kg and 0.01 – 0.12 mg/kg respectively) and As concentrations (0.11 – 0.57 mg/kg; 0.01 – 0.04 mg/kg and 0.01 – 0.02 mg/kg respectively) in the root, stem and leaves at the different leachate treatments. Fe was reported highest concentration in Akasia grown in hydroponic system at a range of 1.75 – 3.08 mg/kg in root, 0.29 – 0.78 mg/kg in stem and 0.20 – 0.55 in leaves. The trend of heavy metal and macro nutrient accumulation from leachate wastewater by *A. mangium* in hydroponic system in decreasing order were as listed below:

$$Fe > NH_3\text{-}N > CN > As$$

Statistically strong association was observed between As in stem and As in root, As in leaves and As in root and between As in stem with As in leaves ($r=0.94$, $r=0.88$ and $r=0.94$, $p<0.05$), respectively. Low correlation was found between Fe in stem with Fe in root ($r=0.29$, $p<0.01$) and Fe in leaves with Fe in root ($r=0.24$, $p<0.05$). In the case of CN, a strong Pearson’s correlation was observed in CN in stem and leaves with CN in root ($r = 0.97$ and $r=0.84$, $p<0.05$ respectively). A strong correlation was also found in between CN In stem and CN concentration in leaves of Akasia. In NH_3-N uptake, a strong Pearson’s correlation was observed in NH_3-N in leaves with NH_3-N in root ($r = 0.84$, $p<0.05$) and in between NH_3-N in leaves with NH_3-N in stem ($r=0.87$, $p<0.05$).

Akasia plants grown via hydroponics system in 25% TL (0.24% N-content) recorded significantly highest accumulation of As, Fe, CN and NH_3-N. Application of leachate as strong as above 50% TL had also affected the As, Fe, CN and NH_3-N accumulation in Akasia negatively. Visual symptoms of metal toxicity were observed in Akasia plants treated with high concentrations of the heavy metals whereby the plants were attacked by white rot fungi in the root zone (Plate 4.16). Therefore, the research was discontinued.

Plate 4.16: Akasia roots grown hydroponically attacked by White rot fungi

4.11.4 Uptake of heavy metals by hydroponically grown Akasia

In Fe uptake by plant tissues, Akasia showed significantly higher BCF ($p<0.05$) in 25% TL whereas in As bioaccumulation, significantly higher BCFs were recorded by Akasia plants grown in 25% TL, 50% TL and 100% IF (Figure 4.35). The BCF value in

As and Fe treatment, however, showed reduction after the treated leachate concentration of 50%. In Cyanide reduction by plant cells, higher BCF ($p<0.05$) was observed in the treatment of 25% TL. The treatment 25% TL gave an increased BCF value of 6.3% compared to inorganic fertilizer treatment. The BCF value subsequently reduced after the treated leachate concentration of 50%. In NH_3-N accumulation, plants treated with 25% TL recorded highest BCF among the other treatments applied to hydroponic culture of Akasia.

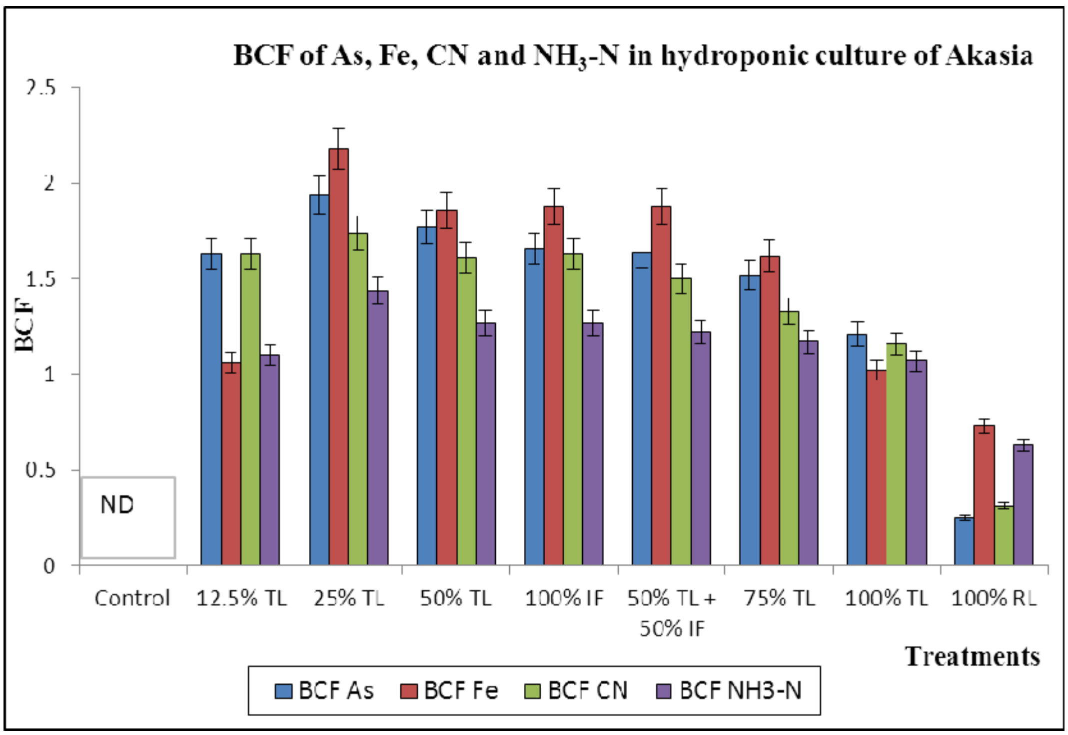


Figure 4.35: Bioconcentration factor (BCF) of As, Fe, CN and NH_3-N in hydroponically grown Akasia under different leachate treatment. Bar indicates standard error (n=4)

The range of BCF in NH_3-N and CN accumulation in Akasia in this study was 0.63 – 1.44 and 0.31 – 1.74 respectively in comparison to As-accumulation which recorded higher BCF value in the range of 0.25 – 1.94. Fe accumulation in Akasia reported Bioconcentration Factors in the range of 0.73 – 2.18.

Akasia roots in hydroponic culture was observed to be effective in taking up large amounts of As, Fe, CN and NH_3-N but restricted its translocation to other plant parts at the same time. The translocation factor (TF), the ratio of As, Fe, CN and NH_3-N in shoot to roots of Akasia indicates internal metal transportation (Deng et. al., 2004). TF was limited to below one in all the treatments applied to wastewater as shown in Figure 4.36 below.

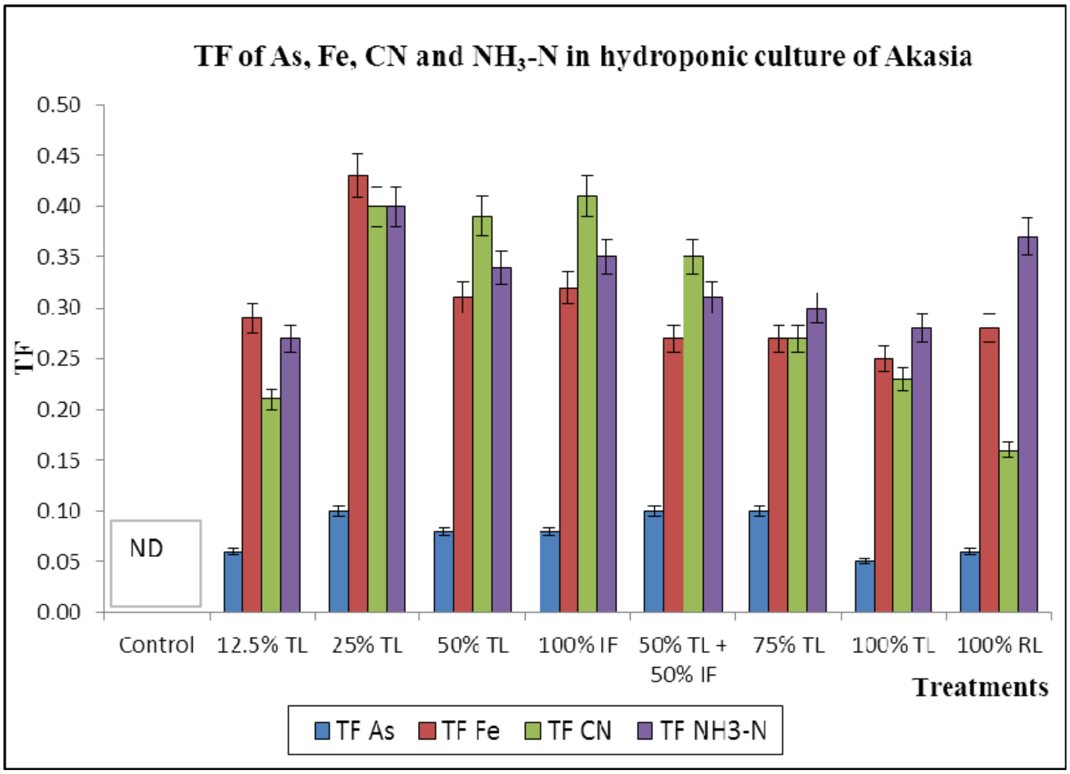


Figure 4.36: Translocation factor (TF) of As, Fe, CN and NH_3-N in hydroponically grown Akasia under different leachate treatment. Bar indicates standard error (n=4)

The ion selectivity of metals by the Akasia plants were Fe> As> CN> NH_3-N based on bioconcentration of selected parameters in plant roots. Batty and Younger (2002) stated that the majority of Iron was stored in and around the roots of the macrophyte *Phragmites australis* grown in mine water treatment wetlands which helped allay fears of possible release of contaminants during seasonal die-back or emergent shoots and leaves. Translocation ability of Akasia plants were in the order of Fe> NH_3-N > CN> As.

Quantity of elements that can be accumulated by Akasia plants grown hydroponically has also been shown to correlate well with concentration of heavy metals in the water according to Pearson's correlation test. Fe in root and Fe in wastewater solution showed r= 0.74, p<0.05; and CN in root and CN in leachate solution showed r = 0.82, p<0.05; and NH_3-N in root and NH_3-N in solution recorded r= 0.69, p<0.05.

The results also showed that the growth rate of treated wetland plants was significantly higher than those in the control tanks. Total harvested biomass from treatment tanks was also much higher than from control tanks (Table 4.25). The pollutants uptake efficiency of treatment plant samples is in the sequence of root> stem> leaves for all the selected parameters.

Table 4.25: *A. mangium* Plant Dry Matter (PDM in g) at final harvest (week 5)

Treatments	PDM (g) of hydroponically grown Akasia		
	Roots	Stems	Leaves
Control(dH_2O)	0.14±0.00	0.07±0.01	0.31±0.06
12.5 % TL	0.18±0.03	0.16±0.03	0.40±0.03
25% TL	0.26±0.10	0.21±0.04	0.55±0.02
50 % TL	0.19±0.08	0.18±0.04	0.51 ±0.07
100 % IF	0.22±0.03	0.18±0.02	0.53±0.04
50 % TL + 50 % IF	0.18±0.02	0.16±0.00	0.47 ±0.01
75 % TL	0.15±0.00	0.16±0.01	0.47 ±0.02
100 % TL	0.13±0.05	0.12±0.01	0.35±0.02
100 % RL	0.11±0.02	0.05±0.01	0.23±0.06

Table 4.26 displays the Pearson's correlation test between the root and stem, root and leaf, and stem and leaf of As, Fe, CN and NH_3-N in *A. mangium*.

Table 4.26: Significant difference (P) of correlation between the root and stem, root and leaf, and stem and leaf of As, Fe, CN and NH_3-N in hydroponic culture of *A. mangium* according to the Pearson correlation test (r)

<table>
<tr><th rowspan="2">Pearson correlation test in contaminant uptake in plant parts</th><th colspan="2">As</th><th colspan="2">Fe</th><th colspan="2">CN</th><th colspan="2">NH3-N</th></tr>
<tr><th>r</th><th>p</th><th>r</th><th>p</th><th>r</th><th>p</th><th>r</th><th>p</th></tr>
<tr><td>Root and stem</td><td>0.94</td><td rowspan="3"><0.05</td><td>0.72</td><td><0.05</td><td>0.97</td><td rowspan="3"><0.05</td><td>0.77</td><td rowspan="3"><0.05</td></tr>
<tr><td>Root and leaves</td><td>0.88</td><td>0.39</td><td rowspan="2"><0.01</td><td>0.84</td><td>0.93</td></tr>
<tr><td>Stem and leaves</td><td>0.94</td><td>0.57</td><td>0.83</td><td>0.87</td></tr>
</table>

* r= Correlation; p= Probability

Highest nutrient removal from wastewater samples through uptake by the *A. mangium* was recorded by 25% TL treatment with a removal efficiency of 72.0% As, 75.3% Fe, 67.0% CN and 52.2% NH_3-N, respectively. The results provided strong confirmation that constructed wetland is efficient for the decontamination wastewater polluted with heavy metal and it is also a cost-economic system for the preservation of water environment from heavy metal contamination for it offers operation and maintenance at a lower cost.

4.12 Comparative Study of Contaminant Bioaccumulation based on N-content of Treatments in Hydroponic Culture

In Kenaf and Akasia, highest growth measurement was recorded by treatments containing 0.24% N-content. Fe, As, CN and NH_3-N accumulation in Kenaf and Akasia plants increased with the increase in N-content treatments but after the concentration of 0.48% N-content (50% TL and 100% IF), the bioaccumulation of contaminants or metals decreased. Bioconcentration of As, Fe, CN and NH_3-N were observed highest in the treatment 25% TL (0.24% N-content) in Kenaf. In Akasia, highest bioconcentration of As, Fe, CN and NH_3-N was also recorded by 0.24% N-content.

Comparison among 100% IF and 50% TL treatments applied to Kenaf and Akasia, both containing 0.48% N-content, the test plants proved a higher growth of leave length, stem height, root length and total plant dry biomass for 100% IF. In hydroponically grown Kenaf, better accumulation of Fe and CN was observed in 100% IF treatment compared to 50% TL treatment. As and NH_3-N concentration in both treatments was equivalent. The bioconcentration of As, Fe and CN in Akasia also recorded higher BCF values for plants treated with 100% IF compared to plants treated with 50% TL.

Among 50% TL + 50% IF and 75% TL treatments where by both contained 0.72% N-content, growth measurement of leaves, stems, roots and plant dry matter showed a better results with application of 50% TL + 50% IF treatment in Kenaf and Akasia. The bioaccumulation of As, CN and NH_3-N also proved a higher bioconcentration in Kenaf plants at 50% TL + 50% IF. The bioaccumulation of Fe was equivalent in both the 0.72% N treatments in Kenaf. Removal percentages of Fe, As, CN and NH_3-N from leachate wastewater also was recorded lower in 75% TL comparable to 50% TL + 50% IF. BCF of all four parameters tested was significantly higher in 50% TL + 50% IF.

As for Akasia, 50% TL + 50% IF treatment recorded a better growth of plant parts compared to 75% TL. Higher bioconcentration of As, Fe, CN, and NH_3-N was found in the 50% TL + 50% IF treatment compared to 75% TL in hydroponic culture of Akasia. Removal percentages of Fe, As, CN and NH_3-N from constructed wetland also was recorded lower in 75% TL comparable to 50% TL + 50% IF. BCF of all 4 parameters tested was significantly higher in 50% TL + 50% IF.

