UNIVERSITY OF NAIROBI

INVESTIGATION OF MUTAGENICITY OF SOLAR DISINFECTED (SODIS) WATER STORED IN PLASTIC BOTTLES

By:

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(I56/76301/2009)

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2015

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements

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DEDICATION

This thesis is dedicated to my husband Samson, our children Claire, Faith, Joshua, Emmanuel and Patience, my parents, brothers and sisters who have always been a source of inspiration.

*You are the light of the world. A town built on a hill cannot be hidden. Neither do people light a lamp and put it under a bowl. Instead they put it on its stand and it gives light to everyone in the house. In the same way, let your light shine before others, that they may see your good deeds and glorify your Father in heaven (**Matthew 5:14-16**).*

Almighty Lord, I care not for worldly riches, but I would like to join the fold. Tell me, is my name written in the book of Thy kingdom? You are a perfect potter and I am the clay. Mould me Lord. This is what I pray.

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ABSTRACT

Waterborne illnesses associated with poor sanitation are a major problem in Africa's major slum, Kibera. Microbiologically the Solar Disinfected (SODIS) water is safe but the potential mutagenicity of such water is of great concern. When water contained in polyethylene terephthalate bottles is treated using solar, the additives (plasticizers) in the plastic bottle may easily hydrolyze, photochemically degrade and leach into the disinfected water in minute quantities that may be responsible for mutagenicity. In this study, five phthalate esters (dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate, benzyl butyl phthalate and bis-2-ethylhexyl phthalate) and bis-2-ethylhexyl adipate residues was liquid- liquid extracted from samples using organic solvents while detection and quantification were done at intervals using a Gas chromatograph mass spectrometer (GC/MS). The study simultaneously evaluated the mutagenicity of the unconcentrated SODIS samples using the Ames Microplate Mutagenicity test with tester strains TA98 and TA100 with and without metabolic activation. A sample was termed mutagenic if it recorded an average mutagenicity ratio ≥ 2.00. Samples were collected and analyzed for total coliform group of bacteria using the Multiple Tube Fermentation Technique. The complete test confirmed presence of fecal coliforms in the samples which upon a six hour exposure to solar reported an 87.37% antibacterial effect. Reported Mutagenicity ratio ranged from undetectable limits to 4.69±0.88 with samples having metabolic activation (72%) generally reporting a higher mutagenicity ratio than the corresponding samples without. Approximately 58% of samples with tester strain TA100 recorded a higher sensitivity to the Ames test than those with TA98. Household water registered the highest levels of both butyl benzyl phthalate (28.27±0.00µg/L) and bis-2-ethylhexyl adipate (152.97±0.00µg/L) at the 30th and 60th days respectively. The Most significant Pearson's correlations (0.99) were reported between levels of toxicity of Kibera house water and bis-2-ethylhexyl adipate at the 90th day. Results of this study reveal that the amount of plasticisers that leak from the PET bottles into SODIS water are generally low and calculated average daily intake of individual analytes are generally lower than reference doses. Hence in isolation, individual phthalate ester or bis-2-ethylhexyl adipate concentration cannot be entirely held responsible for the reported mutagenicity of the water. With respect to mutagenicity SODIS water stored in PET bottles is safe to use up to and including the 30th day after which such bottles should be discarded and replaced with new ones. Sustained monitoring of toxicity levels of drinking water stored in PET bottles between the 30th and the

60^{th}day using automated or robot scoring is recommended to establish the exact day beyond which the bottle is unsafe to use.

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENTS iv
ABSTRACT v
LIST OF TABLES xii
LIST OF FIGURES xiv
ABBREVIATIONS xvi
CHAPTER 1: INTRODUCTION 1
1.1 Background 1
1.2 Statement of the Problem 3
1.3 Justification 4
1.4 Objectives of the Study 5
1.4.1 Overall Objective 5
1.4.2 Specific Objectives 5
CHAPTER 2: LITERATURE REVIEW 6
2.1 Background 6
2.2 Conventional Methods for Microbiological Treatment of Drinking Water 7
2.2.1 Boiling 7
2.2.2 Chemical Disinfection 7
2.2.3 Microbiological Filtration 8
2.2.4 Flocculation and Disinfection 8
2.2.5 Safe Storage 9
2.3. Water Microbiology 9
2.3.1 The Gram Reaction (Staining) 11
2.3.2 Gram Positive and Gram Negative Cell Walls 11
2.4 Solar Disinfection (SODIS) 12

