

UNIVERSITY OF NAIROBI
Department of Civil Engineering



**Phytoremediation of Polychlorobiphenyls
(PCB's) In Landfill E-waste Leachate with
Water Hyacinth (*E.crassipes*)**

MSc Research Thesis

By

Erick Omondi Auma

F56/65391/2010

A thesis submitted in partial fulfillment for the award of degree of Master of Science in Civil Engineering in the Department of Civil and Construction Engineering, University of Nairobi

October, 2014

DECLARATION

This thesis is my original work and has not been presented for a degree in the University of Nairobi or in any other University.

Signature: Date:

Erick Omondi Auma
F56/65391/2010

Approval by the University Supervisor

This Research Project has been submitted with my approval as the University Supervisor.

Signature: Date:

Dr. P. K Ndiba
Department of Civil and Construction Engineering
University of Nairobi.

Signature: Date:

Dr P.G. Njuru
Department of Environmental Science and Technology
Southern Eastern Kenya University

Approval by the Chairman of the Department

This Research Project has been submitted with my approval as the Chairman of the Department.

Signature: Date:

Prof. S.K Mwea
Chairman, Department of Civil and Construction Engineering
University of Nairobi

ABSTRACT

The presence of e-waste in a landfill can release persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), into the environment. PCBs are a family of more than 200 chemical compounds (congeners), each of which consists of two benzene rings and one to ten chlorine atoms. This study investigated use of water hyacinth (*Eichhornia crassipes*) for phytoremediation of landfill leachate waste containing PCB. Landfill leachate was simulated in the laboratory by spiking water samples with PCB to obtain concentrations of 5, 10 and 15 $\mu\text{g/L}$, which were in one to two orders of magnitude above the US Environmental Protection Agency (EPA) limit of 0.5 $\mu\text{g/L}$ or 0.5 ppb. Water hyacinth plants were grown in 2 L samples of the PCB spiked water for 15 days and evaluated for tolerance and bioaccumulation of PCB. Phytoremediation of PCB spiked water by the plants was evaluated by measuring the change in concentration of PCB. The plants tolerated PCB concentrations in the range of 5 to 15 $\mu\text{g/L}$ without depicting any serious adverse effect except for change in root color and an initial wilting of peripheral leaves. Water hyacinth reduced the concentration of PCBs in the leachate over 15 days from 15 to 0.42 $\mu\text{g/L}$ for the 15 $\mu\text{g/L}$ initial concentration sample and to below the GC/MS detection limit of 0.142 $\mu\text{g/L}$ for the 10 and 5 $\mu\text{g/L}$ initial concentration samples. Bioaccumulation of PCB in the plant tissue was evaluated through solid phase extraction and testing of samples for PCB with GC/MS. Bioaccumulation of PCBs at a concentration of 0.179 $\mu\text{g/g}$ was observed in the water hyacinth roots for the 15 $\mu\text{g/L}$ sample but none was detected for the lower initial PCB concentration and shoots. The study demonstrated potential of water hyacinth plants in phytoremediation of PCBs in e-waste leachate.

ACKNOWLEDGEMENT

I am highly indebted to a number of people for their help and support during my research. My first appreciation and gratitude goes to my supervisors; Dr. P.K Ndiba and Dr. P.G Njuru. Your consistent guidance, insightful advice and endless encouragement made this research possible. You also showed confidence in me both academically and personally which motivated me to complete this work.

I am sincerely grateful to my wife Sarah for the moral support and motivation she accorded me during the demanding period of the research work. I am also very grateful to my dear parents for their continuous long-term support and understanding especially during this research work.

To all those who helped in documentation, sourcing of chemicals and laboratory testing and analysis of data, I am grateful. My fellow students who were a source of encouragement and positive criticisms are highly appreciated.

Above everything else, I feel greatly indebted to the Almighty God who has given me strength, favour and provisions necessary for this work. I saw His outstretched hand of guidance and steadfast grace that was sufficient to me on this challenging road of gathering and disseminating knowledge to mankind.

DEDICATION

This Research work is dedicated to my daughter, Aurel as a motivation to her in pursuing education even to a higher level than this.

Table of Contents

ABSTRACT	ii
DEDICATION.....	iv
LIST OF FIGURES	viii
LIST OF TABLES.....	ix
ABBREVIATIONS AND ACRONYMS.....	x
CHAPTER 1	1
1.0 INTRODUCTION	1
1.1 Background Information.....	1
1.2 Problem Statement.....	3
1.3 Objectives	4
1.4 Scope of Study	4
2.0 LITERATURE REVIEW	6
2.1 General Overview of PCB's Contamination	6
2.2 Properties of PCBs.....	7
2.2.1 Chemical Composition	7
2.2.2 Chemical Structure	7
2.2.3 Purity and Impurities	8
2.2.4 Physical and Chemical Properties of PCB's.....	8
2.3 Sources and Uses of PCBs.....	11
2.3.1 Uses of PCB's.....	11
2.3.2 Sources of PCBs in the Environment	13
2.4 Regulatory Framework for PCBs	15
2.5 Kinetics and Metabolism of PCBs.....	16
2.6 Effects of PCBs on Organisms in the Environment	18
2.7. Remediation by Surfactants.....	18
2.7.1. Types of Surfactants and their Mode of Action.....	19

2.8 Use of Constructed Wet Lands in Waste Water Treatment.....	20
2.8.1 Water Hyacinth.....	22
2.8.2 Water Hyacinth Nutrient Requirements	23
2.9 Phytoremediation.....	24
2.9.1 Types of Phytoremediation.....	25
2.9.2 Phytoremediation by Water Hyacinth	28
2.9.3 Use of Water Hyacinth in Pytoremediation of Organic Contaminants ..	29
CHAPTER 3	31
3.0 METHODOLOGY	31
3.1 Introduction.....	31
3.2. Water Hyacinth Planting, Growth and Maintenance	31
3.3 Supplement Nutrient Level Determination.....	32
3.4 Preparation of Model Leachate Solution	33
3.5 Experiment Set-Up	33
3.5.1 Assessment of Water Hyacinth PCB Tolerance by Growth.....	33
3.5.2 Phytoremediation	34
3.5.3 PCB Bioaccumulation	35
3.6 PCB Extraction Process	35
3.7 Mass Spectrometer Calibration.....	36
3.8 Measurement of PCB Levels	39
4.0 RESULTS AND DISCUSSIONS.....	42
4.1 Water Hyacinth Tolerance to PCB	42
4.1.1 Plant Growth by Length.....	42
4.1.2. Growth by Weight	45
4.2 Observations Made During Plant Growth in PCB Spiked Water.....	45
4.3 Water Hyacinth Phytoremediation of PCB.....	46
4.4 Bioaccumulation	48

CHAPTER 5 52

5.0. CONCLUSIONS AND RECOMMENDATIONS 52

5.1 CONCLUSIONS 52

5.2 RECOMMENDATIONS 52

REFERENCES 54

APPENDICES 61

Appendix A – Water Hyacinth Plants Growth Data 61

Appendix B - Photographs Water Hyacinth Plants Grown During the Study. 63

LIST OF FIGURES

Figure 2.1: PCB Chemical Structure	8
Figure 2.2. Schematic Representation for Surfactant Properties in Aqueous Solution..	20
Figure 3.1: Chromatogram Standard	37
Figure 3.2. Mass Spectrum of the PCB	38
Figure 3.3. Library Confirmation of PCB.....	38
Figure 3.4. PCB Standard Curve.....	40
Figure 4.1: Growth of Shoot in PCB Free Environment	42
Figure 4.2: Growth of Roots in PCB Free Environment	43
Figure 4.3: Growth of Shoots in PCB Spiked Environment.....	44
Figure 4.4: Growth of Roots in PCB Spiked Environment	44
Figure 4.5: PCB Spiked in water sample I Chromatogram	49
Figure 4.6: PCB Spiked in water sample II Chromatogram	49
Figure 4.7: Chromatogram for PCB in Roots	50

LIST OF TABLES

Table 2.1: Major Trade Names for PCBs	9
Table 2.2: PCB Uses.....	12
Table 2.3: PCB Sources in Waste Material and Recycling Operations.....	14
Table 2.4: Current Authorized Uses of PCBs.....	15
Table 2.5: Types of Phytoremediation for Organic Constituents (ITRC, 1999).	26
Table 3.1: Optimal Nutrient Requirement for Water Hyacinth.....	32
Table 3.2: Standard Calibration Curve for PCB	39
Table 4.1: Summary of Growth figures PCB Spiked Environment	45
Table 4.5: Summary of Phytoremediation and Bioaccumulation Test Results	46

ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
BCD	Base Catalysed Decomposition
BFR	Brominated Flame Retarders
BTEX	Benzene, Toluene Ethylbenzene, and Xylenes
CB	Chlobiphenyls
CMC	Critical Micelle Concentration
COD	Chemical Oxygen Demand
DDT	Dichloro Diphenyl Trichloroethane
DEQ	Department of Environmental Quality
DNT	Dinitrotoluene
DRE	Destruction Removal Efficiency
EIP	Environmental Improvement Program
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
GC/MS	Gas Chromatography / Mass Spectrometry
GPCR	Gas Phase Chemical Reduction
HCB	Hexachloro Benzene
HMX	High Melting Explosive
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IRPTC	International Register of Potentially Toxic Chemicals
ITRC	Industrial Toxicology Research Centre
K	Potassium
LC	Lethal Concentration
LCD	Liquid Crystal Display

LD	Lethal Dose
MCD	Mechano Chemical Dehalogenation
MSW	Municipal Solid waste
N	Nitrogen
OSHA	Occupational Safety and Health Administration
P	Phosphorus
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychloro Biphenyls
PCDF	Polychlorinated Dibenzo-p Dioxins
POP	Persistent Organic Pollutant
PTFE	Polytetrafluoroethene
R&D	Research and Development
TCP	Tetrachlorophenol
TNT	Trinitrotoluene
TSCA	Toxic Substances Control Act
USNIOH	United States National Institute for Occupation Safety and Health
VOC	Volatile Organic Compounds
WHO	World Health Organization

CHAPTER 1

1.0 INTRODUCTION

1.1 Background Information

Excessive generation of municipal solid wastes (MSW) has been identified as one of the most serious environmental problems in the world. Up to 95% of the MSW collected worldwide are disposed off in landfills (Kurniawan et al., 2006), where they undergo a series of physicochemical and biological changes. Degradation of the wastes to various levels combines with percolated rainwater to generate a highly contaminated liquid known as “leachate.”

Persistent organic pollutants (POPs) are chemical substances that are extremely stable in the environment and are known to accumulate in biological tissue thereby posing risk to both human health and the environment. POPs originate from a variety of human activities especially agricultural and industrial activities, and electronic waste or e-waste. POPs can be conveyed for thousands of miles through air or water currents and may be found in remote ecosystems far from their source including where they have never been used (EUROPA, 2007). Polychlorinated biphenyls (PCBs) are POP's comprising a family of more than 200 chemical compounds (congeners), each consisting of two benzene rings and one to ten chlorine atoms. PCBs are used extensively in industry as coolants and lubricants in transformers, capacitors, and other electrical appliances because of their chemical inertness, resistance to heat, non-flammability, low vapour pressure, and high dielectric constant. Products containing PCBs include old fluorescent lighting

fixtures, electrical appliances containing PCB capacitors, old microscope oil, and hydraulic fluids.

Polychlorinated biphenyls (PCBs) in landfill leachate are of environmental concern because they are toxic, persistent and bio-accumulative. The use and disposal of such substances in landfill is not a sustainable practice. However, for different social and economic reasons such substances are still in use and are regularly released and disposed of to the environment through landfills.

The characteristics of a landfill leachate depend on a number of factors including the nature and origin of solid waste. In the case where e-waste is disposed of in a landfill, the leachate will consist of heavy metals and persistent organic pollutants. The presence of organic pollutants in leachate is one of the reasons why regulatory bodies do not favor treatment of leachate with municipal sewage (Zenon Environmental Systems, 1989).

Many advanced soil remediation techniques exist in both industrialized and developed countries. They include gas phase chemical reduction (GPCR), mechano chemical dehalogenation (MCD) and thermal desorption. Some promising techniques such as base catalyzed decomposition (BCD) and sonic technology are still under development either at the laboratory stage or at the pilot study. However, due to the financial constraints, many advanced technologies are unlikely to be adopted by the developing countries. Consequently, finding suitable and affordable remediation technologies is critical in solving problems in the developing countries.

Constructed wetlands are commonly used to treat municipal wastewater, acid drainage, agricultural run-off, animal wastes, and industrial wastewater. Water hyacinth plants in constructed wetlands are efficient in removal of a vast range of pollutants including suspended materials, biochemical oxygen demand (BOD), nutrients, organic matter, heavy metals and pathogens in a process known as phytoremediation. Since water hyacinth is an invasive plant of environmental concern, its use in phytoremediation of PCB's will offer a dual solution to environmental concerns. This study evaluated phytoremediation of e-waste in landfill waste using water hyacinth plants, as a simple and economic method of treatment of e-waste landfill leachate.

1.2 Problem Statement

Landfilling is currently the most common method of e-waste disposal in developing countries. Landfills receive source-wastes that generate both heavy metals and persistent organic pollutants. The use of phytoremediation technologies in treating industrial and municipal landfill leachates with particular attention to heavy metals has been shown to be viable (Keith et al., 2006, Akpor et al., 2010). Subsequently, the use of plants in combination with physical and chemical treatment methods to treat heavily polluted wastewaters has been recommended (Liehr et al., 1996). However, persistent organic pollutants such as PCBs have received little attention although they pose a great threat to the environment.

Because landfills are a depository for many items containing POPs, it is possible that PCBs from e-waste pollutants could accumulate in landfills and constitute a long-term source. For example, there is a high possibility that

leachate from Dandora dumpsite in Nairobi, which receives a large volume of dismantled electronic waste, may leach into the ground water directly or flow into the nearby Nairobi River. Pollution of the river could have adverse impacts on human health directly through consumption of the polluted water downstream or indirectly through consumption of plants irrigated using the river water downstream. At the same time, various institutions and organizations are stuck with electronic waste in their stores due to lack of appropriate disposal method. Therefore, there is need to develop simple, economic and sustainable methods of treatment and disposal of e-waste leachate from a landfill.

1.3 Objectives

The overall objective of this research was to investigate use of water hyacinth in phytoremediation of PCB in e-waste landfill leachate.

The specific objectives are:

- 1) Establish tolerance of water hyacinth to PCB pollution by assessing plant growth and vigor in PCB spiked water.
- 2) Evaluate phytoremediation of PCB in water by water hyacinth plants.
- 3) Establish bioaccumulation of PCB in plant tissue.