Comparison analysis was also carried out for 100% TL (0.96% N-content) and 100% RL (0.98% N-content) treatments. Growth of both Kenaf and Akasia plants proved to show better results with 100% TL. Same goes for Bioconcentration Factor of Fe, As, CN and NH_3-N in the two test plants which recorded a higher accumulation in plants treated with 100% TL.

Control treatment (Zero N-content) recorded a lower Kenaf and Akasia growth rate compared to 100% IF (0.48% N-content) and also 100% RL. Bioconcentration values of Fe, As, CN and NH_3-N was shown to be higher in 100% IF and 100% RL for both the test plants compared to Control. Treated leachate at 12.5% (0.12% N-content) gave a better record of plant growth and uptake of heavy metals compared to 100% TL and 100% RL in hydroponic system. Akasia and Kenaf growth in hydroponic culture was observed to show better accumulation percentage in plants treated with fertilizer amendment among the treatments with the same N-content. The Control treatments gave an insignificant constant bioconcentration of contaminants in Kenaf and Akasia plant cells.

4.13 Comparative Study on Efficiency of Fe, As, CN and NH_3-N Uptake and Bioaccumulation between the Two Test Plants: Kenaf and Akasia

4.13.1 Kenaf and Akasia grown in Pot-culture system

Based on Relative Growth Rate of the two test plants used in this study, Akasia showed a significantly higher growth rate of plants' root, stem, leaves and total plant dry matter compared to Kenaf ($p<0.05$). Among the nine treatments applied for this study, Akasia showed a 17.5% of higher RGR compare to Kenaf in pot culture. On the average, Akasia recorded higher removal percentage of Fe, As and CN concentrations from the soil polluted with landfill leachate. On the other hand, Kenaf plants in 'pot-culture' were shown to be better accumulators of Nitrogen macronutrient, which is NH_3-N. Kenaf and Akasia growth were recorded highest at the optimum leachate concentration of 25% TL. At this optimum treatment concentration, Akasia plants grown in pot-culture recorded higher Bioconcentration factor of As, Fe, and CN and NH_3-N compare to Kenaf. This is in line with a study conducted by Majid et. al. (2011) whereby high BCF (11.28) of Cd was found in the A. mangium plants grown in copper contaminated soil. The Kenaf plants were also able to translocate As, Fe and CN better in comparable to Akasia at the optimum leachate concentration (25% TL) applied to both the test plants.

Table 4.27 was extracted from a study carried out by Robinson et. al. (2006) for As accumulation by plants in Taupo Volcanic Zone, New Zealand.

Table 4.27: The geometric means and standard deviation ranges of the As concentrations waters (mg/L), as well as soils, sediments, aquatic and terrestrial plants (mg/kg dry weight) from the Taupo Volcanic Zone

Sample Type	No. of samples	Geomean [As] and S.D. range
Soils	4	50 (33-79)
Sediments	23	38 (16-93)
Terrestrial plants	36	<0.5 (30 samples) -11
Waters	23	0.021 (0.005 – 0.078)
Aquatic plants	184	125 (12-1222)

*Source: Robinson et. al., 2006

In the current study, Kenaf and Akasia recorded a higher mean As concentration of 16.2 and 22.8 mg/kg plant dry weight, respectively compared to the terrestrial plants as reported in Table 4.29 (<0.5 mg/kg of native ferns dry weight). Hydroponic culture of Kenaf and Akasia bioconcentrated 0.24 and 0.36 mg/kg in plat dry biomass, respectively which is relatively lower compared to watercress and few other aquatic plants. The variance in the results may be in accordance to different characteristic of wastewater (leachate and volcanic waste), soil condition, weather differences in tropical and temperate climate, plant species and duration of study.

Phytoremediation process using Akasia plants recorded the highest bioaccumulation rate constant for Fe of 0.04 day^{-1} and half-life 22.7 days. The bioaccumulation rate and half life of CN and NH_3-N accumulation in Akasia were 0.03 day^{-1}, half-life of 25.2 days and 0.03 day^{-1}, half-life of 50.4 days respectively. The bioaccumulation rate and half life of Fe, CN and NH_3-N accumulation in Kenaf were 0.03 day^{-1} with half-life of 21.9 days, 0.02 day^{-1} with half-life of 34.2 days and 0.02 day^{-1} with half-life 51.4 days, respectively. Akasia and Kenaf showed similar bioaccumulation rate constant of 0.02 day^{-1} for As uptake by the test plants but Akasia recorded a shorter half-life of 32.1 days compared to Kenaf (36.3 days). Till to date, phytoremediation data analysis using First-order kinetics for heavy metal uptake was less discovered, therefore references to support this model is next to nil. Most of the metal adsorption properties of plants were best described by the pseudo second order kinetics (Keskinkan et. al., 2004; Srivastava et. al., 2006). *A. mangium* are fast growing trees and potentially used for 'Phytocapping' which has been recognised as an efficient, cost-economical and environment-friendly mechanism for landfill leachate remediation in Queensland, Australia (Venkatraman and Ashwath, 2007). In this system, soil cover acts as a 'storage' and plants act as 'bio-pump and filters'.

Based on the comparison study conducted on some of the models used as reference, among them RGR; Accumulation percentage; BCF and TF; and First-order kinetics contaminants (bioaccumulation rate constant and half-life of contaminants in plants), it is proven that *A. mangium* is a better phytoremediator of Fe, As, CN and NH_3-N compared to *H. cannabinus* L. in pot-culture.

4.13.2 Kenaf and Akasia grown in Hydroponic culture system

Metals bioconcentrated in plant tissues can cause toxic effects, particularly during translocation to above ground tissues (Weis and Weis, 2004). Most experiments studying on the effects of metal accumulation upon plants have focused on growth as the response to the pollutants, though effects can be measured at the biochemical and cellular level (Weis and Weis, 2004). Growth of test plants' cultured hydroponically for the purpose of this research was studied based on total plant height and plant dry matter at final harvest. Akasia plants grown in leachate polluted wastewater showed a better growth of 35.4% compared to Kenaf plants in hydroponic system. Besides that, Kenaf plants resembled a better survival of 7 weeks in the system compared to Akasia plants which started showing signs of mortality at week 5. But all readings of plant growth were measured at week 5. Total plant dry biomass of Akasia grown in different concentrations of leachate treatments was recorded at an average of 5.2 g compared to Kenaf which gave a reading of 4.7 g ($p<0.05$).

Kenaf and Akasia growth in hydroponic system were recorded at optimum leachate concentration of 25% TL. At this optimum treatment concentration, Akasia plants grown in hydroponic system recorded higher bioaccumulation (based on BCF values) of As, Fe, and CN and NH_3-N in its plant tissues. Akasia plants potrayed better contaminant exclusion mechanism as the Kenaf plants were able to translocate As, Fe, CN and NH_3-N better comparable to Akasia at different leachate concentration (25% TL) applied to both the test plants.

At the optimum leachate concentration (25% TL) in wastewater, Akasia recorded higher removal efficiency percentage of As, Fe and NH_3-N concentrations from the wastewater polluted with landfill leachate. Both Akasia and Kenaf showed similar uptake efficiency for CN. According to a study conducted by Mishra and

Tripathi (2008), accumulation of Fe in *E. crassipes* (water hyacinth) from wastewater contaminated with three different concentrations of 1.0, 2.0 and 5.0 mg/L of Fe in laboratory experiment ranged between 15.3 and 22.5 g/kg. This is greater than accumulation of Fe by Kenaf and Akasia in hydroponic culture which was in the range of 0.32 – 2.48mg/kg and 0.2 - 3.08mg/kg, respectively.

From the comparison study conducted based on some of the models used as reference: Plant growth; Removal efficiency; BCF and TF of metals and macronutrient; it is proven that *A. mangium* is a better phytoremediator of Fe, As, CN and NH_3-N in comparison to *H. cannabinus* L. in hydroponic system. The mechanism of contaminant removal in this study was observed as rhizofiltration.

Substrate interactions remove most of the toxicants from contaminated water in a constructed wetland, whereby plants funstion as a “polishing system” (Deng et. al., 2004). The *H. cannabinus* and *A. mangium* played a vital role in metal reduction via various mechanisms such as filtration, adsorption, and cation exchange, and through plant-induced chemical changes in the rhizosphere (Nouri et. al., 2009). Metal accumulation by wetland plants differed among species, populations and tissues (Deng et. al., 2004). Metals accumulated by the test plants in this study were mainly distributed in plant root tissues, recommending that an exclusion strategy for metal tolerance significantly exists in them (Deng et. al., 2004). Roots exudates were considered a source for carbon to stimulate bacterial activity (Weis and Weis, 2004).

Plant species differ widely in their ability to accumulate heavy metals. The general trend as discussed earlier demonstrated that the root tissues absorbed significantly higher concentrations of metals than shoots, thus evidenced high plant availability of the substrate metals and its restricted mobility once inside the plant (Deng et. al., 2004). This is in congruence with previous studies as carried out by

Cardwell et. al. (2002) and Fitzgerald et. al. (2003). Results also showed that the higher the nutrient concentration applied, the higher the nutrient uptake by *A. mangium* and *H. cannabinus*. In a wetland system, nutrient is lost through microbial decomposition, nitrification/ denitrification, plant uptake, and evapotranspiration.

One of the pollutants reduction mechanisms reported is evapotranspiration. Evaporation is defined as atmospheric water losses from a wetland that takes place from the water and soil. Transpiration is the water losses from emergent portions of plants. The combination of both evaporation and transpiration processes is termed as evapotranspiration (Sim, 2003). Daily transpiration is advantageously associated to mineral adsorption. Daily transpiration is applicable as an index of the water purification capability of plants. In a wetland system, precipitation and evapotranspiration affects the water flow through the system (Idris et. al., 2010). Evapotranspiration process increases contact times and slow water flow. In most of the areas, precipitation and evaporation are apparently destined to have least impacts on constructed wetlands (Idris et. al., 2010). If the wetland type is typically shallow open water, precipitation/evaporation ratios fairly relative to water balance. However, transpiration losses from photosynthetically active plants become important and noticeable in large and dense distribution of tall plants (Idris et. al., 2010).

4.14 Comparative Study on Efficiency of Fe, As, CN and NH_3-N Uptake and Bioaccumulation between Two Different Systems of Plant Growth: Pot culture System and Hydroponic Culture System

Test plants in the hydroponic culture study were compared in terms of Growth instead of Growth rate as plants in hydroponic culture had low survival rate (5 weeks of growth). Test plants in the Pot-culture were compared based on growth rate and experiment was carried out for a period of 4 months. This phenomenon can be best explained as the test plants used were terrestrial plants whereby they survive better planted in soil (pot-culture system). Metal concentrations in plant tissues varied among different species with the same soil or wastewater condition signifying their different capacities for metal accumulation (Deng et. al., 2004).

Trees growth irrigated with landfill leachate varied greatly between Kenaf and Akasia in this current study and Populus and Salix study conducted by Zalesny Jr. and Bauer (2007). Kenaf and Akasia exhibited greater plant height, leaf and root dry mass compared to Populus and Salix. The variation in measured trait could be attributed to different characteristics of leachate and differences in phytoremediation capabilities among genotypes of tress. Differences in soil also have contributed substantially to element concentrations in plants (Bockheim and Crowley, 2002).

Based on the removal efficiency test conducted, Kenaf in Pot-culture was able to uptake higher percentage of Fe (90.5%) and As (78.6%) and CN (79.6%) and NH_3-N (98.9%) compared to Kenaf in hydroponic culture where Fe cumulative accumulation was 72.4%, As was 60.0%, CN was 64.0% and NH_3-N was 46.1% at final harvest. Removal efficiency of NH_3-N in pot culture was greater by 52.8% compared to hydroponic culture. This is best explained by the bioavailability of the metals is low in wetland system compared to terrestrial systems with oxidized soils (Abou-Shanab et. al., 2007). This is because wetland sediments are primarily identified as a sink for metals and may contain very high concentrations of metals in a reduced state in the anoxic zone (Weis and Weis, 2004). Kenaf in pot-culture proved to be a better accumulator of NH_3-N. This may be due to the presence and activities of microbes in the root nodules of Kenaf which is supported by the soil medium (Khan et. al., 2009). Besides that, Mycorrhizae (symbiotic fungi connected with plant root system) contribute an interface between the plant roots and the soil thereby increases the absorptive surface area of root hairs. They are also productive at accumulating metals that may be present at toxic concentrations in the soil (Weis and Weis, 2004; Meharg and Cairney, 2000).

The presence of metal-rich rhizoconcretions or plaque on the roots is a extraordinary feature of roots of some wetland plants (Weis and Weis, 2004). These structures are made up mainly of iron hydroxides and also other metals such as manganese that are accumulated and crystallised on the root surface. The metals are transported from the reduced anoxic sediments of estuarine and bioaccumulated in the oxidized microenvironment around the roots (Weis and Weis, 2004). There have been contradictory studies as to whether the occurence of the plaque augments or depreciates the absorbtion of metal by the plants. Amount of zinc taken up by *Aster tripolium* (Otte et. al., 1989) and the amount of manganese taken up by *P. australis* (Batty et. al., 2000) was reduced due to the presence of these concretions. The mechanism could be most probably been through the plaque acting as a physical barrier, although the barrier was not effective at low pH conditions (Weis and Weis, 2004).

In the case of Akasia plant, Fe (94.6%), As (98.3%), CN (83.7%) and NH_3-N (98.5%) reduction from soil recorded higher bioaccumulation percentage compared to Akasia in wastewater. Nevertheless, Akasia and Kenaf grown in soil polluted with NH_3-N from leachate source showed an equivalent efficiency of contaminant removal of 98%. The contaminant reduction efficiency in soil was greater than wastewater most probably because *A. mangium* is a leguminous tree whereby the root nodules' and microorganism activity is more enhanced in soil medium compared to wastewater.

At optimum condition of growth and phytoremediation process in this study (25% TL), Kenaf in pot culture recorded significantly higher bioconcentration of toxic metals, As, Fe and CN and Macronutrient N (NH_3-N) in their plant cells compared to hydroponic culture. Significant presence of contaminant uptake was observed in plant roots from contaminated soil as discussed in earlier paragraphs. Nevertheless, hydroponically grown Kenaf was able to translocate Fe, As and CN in aerial plant parts better than Kenaf in vegetative culture, a better translocator of NH_3-N. In the event of Akasia in hydroponic system, the scenario is similar to Kenaf plants.

Akasia in pot culture too recorded significantly higher bioconcentration of As, Fe, CN and NH_3-N in their plant cells compared to hydroponic culture. Significant presence of selected toxic metals and Nitrogen uptake was observed in plant roots from contaminated soil. But in the case of Translocation factor, the case study reported that Fe and CN retention in Akasia stem and leaves were significantly higher in hydroponic culture compared to pot culture. Translocation of Fe and NH_3-N in aerial plant parts were greater in soil culture compared to hydroponic culture.

In general, it can be concluded that Kenaf and Akasia plants showed a better survival rate in pot culture as they were naturally terrestrial plants. The hydroponic culture system was conducted as a novel trial of terrestrial plants cultivation in a different system to study the uptake efficiency of selected parameters of contaminant. Though pollutant removal efficiency was recorded higher for rhizofiltration mechanism, but phytoextraction and phytostabilisation was proven to be a better mechanism in terms of biological treatment of landfill leachate using plants.