2.4.1. SODIS Protocol 13

2.4.2 Trends in Application of SODIS 15

2.4.3 Factors affecting the efficiency of SODIS 15

2.5 Genotoxic compounds in SODIS water 16

2.6. Synthesis of Polyethylene Terephthalate 16

2.6.1 The Manufacture of PET Bottles for Drinking Water 17

2.7 Phthalates 18

2.7.1 Mode of Action of Plasticisers 21

2.7.2 Global Production of Phthalates 22

2.7.3 Uses of Phthalates 23

2.7.4 Sources of Human and Environmental Exposure to Phthalates 25

2.7.5 Occupational Exposure to Phthalates 30

2.7.6 Degradation of Phthalates 31

2.7.7 Toxicity of Phthalate Acid Esters and their Metabolites 32

2.7.8 Exposure Limits/Toxicity Reference Values of Some Common Plasticisers 39

2.8 Adipates 41

2.9 Mutagenicity Tests 42

2.9.1 Ames Mutagenicity Test 42

2.10 Supernatant 9000g (S9) 46

CHAPTER 3:MATERIALS AND METHODS 47

3.1 Study Area- Kibera Slums, Nairobi, Kenya 47

3.2 Glassware Cleaning 47

3.3 Chemicals 48

3.4. Equipment and Apparatus Used 48

3.5 Sampling and Sample Treatment 49

3.6 Determination of Physicochemical and Chemical Parameters of Samples 49

3.6.1 Determination of Conductivity 49

3.6.2 Determination of Total Dissolved Solids and Salinity 49

3.6.3 Determination of pH 50

3.6.4 Determination of Temperature 50

3.6.5 Determination of Turbidity and Colour 50

3.6.6 Determination of Total Suspended Solids (TSS) 50

3.6.7 Determination of Sulphate Ions in the Samples 51

3.6.8 Determination of Fluoride Ion Concentration 51

3.6.9 Determination of Chemical Oxygen Demand (COD) 52

3.6.10 Determination of Dissolved Oxygen (DO) 52

3.6.11 Determination of Dissolved Chloride Ions 53

3.6.12 Determination of Iron Content 53

3.6.13 Determination of Nitrogen Content 54

3.7 Microbiological Analysis of Samples 54

3.7.1 Presumptive Test for Coliform Group of Bacteria 54

3.7.2 Confirmed Test for Coliform for Coliform Group of Bacteria 55

3.7.3 The Complete Test for Coliform Group of Bacteria (Gram Staining) 55

3.8 Exposure and Storage Conditions 56

3.9 Ames Mutagenicity Test 56

3.9.1 *Salmonella typhimurium* Culture Preparation 56

3.9.2 Determination of Optical Densities (OD) of Cultures 57

3.9.3 Modification of Ames Mutagenicity Test using Induced Rat Liver 57

3.9.4 Dilution of Test Samples 58

3.9.5 Preparation of the 24 Well Exposure Plates 58

3.9.6 Transfer of Exposure Cultures to 384- Well Plates 59

3.9 7 Plate Scoring 59

3.10 Chemical Analysis 60

3.10.1 Preparation of Analytical Standard Stock Solutions 60

3.10 2 Method Validation 61

3.10.3 Sample Extraction 61

3.10.4 Sample Clean up 62

3.10.5 Identification and Quantification of Plasticisers 62

3.10.6 Working Conditions of Gas Chromatograph/Mass Spectrometer 64

3.11 Quality Control and Quality Assurance 64

3.12 Data Analysis 64

CHAPTER 4: RESULTS AND DISCUSSION 65

4.1. Microbiological Analysis of Samples 65

4.1.1 Presumptive Test for Coliform Group of Bacteria 65

4.1.2. Confirmed Test for Coliforms 68

4.1.3 Completed Test for Coliform Bacteria 70

4.2. SODIS Test 73

4.3. Levels of Physicochemical Parameters in Samples 74

4.4 Mutagenicity/Toxicity Levels in Samples 79

4.5 Chemical Analysis of Water Samples 84

4.5.1 Recovery, Identification and Quantification of PAEs 84

4.5.2 Levels of Bis-2-ethylhexyl Adipate (DEHA) in Samples 85

4.5.3 Levels of Benzyl Butyl Phthalate (BBP 85

4.5.4 Levels of Di-*n*-butyl Phthalate (DBP) 86

4.5.5 Levels of Diethyl Phthalate (DEP) 87

4.5.6 Levels of Bis-2-ethylhexyl Phthalate 88

4.5.7 Levels of Dimethyl Phthalate (DMP) 89

4.6. Correlations between Levels of Selected Plasticisers and Levels of Toxicity of SODIS Water Samples Using Tester Strains TA98 and TA100 96

4.6.1 Correlations between Levels of Bis- 2-ethylhexyl Adipate (DEHA) and Levels of Toxicity of SODIS Samples 97

4.6.2 Correlations between Levels of Benzyl butyl phthalate (BBP) and Levels of Toxicity of SODIS Samples 98

4.6.3 Correlations between Levels of Di-*n*-butyl Phthalate (DBP) and Levels of Toxicity of SODIS Samples 98

4.6.4 Correlations between Levels of Diethyl Phthalate (DEP) and Levels of Toxicity of SODIS Samples 99

4.6.5 Correlations between Levels of Bi-2-ethylhexyl Phthalate (DEHP) and Levels of Toxicity of SODIS Samples 100

CHAPTER 5 :CONCLUNSIONS AND RECOMMENDATIONS 102

5.1 Conclusions 102

5.2 Recommendations 104

5.2.1 Research Recommendations 104

5.2.2 Policy Recommendations 105

REFERENCES 107

APPENDICES 129

LIST OF TABLES

Table 2.1 Existing bacterial contamination regulations and guidelines for drinking water..... 10

Table 2.2: Genotypes of the Ames MPF™ *Salmonella typhimurium*..... 44

Table 4.1: Average MPN Index/100ml of Water Samples N=3±S.D..... 67

Table 4.2: Levels of Selected Physicochemical and Chemical Parameters of Water Samples N=3±S.D..... 78