1.4 Scope of Study

This study focused on the phytoremediation ability of water hyacinth (*Eichhornia crassipes*) on POPs in e-waste leachate. The study assessed the ability of the water hyacinth to tolerate, extract, and bio-accumulate POP pollutant PCB from e-waste leachate. The study used a model leachate

solution of the study pollutants prepared in the laboratory using PCB101 congener of the PCBs.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 General Overview of PCB's Contamination

Landfilling of the wastes presents significant contamination concerns as toxins can leach into groundwater and soil. These pollutants include brominated flame retardants (BFR) found in TVs, circuit boards, connectors and cables; Polychlorinated biphenyls (PCB's) found in transformers and capacitors; lead, found in cathode ray tubes and circuit boards, and other heavy metals in LCD monitors. Townsend et al. (2003) showed that lead leached from waste electronic devices at concentrations greater than EPA regulatory toxicity limit of 0.5 µg/L. Smaller devices such as mobile phones and remote controls tended to leach higher concentrations of lead than larger devices.

The presence of BFR and PCB's in landfill leachate is of concern because they are toxic, bioaccumulative, and persistent in the environment. Levels of BFR and PCB in the environment and in humans are on the increase; for example, the levels found in United States (US) human breast milk are many times higher than levels found in other parts of the world (Shyamala, 2011). During the 1975 to 1995 period, PCB concentrations in the Upper Mississippi, US, ranged from 0.07 to 33.0 mg/kg for common carp, exceeding U.S. Food and Drug Administration guideline of 2 mg/kg PCB in fish tissue (Lee and Anderson, 2012). The U.S. Environmental Protection Agency (EPA) limit for PCBs in drinking water systems is 0.5 ppb or 0.5 µg/L (US EPA, 2012).

Sources of PCBs to the environment include emissions from manufacturing and emissions from the use of products. Since landfills are a depository for many items containing PCBs such as e-waste as well as textiles, furniture, and construction products, it is possible that PCBs could accumulate in landfills and form a long-term source. PCBs in landfill leachate can also be released back to the environment through sewage sludge if the leachate is sent to a wastewater treatment plant (Townsend et al., 2003).

2.2 Properties of PCBs

2.2.1 Chemical Composition

PCBs are chlorinated hydrocarbons that are manufactured commercially by the progressive chlorination of biphenyl in the presence of a suitable catalyst such as iron chloride. Depending on the reaction conditions, the degree of chlorination can vary between 21 and 68% (w/w). The yield is always a mixture of different compounds and congeners. Thus, 209 different chemical components may exist, but only about 130 of these are likely to occur in commercial products or mixtures of these compounds (Bretherick, 1986). Individual PCBs have been synthesized for use as reference samples in the identification of gas-liquid chromatographic peaks, for toxicological investigations, and in order to study their metabolic fate in living organisms.

2.2.2 Chemical Structure

The basic chemical structure of PCBs includes two benzene rings (known as the biphenyl) and between 1 and 10 chlorine atoms substituted on each of the benzene molecules. Figure 2.1 shows the basic structure of PCBs, where the numbers 2-6 and 2'-6' represent possible substitution locations for chlorine. There are 209 individual

PCB compounds, also known as congeners (Flynn, 1997). Typically, PCBs occur as mixtures of congeners or Aroclors (Bernhard and Petron, 2001). Aroclors are identified by number such as 1254, where the last two digits represent the percent content of chlorine; higher Aroclor numbers reflect higher chlorine content (ATSDR, 2001).

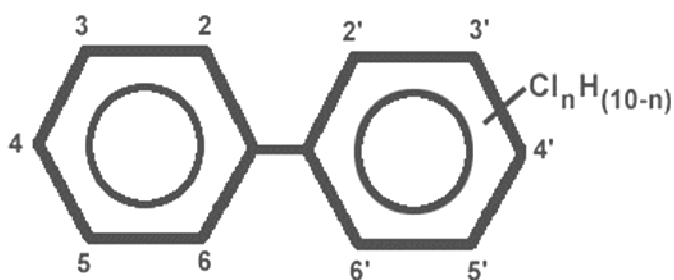


Fig. 2.1 Polychlorinated Biphenyl (PCB) Chemical Structure

2.2.3 Purity and Impurities

Commercial PCBs (Table 2.1) are sold based on their physical properties, and not their chemical composition. Different batches may vary somewhat in their composition. The impurities known to be present in commercial PCBs include chlorinated naphthalenes and small quantities of the highly toxic polychlorinated dibenzofurans (PCDFs). There are no authenticated reports of the presence of polychlorinated dibenzo- p-dioxins (PCDDs) in commercial PCBs (CEC, 1984).

2.2.4 Physical and Chemical Properties of PCB's

According to CEC (1984), individual pure congeners of PCB are colorless, often crystalline compounds, but commercial PCBs are mixtures of these congeners with a clear, light yellow or dark color, and range from oily liquids to waxy or hard solids. They do not crystallize at low temperatures, but turn

into solid resins (Sax, 1984). Because of the chlorine atoms in the molecule, the compounds have a fairly high density. In practice, PCBs are fire resistant, and have a fairly high flash-point (170 - 380 °C). They form vapours that are heavier than air, but they do not form an explosive mixture with air (Sax, 1984).

The electrical conductivity of PCBs is very low, and their resistance to thermal breakdown is extremely high. These properties make them suitable for use as cooling liquids in electrical equipment.

Table 2.1 Trade Names of Major PCBs (EPA Polychlorinated Biphenyls Inspection Manual, 2004)

1) Apirolio (t,c)	2) Disconon (c)	3) PCBs
4) Areclor (t)	5) Dk (t,c)	6) Phenoclor (t,c)
7) Aroclor	8) Duconol (c)	9) Polychlorinated biphenyl
10) Arubren	11) Dykanol (t,c)	12) Polychlorobiphenyl
13) Asbestol (t,c)	14) EEC-18	15) Pydraul
16) Askarel	17) Elemex (t,c)	18) Pyralene (t,c)
19) Bakola 131 (t,c)	20) Eucarel	21) Pyranol (t,c)
22) Biclor (c)	23) Fenchlor (t,c)	24) Pyroclor (t)
25) Chlorextol (t)	26) Hivar (c)	27) Saf-T-Kuhl (t,c)
28) Chlorinated Biphenyl	29) Hydol (t,c)	30) Santotherm FR(a)
31) Chlorinated Diphenyl	32) Inclor	33) Santovac 1 and 2
34) Chlorinol	35) Interteen (t,c)	36) Siclonyl (c)
37) Chlorobiphenyl	38) Kanechlor (t,c)	39) Solvol (t,c)
40) Clophen (t,c)	41) Kennechlor	42) Sovol
43) Chlorphen (t)	44) Montar	45) Therminol FR(a)
46) Delor	47) Nepolin	
48) Diaclor (t,c)	49) No-Flamol (t,c)	
50) Dialor (c)	51) PCB	

Notes :(a) Previous products (FR-series) used as heat transfer fluids contained PCBs, but current products do not contain PCBs
 (t) Used in transformers
 (c) Used in capacitors

PCBs are chemically stable under normal conditions. They are very resistant to a range of oxidants and other chemicals, and they remain chemically unchanged even in the presence of oxygen, or some active metals, at high temperatures (up to 170 °C), and for protracted periods (IRPTC, 1985).

PCBs have a relatively low vapor pressure. Despite their low volatility, PCBs do volatilize from both soil and water partly because of their hydrophobicity. Atmospheric transport may therefore be a significant contributor to their widespread presence (DEQ, 1997). Once emitted, PCBs can be transported long distances in air, and then re-deposited by settling or scavenging by precipitation. This cycling process continues indefinitely and is referred to as the grasshopper effect (EPA, 2001). It is estimated that there are currently 1,000 tons of PCBs cycling through the atmosphere over the U.S. (HSDB, 2003). Studies performed at Lake Michigan show that 80 percent of the PCBs entering the lake come from the air (Delta Institute, 2000). Additional evidence of the atmospheric deposition of PCBs is the presence of PCBs in sparsely populated areas of Canada and in Arctic polar bears, which are both far from point sources of PCB contamination (Fiedler, 1997).

PCBs are non-polar and therefore are only slightly soluble. This characteristic inhibits the transport of PCBs from soil to water (groundwater or surface water) and makes them bind strongly to soils. PCBs can be transported to surface water via entrainment of contaminated soil particles in surface water runoff. In water, a small portion of PCBs will dissolve, but the majority will bind to organic particles and bottom sediments (Nagpal, 1992). Although PCBs have a strong affinity for sediment, small amounts of PCBs are released from sediments into water over time (ATSDR, 2000). Once in the water,

PCBs are also taken up by small organisms and fish. PCBs accumulate in the fatty tissue of these organisms. Although PCBs are practically insoluble in water they dissolve easily in hydrocarbons, fats, and other organic compounds and they are readily adsorbed by fatty tissues (Sax, 1984). The partition coefficient ($\log K_{ow}$) values for all 209 PCB congeners range from 4.46 to 8.18.

2.3 Sources and Uses of PCBs

2.3.1 Uses of PCB's

The industrial usefulness of PCBs depends on their chemical inertness, resistance to heat, non-flammability, low vapor pressure (particularly with the higher chlorinated compounds), and high dielectric constant (Sax, 1984). In the U.S., the most commonly used Aroclors were: 1221, 1232, 1242, 1248, 1254, and 1260 (DEQ, 1997). These and other Aroclors were used in a variety of materials to enhance insulative properties, improve physical and chemical resistance, and act as plasticizers, coolants, and lubricants. Approximate usage of PCBs in the US is summarized as follows (EIP Associates, 1997):

- i. Closed system and heat transfer fluids (transformers, capacitors, fluorescent light ballasts, etc.): 60%
- ii. Plasticizers: 25%
- iii. Hydraulic fluids and lubricants: 10%
- iv. Miscellaneous uses: 5%

The primary applications of PCBs as well as other applications are presented in Table 2.2.

Table 2.2 Uses of PCB

Primary Applications	
Dielectric fluids and transformers	Used as insulating material, coolant, and for fire-resistant properties. Potential sources would be facilities which used, stored, and serviced electrical equipment and which used significant amounts of electricity e.g. electrical transmission and distribution facilities; electrical equipment maintenance facilities and salvage yards; rail yards; and manufacturing facilities (sawmills, pulp and paper mills, chemical manufacturing, shipyards, primary and secondary metals smelting and refining)
Capacitors	Present in industrial facilities, industrial machinery both fixed and mobile, and consumer products. Includes larger power-factor correction capacitors associated with transformers, manufacturing facilities, and commercial buildings (usually near high power-usage equipment such as computer rooms and heating and cooling units); and smaller electric motor-start capacitors used in industrial equipment and appliances such as hair dryers, air conditioners, refrigerators, power tools, and submersible well pumps.
Electromagnets	Oil-cooled electromagnets are constructed with coils immersed in transformer oil to prevent over-heating and shorting. Used in cranes for picking up metal and for metal separation in recycling operations (metal scrap yards, tire shredding, concrete crushing, slag operations, etc.).
Fluorescent light ballasts	PCB-containing capacitors were used in fluorescent light ballasts. PCB-containing asphaltic resin (potting material) was also utilized as insulating material for some ballast.
Miscellaneous electrical equipment	Switches, voltage regulators, circuit breakers, reclosers, rectifiers, and some oil cooled electric motors.
Heat transfer systems	Where oil is circulated through a non-contact system as a heat transfer medium for heating, cooling, and maintaining uniform temperature throughout a system or manufacturing process.
Hydraulic fluids	Any application of hydraulic oil such as industrial equipment and machinery, commercial equipment, automotive brake fluid, etc.

Plasticizers	Used in polyvinyl chloride plastic, neoprene, chlorinated rubbers, laminating adhesives, sealants and caulking, joint compounds (concrete).
Lubricants	Cutting oils, compressors, electrical equipment, oil-impregnated gaskets and filters; also currently present in low concentrations in recycled oil. Also used in vacuum pumps at high tech and electronics manufacturing facilities, research labs, and wastewater treatment plants.
Other applications	
Dust control (de dusting agents)	Present in dust control formulations, and used oil historically used for dust suppression.
Pesticides	As an extender to extend the life of pesticides.
Fire retardants	Coatings on ceiling tiles, and textiles including ironing boards and yarn.
Paints, coatings	As plasticizers in paint, corrosion resistant paints for various applications including military/navy ships, epoxy resins on metal surfaces, and waterproofing coatings for various applications.
Carbonless copy paper	Used as an ink pigment carrier (microencapsulation of dye); when the top sheet was pressed down, ink and PCB oil were transferred to the copy.
Printing inks	Ink for newsprint and as a dye carrier; also used as a solvent for deinking newsprint for recycling.
Investment casting waxes	Used as wax extenders.
Wood treatment	May be present as an impurity in pentachlorophenol (Warrington, 1996).

Sources: ATSDR (2000), DEQ (1997), EIP Associates (1997), UNEP Chemicals (1999)

2.3.2 Sources of PCBs in the Environment

Large volumes of PCBs have been introduced to the environment through burning of PCB-containing products, vaporization from PCB-containing coatings and materials, releases into sewers and streams, improper disposal of

PCB-containing equipment in non-secure landfill sites and municipal disposal facilities, and by other routes such as ocean dumping (ATSDR, 2001). A summary of sources of PCBs is presented in Table 2.3.

Table 2.3: PCB Sources in Waste Materials and Recycling Operations

Material / Operation	Comments
Scrap metal recycling	Transformer shell salvaging; heat transfer and hydraulic equipment; and fluff. Also present in non-ferrous metal salvaging as parts from PCB containing electrical equipment, and oil & grease insulated electrical cable.
Auto salvage yards, auto crushing	Hydraulic fluid, brake fluid, recycled oil, capacitors, and oil-filled electrical equipment such as some ignition coils.
Repair activities	Shipyards, locomotive repair, heavy equipment repair facilities, auto repair, repair of manufacturing equipment, etc.
Used oil	May be present in used oil from various sources including auto salvage yards, automotive and heavy equipment repair shops, hydraulic equipment repair, industrial machinery repair, etc.
Recycled paper	Paper may contain PCBs where carbonless copy paper has been used in recycling. However, PCB concentrations have decreased over time as the volume of unrecycled carbonless copy paper is reduced. Recycled paper containing PCBs has been used for food packaging (CWC, 1997). PCB concentrations in food packaging are restricted to 10 ppm unless an impermeable barrier is present between the packaging and food product (FDA, 2003).
Effluent	PCBs may be in wastewaters from manufacturing facilities and equipment such as chemical and pesticide facilities, pulp and paper mills, cooling waters from vacuum pumps and electric power generation facilities where leaks have occurred, and condensate from vacuum pumps and natural gas pipelines.
Asphalt roofing, and roofing felt	Anticipated at generally very low concentrations where used oil containing PCBs has been used in asphalt mix.