Based on literatures, it is reported that the roots uptake greater concentration of metals or other toxicants (at some cases 100 times more) in comparison to the shoots (Malik, 2007). The comprehensive research carried out illustrated that this phenomenon is because of the differences in the biochemistry for uptake in the two tissues. The smaller, harder cations often bind preferentially to the smaller atoms such as N and O in the roots. When translocated to the shoots, they bind to more complex compounds such as oxalates or phytochelatins (Malik, 2007). Metal translocation into shoots appeared to be very limited in both the test plants; therefore harvesting plants will not be an efficacious source of metal removal (Deng et. al., 2004) in the Pot-culture and wetland system. Despite that, in the view of toxicology, harvesting plants could be a worthwhile property, as metals would not pass into the food chain via herbivores, and consequently avoid potential risk to the environment (Nouri et. al., 2009). Dimitriou et al. (2006) stated that there are several landfills in Sweeden where the leachate is treated by irrigation of willows. Plant vitality and growth are important factors controlling treatment efficiency (Dimitriou et. al, 2006) if phytoremediation system was chosen for remediation of leachate.

Results also showed that plant maturity played an important role in metal removal by the test plants (Choo et. al., 2006). Statistical analysis showed that the difference in the accumulation of Fe, As, CN and NH_3-N by test plants of different maturity is significant in leaves, stem as well as roots at a confidence interval of 95%. Plants with higher maturity stage had higher capacity for metal accumulation because they had greater biomass which could be interrelated with higher number of active metal binding sites or absorption sites. The increase in metal uptake by plant tissues of different maturity can also be possibly attributable to increased permeability and metabolic activities associated with increasing age (Choo et. al., 2006). Amount of N_2 fixed by plants too increases with age due to the corresponding increase in N accumulation (Mercado Jr. et. al., 2011).

Mature leaves of *H. cannabinus* L. and *A. mangium* were recognized to own large numbers of degenerated sap cells that are identified to be important in metal accumulation (Choo et. al., 2006; Lavid et. al., 2001) thus explaining the higher Fe, As, CN and NH_3-N concentrations in the older leaves. Regardless of plant age, the roots bioconcentrated the highest concentration of Fe, As, CN and NH_3-N which was on the average more than 50% of the total amount accumulated (Choo et. al., 2006), followed by stem and leaves.

4.15 Development of Decision Support Software: e-PMS

A Decision Support Software (DSS) was developed for Phytoremediation process. The software model was entitled e-Phytoremediation Modeling System version 1.1 (e-PMS). The software was developed using the platform of Visual Basic Edition 2005 programming language. The e-PMS DSS system was designed based on a list of calculation models associated with phytoremediation processes: Kinetics, Relative Growth Rate (RGR), Bioconcentration Factor (BCF) and Translocation Factor (TF). In addition, e-PMS is also an interactive portal with creative graphics where users are able to learn the scientific classification and details as well as view photos of test plants. Users may also get hands on of new technical terms in phytoremediation process through the output of Glossary. Layouts of the model are as shown below. Figure 4.37 shows the program's title page.

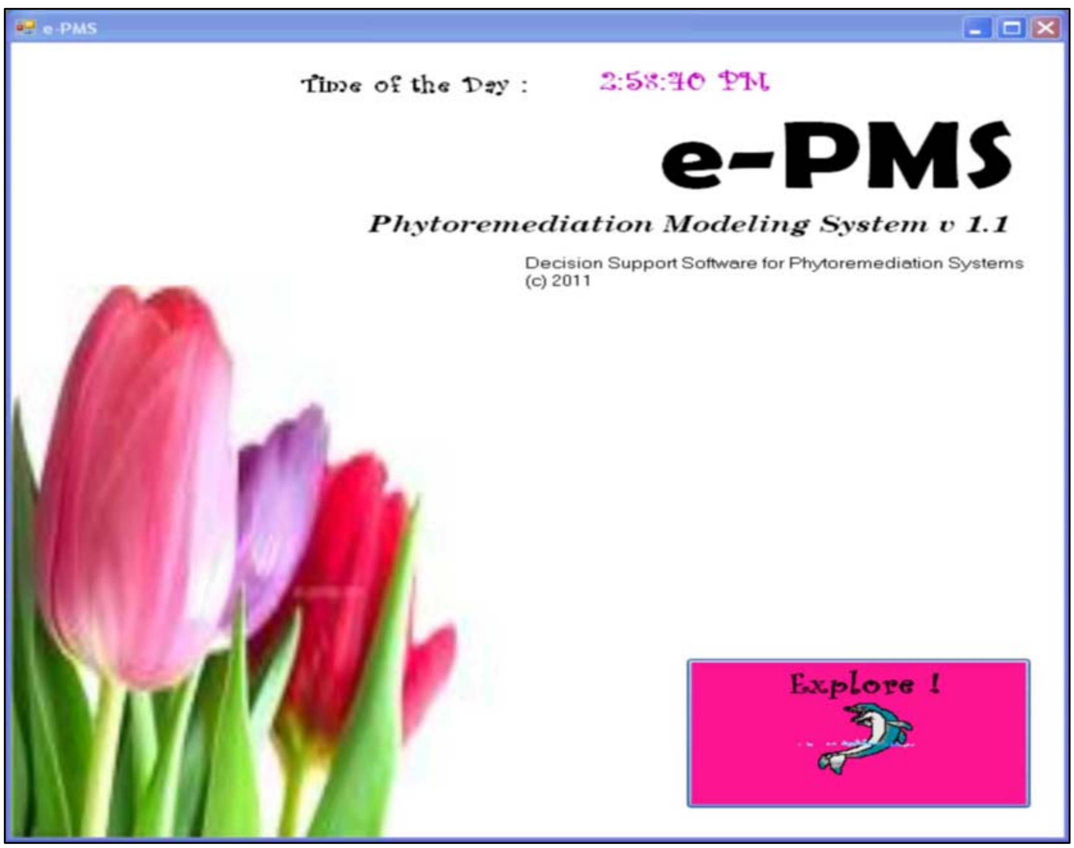


Figure 4.37: e-Phytoremediation Modeling System title page. The 'Time of the Day' menu displays time. The user needs to click on the 'Explore' button to proceed to log on to the system

There is a display of time next to the label 'Time of the Day' at the top of the form which shows up immediately when the application starts. The 'Explore' button provides access to the following windows of system log-in (Figure 4.36).

The 'Log In to e-PMS' window is as shown in Figure 4.38. Users need to enter an assigned username and password. The system requires the users to enter password which contains a maximum of 6 characters including numerical and alphabet via a pop-up alert message. The 'Log In' button allows users to access to the e-PMS system. Otherwise, users may click on the 'Quit' button to exit from the system.

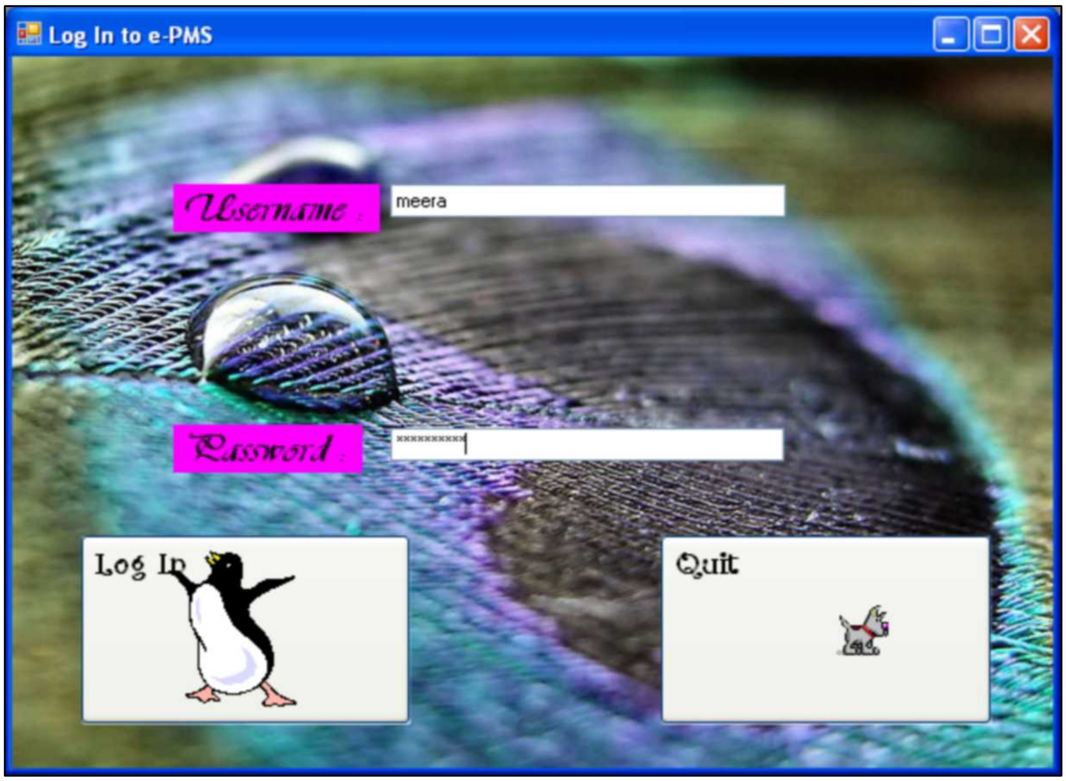


Figure 4.38: The Log In page. The user needs to click on the 'Log In' button to proceed to log on to the system

The 'Test Plant Input' form is designed to display the local and binomial name of the test plants (Figure 4.39A and Figure 4.39B). e-PMS version 1.1 provides inputs on the two test plants used in the previous study, Kenaf and Akasia.

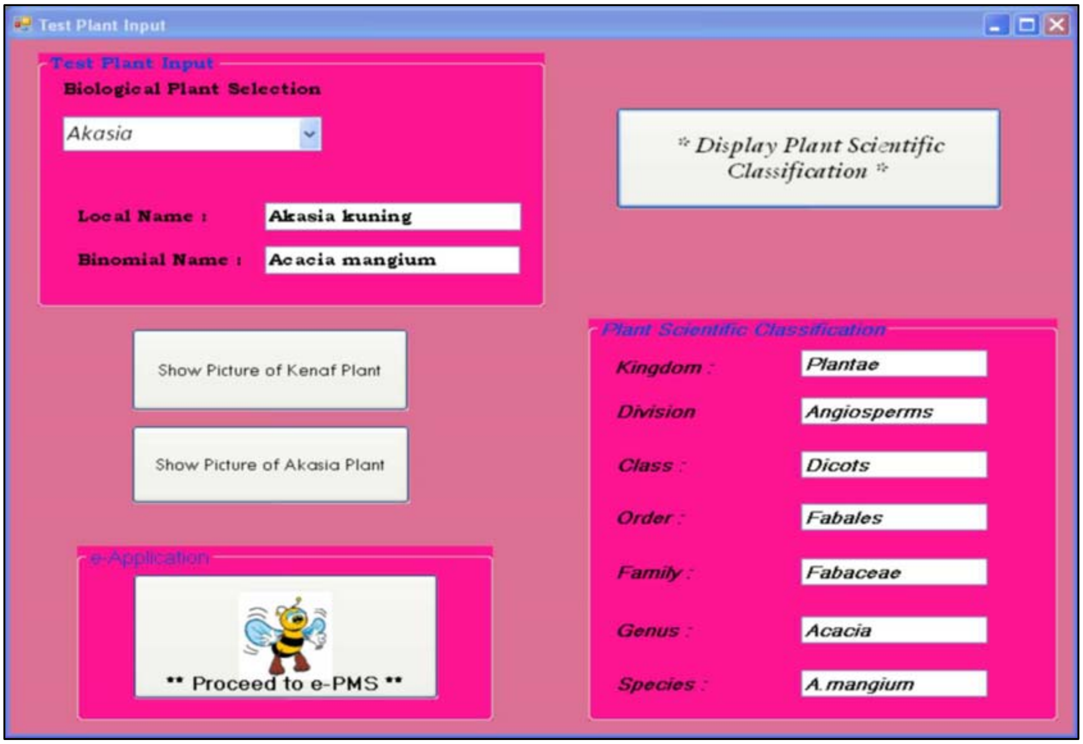


Figure 4.39A: Test Plant Input Interphase: Display for Akasia

Figure 4.39B: Test Plant Input Interphase: Display for Kenaf

Users may select the test plants from the drop down menu at 'Biological Plant Selection'. Immediately, Local name and the Binomial of plant selected will be displayed below the selection. When the user clicks on the button 'Display Plant Scientific Classification', the scientific classification of the selected plant is displayed in the group box on the right panel according to Kingdom, Division, Class, Order, Family, Genus, and Species. Users may also view the photographs of the test plant selected by clicking on the buttons 'Show Picture of Kenaf or Akasia Plant'. Users may proceed to the e-application of the Phytoremediation Modeling System by clicking on the button 'Proceed to e-PMS' in the e-Application panel at the bottom of the form.

Window of Photo_Kenaf (Figure 4.40) shows up when users click on button 'Show Picture of Kenaf Plant' on the Test Plant Input form.

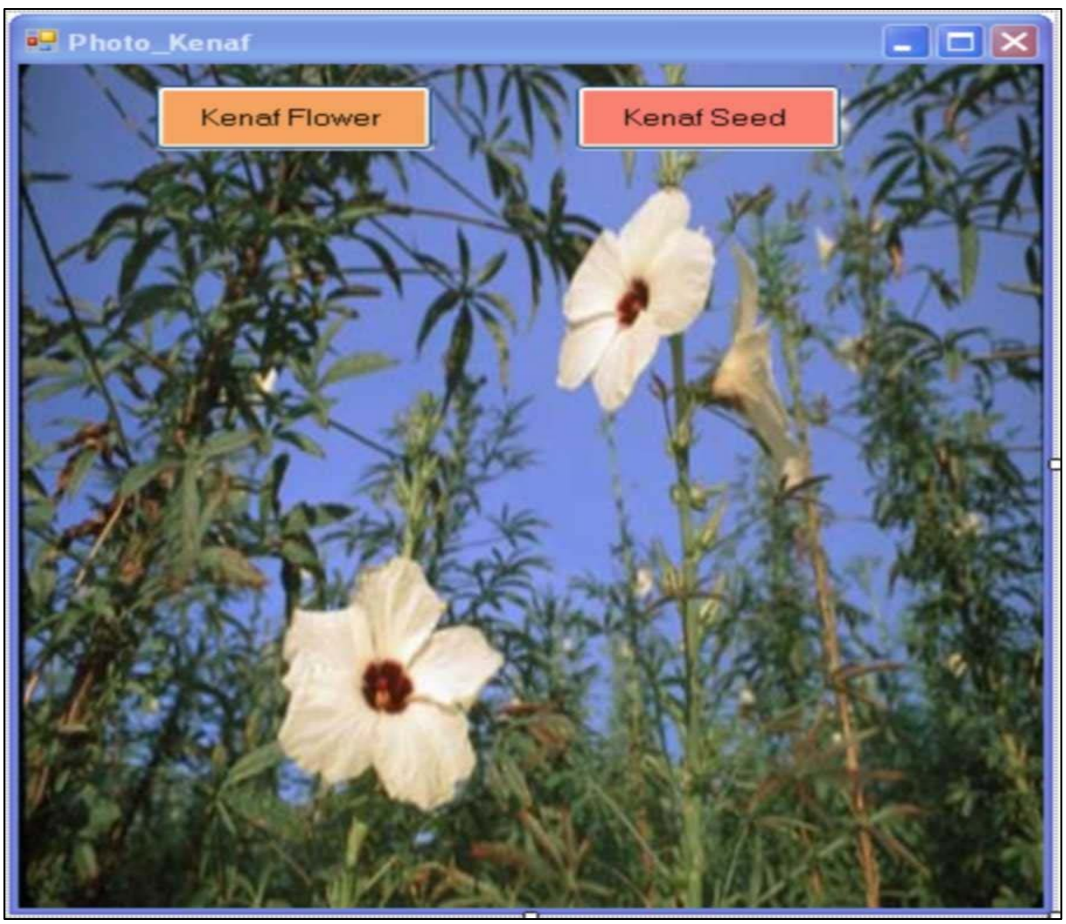


Figure 4.40: Photograph of Kenaf plant

Users may also view the pictures of Kenaf flower and Kenaf seeds by clicking on the option buttons at the top part of the window form. Photograph of Kenaf flower (Figure 4.41) and Kenaf seed (Figure 4.42) is displayed when users access to buttons ‘Kenaf Flower’ and ‘Kenaf Seed’ respectively from the window Photo_Kenaf.