Table 4.3: Optical Densities of Tester strains Used..... 79

Table 4.4: Mutagenicity Ratio (levels) in SODIS Samples using TA98 and TA100 Tester strain with and without metabolic Activation N=3 ±S.D..... 82

Table 4.5.1: Correlation Coefficients for Selected Phthalates..... 84

Table 4.5.2 Levels of Bis -2-ethylhexyl adipate in SODIS Water Samples (µg/L±S.D, N=3) 85

Table 4.5.3: Levels of Benzyl butyl phthalate in SODIS Water Samples (µg/L±S.D, N=3)... 86

Table 4.5.4: Levels of Di-*n*- butyl phthalate in SODIS Water Samples (µg/L±S.D, N=3)..... 87

Table 4.5.5: Levels of Diethyl phthalate in SODIS water Samples (µg/L±S.D, N=3)..... 87

Table 4.5.6: Levels of Bis-2-ethylhexyl phthalate in SODIS water Samples..... 88

Table 4.5.7: Levels of Dimethyl phthalate in SODIS water Sample (µg/L±S.D, N=3)..... 89

Table 4.5.8: Levels of Pentachlorophenol (µg/L) in SODIS water Samples..... 93

Table 4.5.9: Average daily intake of different PAEs in the non-refill SODIS samples (µg/kg/day)..... 94

Table 4.5.10: Levels of PAEs and DEHA in Kibera House Water (µg/L) ±S.D, N=3 BDL=Below Detection Limit..... 129

Table 4.5.11: Levels of PAEs and DEHA in Distilled Water (µg/L)±S.D, N=3 BDL=Below Detection Limit..... 129

Table 4.5.12: Levels of PAEs and DEHA in Nairobi Dam Water (µg/L)±S.D, N=3 BDL=Below Detection Limit)..... 130

Table 4.5.13: Levels of PAEs and DEHA in Undugu Shallow Well Water (µg/L) ±S.D, N=3 BDL=Below Detection Limit..... 130

Table 4.5.14: Levels of PAEs and DEHA in Kibera Municipal Tap Water (µg/L)±S.D, N=3 BDL=Below Detection Limit 131

Table 4.6.1: Correlations between levels of bis-2-ethylhexyl adipate (DEHA) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 132

Table 4.6.2: Correlations between levels of Benzyl butyl phthalate (BBP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 133

Table 4.6.3: Correlations between levels of Di-n-butyl phthalate (DBP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 134

Table 4.6.4: Correlations between levels of Diethyl phthalate (DEP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 135

Table 4.6.5: Correlations between levels of bis-2-ethylhexyl phthalate (DEHP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 136

Table 4.6.6: Correlations between levels of Dimethyl phthalate (DMP) and levels of toxicity of SODIS samples using tester strains TA100 and TA98 (N=3) 137

Table 4.6.7: Correlations between levels of Pentachlorophenol (PCP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3) 138

Table 4.6.8: Correlations between TA 98 and TA100 *Salmonella typhimurium* tester strains, N=3 139

LIST OF FIGURES

Figure 2.0: General Phthalate Structure 18

Figure 2.1: Dimethyl phthalate 19

Figure 2.2: Diethyl phthalate 19

Figure 2.3: Di ethyl terephthalate 19

Figure 2.4: Bis-2-ethyl hexyl phthalate 19

Figure 2.5: Benzyl butyl phthalate 20

Figure 2.6: Di-*n*-butyl phthalate 20

Figure 3.0: Calibration curve for diethyl phthalate 63

Figure 4.1; Kibera Household Water (1ml) at the presumptive stage indicating colour change from pink to yellow 66

Figure4.2; Municipal tap water (1ml) at the presumptive stage indicating colour change from pink to yellow 66

Figure 4.3; Nairobi Dam water (1ml and 0.1ml tubes) at the presumptive stage showing colour change from pink to yellow 67

Figure 4.4: Kibera house water at the confirmatory stage; Green metallic sheens indicate presence of Coliform bacteria 69

Figure 4.5:Undugu well water at the confirmatory stage ;Green metallic sheens indicate presence of Coliform bacteria 70

Figure 4.6: Gram negative Coliforms from Undugu well water (mg × 1000) at the completed bacterial test stage 71

Figure 4.7: A Section of the SODIS samples displayed on a roof 73

Figure 4 8.1: Levels of Different PAEs in' SODIS' Distilled water 91

Figure 4.8.2; Levels of Different PAEs in' SODIS' Municipal tap water 91

Figure 4.8.3: Levels of Different PAEs in SODIS Kibera House Water 92

Figure 4.8.4: Levels of Different PAEs in' SODIS' Undugu well water 92

Figure: 4.8.5: Levels of Different PAEs in' SODIS' Nairobi Dam water 93

Figure 4.13: Major steps in the Ames mutagenicity test 140

Figure 4.14: Pentachlorophenol 148

ABBREVIATIONS

AAP	American Academy of Pediatrics
ACGIH	American Conference of Industrial Hygienists
APHA	American Public Health Association
ATSDR	Agency for Toxic Substances and Disease Register
AWWA	American Water Works Association
BBP	Butylbenzyl phthalate
CAS	Chemical Abstract Service
CBS	Central Bureau of Statistics
CEH	Committee on Environmental Health
COD	Chemical Oxygen Demand
CV	Crystal Violet
DBP	Dibutyl Phthalate
DEHA	Di (2-ethylhexyl) Adipate
DEHP	Diethylhexyl terephthalate
DEHT	Diethylhexyl terephthalate
DEP	Di-ethyl Phthalate
DMP	Dimethyl phthalate
DNA	Deoxyribonucleic acid