Building demolition	Electrical equipment, joint caulking, oil & grease insulated cable, surface coatings as flame retardant and waterproofing.
Landfills	Municipal and industrial solid waste; virtually all potential sources could be present, including waste materials and soils from remediation sites.
Wastewater treatment plant sludge	Derived from atmospheric deposition and storm water, water supply systems, leaks and spills, leaching from coatings and plastics containing PCBs, PCBs in food and human waste.

Sources: ATSDR (2000), DEQ (1997), EIP Associates (1997), UNEP Chemicals (1999)

2.4 Regulatory Framework for PCBs

In the US, PCBs were regulated under a series of EPA actions culminating with a ban in 1979 on manufacturing, processing, distribution, and use of PCBs under the Toxic Substances Control Act (TSCA). Items such as transformers and hydraulic fluids were identified as high-risk sources and were targeted for accelerated phase-out. EPA anticipated that other lower-risk sources would eventually be removed from circulation as various products reached the end of their useful lives. Certain current uses of PCBs are authorized under 40 CFR Part 761 as summarized in Table 2.4.

Use	Comments
Transformers	Authorized use at any concentration though restrictions and regulatory requirements increase with higher PCB concentration thresholds
Railroad Transformers	Transformers used in locomotives and self propelled railcars. Authorized use at <1,000 ppm;<50 ppm if transformer coil is removed at any time.

Heat transfer & hydraulic systems, mining equipment	Authorized use at <50 ppm
Natural gas pipelines	Authorized at < 50 ppm, or at > 50 ppm with additional requirements. PCBs may be present in natural gas compressors, scrubbers, filters, and in condensate.
Research & Development	Authorized primarily for purposes relating to environmental analysis, management, and disposal of PCBs. R&D for PCB products is prohibited.
Scientific Instruments	Examples include oscillatory flow birefringence & viscoelasticity instruments for the study of the physical properties of polymers, microscopy mounting fluids, microscopy immersion oil, and optical liquids.
Carbonless copy paper	Use of existing carbonless copy paper is permitted; manufacturing of new carbonless copy paper is not authorized.
Electromagnets, switches, voltage regulators, circuit breakers, reclosers, cable	No restrictions on existing use; restrictions on PCB concentrations if serviced and oil is removed or replaced.
Porous surfaces	EPA considers building materials, such as concrete, porous with respect to PCB leaks and spills. Porous building materials may be left in place following spills provided various conditions are met. Older industrial machinery often was designed to slowly leak (PCB-containing) hydraulic oil as a lubricant.
Source: EPA (2002)	

2.5 Kinetics and Metabolism of PCBs

Animal studies involving mainly oral, inhalation, and dermal exposures to PCB mixtures and individual congeners show in general that PCBs are rapidly absorbed, particularly by the gastro-intestinal tract after oral exposure. It is clear that absorption does occur in humans, but information on the rates of absorption of PCBs in humans is limited (US NIOSH, 1976). From the available studies, the data on the distribution of PCBs, suggest a biphasic

kinetic process with rapid clearance from blood, and accumulation in the liver and the adipose tissue of various organs (Gosselin et al., 1984). There is also evidence of placental transport, fetal accumulation, and distribution to milk. In some studies with humans, the skin was a tissue with a high concentration of PCBs but the concentration in the brain was lower than would be expected based on the lipid content (IARC, 1972).

Mobilization of PCBs from fat appears to depend largely on the rates of metabolism of the individual PCB congeners. Excretion depends on the metabolism of PCBs to more polar compounds, such as phenols, conjugates of thiol compounds, and other water-soluble derivatives (US NIOSH, 1976).

Metabolic pathways of PCB's include hydroxylation, conjugation with thiols and other water-soluble derivatives, some of which can involve reactive intermediates, such as the arene oxides. The rates of metabolism have been shown to depend on the PCB structure and reflect both the degree and position of the chlorine substituent. The polar metabolites of the more highly chlorinated PCBs appear to be eliminated primarily in the faeces, but excretion in the urine can also be significant. An important elimination route is via breast milk. Certain PCB congeners can also be eliminated via the hair (Gosselin et al., 1984).

The available kinetic studies indicate that there is a wide divergence in biological half-life among the individual congeners and this can reflect differences in structure-dependent metabolism, tissue affinities, and other factors, affecting mobilization from storage sites (IARC, 1972).

Persistence in tissues is not always correlated with high toxicity and differences in toxicity between PCB congeners may be associated with specific metabolites and/or their intermediates (Gosselin et al., 1984).

2.6 Effects of PCBs on Organisms in the Environment

PCBs are universal environmental contaminants and are present globally in most environmental compartments, both abiotic and biotic. Since many countries have controlled both use and release, new input into the environment is on a much smaller scale, compared with the past. However, the available evidence suggests that the cycling of PCBs is causing a gradual redistribution of some congeners towards the marine environment. There is a trend for the highest chlorinated congeners to accumulate preferentially. While a large proportion of the PCBs are adsorbed onto particulates in sediment, it is still bio available to organisms and will continue to be accumulated in the higher trophic levels (US NIOSH/OSHA, 1981).

2.7. Remediation by Surfactants

Surfactants are employed extensively to remove PCBs in soil washing and flushing processes. Surfactants have hydrophilic and hydrophobic groups in one molecule, which make them exhibit affinity to both water and oily liquids like PCB's. Surfactants are used in daily life and by many kinds of industries because of properties such as surface tension reduction, solubility enhancement wettability and foaming capacity (Tsuji, 1998). PCBs are mainly bound to the hydrophobic portion of the soil organic matter by nonspecific hydrophobic mechanisms, while metal ions are bound to the surface of soils by specific chemical interactions.

Several studies on desorption of hydro organic carbons such as PCBs, PAHs, Hexachlorobenzene (HCB), and tetrachlorophenol (TCP), using surfactants describe the main desorption mechanism as the partitioning of organic contaminants into hydrophobic micelles by hydrophobic interaction (Jafvert et al., 1995; Park and Boyd, 1999; Chu and Chan, 2003). The mechanisms by which metals are removed are slightly different, from those undergone by PCBs, and depend on the types of metals and surfactants. In a study where surfactants were used to remediate Cd from contaminated soil, enhancement of metal removal occurred mainly below critical micelle concentration (CMC) and increased slowly above the CMC (Doong *et al.*, 1998). This finding suggested that an ion exchange mechanism was occurring during Cd extraction. Mulligan et al. (1999) also investigated the mechanism for metal removal from soil by bio surfactants. The removal occurred through extraction from the soil surface by the surfactant, followed by complexation with the metal.

2.7.1 Types of Surfactants and their Mode of Action

Surfactants are classified as either ionic or nonionic, depending upon the character of their hydrophilic group. The hydrophobic group establishes the degree of hydrophobic property of the surfactant, based on the length of the hydrocarbon chain. The most popular hydrophobic group used in surfactants is a hydrocarbon that has a total of 10 to 20 carbon atoms. Surfactants can be made synthetically using fatty acids, paraffins, alkylbenzenes, alcohols, and polyoxypropylenes as a source of hydrophobic groups (Swisher, 1970). The typical types of synthetic surfactants include alkylbenzenesulfonates (anionic),

alkyltrimethyl-ammonium salts (cationic), alkylcarboxybetaine (zwitterionic), and polyoxyethylene alkyl ether (nonionic) (Tsuji, 1998).

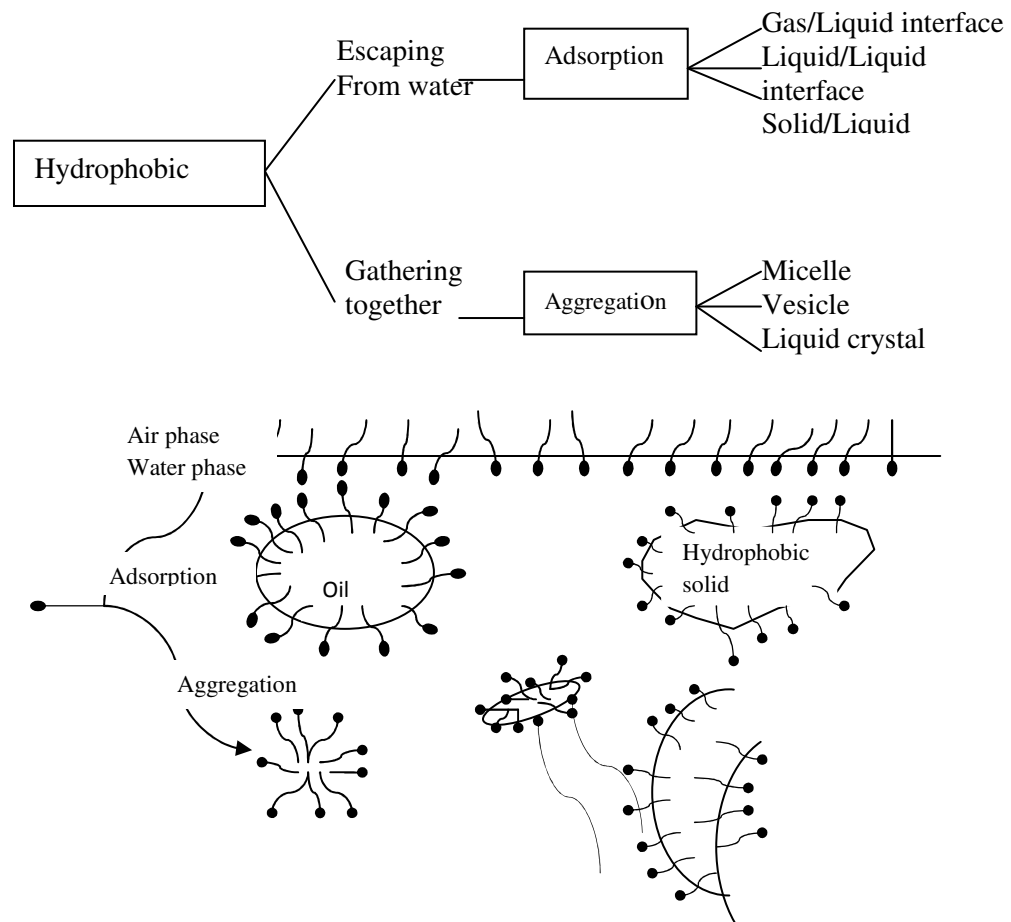


Figure 2.3 Schematic Representation for Surfactant Properties in Aqueous Solutions (Tsuji, 1998)

2.8 Use of Constructed Wet Lands in Waste Water Treatment

Several studies on use of constructed wetland to treat landfill leachate in New York were conducted in a greenhouse with half of the beds planted with phragmites and half left unplanted (Liehr et al., 1996). For a 15-day residence time, the effluent had COD of 480 mg/L, BOD₅ of 60 to 136 mg/L and Fe of 48 to 57 mg/L. BOD and nutrient removals were greater in planted than in unplanted beds.

Biological techniques used to remediate metal contaminated soil essentially involve the use of plants and organisms to remediate these soils (Umeoguaju, 2009). They include phytovolatilisation, phytostabilisation, phytofiltration, and phytoextraction processes. Constructed wetlands have proven to be a very effective method for the treatment of municipal wastewater. For a small community with limited funds, constructed wetlands are an attractive wastewater treatment option. Rural municipalities have access to adequate inexpensive land, and wetlands blend into a natural landscape setting. Once the wetlands are designed and constructed, annual maintenance costs are low (Renee, 2001).

The search for hyper accumulators in wetlands has led to intensive screening of many plant species. Metal resistance in plants varies among genera, species, and clones; some herbaceous species, such as grasses and mustards, have been found to be capable of accumulating significant amounts of metal in their tissues (Kuzovkina et al., 2004). Metals affected ciliate populations in activated sludge plants negatively depending on bioavailable concentration and nature of the heavy metal (Gonzalez et al., 2005).

Trace element removal by wetland vegetation can be greatly enhanced by selection of appropriate wetland plant species. The selection is based on the types of elements to be remediated, the geographic location, microclimate, hydrological conditions, soil properties, and known accumulation capacities of the species. Knowledge of the capabilities of different wetland plant species to absorb and transport trace elements under different conditions is important (Liao et al., 2004). One such plant is the vascular aquatic plant, water

hyacinth, which is commonly found in tropical and subtropical regions of the world. Water hyacinth is considered to be an ideal candidate for use in the rhizofiltration of toxic trace elements from a variety of water bodies (Liao et al., 2004).

Wetlands are constructed as either surface flow or subsurface flow systems. Surface flow systems require more land, but are generally easier to design, construct and maintain. They consist of shallow basins with emergent and submergent wetland plants that tolerate saturated soil and aerobic conditions. Water flows in at one end of the basin, moves slowly through, and is released at the other end (Renee, 2001).

Maine et al. (2006) reported the use water hyacinth system for the treatment of wastewaters from a tool factory in Santo Tome, Argentina. Tua (2006) reported the use of water hyacinth-based constructed wetlands for the treatment of fish processing wastewater in Vietnam. Perdomo et al. (2000) reported experimental use of water hyacinth for dairy wastewaters treatment in Uruguay. The system performed well but parallel system with *Typha* spp. exhibited better removal effect. Kalibbala et al. (2002) used ponds with water hyacinth for tertiary treatment of brewery wastewater in Uganda.

2.8.1 Water Hyacinth

Water hyacinth is a free-floating perennial water plant that forms large, dense mats on the water surface. Each plant consists of several broad, leathery leaves, spongy inflated petioles (leaf stalks), a crown and a mass of fine, hairy roots. Water hyacinth shows considerable variation in both leaf and flower

form. Leaves are smooth, hairless and glossy. They are generally a bright green colour and can be tinged a rusty yellow on their edges.

Seeds are 1 to 1.5 mm long and roughly egg-shaped, with ridges from end to end. They are long-lived and may survive in mud for up to 20 years. The seeds are dispersed by birds and can remain viable for 15-20 years. However, the main method of reproduction is vegetative, through stolons. A single plant under ideal conditions can produce 3,000 others in 50 days, and cover an area of 600 sq metres in a year.

Roots are fibrous and featherlike. In deep water, they may trail below the plant and can be up to 1 m in length. In shallow water, the roots may take hold in the substrate of mud or sediment.

Flowers are 4 to 7 cm across, funnel-shaped, light bluish-purple or dark blue with a yellow centre and have six distinct petals. The upper petal is darker purple with a yellow mark in the centre. Flowers can self-fertilize and are formed on upright stems with between 3 and 35 but commonly 8 flowers on each spike. Water hyacinth can show variation in form depending on growth conditions (Julien et. al, 1999).