Figure 4.41: Form showing photograph of Kenaf flower

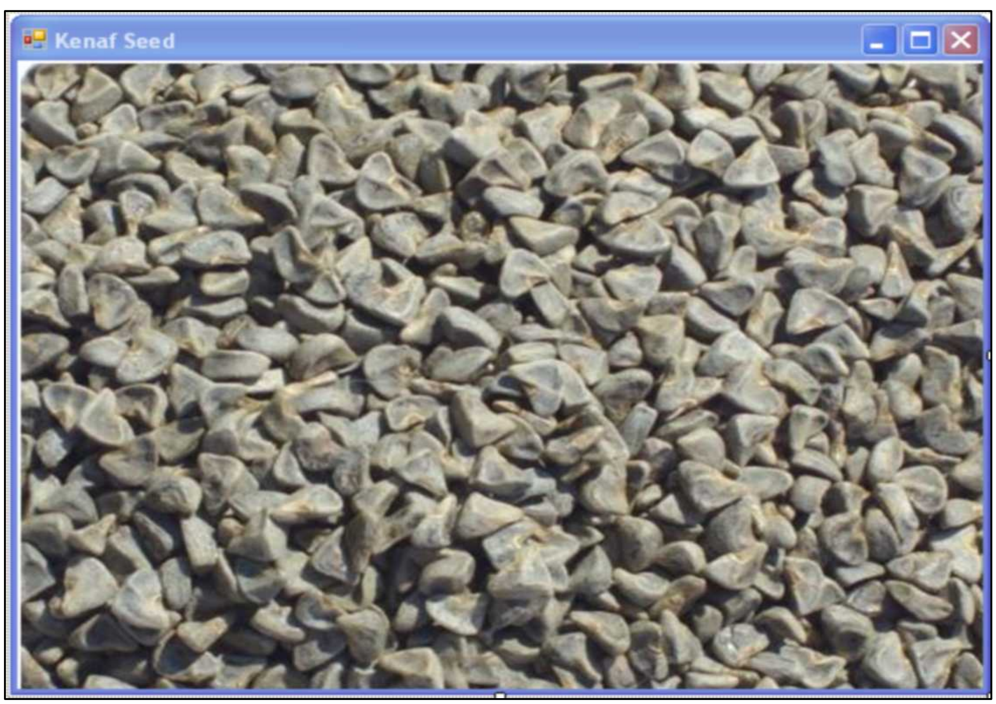


Figure 4.42: Display of Kenaf seeds photograph

Users may view the photograph of Akasia plant (Figure 4.43) by accessing through button 'Show Picture of Akasia Plant' on the Test Plant Input form. Additionally, photograph of Akasia flower (Figure 4.44) and Akasia seed (Figure 4.45) will be displayed when users access to buttons 'Akasia Flower' and 'Akasia Seed' respectively from the window form of Photo_Akasia.

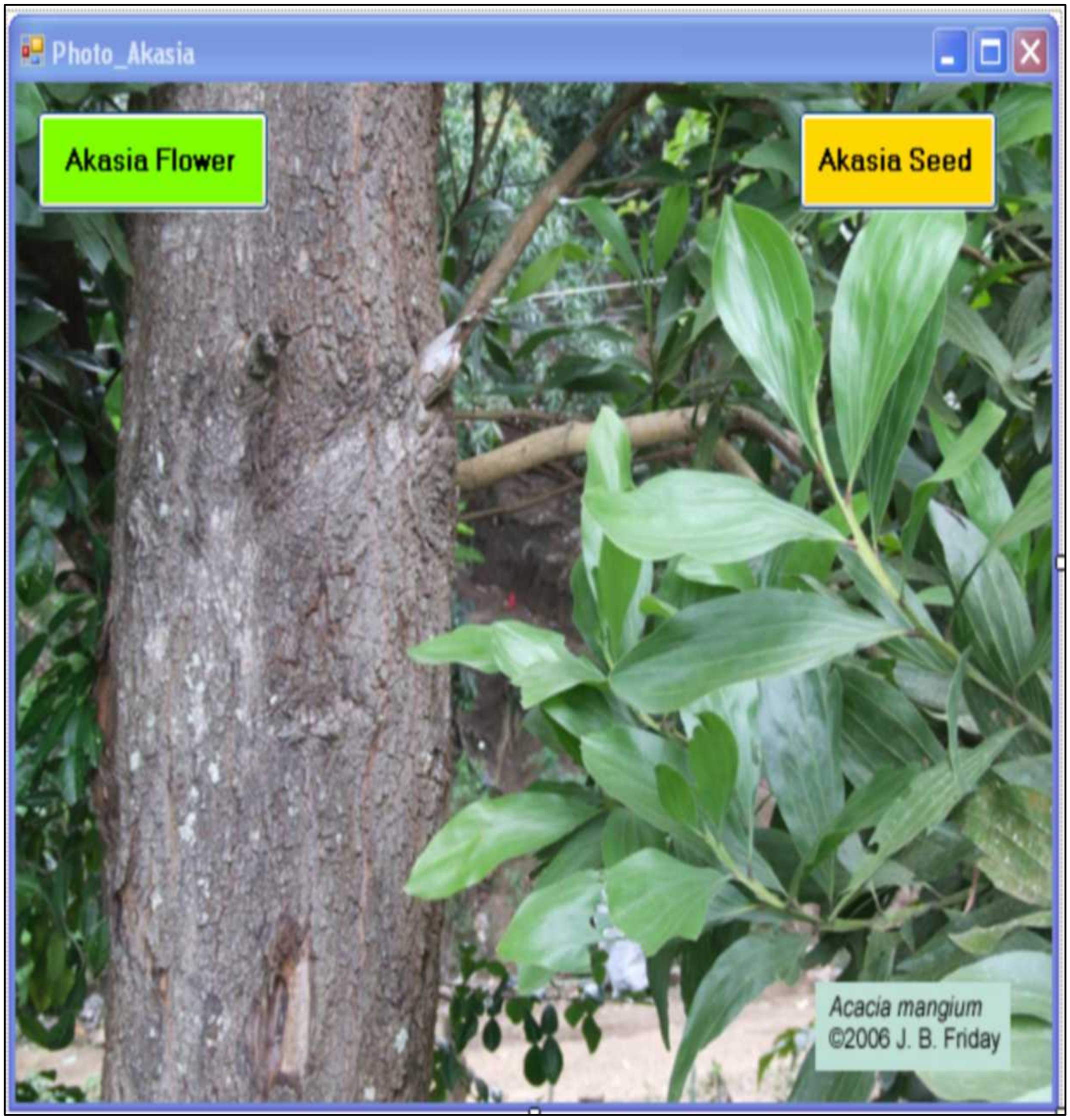


Figure 4.43: Photo of Akasia plant displayed when users click on the button 'Show Picture of Akasia Plant' on the Test Plant Input form.

Figure 4.44: Window displaying photograph of Akasia flower

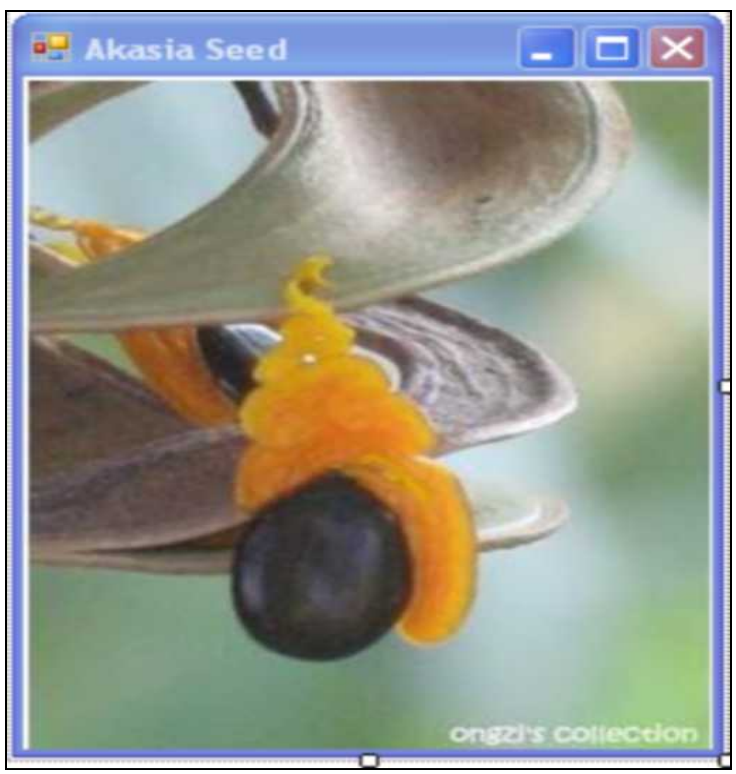


Figure 4.45: Window displaying photograph of Akasia seed

Window Phytoefficiency Models as shown in Figure 4.46 is displayed by accessing through the ‘Proceed to e-PMS’ button on the Test Plant Input window shown above. This window was designed to display the list of Phytoefficiency Models proposed in the e-Phytoremediation Modelling System (e-PMS) DSS version 1.1. The PhytoEfficiency Models window provides access to several windows of input data and model parameters. Users may also select the type of wastewater studied, i.e. Industrial wastewater, domestic, landfill leachate, POME etc. from the drop down menu under label ‘Type of Wastewater’ at the right panel of the window. Users may log in to the respective windows of mathematical models by clicking on the buttons by the name of the models applicable.

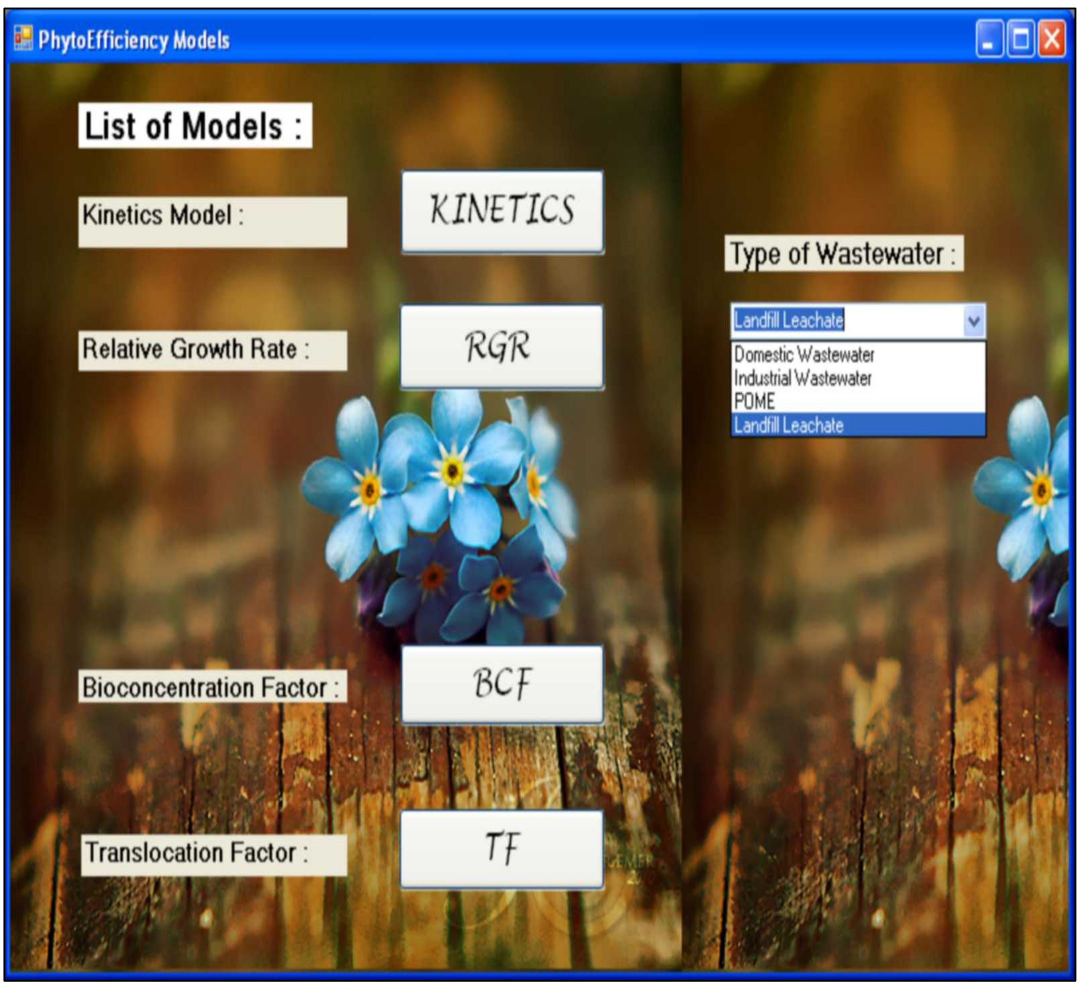


Figure 4.46: Window displaying list of e-PMS models and selection of type of wastewater

Figure 4.47 shows the Kinetics mathematical model based on First Order Kinetics of Yeung et. al. (1997). The model estimated the metal/contaminant bioaccumulation rate constant (k) and half-life ($t_{1/2}$) of metal in plant relative to treatments applied. This model was based on the assumption that the bioaccumulation rate of metals is positively correlated with the pool size of metal in soil (Abioye et. al., 2010).