DWEL	Drinking Water Equivalent Level
DWSHA	Drinking Water Standards and Health Advisories
E.*coli*	*Escherichia Coli*
ECHA	European Chemicals Agency
ECMO	Extracorporeal Membrane Oxygenation
EU	European Union
FCSPA	Faculty of Consulting Physicians of South Africa
GC	Gas Chromatograph
HAA	Hormonally Active Agent
HPLC	High Performance Liquid Chromatography
HTH	High Test Hypochlorite
I.V	Intravenous
IARC	International Agency for Research on Cancer
IOM	Institute of Occupational Medicine.
IR	Infrared
MCL	Maximum Contaminant Level.
MCLG	Maximum Contaminant Level Goal.
MEHP	Mono ethylhexyl phthalate
MHRD	Ministry of Human Resource Development

MNP	Most Probable Numbers
MS	Mass Spectrometer
NAS	National Academy of Sciences
NICU	Neonatal Intensive Care Unit
NIOSH	National Institute of and Health Occupational Safety
NMEICT	National Mission on Education through Information and Technology
NMP	Normal Melting Points
NTP	National Toxicology Program
NTU	Nephlemetric Turbidity Units
OPPTS	Office of Prevention, Pesticides and Toxic Substances.
OSHA	Occupational Safety and Health Administration
PAEs	Phthalic Acid Esters
PCP	Pentachlorophenol
PET	Polyethylene terephthalate
RfD	Reference Dose
S9	Supernatant 9000g
SBSE	Stir bar sorptive extraction
SD	Sprague-Dawley
SODIS	Solar Disinfection

SPE	Solid Phase extractors
SPSS	Statistical Programme for Social Scientists
TBE	Tetrabromoethane
TLV	Threshold Limit Value
TSS	Total Suspended Solids
UV	Ultra Violet
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Water in sufficient quantity and good quality is vital for life. Much of the fresh water accessible to man constitutes 1% of the world fresh water readily accessible for direct human use and has been polluted (Revenga and Greg, 2000). The polluted water presents a global threat to human health and wellbeing. As a result, a large portion of the world's population does not have access to microbiologically safe sources of water for drinking. WHO/UNICEF, (2000) estimated that 1.1 billion people do not have access to safe drinking water. Consumption of unsafe water continues to be a major cause of 2.2 million diarrheal disease mortality and morbidity annually, especially children under five years of age (WHO, 2004).

Feacal contamination of raw and treated water is a persistent problem. This is largely exacerbated by increasing populations in both urban and rural areas, increasing pollutant transport into ground and surface water due to deforestation, recurrent disastrous weather events and global climate change (Meierhofer and Wegelin, 2002).

Physical and chemical methods are used for on-site or offsite treatment to remove microbial contaminants from drinking water at household level. Physical water treatment techniques include boiling, solar ultraviolet radiation, sedimentation and filtration; while the chemical techniques include coagulation, flocculation, ion-exchange and oxidation by chlorination and ozonation (Ibáñez *et al.*, 2007). However, most of these methods are expensive and out of reach to majority of the poor in developing countries such as Kenya.

Nairobi is the commercial, industrial and capital city of Kenya and currently has a population of about 3,138,369 people (KNBS and ICF Macro, 2010). Due to rural-urban migration, the development of basic sanitation and other infrastructure is well below capacity to meet the needs of the city. This has resulted in mushrooming of informal settlements in the City and its environs. Nairobi hosts Kibera, one of the largest slums in Africa, with a population density of over 4000 people/hectare. There is no proper infrastructure in the slum, nor any safe means of disposal of solid waste and sewage. Due to scarcity of toilet facilities, residents have resorted to the use of plastic bags for excrement, which are then thrown on roof tops or nearby streams (Graf *et al.*, 2008). The risk of faecal contamination of food and water in Kibera and other informal settlements is high. Recently new residential estates for the Kibera people have been put up by the government. This has gone a long way to alleviate the sanitation problems. However this project is not yet complete.

Higher levels of microbial contamination are also associated with storage vessels having wide openings such as buckets and pots which increase vulnerability due to introduction of hands, cups and used dipper residues that carry faecal contamination (Quick *et al.*, 1999). Prolonged storage times, higher levels of airborne particulates, inadequate hand washing, and the use of aged stored water to prepare food play a major role in water contamination as well as increased risk of waterborne diseases (Sobey, 2002).