Water hyacinth infestations increase most rapidly by the production of new daughter plants. During high water flows and flooding, infestations can break up and be moved to new locations.

2.8.2 Water Hyacinth Nutrient Requirements

The growth of water hyacinth is correlated to the availability of nutrients in the water. The more nutrients, especially N and P, available the more luxuriant

the growth of the plant (Reddy et al., 1989; Reddy et al., 1990; Gopal, 1987). Under ideal situations the time to double the biomass of water hyacinth plant is 6 - 14 days and as soon as the available water surface is covered, the plants start to compete for available resources, like sunlight and grow taller (Penfound and Earl, 1948). Reddy et al. (1989) and Reddy et al., (1990) found that very high levels of N and P can have a detrimental effect on the growth of the plants and production of daughter plants.

Studies carried out by Reddy and Toker (1983) found that water hyacinth yielded optimum results of biomass yield where N:P ratio in water medium was 2.3 - 5. Reddy et al., (1989) found optimal tissue-N concentration for water hyacinth at 16 mg N g-L.

According to Reddy et al. (1989), water hyacinth biomass quantity and quality were significantly affected by supply of potassium. A supply of 12-52 mg K liter-L resulted in maximum productivity. The optimal tissue-K content required to achieve maximum productivity was 22 mg K liter-L.

2.9 Phytoremediation

Presently, over 400 species of plant are identified to have potential for remediation of water sources (Lone et al., 2008). Phytoextraction can also be used for the recovery of precious metals such as gold, silver, platinum, and palladium, which indicates the wide possibilities of the phytoremediation technology with regards to mining (Torresdey et al., 2005). The ability of water hyacinth (*Eichhornia crassipes*) to absorb and translocate cadmium lead, copper, zinc, and nickel in wetlands has been investigated by Liao and Chang (2004). According to the study, concentrations of extractable Fe, Mn,

Zn, Ni and Cd were 0.491, 0.104, 0.079, 0.032, and 0.071 ppm, respectively, before phytoremediation, but 0.298, 0.080, 0.061, 0.028, and 0.063 ppm respectively, after 24 h from phytoremediation, and 0.091, 0.026, 0.020, 0.014, and 0.033 ppm, respectively, after 48 h.

Phytoremediation of heavy metal in water accumulated by aquatic macrophytes have been investigated Kumar (2008). The study used roots, stems and leaves of native aquatic plants (biomonitors) represented by seven species: *Ipomoea aquatica*, *Eichhornia crassipes*, *Typha angustata*, *Echinochloa colonum*, *Hydrilla verticillata*, *Nelumbo nucifera* and *Vallisneria spiralis*. The result showed the greatest and lowest accumulation of heavy metals was in *N. nucifera* and *E. colonum*, respectively. The detected values of cadmium and lead were within normal range, while that of cobalt and nickel were within the critical range. Zinc and copper showed the highest accumulation at alarming toxicity levels (Kumar et al., 2008).

2.9.1 Types of Phytoremediation

The US EPA Phytoremediation Resource Guide (EPA, 1999) gives the definition of six types of phytoremediation and their applications. Phytoaccumulation, also called phytoextraction, refers to the uptake and translocation of metal and other contaminants in the soil by plant roots into the above ground portions of the plants. Certain plants called hyper accumulators absorb unusually large amounts of pollutants in comparison to others and to the ambient metals concentration. These plants are selected and planted at a site based on the type of contaminants present and other site conditions. The second form of phytoremediation involves the breakdown of the contaminants taken up by the plants through metabolic processes within the plant, or the

breakdown of contaminants external to the plant through the effect of compounds such as enzymes that are produced by the plants. This type of phytoremediation is called phytodegradation or phytotransformation (Table 2.5.). Pollutants are degraded, used as nutrients and incorporated into the plant tissues. In some cases metabolic intermediate or end products are re-released to the environment.

Table 2.5 Types of Phytoremediation for Organic Constituents (ITRC, 1999)

Types of Phytoremediation	Process involved	Contaminant treated
Phytostabilization	Plants control pH, soil gases, and redox conditions in soil to immobilize contaminants. Humification of some organic compounds is expected.	Expected for phenols, chlorinated solvents (tetrachloromethane and trichloromethane) and hydrophobic organic compounds
Rhizodegradation	Plant exudates, root necrosis, and other processes provide organic carbon and nutrients to spur soil bacteria growth by two or more orders of magnitude. Exudates stimulate degradation by mycorrhizal fungi and microbes. Live roots can pump oxygen to aerobes while dead roots may support anaerobes.	Poly aromatic hydrocarbons, BTEX, and other petroleum hydrocarbons, perchlorate, atrazine, alachlor, polychlorinated biphenyl (PCB), and other organic Compounds
Rhizofiltration	Compounds taken up or sorbed by roots or sorbed to algae and bacteria	Hydrophobic organic chemicals
Phytodegradation	Aquatic and terrestrial plants take up, store, and biochemically degrade selected organic	Munitions(TNT,DNT,HMX, nitrobenzene, picric acid, nitrotoluene), atrazine, halogenated

	compounds to harmless byproducts, products used to create new plant biomass, or byproducts that are further broken down by microbes and other processes to less harmful products. Reductive and oxidative enzymes may be used in series in different parts of the plant.	compounds (tetrachloromethane, trichloromethane, hexachloromethane, carbontetrachloroethene, dichloroethene), DDT and other chlorine and phosphorus based pesticides, phenols, and nitrates.
Phytovolatilization	Volatile organic compounds are taken up and transpired. Some recalcitrant organic compounds are more easily degraded in the atmosphere (phyto degraded).	Chlorinated solvents (tetrachloromethane and trichloromethane), organic VOCs, BTEX, MTBE

In the case of polluted soils and ground water, another form of phytoremediation called phytostabilization enables certain plant species to immobilize contaminants through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone and physical stabilization of soils. Once the plant has taken up the pollutants, phytovolatilization helps the plant in transpiration of the contaminant, with release of either the contaminant or often a modified form of the contaminant to the environment.

Plants can also biodegrade the pollutants within the soil through microbial activity that is enhanced by the presence of the rhizosphere. Microorganisms such as yeast, fungi, and/or bacteria, consume and degrade or transform organic substances for use as nutrients. This form of phytoremediation is called rhizodegradation. In some cases, the plant roots may adsorb or

precipitate the contaminants when contaminants are in solution surrounding the root zone. This type of phytoremediation is known as rhizofiltration (ITRC, 1999).

2.9.2 Phytoremediation by Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) has drawn attention as a plant of rapid growth and high biomass production, and, therefore, having potential for removing pollutants from domestic and industrial waste effluents. The ability of water hyacinth to remove nutrients and other chemical elements from sewage and industrial effluents has been demonstrated (Saltabas and Akcin, 1994). Removal of chromium, copper and nickel, the suggested that water hyacinth may be used for industrial effluents. Haselow et al. (1992) indicated that managed growth and harvesting of water hyacinth can be utilized for water quality improvement in lakes, but only if the plants are mechanically removed from the system. Aoyama and Nishizaki (1991) evaluated the practical use of water hyacinth grown in natural water channels or ponds for water purification. They recommended a shorter harvesting interval to obtain more yield of the biomass.

Jebanesan (1997) indicated that water hyacinth was effective in the treatment of dairy wastewater. In his study, turbidity decreased in undiluted effluent by 30.48 %, but after dilution, turbidity also decreased by 48.59 % during the treatment period. Total solids showed a pronounced reduction in both diluted and undiluted effluents ($p < 0.05$) in the *Eichhornia* sp. Different nutrients like potassium, sodium ($p < 0.05$) and nitrate, sodium showed the highest reduction of 83.74 %. In the effluent control, both BOD and COD did not show much variation.

2.9.3 Use of Water Hyacinth in Pytoremediation of Organic Contaminants

The use of water hyacinth in wastewater treatment was studied extensively in 1970s and early 1980s. However, little scientific literature information appeared because the systems studied were not economical (Sengupta et al., 2008). Stewart et al (1987) pointed out that while the concept of using water hyacinth for treatment of wastewater had been extensively investigated and reviewed for nearly 20 years, there was still little information regarding the operational needs associated with large scale operations. Also, the use of water hyacinth is extremely temperature-dependent and is restricted to subtropical and tropical regions.

Water hyacinth has been employed for wastewater treatment in many parts of the world. Part of the gaseous oxygen produced by photosynthetic activity of the green leaves is translocated to the stems and roots and to the water body; this oxygen is used by the aerobic and facultative bacteria in biodegrading organic matter contained in the wastewater (Nevana et al., 2006).

Nor (1994) found *Eichhornia crassipes* has tremendous capacity to absorb phenolic compounds and Cu and Zn simultaneously. The presence of Cu or Zn resulted in decreased phenol uptake during the first 0.5 day, while the presence of both Cu and Zn resulted in higher phenol uptake. After one-day exposure, however, little or no differences could be discerned. Similarly, absorption of Cu and Zn in the presence of phenols did not seem to be adversely affected by different combinations of Cu and Zn concentrations. In fact, some synergistic effect could be detected between Cu and Zn absorption. Bioassays involving filtered *Eichhornia* root extract indicated that phenol was

also removed by the aqueous root extracts indicating that cellular constituents of roots were involved in the process.

Cyanide degradation by water hyacinths in solutions containing 3 - 300 mg/L cyanide was investigated in batch tests (Granato, 1993). Water hyacinth was more efficient in removal of free cyanide in the first eight hours, compared to cyanide controls. Gold mill synthetic effluents containing free cyanide, 9 to 20 mg/L; thiocyanate, 14 to 23 mg/L; and metalocyanides of iron, copper and zinc was fed to a continuous laboratory scale unit; copper and thiocyanate remained unchanged in the solution.

Water hyacinth was found to grow in oil-refinery wastewater after the water had undergone an initial treatment of oil-separation, flotation and aeration (Tang and Xian-wen, 1993). The optimum COD under which *E. crassipes* can be used to oxidize oil-refinery wastewater was found to be between 65 and 131 mg/L and the highest possible COD as 262 mg/L. *E. crassipes* exceeded un-vegetated control reduction of turbidity by 32%; phenol oil, 18%; COD, 8 - 13%; nitrogen, 6 - 18%; phosphorus, 12 - 24%; and several heavy metals 3 - 54%. In addition, its oxygenated root zone helped to bring about flotation and flocculation of oil residue, increased aerobic degradation, and developed large microbial population to stimulate decomposition.

CHAPTER 3

3.0 METHODOLOGY

3.1 Introduction

To achieve the objectives of this study, growth of water hyacinth plants in PCB polluted water was simulated under laboratory conditions. The removal of PCB from the water by young water hyacinth plants grown in water spiked with different concentrations of PCB was measured with GC/MS and compared with control in uncontaminated water. PCB removal was evaluated for three initial concentrations of PCB and an exposure period of 12 days. More frequent periodic sampling was not practical because of the large sample required for each test and the cost of analysis, was about US\$100 per sample. Bioaccumulation of PCB in the plant tissues, roots stem and leaves was measured for each of the PCB concentrations.

3.2. Water Hyacinth Planting, Growth and Maintenance

Young water hyacinth plants of similar size, health and foliage color and an average height 15 ± 2 cm were obtained from Nairobi Dam. The plants were transported in large open buckets in the water of origin to ensure smooth transition from the dam environment to the laboratory environment. The buckets were half filled with the water and packed with water hyacinth forming a canopy to control splashing during transport.

During the study period, experimental plants that were not in use were kept floated in the nutrient boosted water and exposed to the natural environment in an open area conducive for plants growth. The plants were enclosed in a lockable welded wire mesh cage with the shoots exposed.

3.3 Supplement Nutrient Level Determination.

To obtain vibrant growth of water hyacinth plants, essential nutrients of NPK fertilizer were added to tap water used to grow the plants. The N:P ratio is important for the growth of water hyacinth because phosphorus uptake is directly proportional to nitrogen availability (Reddy et al., 1990). Reddy and Toker (1983) found N:P ratio of 2.3 to 5 in a water medium yielded optimum growth of water hyacinth biomass.

Confirmatory tests for the nutrient dosage were carried out by dosing 17:17:17 NPK fertilizer 0.6 g/L below and above the recommended dosage of 3.2 g/L (Reddy et al 1990). The plants were left to grow for a period of 15 days and the dosage yielding optimal performance was established for adoption in the study (Table 3.1).

Table 3.1 Optimal Nutrient Requirement for Water Hyacinth

Sample	NPK Dosage (g/L)	Shoot			Roots		
		Original height (mm)	Final height (mm)	Percent growth (%)	Original length (mm)	Final length (mm)	Percent growth (%)
1	2.8	155	172	11.0	2	57	275
2	3.2	159	181	13.8	2	78	375
3	3.8	157	178	13.4	2	44	220

The 3.2 g/L dosage yielded the best overall growth recorded at 13.8% for the shoot and 375% for the roots. Furthermore, healthier foliage was observed in the plants in the water with this dosage.

3.4 Preparation of Model Leachate Solution

A representative leachate solution that contained selected congeners of PCB was prepared. Concentration of PCB was varied for different containers in the increasing orders of magnitude. PCB solution with concentrations of 5, 10, and 15 µg/L were placed in containers 1, 2 and 3, respectively. These concentrations were one to two orders of magnitude greater than the US EPA guideline limit of 0.5 µg/L for PCB (EPA, 2007).

The PCB congener, Penta CB, commonly referred to as CB101, was used for this investigation. 20 ml of 2, 2', 4, 5, 5'-Pentachlorobiphenyl (PCB No.101 solution, analytical standard for environmental analysis, 10 ng/µL in isooctane) was procured from Kobian Scientific Kenya Limited. The chemical remained under custody of the supplier until the set up for its use was completed.

PCBs disperse poorly in water and, therefore, require the aid of a surfactant to improve solubility. Methanol was used as the surfactant. Three representative batches of leachate concentrations were prepared by varying volumes of PCB spiked to 5 g of soil powder and varied volumes of analytical grade of methanol. The matrix was thoroughly mixed in a Pyrex glass tube by shaking for three minutes and then brought to two liters volume with tap water for each container (e.g. Chu et al., 2003).

3.5 Experiment Set-Up

3.5.1 Assessment of Water Hyacinth PCB Tolerance by Growth

The tolerance of water hyacinth to PCB was evaluated by comparing the extent of growth in a polluted environment to that of non polluted free -

nutrient enriched water. Three similar plants were grown in 5, 10, and 15 $\mu\text{g/L}$ concentrations of PCB 101 and their growth evaluated by measuring the length of a single marked shoot (petiole) and the fiber roots in every container and comparing it to that of control (PCB free environment). Measurements of the lengths of roots and shoot were taken every three days over a period of twelve days. Additionally, plants were observed for occurrence of dented growth, wilt and rust coloration.