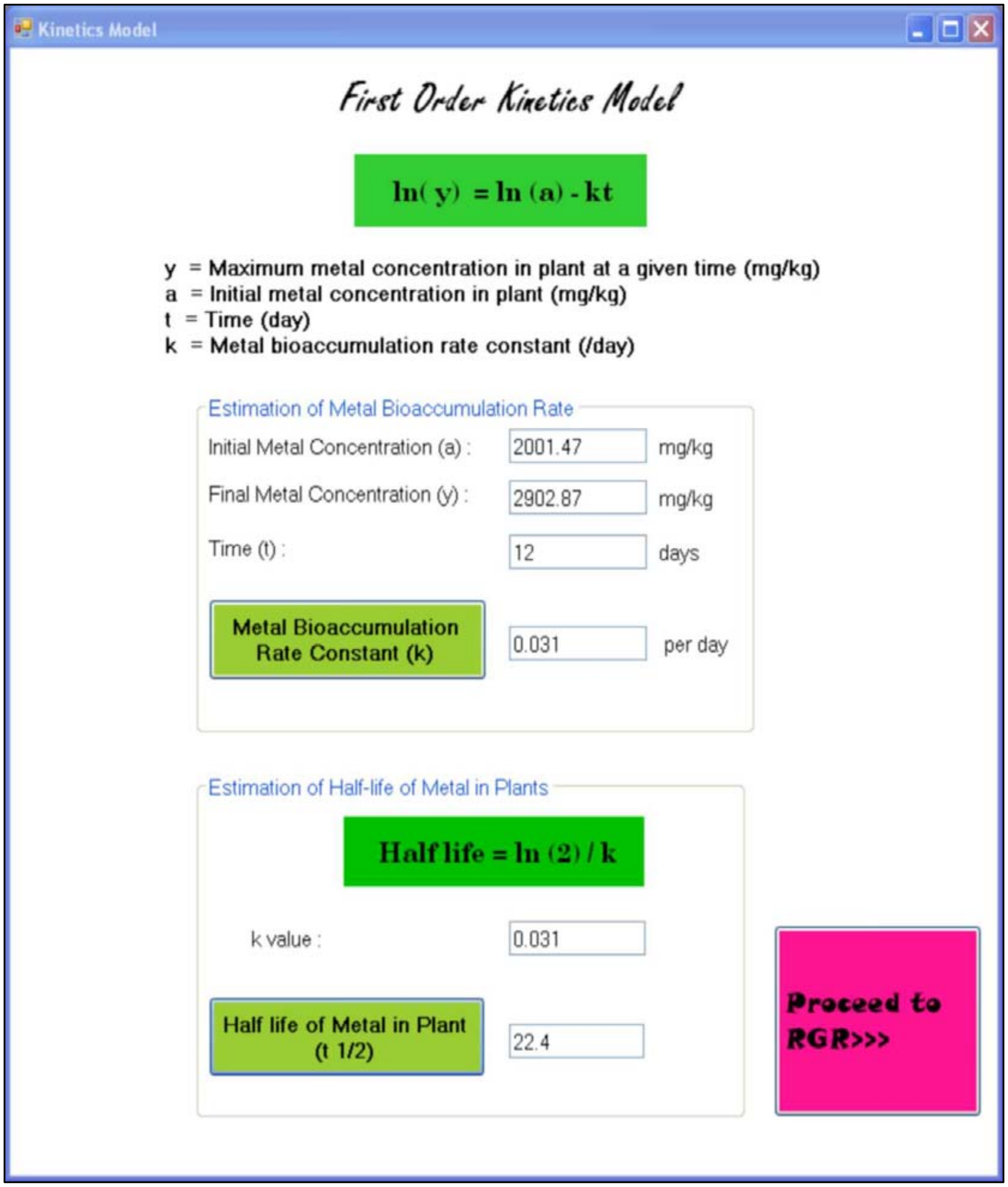


Figure 4.47*:* First Order Kinetics Model component window

Users need to enter his/her own data input on initial metal concentration (a), final metal concentration in plant (y) including the time period the study took place (t) in days to compute bioaccumulation rate constant and half-life values. The (k) value at the panel of “Estimation of Half-life of Metals on Plants” will be automatically generated based on the output of “Estimation of Metal Bioaccumulation Rate” textbox. User may click on the button ‘Proceed to RGR >>>’ to access to the following RGR model (Figure 4.48).

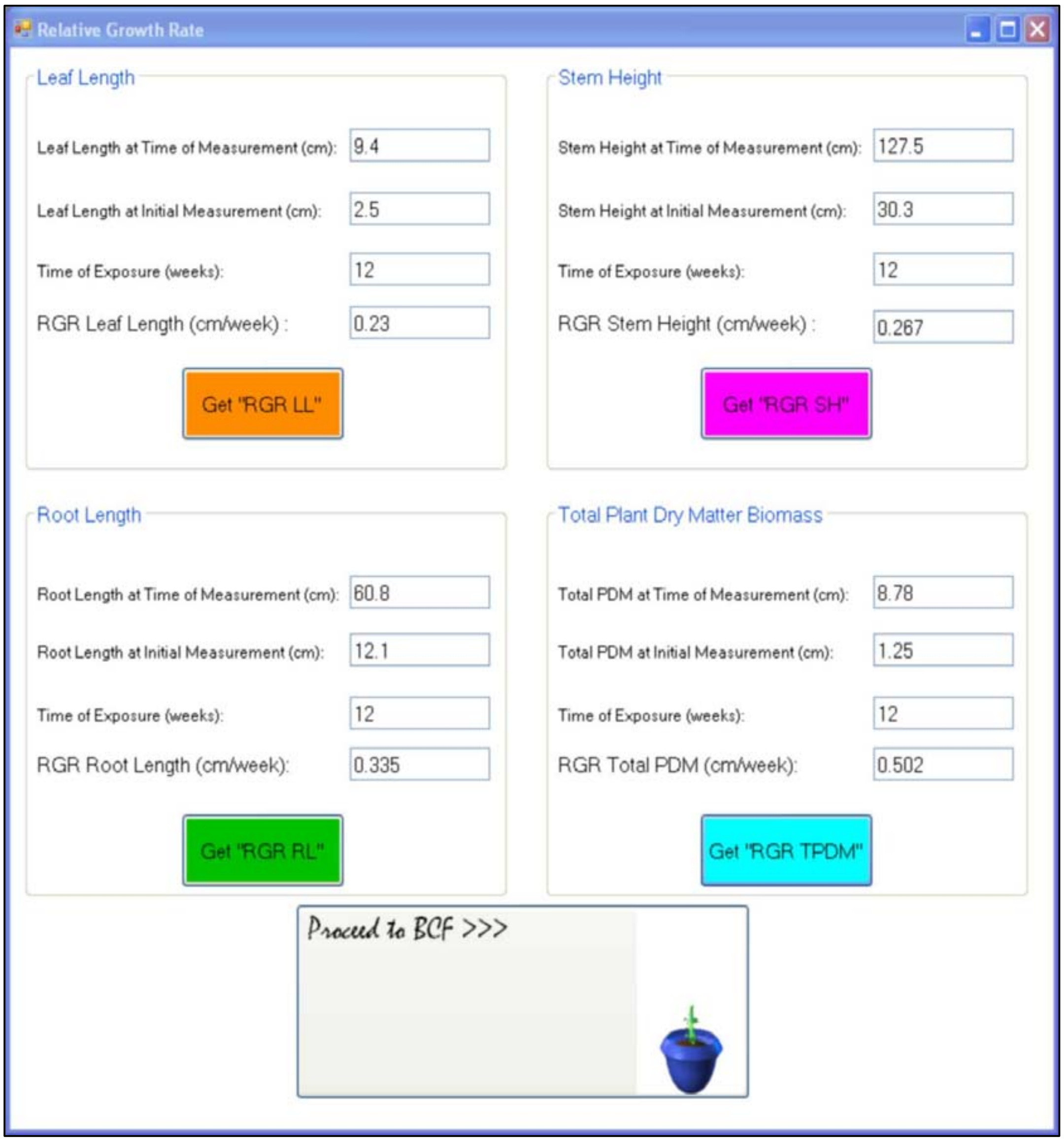


Figure 4.48*:* Form of Relative Growth Rate model component window

Among the variables inputs of Relative Growth Rate model in Figure 4.48 are RGR of Leaf Length (RGR LL), RGR of Stem Height (RGR SH), RGR of Root Length (RGR RL), and RGR of Total Plant Dry Matter Biomass (RGR TPDM). Users need to key in inputs on initial and final parameter of plant variables, i.e. leaf length as well as time of exposure in weeks. The RGR value of plant parameters will be computed by the system by clicking on the respectively colored buttons available. Button 'Proceed to BCF' is available at the bottom of the window for accessing BCF model (Figure 4.49).

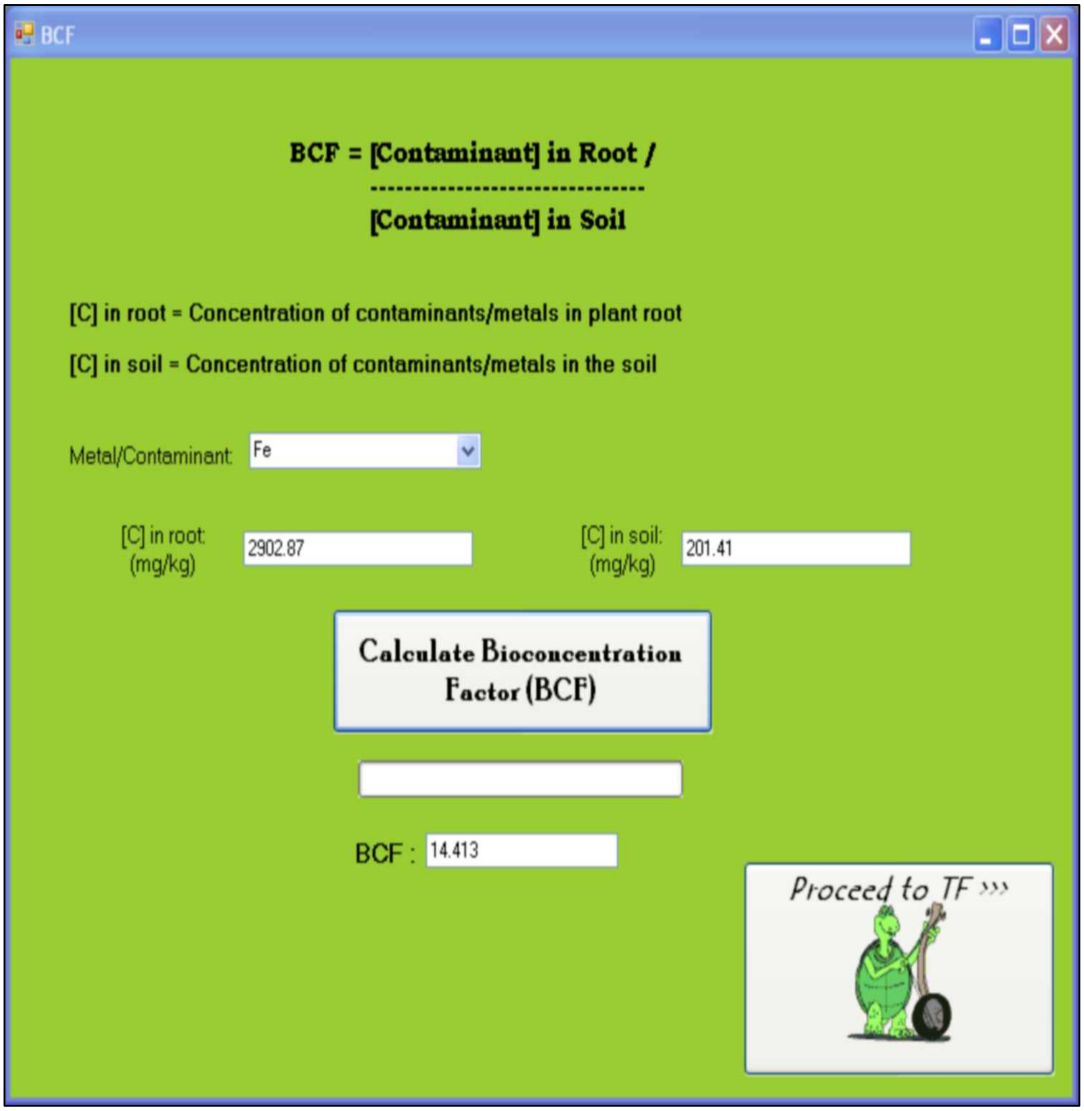


Figure 4.49*:* Bioconcentration Factor (BCF) model component window

BCF window computes the value of Bioconcentration Factor from the inputs of users. Definition of BCF model is provided at the top panel of window. Users may select metal/contaminant from the drop down menu available. Users need to feed in concentration of metal selected in plant root and soil. The system will compute BCF value at the text box provided next to the label: BCF. There is a display of progress bar below the button 'Calculate BCF'. Clicking on the button 'Proceed to TF>>>' gets user to move on to TF model as shown in Figure 4.50.

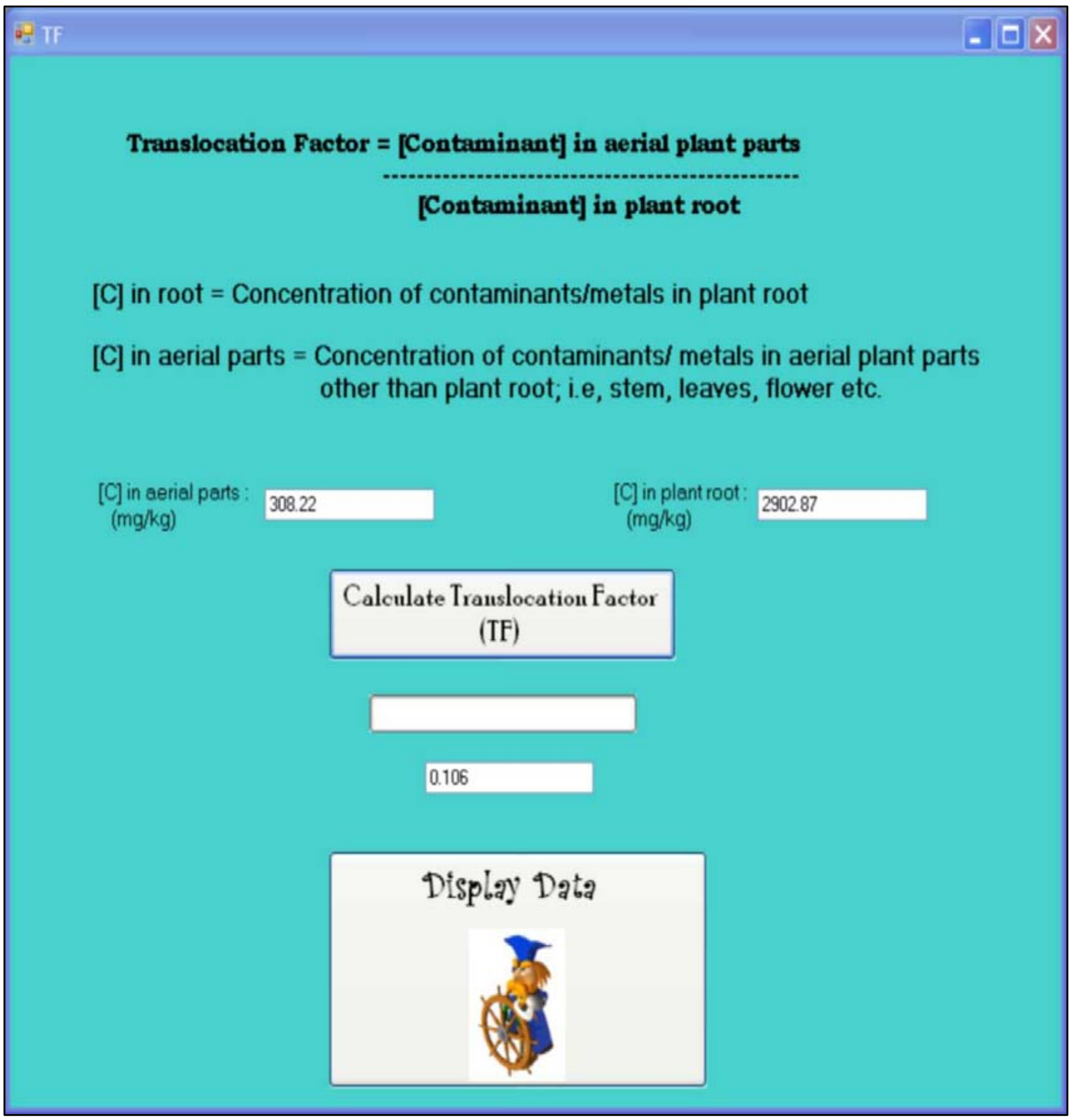


Figure 4.50*:* Translocation Factor (TF) model component window

In Translocation factor (TF) model window, users need to key in the value of metal concentration in aerial plant parts in order for the system to compute the value of TF. The value of metal concentration in plant root will be auto generated from the output of BCF window. Button 'Display Data' enable users to view the data/input from all the four mathematical models in the previous window forms.