Solar Disinfection (SODIS) offers a simple and affordable alternative for treatment of such waters on domestic scale, especially for the rural and urban poor who do not have access to municipal water supply. Based on SODIS protocol, contaminated water is placed in transparent polyethylene terephthalate (PET) bottles which are then exposed to bright sunlight for at least 6 hours before use (Meierhofer and Wegelin, 2002). Solar UV deactivates or kills pathogens, providing an affordable source of safe drinking water. Since used PET bottles are readily available especially to urban populations, SODIS is a practical solution for access to

safe drinking water. Studies have indicated up to 40% decrease in incidence of water borne diseases in areas where SODIS has been used in Kenya (Conroy *et al.*, 1996, 2001). Similar results were reported by Hobbins (2003) and Rose *et al.* (2006) in Bolivian countryside and Southern India respectively. However, concerns have been raised about the probable genotoxic effect of SODIS treated water. A preliminary survey by Ubomba *et al.*, (2010) detected genotoxicity in water stored in PET bottles after two months for both SODIS treated water and that stored in PET containers. The study identifies quantities of phthalate esters in SODIS water and scores levels of toxicity of the non-refill samples stored in polyethylene terephthalate bottles manufactured and commonly used within the Nairobi Metropolitan using Ames mutagenicity assay with tester strains TA98 and TA100 with and without metabolic activation. Assessment time was between 6 hours and 90 days.

1.2 Statement of the Problem

Increased human population and environmental pollution being major contributors of water scarcity and pollution in most cities and slums has led to use of solar Disinfection (SODIS); a cheap method which mainly requires PET containers and solar energy. Although SODIS has become a useful tool for people without access to safe drinking water in developing countries, contributing enormously to the struggle against water borne diseases, application of this versatile disinfection technology is faced by uncertainty due to possible health hazards associated with the deterioration of the plastic bottle after prolonged use. Plasticiser phthalates or adipates such as di-2-ethylhexyl adipate (DEHA) used in the manufacture of polyethylene terephthalate bottles are hormonally active agents, suspected carcinogens and confirmed endocrine disruptors. These compounds have been documented to cause adverse toxic effects to vital body organs such as the liver, kidney and reproductive system. When water contained in polyethylene terephthalate bottles is treated using solar, the additives in the plastic bottle may easily hydrolyse, photochemically degrade and leach into the water in minute quantities

that may be responsible for mutagenicity This research aimed at characterizing and quantifying selected phthalic acid esters and DEHA in SODIS water and employing the Ames microplate mutagenicity test with help of both TA98 and TA100 tester strains to evaluate the potential mutagenicity of the un-concentrated SODIS water samples over a period of 90 days using polyethylene terephthalate bottles manufactured and used in Kenya.

1.3 Justification

Phthalates being endocrine disruptors and suspected carcinogens their concentrations in portable water are of great health concern. Link between mutagenicity of drinking water and increased cancer risk has been revealed in some epidemiological studies (Koivusalo *et al.* 1995; Haider *et al.* 2002; Lah *et al.*2005a) hence continuous evaluation of toxicity levels is very vital. Since the PET bottles are rich in these phthalates there is need to know how much phthalate leakage there is in solar disinfected water in relation to the level of toxicity of such water which is widely used in most slums. The results of this investigation will assist in alerting frequent SODIS water consumers, the health officials, and the general public on the safety period of using the locally available SODIS PET bottles. Dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate, benzyl butyl phthalate and bis-2-ethylhexyl phthalate are among the six phthalate acid esters classified as priority pollutants by the United States Environmental Agency (USEPA) hence investigation/monitoring of their presence in SODIS water can serve as a valuable record of contamination of drinking water. This study represents the first survey in which phthalate residues in SODIS water samples have been investigated and their levels correlated with the mutagenicity of the samples in the region.

1.4 Objectives of the Study

1.4.1 Overall Objective

The overall objective of this study was to evaluate the mutagenicity of Solar Disinfected (SODIS) water stored in polyethylene terephthalate bottles over a long of time using the Ames mutagenicity test and subsequently to correlate the outcome of the results with levels of selected plasticizer residues in the water samples.

1.4.2 Specific Objectives

The specific objectives of the study were to:

i. identify and quantify selected organic compounds (five phthalic acid esters and bis-2-ethylhexyl adipate) leaching from PET bottles at weekly/monthly intervals

ii. assess the mutagenicity of SODIS water using Ames microplate format mutagenicity assay

iii. Evaluate the antibacterial effectiveness of solar disinfection on water quality.

iv. correlate toxicity levels with levels of phthalate residues in SODIS samples

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

Waterborne illnesses associated with unsafe drinking water and poor sanitation is a major cause of death in the developing world (WHO, 2004).

World Health Organization (WHO) declares that in developing countries, 80% of all human diseases are water borne (Abera *et al.*, 2011). Pathogenic microorganisms responsible for waterborne diseases include bacteria such as *E. coli* and viruses (Ibañez *et al.*, 2007). Although a variety of methods are available for treating drinking water, many people do not have access to these life-saving technologies (McGuigan *et al.*, 1998) and as a result drinking water in many countries does not meet the WHO set standards (Gyamfil *et al.*, 2012). Poor hygiene practices often are also significant sources of infection. Central disinfection of piped water supplies is becoming more common; however, these systems often fail to provide adequate disinfection due to inconsistent dosing and deteriorated distribution networks.