During the exposure period, the plants growth, health and vigor were noted. On the fifteenth day and after sampling for PCB tests, the plants were lifted off the solution, rinsed in distilled water and allowed to drip for five minutes (Yongchul et al., 1999). They were then weighed using Denver XL-+810 electronic balance. The difference in weight was used to establish the overall growth by change in mass. Growth parameters of the plants were used to determine the tolerance of the plants in different concentrations of PCB. The stems and rinsed roots were stored for bioaccumulation tests.

3.5.2 Phytoremediation

A set of three, two liter containers, were thoroughly washed in tap water and rinsed with distilled water. The containers were filled with 5, 10 and 15 $\mu\text{g/L}$, PCB leachate solutions prepared previously and enriched with essential nutrients by adding NPK 17.17.17 at 3.2 g/L for vibrant growth of the hyacinth.

Selected young and healthy water hyacinth plants were weighed on LIBROR AEG-220 electronic balance to obtain their initial weight before exposure to the PCB matrix solution. The lengths of shoot and roots were then measured

and the plants placed in the PCB matrix solutions in a protected area, open to the natural environment and monitored.

The test plants were left to grow in the solution for a retention period of 15 days, which is close to the 15 days recommended for wetlands (Leir et al., 1996). On the twelfth day, 10 ml of the matrix solution was sampled and stored at 4°C for extraction and PCBs analysis. Analyses for PCB were carried out at the Chemistry Department of Jomo Kenyatta University of Agriculture and Technology (JKUAT) using Konik 400B GC/MS instrument equipped with a capillary column and automatic injector equipment.

3.5.3 PCB Bioaccumulation

The plants removed from the solution at the end of the twelfth day were prepared for bioaccumulation tests following USEPA SW-846 Method 3540C. The plant materials were cut into smaller pieces of approximately 15 mm sizes and oven dried for 48 hours at room temperature to control possible volatility. To ensure uniform distribution of the bioaccumulated pollutants in the various samples, the dry materials were ground into powder using a mortar and pestle. About 5 g of the ground samples were weighed and extracted with methylene chloride-acetone (1:1) using automated soxhlet extractor. The extract was then subjected to sulfuric acid for clean-up. Subsequently, two millimeters of the aliquot of the extract was injected into a GC/MS (Model) for PCB analysis (Alma et al., 2010).

3.6 PCB Extraction Process

Water samples were extracted and analyzed using EPA Method 608 while plant material were extracted and analyzed using USEPA SW-846 Method

3540C. Solid samples were weighed before extraction using a bath type Soxhlet extractor unit No.3456A from IKEDA Scientific Company Limited. The extract was then filtered overnight in a filtration column comprised of filter paper; anhydrous sodium sulphate and activated charcoal to remove color. The extract was then concentrated to smaller volumes of about 1 mL in a rotary vacuum evaporator - RE100 and finally stored for analysis.

Extraction of liquid samples was carried out by measuring 500 mL of the sample in a graduated measuring cylinder and transferring to a reparatory funnel for liquid-liquid extraction using a 1:1 ratio hexane: acetone mixture and organic layer separated from water layer. The organic layer was then transferred to a filtration column and left overnight for color removed. After extraction, the liquid extracts were stored at 4°C until analysis.

3.7 Mass Spectrometer Calibration

Three concentrations of calibration standards were prepared by adding volumes of stock standard 10 ng/uL PentaCB 101 (2, 2', 4, 5, 5'-Pentachlorobiphenyl) in isooctane to a volumetric flask and diluting to volume with isooctane. One of the external standards was prepared at a concentration above the minimum detection limit (MDL) and the other concentrations corresponded to the expected range of concentrations in the test samples in order to define the working range of the detector.

Using injections of 2-5 µL, each calibration standard was analyzed according to Section 12 of EPA Method 608, and peak height and area responses against the mass injected read from the chromatograms (Figures 3.1 and 3.2) and tabulated in standard curve data (Table 3.2). This information formed the

calibration for measurements of compounds present in the analytes of PCB spiked and plant waste matrix material. The results were interpreted by making reference to the national institute of standards and technology (NIST) PCB library chromatograms (Fig 3.3).

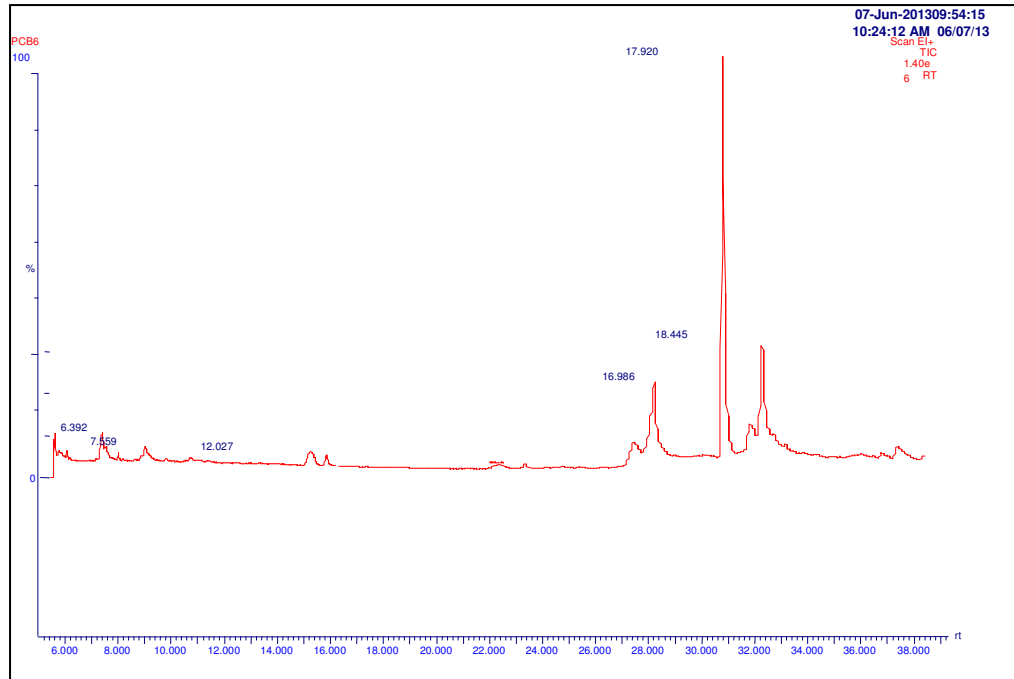


Figure 3.1 Chromatogram Standard - PCB Eluting at Retention Time 17.98 min

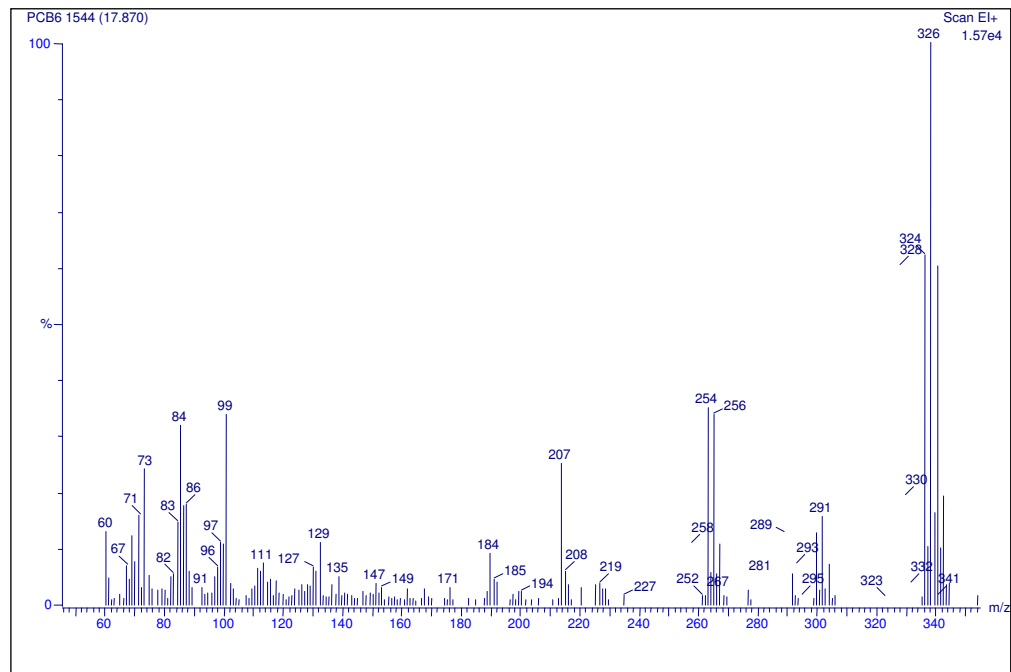


Figure 3.2 Mass spectrum of the PCB

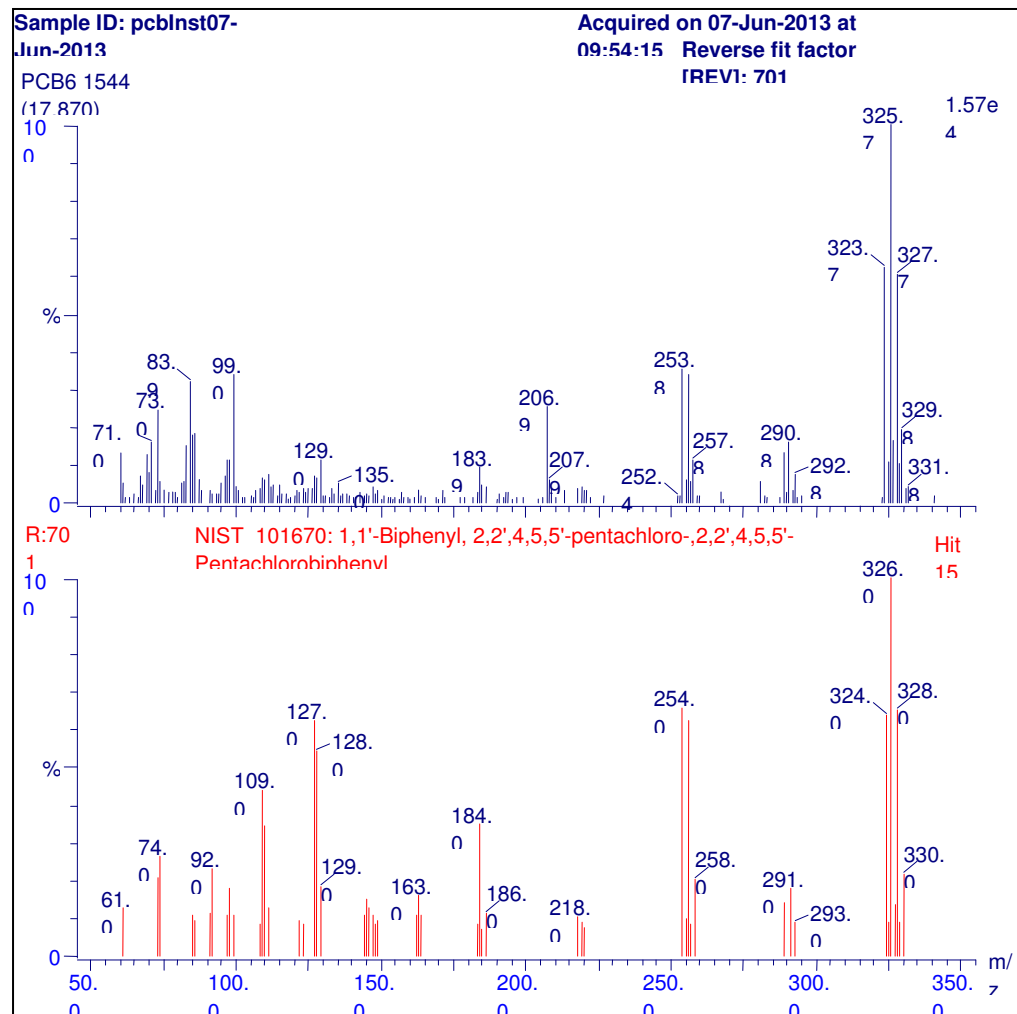


Figure 3.3 Library confirmation of the PCB

Table 3.2 Standard calibration curve Data

Concentration (µg/L)	0	0.5	1	2	3	4
Peak Area (mm ²)	0	110684	206987	443011	701985	893222

The standard calibration data were used to prepare a standard calibration curve for the PCBs shown in Figure 3.4 (Wager et al., 2011).

Six number peaks areas corresponding to injection of pure PCB substance were recorded from the GC chromatograms generated by varying the quantity in μL , injected and the attenuation setting for each chromatogram peaks. A calibration line for the substance (Figure 3.4) was plotted using Vernier's Graphical Analysis program. A linear regression was performed on the line to determine the slope and y-intercept of the line. The concentrations for the unknown mixture were determined by reading the area under the chromatogram peak and the corresponding concentrations.

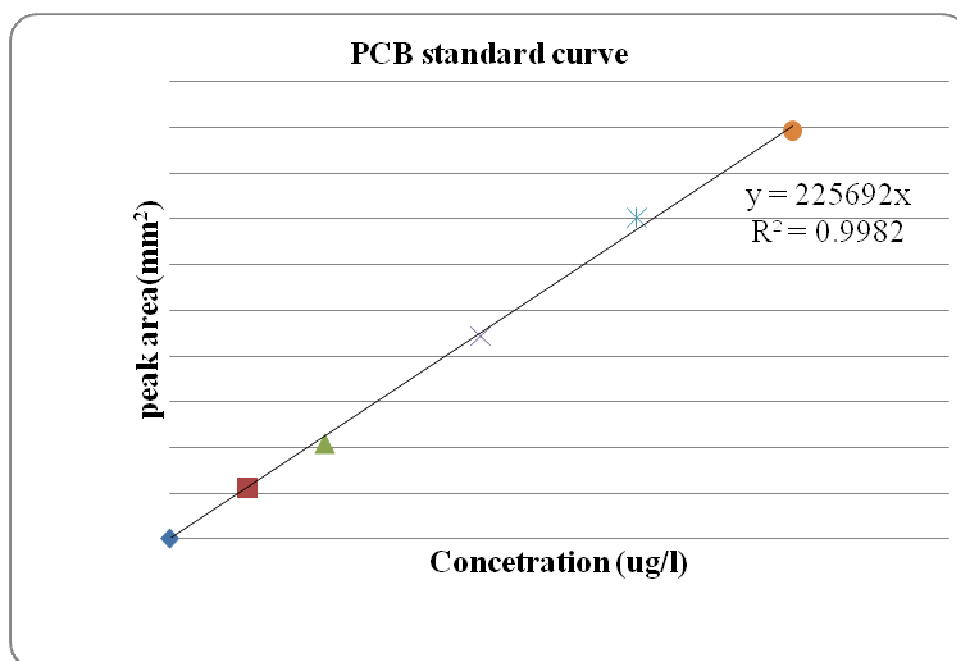


Figure 3.4 PCB Standard Curve

3.8 Measurement of PCB Levels

PCB levels in the extracted samples were initially analyzed using Konik 400B GC/MS at the Kenya Coffee Research Foundation in Ruiru but the detection level of the equipment was found to be inadequate. Consequently, analyses were carried out using Finnigan GC 8000 series with Voyager EI-MS Detector, CE Instrument at JKUAT, which resulted in more distinct peaks.