Data Display window with summarized information is as shown in Figure 4.51. Data from Kinetics, RGR, BCF, TF are displayed when users click on the button 'Display Data' at the upper part of the form. Test plant selected and the type of wastewater and contaminant/metal studied can also be viewed. Besides that, an interactive portal of questions and answers were designed in a new window 'Q & A' as shown in Figure 4.52A.

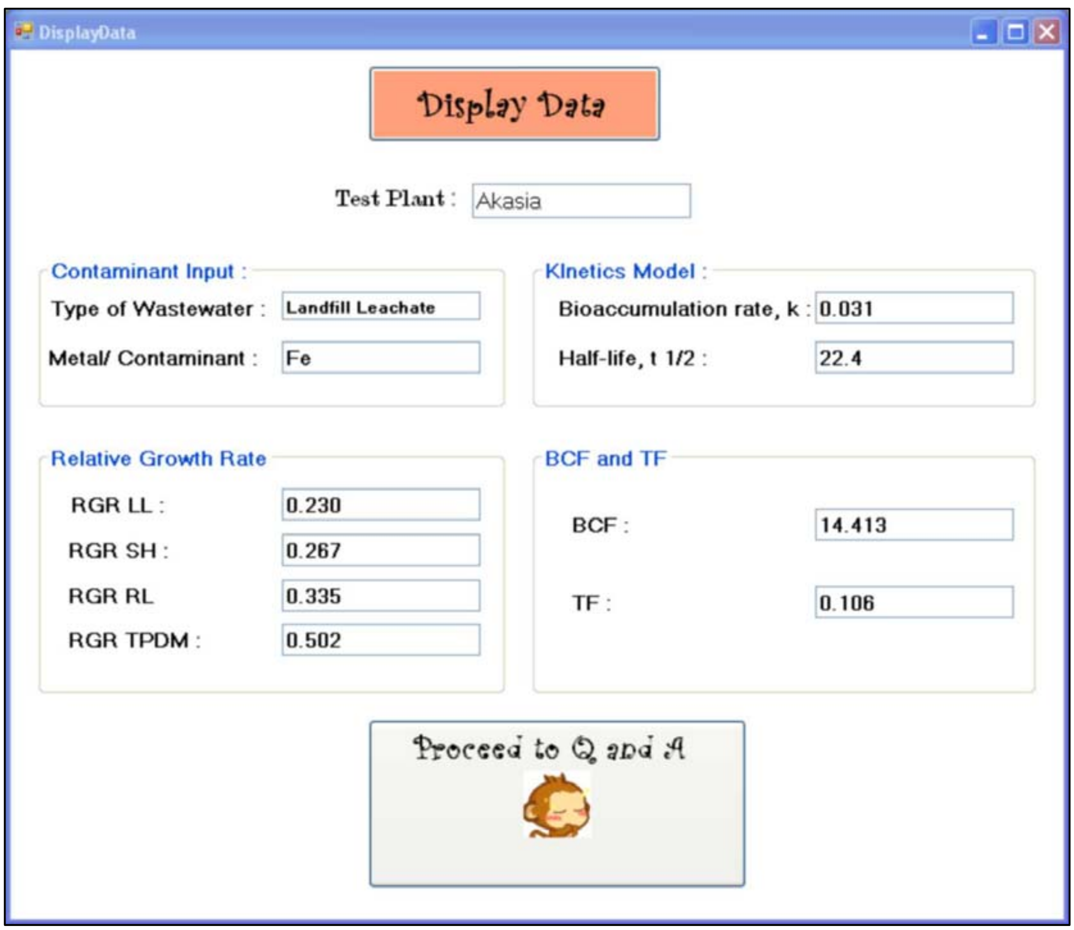


Figure 4.51*:* Information summary window

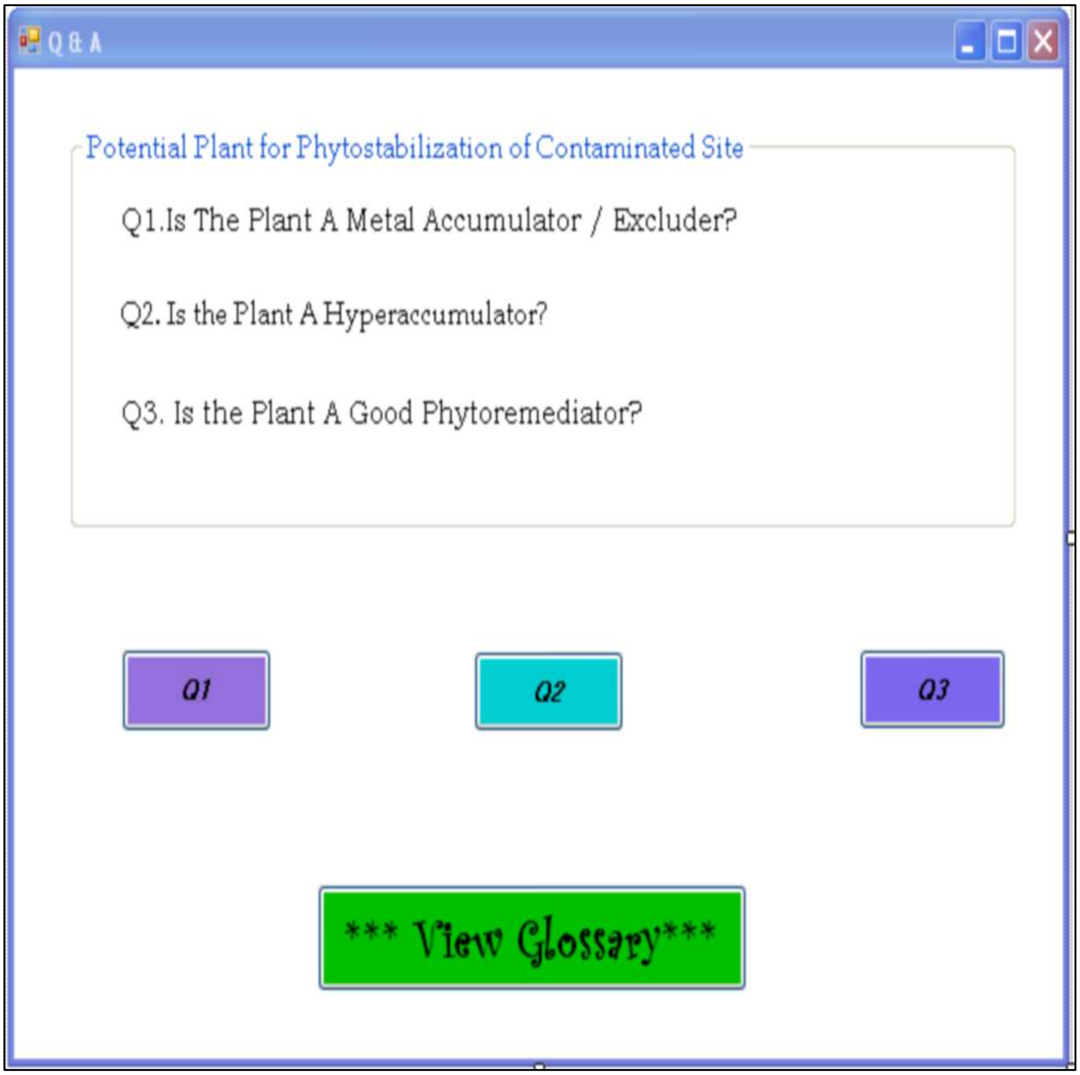


Figure 4.52A*:* Form Question and Answer

Users may click on Button Q1, Q2 and Q3 respectively to get answers to the respective questions posted in the panel of window Q & A (Figure 4.52A). These questions are answered based on standards and definition defined by the system administrator. An example layout of answers to Q1, Q2 and Q3 based on data analysis as displayed in Figure 4.51A is displayed in Figure 4.52B. Button ‘View Glossary’ is provided to proceed to the next form entitled Glossary.

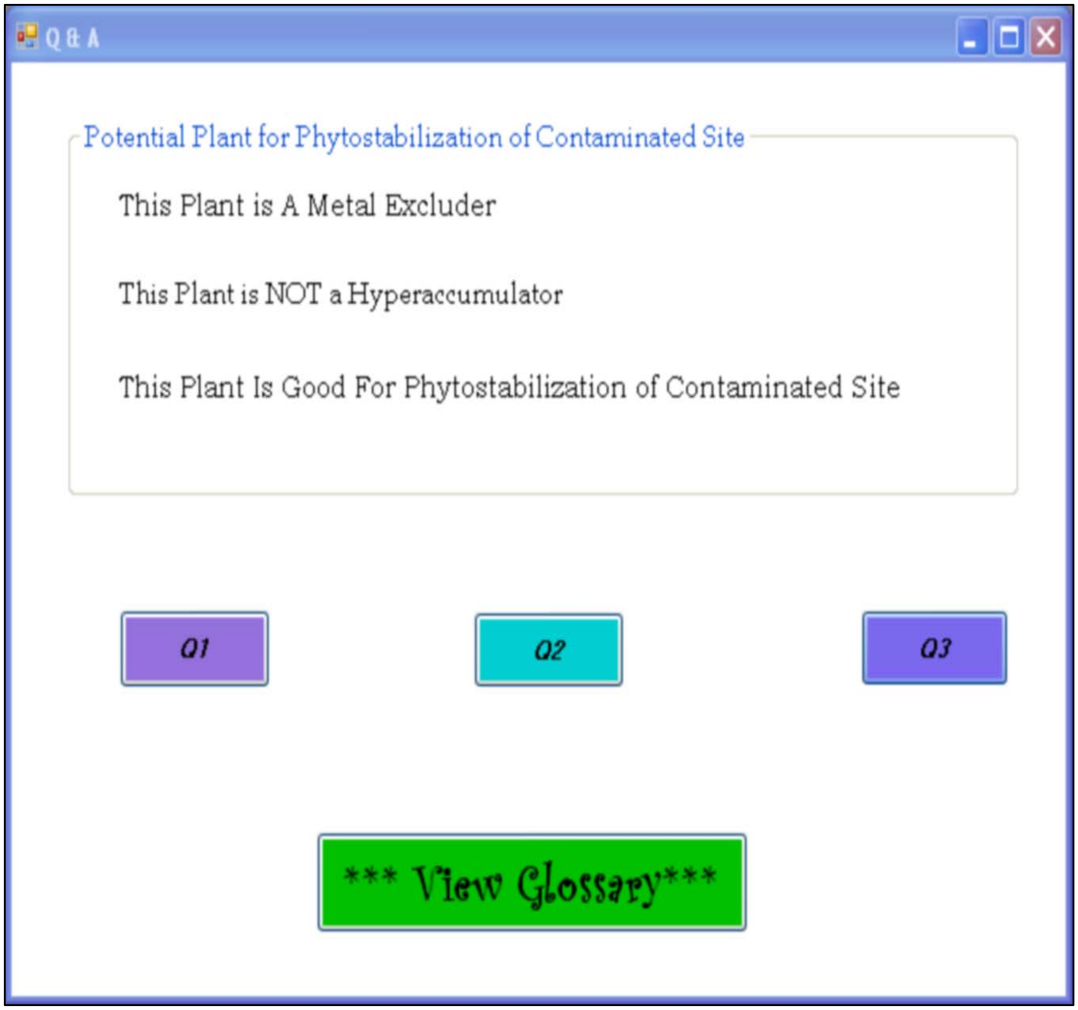


Figure 4.52B: Example of Q and A form based on actual example

The Glossary window (Figure 4.53) provides an e-dictionary service for a list of technical terms provided in the drop down selection menu. Users may view the definition of technical terms of phytoremediation selected in the text box 'Glossary Terms' provided. Some other examples are shown in Figure 4.54 and Figure 4.55.

A Compact Disc containing a published version of the e-PMS version 1.1 software is attached together with the MSc thesis in Appendix D. The software was published in the format of .exe file where it will be able to run independently in any Personal computers or Laptops installed by the users.