In rural and peri-urban areas, without water networks, people often obtain their drinking water from contaminated springs, wells, rivers, or man-made ponds. In such situations, safe drinking water can only be obtained by treating water in the household at the point of use. Unfortunately, the point of use methods currently available such as commercial UV, reverse osmosis, ozonation and boiling are energy intensive and expensive, while chlorination and sand filtration require an inconvenient wait time resulting in their inconsistent application (Ibañez *et al.*, 2007). Furthermore, secondary contamination of drinking water due to incorrect water handling is frequently observed in developing countries. On the other hand,

SODIS is an effective method that uses relatively inexpensive PET bottles (reactors) and natural solar UV. (McGuigan *et al.*, 1998).

2.2 Conventional Methods for Microbiological Treatment of Drinking Water

WHO commissioned a study that identified different products, technologies and approaches used in microbiological treatment of drinking water not excluding SODIS, a number of which have been rigorously assessed for microbiological performance and health impact and have shown to be cost effective (Sobey, 2002).

2.2.1 Boiling

Boiling is the most commonly employed means of treating water at household levels and practiced by more than 500 million people in low-middle income countries (Rosa and Clasen, 2010). World Health Organization recommends bringing water to a rolling boil for just one minute mainly intended as a visual indication that a high temperature has been achieved (WHO, 2004). However, the inconvenience of boiling, the cost and time used in procuring fuel, the potential aggravation of indoor air quality and associated respiratory infections, increased risks of burns has led to other alternatives.

2.2.2 Chemical Disinfection

Most households widely employ hypochlorous acid derived from liquid sodium hypochlorite, solid calcium hypochlorate or high test hypochlorite (HTH) which are readily available and affordable (Rosa and Clasen, 2010). The impact of chemical disinfection in reducing diarrheal diseases has been documented (Arnold and Colford, 2007). However, this method is expensive to the poor especially those living in slums where sanitation is lacking and secondary contamination is high.

In tertiary treatment of urban waste waters chlorine disinfection is widely used to reduce microbial contamination and to prevent the spread of pathogens into the environment. However, chlorine can react with natural organic substances such as humic and folic acids present in surface waters, to release volatile and nonvolatile disinfection byproducts with mutagenic and or carcinogenic activity (Glaze *et al.*, 1993; Meier, 1989).

2.2.3 Microbiological Filtration

In this method high quality ceramic filters are treated with bacteriostatic silver that reduces waterborne protozoa and bacteria by more than 99.999% and 99.999%, respectively (Clasen *et al.*, 2004). "Biosand" filters are simpler but are specifically designed to suspend solids and microbes by means of slime layer that develops within the top few centimeters of sand. If properly constructed, operated and maintained they are capable of removing 99% or more enteric pathogens at household level (Hijnen *et al.*, 2004; Stauber, 2006).

Household filters have a number of advantages compared to other technologies. They operate under a variety of conditions (temperature, pH, turbidity), introduce no chemical into the water and improve the water aesthetically, thus potentially encouraging routine use without extensive intervention to promote behavioural change. High quality ceramic filters treated with bacteriostatic silver have been shown effective in the lab (Clasen *et al.*, 2004).

2.2.4 Flocculation and Disinfection

A particular challenge for most household based treatment technologies is high turbidity (suspended solids) that can use up free chlorine and other chemical disinfectants and cause premature clogging of filters as well as reduce U.V radiation essential in solar disinfection. Flocculation/ coagulation using common substances such as alum can be effective and relatively less costly. Tests of one product have shown reduction in waterborne cysts by more than 99.999% and bacterial by more than 99.999% (Souter *et al.*, 2003).

2.2.5 Safe Storage

Once water has been collected or treated, it is immediately vulnerable to recontamination from microbial pathogens unless protected by safe storage or presence of residual disinfectant such as chlorine. This is principally due to contact with hands during collection, transport or use in the home. An effective solution is use of storage vessel that prevents hand contact by using a narrow mouth for filling with a tight cap and a tap or spout for drawing or pouring rather than dipping into it. Research has shown that stored water supplies in the home for example, frequently contain higher levels of bacteria than water at source (Wright *et al.*, 2003; Clasen and Bastable, 2003).

2.3. Water Microbiology

Water becomes easily contaminated by intestinal pathogens such as coliform group of bacteria, *salmonellae*, *Vibrio* and dysentery causing bacilli. The human faecal material carried in sewage is often dumped in rivers and lakes and this increases water contamination hence, water supply has to be regularly checked for microbial contamination. The coliform group of bacteria are the most reliable indicators of faecal contamination (Park, 2000). Developing countries are worst hit by water related diseases; the leading being the diarrheal diseases, with typhoid, paratyphoid fever, bacillary dysentery, *E. coli* associated diarrhea and cholera being the most common. (Klein's and Prescott, 2002). According to the World Health Organization (WHO), diarrheal diseases account for 4.1% incidences of the total daily burden of disease and are responsible for the death of 1.8 million people every year (Nwachcuku and Gerba, 2004). Bacteria are unicellular microorganisms ubiquitous in nature. Approximately are 1µm (10^{-6} m) in size. Their cell walls are made up of peptidoglycan and reproduce by binary fission. Detection of coliform group of bacteria by MTF technique is easily interfered by high numbers of non coliform bacteria as well as the inhibitory nature of the media leading

sometimes to underestimates of coliform abundance. (Seidler *et al.*, 1981; Evans *et al.*, 1981; APHA, AWWA and WEF 2012).