3.8.1 PCB Chromatogram Analysis

The reference standard of PCB 101 of 0.01 g/L concentration was injected into the Finnigan GC 8000 series with Voyager EI-MS Detector, CE Instrument and the peak area units read. Samples were concentrated to dryness and later reconstituted with 250 mL of HPLC grade of hexane before injection to the GC. An aliquot of the extract was injected into the gas chromatograph (GC) where the analytes were separated by the GC and detected by a mass spectrometer (MS). Since the sensitivity of the MS was low, a double injection was performed by increased volume from one micro liter to two micro liters.

3.8.2 PCB Quantification from Chromatograms

Quantification was based on peak areas from mass chromatograms. To convert the peak areas to mass of analyte, the peak areas were calibrated. The two main strategies were available based on external and internal standards. With external standards, the area of one or more mass chromatogram is calibrated with a known amount of the analyte injected into the GC-MS in a different experiment. Detection limits of a few nanograms can be achieved with this technique. In this study, internal standards which give most accurate quantitative results were used. Ten millimeters of compounds were added to the sample before isolation of the analytes began. After sample extraction and cleanup, only the ratio of response between the analyte and the internal standard was measured. This ratio multiplied by the amount of the internal

standard gave the amount of the analyte injected into the GC-MS system. The amount was converted to concentration using appropriate dilution factors.

CHAPTER 4

4.0 RESULTS AND DISCUSIONS

4.1 Water Hyacinth Tolerance to PCB

The tolerance of water hyacinth to PCB in water was evaluated by comparing the growth of roots and shoots in unpolluted and polluted environments. For both environments, the plants were grown in water enriched with 3.2 g/L of 17:17:17 N:P:K fertilizer to provide nutrients for stimulating growth. The results are presented in the following sections.

4.1.1 Plant Growth by Length

The growth of shoots and roots are presented in Figures 4.1 and 4.2, for PCB free environment and Figures 4.3 and 4.4, for three concentrations of PCB spiked water.

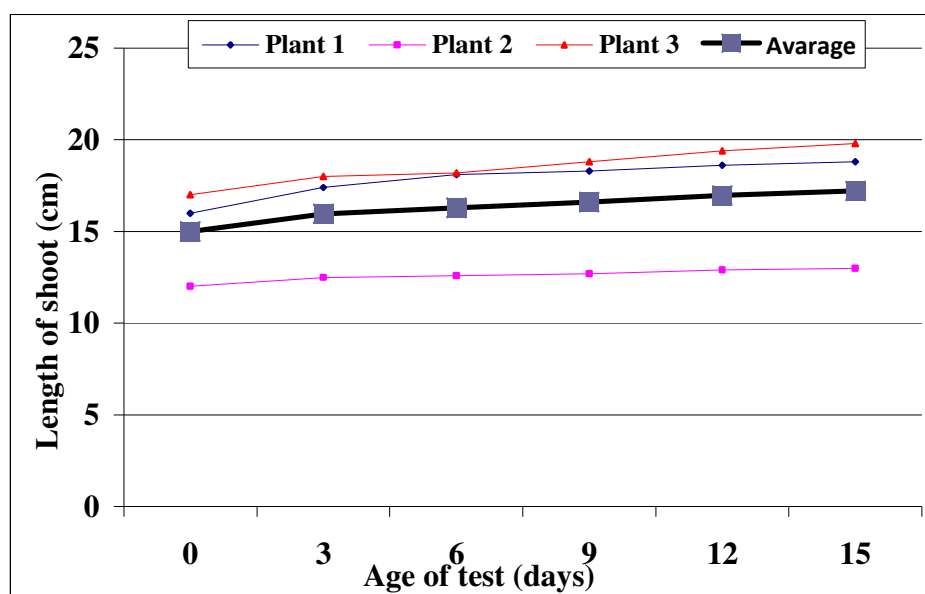


Fig 4.1 Growth of Shoot in PCB Free Environment

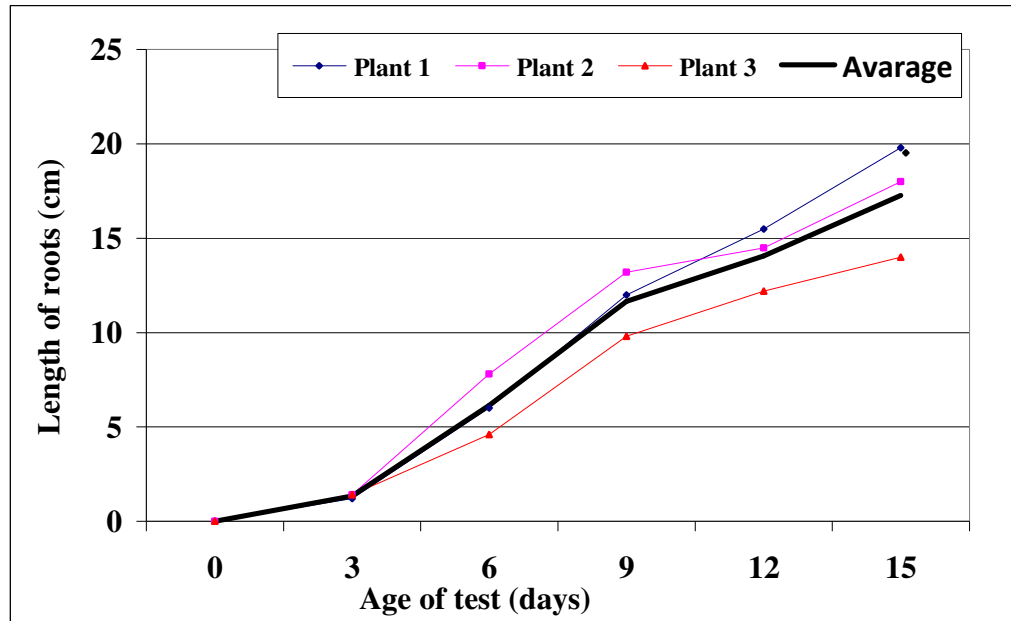


Fig 4.2 Growth of Roots in PCB Free Environment

Shoots grew approximately uniform elongating by 1.0 to 2.8 mm or 10 - 25% growth over 15 days. The roots exhibited a more rapid growth compared to the shoots. The roots grew gradually for the first three days but at an increased rate for the next nine days achieving 12 to 16 mm lengths equivalent to 12 to 18 times increase in length. Consequently, both the roots and shoots showed significant growth with the roots being particularly vibrant.

The growth of roots and shoot of water hyacinth in nutrient enriched water spiked with 5, 10 and 15 $\mu\text{g/L}$ PCB was observed by measuring the roots and shoots after every three days over a period of twelve days. The shoots showed nearly uniform growth of by between 0.8 and 2.2 cm or 9 to 12%. The roots grew from almost nil to between 12 and 14 cm. Similar to the shoots, the roots grew gradually for the first three days and rapidly for the remaining twelve days.

The growth of the plants in both PCB free water and in PCB spiked was similar in pattern and percentage increment.

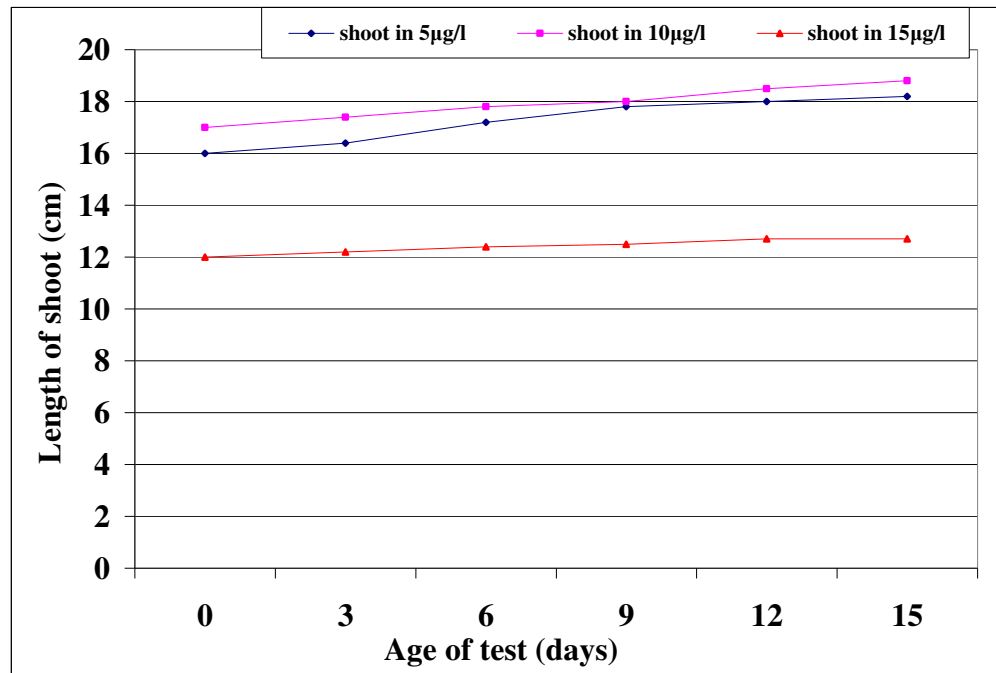


Fig 4.3 Growth of Shoots in PCB Spiked Water

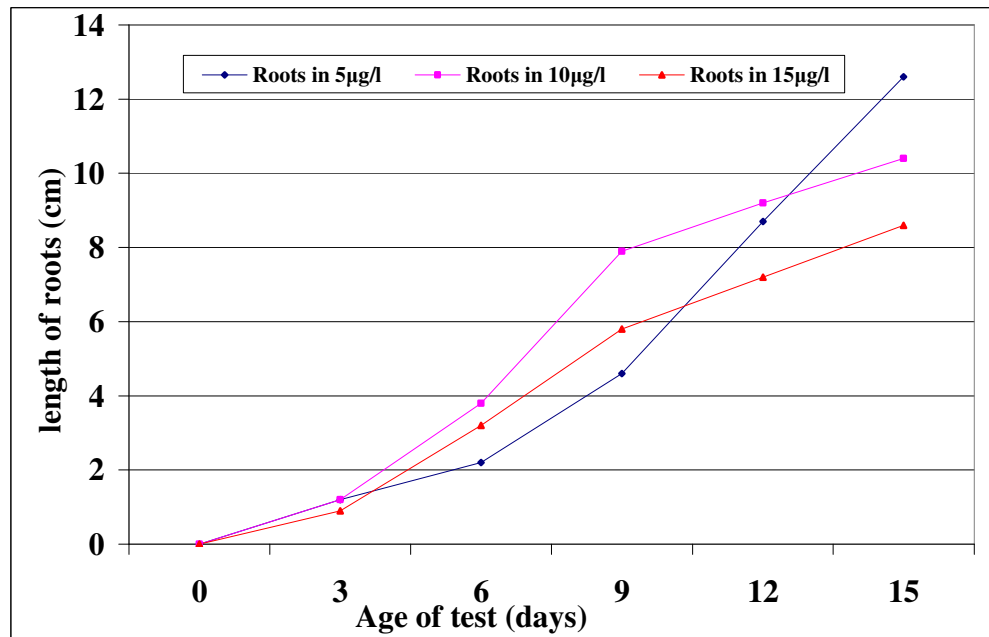


Fig 4.4 Growth of Roots in PCB Spiked Water

Therefore, PCB concentrations in the 5 to 15 µg/L range may not have affected growth of the plants.

4.1.2. Growth by Weight

The growth by weight of water hyacinth was assessed by weighing the plants before and after exposure to nutrient enriched water spiked with 5, 10 and 15 µg/L concentrations of PCB (Table 4.1).

Table 4.1 Growth by Weight for Plants in PCB Spiked Water

Initial PCB concentration (µg/L)	Initial Plant Weight (g)	Final Weight (g)	Growth by Weight (g)	Percent Growth by Weight (%)
5	68.15	75.8	7.65	11.2
10	83.32	91.8	7.48	10.2
15	97.36	106.2	8.84	9.10

The above data shows the plants tolerated the 5 to 15 µg/L PCB concentrations as indicated by 9 to 11% gain in weight. Although the percentage weight gain appears inversely proportional to PCB concentration in the matrix solution, the small difference in growth, different initial weight of plants and use of only three samples make the result inconclusive.

4.2 Observations of Plant Growth in PCB Spiked Water

The growth of the plants was monitored visually within the retention period. The plants grown in 15 µg/L PCB spiked water were adversely affected within the first three days of acclimatization. Although the roots remained vibrant, change in colour to purple was observed. The roots intensity of purple coloration was greater for 15 µg/L than for 5 and 10 µg/L PCB spiked waters. Additionally, older peripheral leaves of plants in 15 µg/L PCB spiked water

showed signs of wilting within the first three days. However, the effect did not seem to extend to other leaves as they remained vibrant throughout the fifteen days retention period. These results indicate some initial stress in the plants which however did not affect their vibrancy. The photos (Plate 1) illustrate the observations.



(a)



(b)

Plate 1: Root colour for (a) PCB free water and (b) 15µg/L PCB spiked water

4.3 Water Hyacinth Phytoremediation of PCB

The results of phytoremediation of PCB by water hyacinth are summarized in Table 4.5. The concentrations of PCB after 12 days for three different initial concentrations are derived from the results of PCB analysis presented in chromatograms in Figure 4.5. Water hyacinth reduced PCB concentrations from 5 and 10 µg/L to concentrations below detection limit of 0.142µg/L and up to an average of 0.42 µg/L for 15 µg/L concentration.