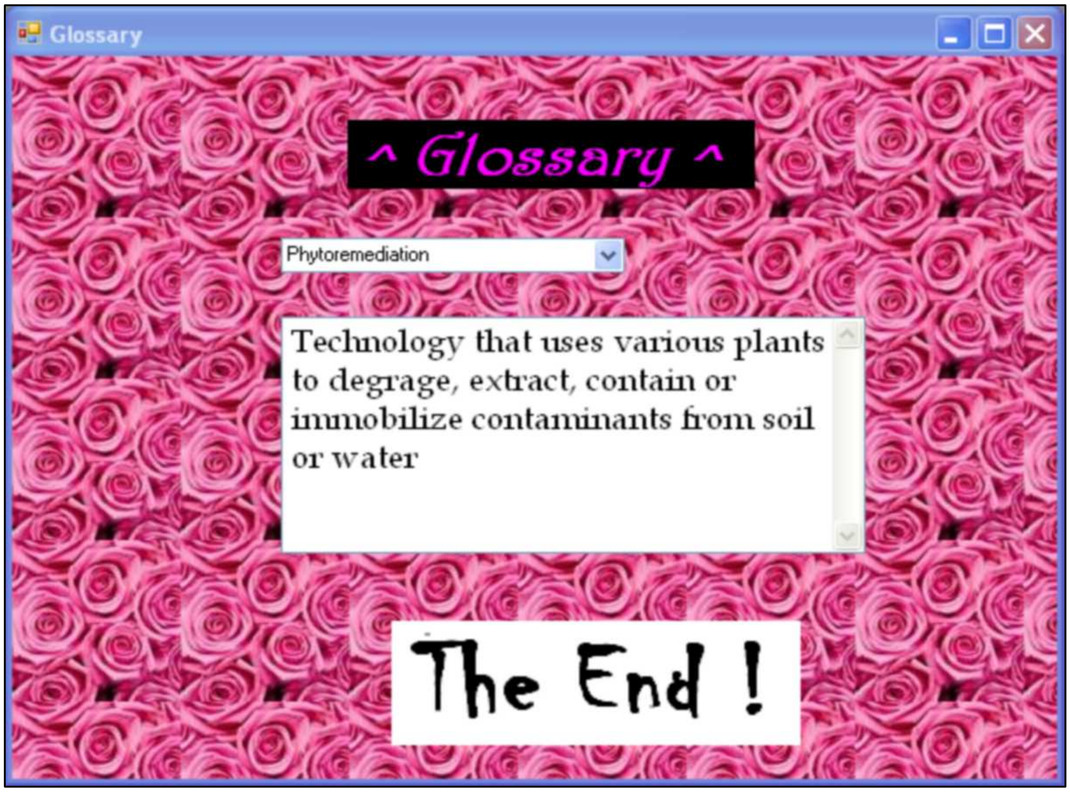


Figure 4.53*:* Display of Glossary Form

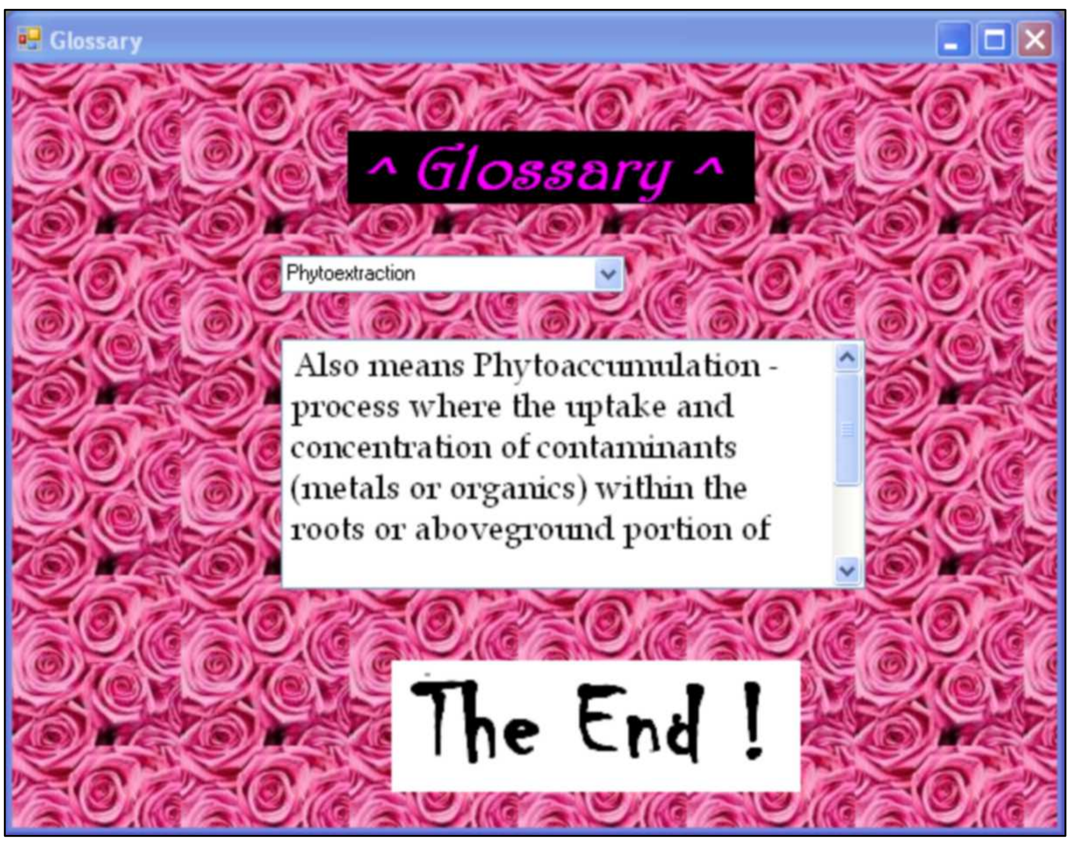


Figure 4.54: Example of glossary term – "Phytoextraction" and its definition

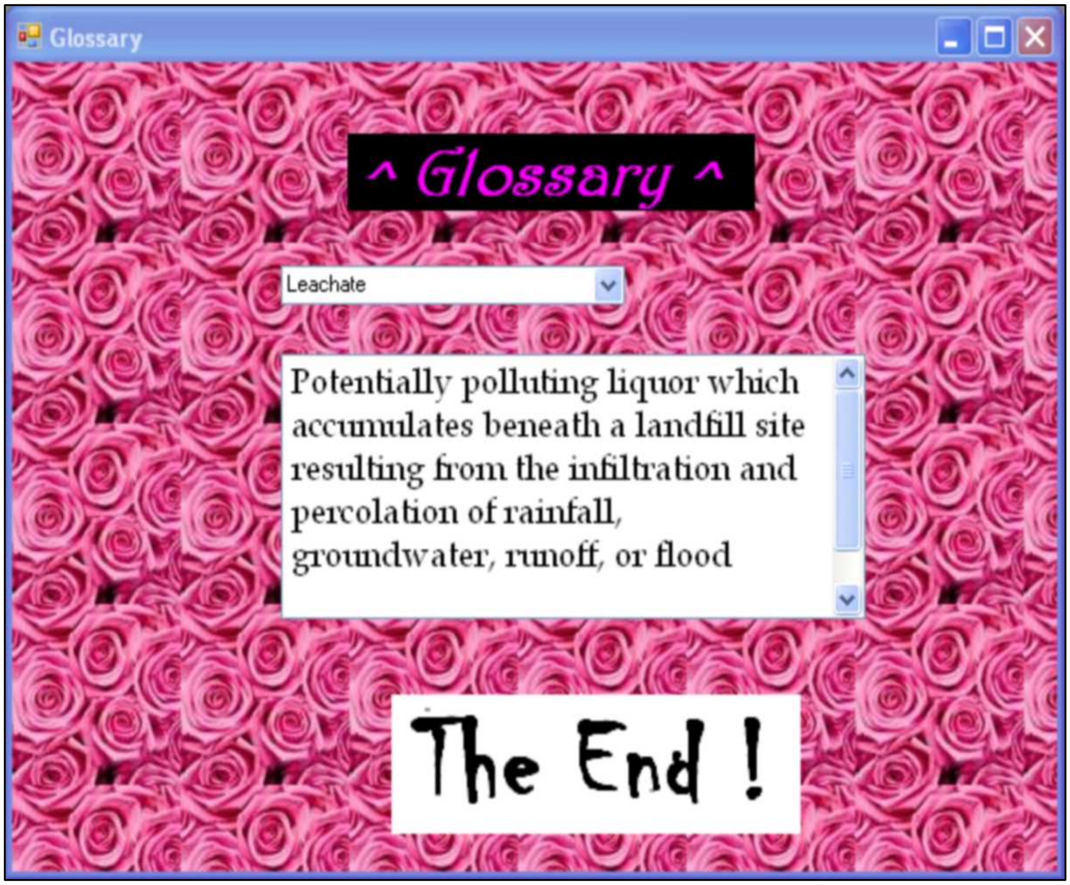


Figure 4.55: Example of glossary term –“Leachate” and its definition

The development of Phytoremediation Modeling System established the beginnings of an integration of biological and artificial intelligence knowledge base for phytoremediation systems. It included: 1) identification, processing, and organization of information required for phytoremediation systems, 2) development of a process and systems model, 3) integration of these models via information processing algorithms into a computerized tool which incorporated biological information with engineering design, and 4) provided a platform for parametric modeling.

The development of e-PMS also serves as a platform for future research directions, i.e selection of test plants which is suitable for treatment of contaminated soil/ water. Based on the models developed for e-PMS, there are several branches which can be developed for future studies:

1. Economic analysis-Cost comparison study
2. Genetically engineering an ideal phytoextraction plant. As noted, a plant with enhanced uptake ability could significantly give a higher RGR and BCF value.
3. In terms of developing a more solid phytoremediation knowledge base, methods should be established for determining correlations between different models. This would provide the ability to predict the outcome for different phytoremediation scenarios without developing parameters from a pilot study.
4. Continued efforts on expanding the process model. Studies should include identifying additional parameters which influence plant uptake. Examples include the impact of root mass, plant age, previous accumulated metals, water velocity, and environmental factors on uptake.

4.16 Post-Harvest Processing of Test Plants

Fuelwood demands, biomass production, soil, water and groundwater pollution and its impact on ecosystem are growing concerns among human population worldwide. Fast growing trees may beneficially meet societal demands ranging from renewable energy to environmental bioremediation and conservation (Wickneswari et. al., 2011). Harvested Kenaf and Akasia will be used efficiently in the processing of pulp, paper and timber. Unlike plants which are of no commercial values, will be harvested and either dumped at landfill or incinerated.

4.16.1 Akasia wood quality

A. mangium and *A. auriculiformis* are the most popular *Acacia* species planted in Southeast Asia for timber and pulp production. The tropical *Acacia* species is estimated to cover close to 600,000 hectares of plantations in the ASEAN region and there is a growing necessity for desirable planting materials, particularly with refined lignin quality for pulping. *Acacia auriculiformis* x *Acacia mangium* hybrid (*Acacia* hybrid) is highly sought after by forest plantation companies because of its superior growth and form compared to the parental species. Lignin is an undesirable component in the conversion of wood into pulp and paper (Wickneswari et. al., 2011).

According to a study conducted by Shibli et. al. (2011), *A. mangium* showed good growth both in height and basal diameter in 50% and 70% sludge. In the field, *A. mangium* was found to be more tolerant and easier to manage. Mortality rate of the species was 30% and the growth was fast. About 4000 individual plants (2 m x 2 m) of *A. mangium* can be planted in a hectare of contaminated land, 75tonnes/ha/year of leaves and stem are expected to be produced and 745kg/ha/year of Zn and 44.6kg/ha/year of Cd can be assumed to be extracted from the soil.

Looking into the potential economical and industrial value of *A. mangium*, therefore further genomics and enzymatic laboratory research is needed to test on the quality of lignin production of A. mangium grown in contaminated sites in accordance to production of quality wood.

4.16.2 Kenaf fibre quality

The Kenaf plant is made up of profitable multicomponents (for instance leaves, stalks and seeds) and within each of the plant components there are variety of efficacious portions, such as fibers and fiber strands, proteins, oils, and allelopathic chemicals. The composition and production of these plant components can be influenced by many factors including planting date, photosensitivity, cultivar, length of growing season, plant maturity and plant populations (Webber and Bledsoe, 2002).

Soil fertility can also significantly influence the Kenaf stalks, leaves, and seed compositions. Webber and associates (2002) carried out a two-year soil fertility study in Oklahoma by using five nitrogen application rates (0, 56, 112, 168, and 224 kg N/ha) on a fine sandy loam soil. It was identified that stalk yield tended to increase as N application rates increased up to 168 kg N/ha. Nonetheless, at 224 kg N/ha a remarkable deprivation in stalk yield occurred compared to the 168 kg N/ha level (Webber, 1996). In the Oklahoma study, excess N application deprived stalk yield and promoted leaf yields (Webber and Bledsoe, 2002). The stalks of Kenaf are processed for pulps and non-pulping products like building materials. The Kenaf plant leaves and stalk (core and bark) can be used as a livestock feed although Kenaf is usually considered a fiber crop. Research determined that Kenaf has high content of protein (Killinger, 1969).

According to the current phytoremediation of Jeram landfill leachate study conducted, concentrations of contaminants were highly accumulated in Kenaf roots in both Pot-culture and Hydroponic-culture. Whereas, literatures stated the use of Kenaf stalks and bark of stalks in the industrial processing. This leads to further laboratory experimental studies on the quality of bark and pulps produced from Kenaf plants grown in contaminated sites.

4.17 General Discussion

Sanitary landfill leachate is usually recognized to be a very high strength wastewater comprising many organic and inorganic components (Aghamohammadi et. al., 2007). Jeram Sanitary Landfill (JSL) leachate contains high concentration of heavy metals such as As, Pb and Fe and metals like CN. Macronutrients of N and Kwere also found in high concentrations compared to Standard. This is in congruence with the findings of Alaribe and Agamuthu (2010) on heavy metals like Fe, As, Pb, Mn, Zn, Cu and Cd in Ampar Tenang landfill leachate. Concentration of Fe from Ampar Tenang landfill site was 15 mg/ L which was 15 times lesser than Jeram landfill leachate (Table 4.28). COD and BOD_5 concentrations of Jeram leachate was 17 and 70 times, respectively higher than Ampar Tenang leachate. Lead concentration in both the landfill leachate was the same.

Table 4.28: Characteristics of Ampar Tenang and Jeram Sanitary Landfill (JSL) leachate

Parameter	**Sampling Sites**	
	Ampar Tenang[1]	JSL[2]
pH	6.5	3.6
Temperature	30	35.5
COD	3150	53165
BOD_5	209	14549
TSS	1718	24450
NH_3-N	-	14
Fe	15.1	223.6
Zn	2.4	4.7
Pb	0.3	0.3
As	0.3	0.5
CN	-	0.4

Source: [1]Alaribe and Agamuthu, 2010; [2]Meera and Agamuthu, 2012

The Ampar Tenang landfill is located in Sepang, southern part of the Selangor state, Malaysia and was in operation since year 2000. The landfill is managed by Alam Flora Sdn. Bhd., a waste management company in Malaysia. The four hectare landfill was designed for approximately 150 metric tons of domestic waste generated daily in the district of Sepang (Alaribe and Agamuthu, 2010).

Jeram Sanitary Landfill leachate has been investigated to be a good fertiliser for Kenaf and Akasia in this study, which is supported by a similar study conducted by Hasselgen (1992), where leachate from older landfills was good fertiliser for short rotation coppice (ie. poplar, willow, maple). Significant increase in biomass yields of Kenaf (47.9%) and Akasia (51.0%) was observed in plants irrigated with landfill leachate compared to control. This is in congruence with a number of studies which have established notable increase in biomass yields of plants in contrast with water or rain irrigated controls (among some examples are willow, poplar and maple) as reported by Duggan, 2005; Brierley et. al. (2001); Tyrrel et. al. (2001) and Martin and Stephens (2001). Productivity of treatment improves with increased production of biomass (Duggan, 2005).

Willow stands have successfully reduced ammonia (97 – 100% removal efficiency) and total nitrogen (43 – 93%) as reported by Duggan (2005) and Hasselgren (1998). In this study, Kenaf in pot culture have successfully reduced As, Fe, CN and NH_3-N levels (79-99% removal efficiency) and Akasia have successfully reduced the above said parameters by 84-99% removal efficiency. Kenaf and Akasia have proven to be good phytoremediators as the results of removal efficiency of pollutants from soil is not of a great variance though willows have been popular plants applied in the field of phytoremediation of landfill leachate polluted soil. In Sweden, willow stands were utilised to achieve good removal efficiencies of problematic landfill leachate elements (Duggan, 2005). The N composition of leachate was successfully reduced by 93% from 1600 kg N ha^{-1} $year^{-1}$ to approximately 100 kg N ha^{-1} $year^{-1}$ over a 10 year period (Duggan, 2005). Akasia accumulated 7.84 – 26.42 kg As ha^{-1} $harvest^{-1}$, 1,410 – 2199.3 kg Fe ha^{-1} $harvest^{-1}$, 6.94 -20.19 kg CN ha^{-1} $harvest^{-1}$ and 164.63 – 394.49 kg NH_3-N ha^{-1} $harvest^{-1}$ in plant tissues during 120 days of growth. Kenaf plant parts accumulated 20.85 kg As ha^{-1} $harvest^{-1}$, 1,476.08 kg Fe ha^{-1} $harvest^{-1}$, 16.15 kg CN ha^{-1} $harvest^{-1}$ and 299.76 kg NH_3-N ha^{-1} $harvest^{-1}$ at optimum leachate concentration of 25% TL.

The experimental study of landfill leachate treatment using Kenaf and Akasia concluded that there were no detrimental effects of applying leachate to the test plants. This study is in contrast to some investigations showing detrimental and phytotoxic effects of applying leachate to willows (Stephens et. al., 2000). This is most probably due to differences in the characteristics and concentrations of leachate applied to plants.