Although the method is labour intensive, time consuming, lacks precision in both qualitative and quantitative terms it is cheap and easy to implement especially when the conditions do not allow the use of the membrane filter technique, such as turbid or colored waters (Evans *et al.*, 1981; Karl, 1998). Table 2.1 Existing bacterial contamination regulations and guidelines for drinking water.

	Total coliform	*E. coli*	Population	Samples/months
United States[a]	0/100 ml (95%) 0/100 ml (100%) 1/1000 inhabitants a consecutive sample from the same site must coliform free	0/100 ml (100%)		1/1000 inhabitants
Canada[b]	0/100 ml (90%) 0/100 ml (100%) < 5000 4 samples/month none should contain more than 10 CFU/100 ml a consecutive sample from the same site must be coliform-f	0/100 ml (100%)	< 5000 5000– 9000	4 samples/month 1/1000 inhabitants
World Health Organization[c]	0/100 ml (95%)	0/100 ml (100%)	> 9000	90+ (1/10,000 inhabitants

***a** US Environmental Protection Agency, 1991.*
***b** Ministe're de la sante', 1996. **c** World Health Organisation*

A Complete test for coliform bacteria is necessary for further confirmation whether the microorganisms are gram negative, nonspore forming and ferment lactose. This is achieved by inoculating a nutrient agar slant a Durham tube of lactose broth accompanied with performance of a gram reaction (Dubey and Maheshwari, 2005; APHA, AWWA and WEF 1998).

Monika *et al.* (2012) carried out a study on water samples collected from five different sources and examined them for microbial contamination using the Multiple Tube Fermentation Techinique. Out of 17 samples tested, 9 were unsatisfactory, one sample was

suspicious and only two were satisfactory whereas five were found excellent. Seven out of the 10 unsatisfactory and suspicious samples tested positive to *E.coli* presence using the Eijkman test. On gram staining and biochemical testing, two indicator organisms were isolated from water samples as *E. coli* and *Enterobacter aerogenes.*

2.3.1 The Gram Reaction (Staining)

Gram staining is a differential technique that distinguishes gram positive and gram negative bacteria. It is based on the microorganism's ability to retain color of the stains used during the procedure depending on the chemical and physical properties of the bacteria's cell wall. Gram-negative bacteria *Escherichia coli* are easily decolorized by alcohol, losing the purple color of the primary stain (crystal violet) while Gram-positive bacteria such as *Staphylococcus aureus* are not decolorized by alcohol hence remain purple (Monika *et al.*,2012). A counter stain is used to impart a pink color to the decolorized gram-negative bacteria. The procedure enables morphology and arrangement of bacteria to be examined using a light microscope (Smith *et al.*, 2005; NMEICT, 2014).

2.3.2 Gram Positive and Gram Negative Cell Walls

Gram-positive bacteria comprise of a thick mesh-like cell wall made up of peptidoglycan, a polysaccharide composed of *N*-acetyl glucosamine and N-acetyl muramic acid. Adjacent layers of peptidoglycan are cross linked by short chains of peptides transpeptidase enzyme, resulting in a rigid cell wall. The thick peptidoglycan layer allows these organisms to retain the crystal violet-iodine complex hence staining the cells purple (Dmitriev *et al.*, 2004; NMEICT, 2014). Embedded in the peptidoglycan layer is Lipoteichoic acid (LTA) which consists of teichoic acids (long chains of ribitol phosphate anchored to the lipid bilayer via a glyceride) acting as regulator of autolytic wall enzymes (muramidases) located in the cell wall that cause disintegration of the cell following injury or death). LTA also has antigenic properties that stimulate specific immune responses when it is released from the cell wall after

cell death due to lysis induced by lysozymal activities, cationic peptides from leucocytes, or beta-lactam antibiotics (Dmitriev *et al.*, 2004; NMEICT, 2014)

While peptidoglycan forms 50-90% of the cell wall of gram positive bacteria in gram-negative bacteria this polysaccharide forms only 10% of the cell wall. Due to this thinness a gram negative bacterium easily loses the crystal violet-iodine complex during decolorization with the alcohol rinse, but is able to retain the counter stain Safranin, hence appear red or pink. This type of cell wall also comprises of a high lipid containing outer membrane that is separated from the cell wall by a periplasmic space (Beveridge *et al.*, 1983; Smith *et al.*, 2005; NMEICT, 2014). The cell wall of Gram-negative bacteria is often a virulence factor that enables pathogenic bacteria to cause disease. The lipopolysaccharide (LPS) or endotoxin component of the cell wall elicits an innate immune response characterized by cytokine production which can produce host toxicity especially in humans. Furthermore, Inflammation may occur as a result of cytokine production. While addition of gram's iodine in the gram staining technique is meant to serve as a mordant (a substance that increases the affinity of the cell wall for a stain by binding to the primary stain, thus forming an insoluble complex which gets trapped in the cell wall) decolorization with 95% alcohol facilitates the dissolving of the lipid outer membrane of the gram negative bacteria so as to expose the peptidoglycan layer as well as increase the porosity of the cell wall.