Table 4.5 Summary of Phytoremediation Test Results

Initial concentration (µg/L)	Sample No.	PCB Concentration in (µg/L)
		Solution
5	1	BDL
	2	BDL
	Average	BDL
10	1	BDL
	2	BDL
	Average	BDL
15	1	0.44
	2	0.40
	Average	0.42

BDL – Below Detection Limit

The GC-MS was used to measure the concentration of the analytes in their complex mixtures. The chromatograms for products derived from the extracted PCB spiked water (Figures 4.5 and 4.6) depict peaks obtained for for two samples from the 15 µg/L PCB concentration test. The peaks at 17.903 minutes (Figure 4.5) and 17.92 minutes (Figure 4.6) were for PCB101 initially spiked in water; the identities of the other peaks were not clear.

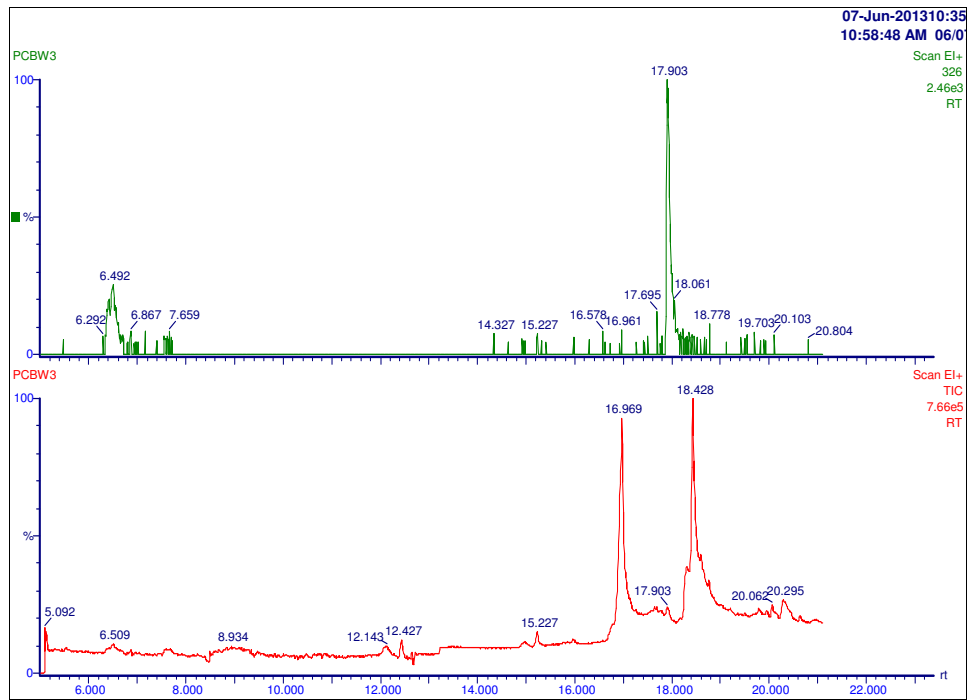


Fig. 4.5 PCB Spiked Water Sample I Chromatogram (15µg/L)

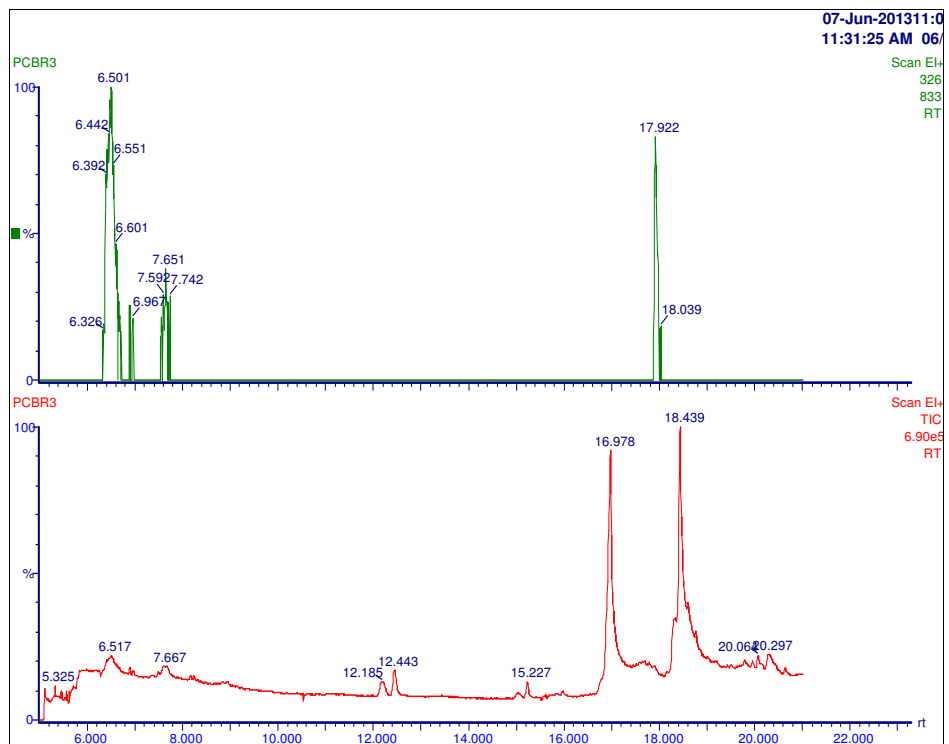


Figure 4.6 PCB Spiked Water Sample II Chromatogram (15µg/L)

4.4 Bioaccumulation

PCB concentration in the extracts for the roots and shoot of plants for 5 and 10 $\mu\text{g/L}$ initial concentrations were below detection limit. Figure 4.7 shows the GC-MS chromatogram for products derived from water hyacinth roots for the 15 $\mu\text{g/L}$ initial concentration solution. The peak at 17.922 minutes was due to PCB101 spiked in water. The identities of the other peaks were not clear as in the case of PCB spiked water. A summary of PCB concentration in the extracts for the roots is presented in Table 4.6. PCB concentration in the extract averaged to 0.54 $\mu\text{g/L}$ translating to 0.179 $\mu\text{g/g}$ of roots. The results indicate a detectible bioaccumulation where the plants were exposed to higher concentrations of PCB above 10 $\mu\text{g/L}$. Detection of PCB in the roots only may be associated with the more vibrant growth of roots compared to shoots and the slow translocation to the shoots. Biodegradation or phytovolatilization of the PCB's might also have played a role.

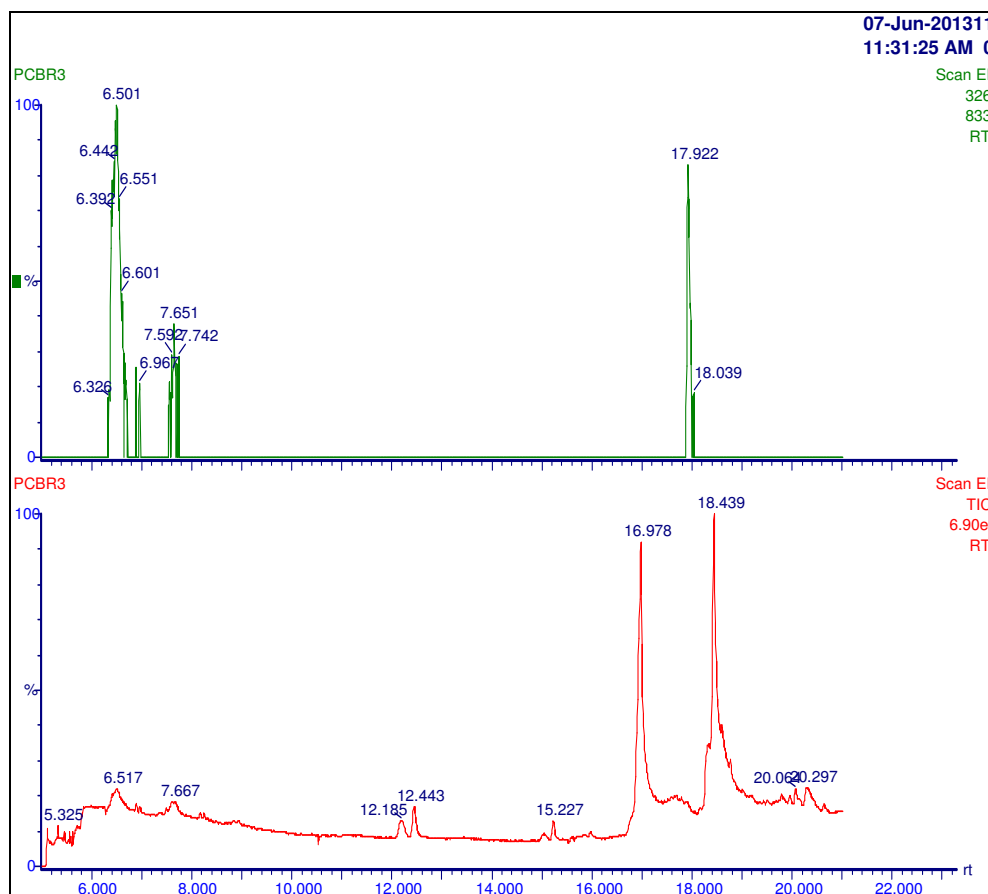


Fig. 4.7 Chromatogram for PCB in Roots Extracts (15µg/L)

Table 4.6 Summary Bioaccumulation Test Results

Initial concentration (µg/L)	Sample No.	PCB Concentration in (µg/L)	
		Leaves	Roots
5	1	BDL	BDL
	2	BDL	BDL
	Average	BDL	BDL
10	1	BDL	BDL
	2	BDL	BDL
	Average	BDL	BDL
15	1	BDL	0.53
	2	BDL	0.55
	Average	BDL	0.54

BDL- Below Detection Limit

4.5 General Discussion

The results of this study demonstrated the ability of water hyacinth to tolerate PCB concentration below 15 µg/L. The result is consistent to the finding of others, for example, Cuningham et al. (1995). Presence of PCB appeared to affect root colour and cause initial wilting of the shoots. The effects could be attributed to the stress in the plants to cope with the pollution, which reveals a probable defense mechanism of the plant. Additionally, there was notable adverse effect on the plants health within the first three days of acclimatization which were later overcome by the plant as vibrant fiber roots developed.

Water hyacinth was shown to phytoremediate PCB concentrations in the range 0 to 15 µg/L. The water hyacinth remained healthy although the growth and development was slightly lower than that of PCB free environment. Therefore, there is need to investigate the ability of water hyacinth to phytoremediate higher PCB concentrations.

The plants under 15 ug/L PCB, bioaccumulated PCB's within its tissues as from below detection limit to an average of 0.179 µg/g in the roots of the plants. While this result signifies the ability of the plant to bioaccumulate, it does not establish whether the plants also biodegraded the PCB's or whether phytovolatilization of the PCB's might have also played a role.

CHAPTER 5

5.0. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study investigated the ability of water hyacinth to tolerate, phytoremediate and bioaccumulate PCB's. The conclusions of the study are:

- 1) Water hyacinth can tolerate a range of PCB concentrations of one to two orders of magnitude above the EPA limit of 0.5 $\mu\text{g/L}$.
- 2) Water hyacinths can phytoremediate PCB's from a simulated landfill leachate PCB matrix solution. In this study, the plants remediated PCB spiked waters from 15 $\mu\text{g/L}$ to 0.42 $\mu\text{g/L}$ for the 15 $\mu\text{g/L}$ PCB concentration and to below the detection limit of 0.142 $\mu\text{g/L}$ for 10 and 5 $\mu\text{g/L}$ respectively within 12 days retention period. Consequently, the water hyacinth has potential for use of as an inexpensive and environmental friendly approach to managing of PCBs in landfill leachate in developing countries.
- 3) Water hyacinths bioaccumulated up to 0.179 $\mu\text{g/g}$ in the roots.

5.2 RECOMMENDATIONS

In this study, water hyacinth showed potential for phytoremediation of PCB in water and, therefore, can serve as an inexpensive and environmental friendly approach in the management of PCB and other POPs in leachate. The recommendations of the study are:

1. Further research to evaluate the plant behavior and ability of tolerance to higher concentrations of PCB above 15 $\mu\text{g/L}$.

2. Evaluation of phytoremediation for PCB concentrations above 15 $\mu\text{g/L}$.
3. Characterization of leachates from major dump sites such as Dandora in Nairobi Kenya to establish potential for release of POP from disposed E-waste.
4. This study investigated phytoremediation of PCBs using commercial PCB product to simulate landfill leachate. Future studies should investigate actual landfill leachate.

REFERENCES

Akpor, O. B. and M.Muchie, (2010).*Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications*.International Journal on physical sciences Vol.5 (12).Pages 1808-1818

Alma, S., Mullaj A., F.Harizaj and J. Shehu, (2010).*Assessment of Heavy Metals Accumulation by Different Spontaneous Plant Species Grown along Lana River, Albania*.Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Tirana, Albania 2010

ATSDR. 2000. *Toxicological Profile for Polychlorinated Biphenyls (PCBs)*. November 2000.Agency for Toxic Substances and Disease Registry Web page: <http://www.atsdr.cdc.gov/toxprofiles/tp17.html>. Web page visited: July 2012.

ATSDR. 2001. *Polychlorinated Biphenyls*. February 2001. Agency for Toxic Substances and Disease Registry Web page: <http://www.atsdr.cdc.gov/tfacts17.html>. Web page visited: February 2012.

Bretherick, L. (1986) *Hazards in the chemical laboratory*. 3rd ed. London, The Royal Society of Chemistry.

CEC (1984) *Classification and labelling of dangerous substances*. Brussels, Commission of the European Communities.

Chu W. and C.Y. Kwan, (2003).*Remediation of contaminated soil by asolvent/surfactant system*.Chemosphere 53 (2003) 9-15Hong Kong Polytechnic University, HunghomKowloon, Hong Kong, China.

Cunningham, S.D.; Berti, W.R. and Huang, J.W. *Phytoremediation of contaminated soils*. Trends in Biotechnology, 1995, vol. 13, no. 9, p. 393-397.

CWC. 1997. *Mill-Based Residual Fiber For Use in Molded Pulp Technology*. Report No. PA-97-2. Clean Washington Center web page: http://www.cwc.org/paper/pa_hm/PA97-2rpt.htm Web page visited: February 2013.

Delta Institute. 2000. *PCBs*. Delta Institute Web page: <http://www.deltainstitute.org/publications/PCBFS.pdf>. Web page visited: March 2012.

DEQ. 1997. *PCB Generic Remedies*. December 1997. Eco-USA. 2002. PCBs. Eco-USA Web page: <http://www.eco-usa.net/toxics/pcbs.shtml>. Web page visited: February 2012.

Dong, K., and Lin, C., (1994). *The purification mechanism of the system of wetlands and oxidation ponds*. In: Proc. 4th Internat. Conf. Wetlands systems for water pollution Control, Guangzhou, China, pg 230-236.