Landfill leachates containing high Electrical Conductivity (0.2 – 0.4 Sm^{-1}) may induce osmotic or ionic stress when sprayed onto trees (Dobson and Moffat, 1993). In Duggan's (2005) study, spray irrigation of leachate onto foliage of sugar maple proofed diminution in rates of photosynthesis by 34% and water use efficiency by 70%. Transpiration rates of sugar maple were generally unaffected wih irrigation of leachate. In contrast, detrimental impact of spray irrigation on foliage was not observed when lower strength leachates were applied (Duggan, 2005) which also explains the decrease in biomass and bioaccumulation of As, Fe, CN and NH_3-N in Kenaf and Akasia treated with leachate concentrations above 50% TL in this study.

It is also important to keep track of where toxins accumulate, with regards to the manner of biomass being treated or used after harvest and burning. Likewise some plant species accumulate contaminants in the leaves instead of the stems, which would be restored to the soil during leaf senescence. A plant species which accumulates toxins in their stems, which will then be harvested, was chosen if removal of the toxicants from the site is a prerequisite (Duggan, 2005). Both Kenaf and Akasia reported bioconcentration of pollutants in plant root compared to aerial plant parts (TF<1) in the study conducted. Therefore there is no need for landfill operators and resource managers to worry on the return of toxins to the environment through leaf, stems, apical buds or flowers. But, if the plant roots were utilised for pulp production, then there is a need to test on the quality of pulp and timber produced prior to industrial processes.

Relative growth rate of *A. mangium* biomass was optimum at leachate treatment 25% TL and decreased at leachate concentration above 50% TL in this current study. The effect of the contaminated site and its relation to *A. mangium* growth and pollutants uptake was also analysed in the Kota Bharu landfill site. The growth of Acacia in landfill site was better than control in terms of tree diameter and produced more branches and leaves. But Akasia plants grown in control site gained plant height faster than contaminated site (Shibli, 2003).

In a study conducted by Zalesny Jr. and Bauer (2007) in Wisconsin, USA, Fe concentration in landfill leachate used for irrigation of Populus and Salix was 13,639 mg/L, which was 60 times the concentration of Fe in Jeram landfill leachate. The Table 4.29 shows the growth of Populus and Salix irrigated with landfill leachate.

Table 4.29: The growth traits of Populus and Salix treated with leachate

Trait	Genus	
	Populus	Salix
Height (cm)	99.74±2.27	117.19±2.26
Leaf dry mass (g)	6.79±0.78	1.16±0.79
Stem dry mass (g)	17.38±1.03	25.67±1.03
Root dry mass (g)	4.49±0.47	8.37±0.47

Source: Zalesny and Bauer, 2007

Comparing the results above with the results observed from the current study, highest root, stem and leaves dry biomass of Kenaf at 6.98, 9.24 and 7.79g, respectively was found at leachate concentration of 25% TL. The highest dry biomass of root, stem and leaves of Akasia were observed at 25% TL (9.21, 11.73 and 9.27 g, respectively). Akasia plants recorded better root and leaf dry biomass compared to Populus and Salix. Kenaf plants recorded one and six times higher leaf biomass compared to Populus and Salix, respectively irrigated with leachate. The differences in Akasia and Kenaf dry biomass among the two different experiments was due to differences in soil and contaminant characteristic, uptake ability of test plants (differences in response towards pollutants) and also plant maturity. The weather condition too was not similar for both the study.

Brassica rapa L. (leafy vegetable) receiving 25% treated leachate from Ampar Tenang Landfill leachate produced longer leaves (23.17±0.6cm) than other treatments (Alaribe and Agamuthu, 2010). The treatment also produced wider leaves which were 1.36 and 3.23 times higher than plants grown in Inorganic fertilizer and control, respectively. This supports the current study carried out with Kenaf and Akasia, whereby 25% TL treatment gave an optimum reading of growth rate of leaf length, stem height and root length. The 25% TL treatment was most probably the optimum in satisfying the nutrient requirement for leaf expansion (Dimitriou et. al., 2006).

Cu, Cd and Zn accumulation in *A. mangium* were significantly ($p \leq 0.05$) influenced by different concentrations of copper treatments (Majid et. al., 2011), where the accumulation of Cu and Cd were higher in the roots and the lowest was in the stems. The scenario was similar to the results of the present study where highest bioaccumulation of As, Fe, CN and NH_3-N was in both *A. mangium* and *H. cannabinus* root. The accumulation rank was in the order of roots > leaves > stem (Majid et. al., 2011) which is slightly different than results that was obtained in this study, where accumulation rank in the order root> stem> leaves. Arsenic was present at concentration of 1.0 mg/kg in the edible parts of *Brassica rapa* L. in 50% TL treatment (Ampar Tenang leachate) as reported by Alaribe and Agamuthu (2010). The result is similar as reported by Kenaf and Akasia treated with 50% TL with As accumulation 0.94 mg/kg and 1.23 mg/kg in plant leaves, respectively. The level of Fe accumulation in Akasia leaves grown in Kota Bharu landfill site was highest ranging between 139.5 – 537.6 mg/kg followed by Cr, Zn, Cu and Cd (Shibli, 2003). On the other hand, Fe in Akasia irrigated with Jeram landfill leachate was in the range of 41.13-64.51 mg/kg, which is about six times lesser than the previous study. This could be probably due to difference in plant maturity and duration of exposure of Akasia plants in the contaminated site. Heavy metal uptake by *A. mangium* grown in landfill site was higher than control plants according to the soil-plant concentration ratio.

Biomass plays a significant role in the accumulation of heavy metals from soil and water. Plants used as a phytoremediator must possess characteristics of high potential capacity to uptake pollutants from soil or water and have large biomass (Ho et. al., 2008). *A. mangium and H. cannabinus* showed high TF and low BCF in soil at higher metal concentrations. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator of contaminated soils (Majid et. al., 2012).

Crops with economical values such as Kenaf, Akasia and others should be proposed in the application of phytoremediation of polluted soil and wastewater in near future. This is because, upon harvesting these plants, there is beneficial use of these plants for industrial processes, i.e. pulp and timber production. Nevertheless, if crops of little economical significance were applied, they would end up polluting the environment again following 'dig and dump' process or incineration.

Salix triandra x viminalis (ordinarily identified as clone "Q83") was grown hydroponically, using the nutrient film technique (NFT) (Duggan, 2005). The researchers studied the impacts of the cultivar on the leachate, and the impacts of the leachate on the cultivar, independently from the soil. They measured the evapotranspiration rate, too, by weighing the tanks. Plant tissues only remained healthy for nutrient solutions with 12.5 and 25% strength leachate solutions (Duggan, 2005) which agreed to the results observed among Kenaf and Akasia grown hydroponically in this study. Trees receiving leachate had a predominantly greater leaf area than water irrigated controls (Duggan, 2005). Phytoremediation of wastewater had been extensively carried out with water hyacinth (*Eichhornia crassipes*) worldwide (Malik, 2007; Liao and Chang, 2004). It would be a novel approach to explore other plants with such characteristics, like fast growing, excellent metal accumulation and aesthetically pleasing for the remediation of wastewater. Besides that, water hyacinth poses threat to water bodies as it is an invasive species (Bhattacharya and Kumar, 2010) and an edible plant as cattle feed.

Development of a Decision Support System, e-Phytoremediation Modeling System (e-PMS) was a novel approach. The model was developed with additional features of graphical user interphases and various calculation models in assessing the performance of phytoremediator plants in comparison to available softwares like DESYRE, RGS and GUI as discussed in Chapter 2 (pages 79-85). In the future directives, it is proposed the development of DSS for phytoremediation processes can be further enhanced with additional features or models for example cost calculations for field application and etc. Besides that, DSS is recommended to be further fine tuned with better graphics or interphases using upgraded programming language like FORTRAN and others.

Phytoremediation is one of the mechanisms for removing hazardous heavy metal besides chemical/ physical remediation, animal remediation and microbial remediation with emphasis on bio-removal aspects (Wu et. al., 2010). The employment of genetic engineering or cell engineering to establish an ideal and expected species will become necessary and popular in the future. Several approaches were proposed including selecting self-pollinated plants, creating infertile polyploid species and carefully choosing easy-controlled microbe species (Wu et. al., 2010). Crop hyperaccumulators (newly termed as "cropaccumulators") by transgenic or symbiotic approach contributes to pragmatic development in the future (Ruiz et. al., 2003). Symbiotic system would be more productive by incorporating both microremediation and phytoremediation. Binding reagent such as metallothioneins (MTs), phytochelatins (PCs) and organic acid will be excreted in larger quantity by symbiotic systems compared to sole plants. This is because the symbiotic microbes are large in amount and provides specific surface area

(Wu et. al., 2010). Plants, however, is appropriate for subsequent post-processing because they possess harvestable stem and leaves aboveground. Hence, the symbiotic mechanism enables soil quality to be upgraded with better acidification, which eventually drives to a better bioavailability, solubility and mobility of heavy metals (Wu et. al. 2010). Genetic engineering technology (such as cell fusion or somatic hybridisation) also demonstrates great capacity. The cell fusion method is of great significance because polyploid plants are usually larger in biomass and more active in transpiration, therefore facilitates in the transportation of heavy metals in root-to-shoot process (Wu et. al., 2010; Geoffrey, 2006).

Phytoremediation too has a number of inbred technical inhibitions. The toxin must be contained within (or must be attracted toward) the actively growing rhizosphere zones of plants (Cunningham and Ow, 1996). Water, nutrient, depth, atmospheric, physical and chemical limitations are among the factors involved. Moreover, the area/ experimental site must be outstretched enough to enable farming techniques to be carried out. Besides that, the phytoremediation process must not present a serious threat to human health or further environmental pollution. Research society engaged in basic and applied sciences should work hand in hand to set forth an effective technology which is low in cost and impact, visually benign, as well as environmentally sound (Cunningham and Ow, 1996). Knowledge of basic plant processes/ metabolic activities on which genetic modification and breeding efforts depend can be further explored and investigated with the expertise of plant biologists.

CHAPTER 5

CONCLUSION

Jeram Sanitary Landfill leachate has a typical young, rural and acidogenic phase characteristics. It has high BOD and COD, indicating presence of highly biodegradable organic materials. The leachate also contained high Suspended Solids, Ammoniacal Nitrogen and Turbidity. Besides that, heavy metals (Pb, Cd, Hg, Cr, As, CN), micronutrients (Fe, B, Mn, Cu, Ni, Zn) and macronutrients (NH_3-N, K, Ca, Mg, P, and S) were also present in high concentrations compared to regulatory standard limit of Regulations 2009 (Department of Environment Malaysia, 2010). Application of phytotechnology using *H. cannabinus* and *A. mangium* was adopted in the bioremediation of Jeram landfill leachate for selected parameters such as Fe, As, CN and NH_3-N. Both the test plants showed high potential to phytoremediate (optimum concentration of 25% treated leachate) soil contaminated with pollutants from landfill leachate, as demonstrated by high uptake ability. High bioaccumulation of As, Fe, CN and NH_3-N was detected in the root but transportation of these parameters were restricted to other plant parts such as stem and leaves. This indicates the uptake of toxic metals by phytoextraction and tolerance of contaminants by phytoimmobilisation mechanism. This phenomenon also minimizes the release of metal again to the environment through decomposition of shredded leaves or incineration of harvested plants.

It was also observed that *H. cannabinus* plants treated with leachate recorded better growth rate of plant root and was taller by 51% than control and 14% better growth than inorganic fertilizer with lots of fibrous roots than other treatments. *A. mangium* is seen to be a potential plant to phytoremediate soil contaminated with heavy metal from landfill leachate as demonstrated by high uptake ability (67.9 – 94.6% of Fe, 80.1 – 98.3% As, 69.6 – 83.7% CN and 94.3 – 98.5% NH_3-N contaminant removal efficiency respectively). Akasia plants treated with leachate recorded 52.0% better growth compared to control and also 13.2% greater plant biomass compared to application of Inorganic fertilizer.

Kenaf and Akasia recorded highest BCF for leachate treatment 25% TL in the average of 2.8 for As, 12.6 for Fe, 3.9 for CN and 8.1 for NH_3-N. Mobility of pollutants in Kenaf and Akasia was restricted to plants roots with TF below 1 for all the pollutants parameters tested. Translocation of NH_3-N in Akasia was exceptional which recorded values above 1 for all the 9 different treatments applied. The mean bioaccumulation rate constant of Fe, As, CN and NH3-N was 0.02/day for Kenaf and 0.03/day for Akasia. Mean half-life of selected pollutants in Kenaf and Akasia were 50.3 days and 45.9 days, respectively.

Kenaf and Akasia in wetland system showed a lower Bioconcentration of As, Fe and CN and NH_3-N in plant tissues compared to pot-culture. Bioremediation of leachate using Kenaf in wetland system displayed a better growth of plant root system compared to control and 100% IF treatments. Hydroponically grown Akasia in leachate contaminated wastewater displayed 1.3 times better growth of plant root system compared to control and 1.2 times taller root length compared to fertilized treatments. In the study of Phytoremediation of Landfill leachate, *A. mangium* is proven to be a better phytoaccumulator of Fe, As, CN and NH_3-N compared to *H. cannabinus* grown in pot-culture and hydroponic-culture due to deep plant root system.

An interactive software named e- Phytoremediation Modeling System (e-PMS) was developed to function as a DSS in analyzing phytoremediator plants using models of the Phytoremediation study. The software also displayed photographs of the Test plants, plant leaves, flower and plant seeds, used as well as scientific classifications of the selected test plants. Question and Answer interphase displayed to determine mechanism of pollutant uptake and whether a selected plant is a good phytoremediator.

The development of e-PMS software was a novel trial carried out as available softwares on Phytoremediation processes are next to nil.

Finally, the objectives have been fulfilled. *A. mangium* recorded to be a better phytoaccumulator of Fe, As, CN and NH_3-N, with removal rate of 70 – 90%. Leachate treated plants showed higher growth biomass and contaminant uptake efficiency compared to control and inorganic treatments. *H. cannabinus* and *A. mangium* performed better in pot-culture compared to hydroponic-culture in the study conducted.

A successful phytoremediation study of contaminants in leachate depends on several factors such as the quantity and quality of leachate, the design of landfill, the test plants selected, the level or degree of treatment needed and final removal methods for residues and effluent. Agroforestry that combines plants with economical value and phytoremediation could be a rising integrated and comprehensive mechanism for sustainable energy, agricultural development, and environmental mitigation worldwide. The use of Kenaf and Akasia for bioremediation reinforces better environmental quality and secondary advantages such as a harvestable product, aesthetic improvements, carbon sequestration and erosion and sediment control.

5.1 Recommendations for Future Research

1. Spray or trickle irrigation of untreated or partially treated leachate onto vegetated land is suggested as a promising option of remediation for contaminated site.
2. Future landfill leachate remediation research should evaluate the effects of landfill leachate on macro- and micro-organisms in the rhizosphere zone of test plants.
3. Assessment of impacts of leachate application on the life cycle and wood quality of Kenaf and Akasia in industrial processes can be evaluated.

4. Exploration of new remediation schemes such as hybrid technologies (i.e. phyto-vapor extraction and phytoland farming) combining phytoremediation and chemical engineering techniques is recommended.

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