EIP Associates. 1997. *Polychlorinated Biphenyls (PCBs) Source Identification*. Prepared by: EIP Associates, October 28, 1997. Prepared for: Palo Alto Regional Water Quality Control Plant. City of Palo Alto web page: <http://www.city.paloalto.ca.us/cleanbay/publications.html>. Web page visited: July 2012.

EPA. 2001. *Frequently Asked Questions about Atmospheric Deposition*. September 2012.

EPA. 2002. *Polychlorinated Biphenyls (PCBs) Manufacturing, Processing, Distribution in Commerce, and Use Prohibitions*. July 1, 2002. 40 CFR Part 761. EPA Office of Pollution Prevention & Toxics web page: <http://www.epa.gov/opptintr/pcb/200240CFR761.pdf>. Web page visited: July 2012

EPA, (2007). *Method 1614 Brominated Diphenyl Ethers in Water Soil, Sediment and Tissue by HRGC/HRMS* Engineering and Analysis Division NW Washington, DC 20460.

FDA. 2003. *Unavoidable Contaminants in Food for Human Consumption and Food Packaging Material, Sub Part B, Tolerances for Polychlorinated Biphenyls (PCBs)*. National Archives and Records Administration, Code of Federal Regulations web page: <http://www.access.gpo.gov/cgi-bin/cfrassemble.cgi>. Web page visited: February 2013.

Fiedler, Dr. Heidelore. 1997. *Polychlorinated Biphenyls: Uses and Releases*. July 1-4, 1997. Proceedings of the Subregional Awareness Raising Workshop on Persistent Organic Pollutants (POPs), St. Peterburg, Russian Federation. United Nations Environmental Program (UNEP) Chemicals web page: <http://www.chem.unep.ch/pops/>. Web page visited: July 2012.

Flynn, L. 1997. *Public Health Concerns about Environmental Polychlorinated Biphenyls (PCBs)*. American Council on Science and Health Web page: <http://www.acsh.org/publications/reports/pcupdate2.html>. Web page visited: February 2012.

Gonzalez, A., S. Díaz, S. Borniquel, A. Gallego, J.C. Gutierrez, (2006). ***Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants***. Res. Microbiol., 157: 108-118.

Gopal, B. (1987) ***Water hyacinth: Aquatic plant studies 1***. Elsevier Science Publishers B.V., The Netherlands

Gosselin, R., Smith, R., Hodge, H., & Braddock, J. (1984) ***Clinical toxicology of commercial products***. 5th ed. Baltimore, Maryland, Williams and Wilkins company.

Gupta, M., K.Anil, Y. Mohammad and K.P. Pandey, (2003). ***Bioremediation: Ecotechnology for the Present Century***. Enviro News letter 9(2). (Online) Available: http://isebindia.com/01_04/03-04-2.html accessed on 5th Dec 2011

Haselow, D.; J. Shuman and W. DeBusk. (1992). ***Effect of aquatic plants on the environment***: The use of water hyacinths and other aquatic plants for nutrient reduction in lakes. Proc. Inter. Sympos. On The Biology and Management of Aquatic Plnts., p. 10.

HSDB.2003. ***Polychlorinated Biphenyls***. Compiled by the National Library of Medicine. Hazardous Substance Data Bank Web page: <http://csi.micromedex.com/Data/HS/HS3945H.htm>. Web page visited: February 2012.

IARC (1972) ***IARC Monographs on the evaluation of carcinogenic risk of chemicals to man***.Lyon, International Agency for Research on Cancer.

Interstate Technology and Regulatory Cooperation (ITRC),(1999).***Phytoremediation Decision Tree***.Available from; www.itrcweb.org.Accessed on 15th Dec.2011.

IRPTC (1985) ***IRPTC file on treatment and disposal methods for waste chemicals***. Geneva, International Register of Potentially Toxic Chemicals, United Nations Environment Programme.

Jafvert, Chad T., Patricia L. Van Hoof, and Janice K. Heath, (1993)."***Solubilization of Nonpolar Compounds by Nonionic Surfactant Micelles***," Water Research, 28: 1009-1017,.

Jebanesan, A. (1997). ***Biological treatment of dairy waste by Eichhornia crassipes Solms***. Environ.Ecol., vol. 15, no.3, pp..521-523.

- Julien MH, Griffiths MW & Wright AD (1999). *Biological control of water hyacinth*. ACIAR Monograph No. 60 . 87 pp.
- Kalibbala, H., Nalubega, and R.N.Kulabako, (2002).*Challenges in the use of constructed wetland in the treatment of industrial wastewater*. University of Dar es Salaam and IWA pages 504-513.
- Keith,C., H. Borazjani, S. Diehl, Y. Su, and B. Baldwin, (2006).*Removal of Copper, Chromium, and Arsenic by Water Hyacinths*.36th Annual Mississippi Water Resources Conference, Mississippi State University 15-19.
- Kumar,J. I.,H. Soni,R. N. Kumar and I. Bhatt,(2008).*Macrophytes in Phytoremediation of Heavy Metal Contaminated Water and Sediments in PariyejCommunity Reserve, Gujarat, India*. Turkish Journal of Fisheries and Aquatic Sciences 8: 193-200 (2008).
- Kurniawan,T. A., W.H. Lo and G. Y. S. Chan (2006).“*Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate*”Journal of Hazardous Materials, vol. 129, no. 1–3, pp. 80–100
- Kuzovkina,Y. A., K. Michael and M. F. Quigley, (2004).*Cadmium and Copper Uptake and Translocation in Five Willow (Salix L.) Species*-International Journal of Phytoremediation, 6(3):269–287; Copyright Taylor and Francis Inc.
- Lee,K.A. and J. P. Anderson, (2001).*Water-Quality Assessment of the Upper Mississippi River Basin, Minnesota and Wisconsin-- Polychlorinated Biphenyls in Common Carp and Walleye Fillets, 1975-95*.”Retrieved fromU.S. Geological Survey(USGS)website:<http://www.rst2.edu/ties/pcbs/university/pdfs/waterqualityPCB.pdf>(17/07/2012
- Liao, S., W. Chang, (2004). *Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan*. J. Aquat. Plant Manage, 42: 60-68.
- Liehr, S. K. and G. M. Sloop,(1996).*Potential for using Constructed Wetlands to treat Landfill Leachate*.Special Report Series No. 17 North Carolina State University.
- Lin, S. and E. J.Poziomek– EPA, (1994).*A field screening method for Polychlorinated Biphenyl Compounds in water*.Research and development report; USEPA Washington DC

Lone, M.I., Z.He, Pj.Stoffella, and X. Yang, (2008).***Phytoremediation of heavy metals polluted soils and water: progress and perspectives***.J. Zhejiang Univ. Sci. B 9(3): 210-220.

Nagpal, N.K., Ph. D. 1992. ***Water Quality Criteria for Polychlorinated Biphenyls***. January 1992. Ministry of Environment, Lands, and Parks, Province of British Columbia web page: <http://wlapwww.gov.bc.ca/wat/wq/BCguidelines/pcbs/index.html>. Web page visited: July 2012.

Nevena Nestic and Ljubinkojovannovic (2006), “ ***Potential Use Of Water Hyacinth (E. Crassipes) For Wastewater Treatment In Serbia***”, [ttp://balwois.com/balwois/admin_istration/full_paper/ffp-623.pdf](http://balwois.com/balwois/admin_istration/full_paper/ffp-623.pdf)

Nishizaki, H. and Aoyama, I. (1993). ***Uptake of nitrogen and phosphate, and water purification by water hyacinth Eichhornia crassipes (Mart.) Solms***. Water Science and Technology, 28, 47–53.

Nor, Y. M., (1994).“***Phenol removal by crassiepes in prescence of trace metals***”Universitissains Malaysia Volume 28, Issue 5, May 1994 pages 1161-1166 Accessed on December 16th 2011

Penfound, W. and T Earle. 1948. ***Biology of the water Hyacinth***. Ecol. Monogr.18:447-472.

Perdomo, S., Bangueses C., and Fuentes J. (1998) ***Potential use of aquatic macrophytes to enhance the treatment of septic tanks liquids***. IAWQ 6th International Specialist Conference on Wetland Systems in Water Pollution Control, 27th September to 2nd October 1998, Sao Pedro, Brazil.

Reddy, K. R. 1988. ***Water Hyacinth Cropping Systems***: I. Production. In Smith, W. H. and J.R. Frank (Eds). Methane from biomass, a systems Approach. Elsevier Applied Science. London. Pp 103-168.

Reddy K. R., and Sutton D.L. (1984) ***Water hyacinths in water quality improvement and biomass production***. J. Environ. Qual., 13, 1-8.

Reddy K.R., and Tucker J.C. (1983) ***Productivity and nutrient uptake of water hyacinth, Eichhornia crassipes***. I. Effect of nitrogen source. Econ. Bot., 37, 237-247.

Renee, L., (2001).***Constructed Wetlands: Passive Systems for Wastewater Treatment***. Technology Status Report prepared for the US EPA Technology Innovation Office under National Network of Environmental Management Studies Fellowship

Saltabas, O. and G. Akcin.(1994). Removal of chromium, copper and nickel by water hyacinth. *Toxicol. Environ. Chem.*, vol. 41, no.3-4, pp. 131-134.

Sax, N.I. (1984) *Dangerous properties of industrial materials*. New York, Van Nostrand Reinhold Company, Inc.

Sengupta, M. and Dalwani, R. 2008 *Phytoremediation – Green for Environmental Clean*. Proceedings of Taal 2007: The 12th World Lake Conference: 1016-1021.

Swisher, R.D. (1970). *Surfactant biodegradation*. Marcel Dekker, Inc., New York, U.S.A Page 469

Tang, S. and X.Lu, (1993). *The use of Eichhorniacrassipes to clean oil-refinery wastewater in china*. *Ecological Engineering* Volume 2, Issue 3, pages 243-251 available on <http://www.sciencedirect.com/science/article/pii/092585749390017A>
Accessed on December 5th 2011

Torresdey G. (1991) *Organic-coated silver nanoparticles in biological and environmental conditions: fate, stability and toxicity*. *J Hazard Mater.* 2014 Feb 28;267:255-63. doi: 10.1016/j.jhazmat.2013.11.067. Epub 2013 Dec 7.

Townsend, T. G., Y.C. Jang, J. H.Ko, B.Pearson, E.Spalvinsand L.Wadanambi (2003). *Assessment of true impacts of E-waste Disposal in Florida*. Annual report #04-0232008, Florida Center for Solid and Hazardous Waste Management

U.S.E.P.A, (2000). *Introduction to Phytoremediation*. National Risk Management Research Laboratory Office of Research and Development

Umeoguaju, F. U., (2009). *Conventional and new ways of remediating soils polluted with heavy metals*. Nigerian Online Publishing Press (<http://scholars.ufumes.com>) accessed on December 17th 2011

UNEP, (1998). *“Inventory of Worldwide PCB Destruction Capacity”* publication from Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

UNEP Chemicals, 1999. *Guidelines for the Identification of PCBs and Materials Containing PCBs*, First Issue, August 1999. United Nations Environmental Program (UNEP) Chemicals web page: <http://www.chem.unep.ch/pops/>. Web page visited: July 2012.

United Nations (1986) *Recommendations on the transport of dangerous goods*. 4thed. New York, United Nations.

U.S. EPA, 2007. *Toxicity and Exposure Assessment for Children's Health* TEACH Chemical Summary Child-Specific Exposure Factors Handbook.

US EPA, Safewater, (2012). "*Consumer factsheet on polychlorinated biphenyls.*" Retrieved from U.S. Environmental Protection Agency website: <http://www.epa.gov/ogwdw/pdfs/factsheets/soc/pcbs.pdf>

US NIOSH (1976) *A guide to industrial respiratory protection*. Vol 3. US National Institute for Occupational Safety and Health, Cincinnati, Ohio, pp. 76-189.

US NIOSH/OSHA (1981) *Occupational health guidelines for chemical hazards*. Vol.3 Washington, DC, US Department of Health and Human Services, US Department of Labor (Publication No. DHHS (NIOSH) 01-123).

Wager, P., M. Schluep, E. Muller, and R. Gloor, (2011). *RoHS regulated Substances in Mixed Plastics from Waste Electrical and Electronic Equipment*. Environmental Science and Technology, American Chemical Society pg 628-635.

WHO/EURO (1987) *PCBs, PCDDs, and PCDFs: Prevention and control of accidental and environmental exposures*. Copenhagen, World Health Organization Regional Office for Europe, pp. 227 (Environmental Health Series No. 23).

Youngchul, K. and K. Wan-Joong, (1999) *Roles of Water Hyacinth and their Roots for Reducing Algal concentration in the Effluent from Waste Stabilization Ponds*. Department of Environmental Engineering, Hanseo University, Seosan, Chungnam, South Korea. Pg 3286.

Zenon Environmental Systems Inc, (1989). *Technical and economic assessment of reverse osmosis for treatment of landfill leachate*. Prepared for Waste Management Branch, MOE, and RAC Project NO 303 RR.

APPENDICES

Appendix A – Water Hyacinth Plants Growth Data

Table A1 Water Hyacinth Plants Growth Data in PCB Free Water Environment.

	Age at test (days)	Height of shoot (cm)	Length of roots (cm)
Container No.1	0	16.0	0
	3	17.4	1.2
	6	18.1	6.0
	9	18.3	12.0
	12	18.6	15.5
	15	18.8	18.0
Container No.2	0	17.0	0
	3	18.0	1.4
	6	18.2	7.8
	9	18.8	13.2
	12	19.4	14.5
	15	19.8	18.0
Container No.3	0	12.0	0
	3	12.5	1.4
	6	12.6	4.6
	9	12.7	9.8
	12	12.9	12.2
	15	13.0	14.0

Table-A2 Water Hyacinth Plants Growth Data in PCB Spiked Water

Container No.	Age at test (days)	Height of shoot (cm)	Length of roots (cm)
1	0	16	0
	3	16.4	1.2
	6	17.2	3.8
	9	17.8	8.2
	12	18.0	12.6
	15	18.2	15.1
2	0	17.0	0
	3	17.4	1.2
	6	17.8	4.1
	9	18.0	9.1
	12	18.5	13.4
	15	18.8	15.6
3	0	12.1	0
	3	12.2	0.9
	6	12.4	4.1
	9	12.5	9.6
	12	12.7	13.8
	15	12.7	16.2

Appendix B - Photographs Water Hyacinth Plants Grown During the Study



Photo 1, experimental set up



Photo 2 showing root development, day 6, container 3



Photo3 showing roots and shoot development on day 6, container 4



Photo 4, roots and shoot development, day 15, container 3



Photo 5, roots and shoot development, day 12, container 3