2.4 Solar Disinfection (SODIS)

This is a simple, affordable and effective water treatment procedure employed to improve the microbiological quality of water at household level. It combines thermal and UV radiation and has been repeatedly shown to be effective for eliminating microbial pathogens and reducing diarrheal morbidity including epidemic cholera (Conroy *et al.*, 2001; Hobbins, 2004).

The concept of SODIS was first presented by Aftim Acra in 1984 after several field investigations that targeted different pathogens using different water qualities and various types of containers under different climatic conditions. A research team EAWAG/SANDEC embarked on comprehensive laboratory experiments in 1991 to assess the potential of this method in the inactivation of microbes which revealed synergies in the inactivation through the combined use of UV-A radiation and increased water temperature (Meierhofer and Wegelin, 2002).

2.4.1. SODIS Protocol

Water that is microbiologically contaminated is placed in transparent polyethylene terephthalate plastic containers and exposed to direct sunlight for a minimum of 6 hours. In cloudy weather, the exposure time is extended to 2 days (Meierhofer and Wegelin, 2002). The containers used for SODIS are put at horizontal depth not exceeding 10 cm when being exposed to the sunlight. At a water depth of 10 cm and moderate turbidity of 26 NTU, UV radiation is reduced to 50% (Sommer *et al.*, 1997).

Polyethylene terephthalate (PET) is the material most commonly used to make the clear plastic bottles in which bottled water is stored. Concerns about health risks related to SODIS water due to leaching of toxic substances from PET bottles are at an increase. Migrating compounds comprise of production residues, hydrolysis and thermal/photo degradation products of PET itself plus additives and in the case of recycled or re-used PET bottles, constituents of beverage previously contained in the bottles such as flavors. Degradation products include aldehydes (such as formaldehyde and acetaldehyde) and plasticizers such as di-(2-ethyl-hexyl-phthalate) DEHP (Nawrocki *et al.*, 2002).

Coloured bottles do not transmit enough UV radiation and therefore are not used for SODIS (Wegelin *et al.*, 2001). Similarly, colour in water increases the time required for inactivating

the pathogens [Reed, 1997]. On the other hand, glass with high content of iron oxide transmits less UV radiation. Furthermore glass bottles frequently break and are not preferred for SODIS (Sommer *et al.*, 1997). Although SODIS bags show high efficiency due to their better surface area-volume ratio, they are not locally readily available and are more delicate, hence difficult to handle than plastic bottles (Rocha, 1985; Sommer *et al.*, 1997).

Roof-tops of buildings often provide a good surface for such exposure. The PET bottles are shaken to enhance aeration in water and this is done only at the beginning of the SODIS process. The bottle need not be moved any more since continuous shaking during solar exposure eventually compromises the efficiency of the process (Kehoe *et al.*, 2001). SODIS uses two components of the sunlight for water disinfection, namely Ultra violet-A radiation and infrared (IR) radiation. The UV-A radiation has a germicidal effect, in that it directly interacts with Deoxyribonucleic acid (DNA), nucleic acids and enzymes of the living cells, changing their molecular structures leading into cell death. This radiation also interacts with oxygen dissolved in water producing free oxygen radicals and hydrogen peroxides which interfere with cell structures and kill pathogens. The IR radiation has a pasteurizing effect. This radiation heats the water raising the temperature which coupled with exposure time eliminates the microorganisms. The combined use of both UV-A radiation and IR radiation produce a synergic effect (Meierhofer 2002). At a water temperature of 50° C a synergic effect of UV-A radiation and temperature occurs and pathogens get destroyed (Sommer *et al.*,, 1997).

Pathogenic microbes such as *Escherichia coli*, *Shigella flexneri*, *Salmonella typhii*, and *Salmonella enteritidis* and *Salmonella partypi* are destroyed by SODIS. Hobbins, (2003); Rose *et al.* (2006) reported a decline in diarrhea by 40% in India following SODIS application. A study carried during a cholera outbreak in an indigenous rural Maasai

community in Kajiado County by Conroy *et al.* (2001) reported that there was an 86% reduction of cholera cases among SODIS users.

2.4.2 Trends in Application of SODIS

Revelations of both laboratory and field research of Aftim Acra and EAWAG/SANDEC research team SODIS have Since 1999 led to local SODIS initiatives and activities launched in several countries including Indonesia, SriLanka, India, Nepal, Pakistan, Uzbekistan, Kenya, Burkina Faso, Thailand, Bolivia, Togo, China, South Africa and Angola (Meierhofer and Wegelin, 2002). In Kenya, SODIS projects have been applied in Maasai land (Kajiado county) and in Kibera (a Nairobi city slum) and the recorded impact has been noted to be positive (Conroy *et al.*, 1996; Graf *et al.*, 2008).

SODIS is particularly useful for emergency water supplies for refugees and in war zones, where conventional water supplies may be unavailable, disrupted or inoperative (Reed, 1997 ; Meierhofer and Wegelin, 2002). It is also applicable for treating water for people in rural villages and urban shanty communities who may have access only to sewerage- contaminated surface water, particularly as a short term remedy during outbreak of waterborne disease(s). In such cases, SODIS is used to disinfect water for preparation of oral rehydration solutions where no reliable safe water supply exists. The main limitations of SODIS are that it is labour intensive; it depends on the weather and climatic conditions as it requires sufficient solar radiation. Moreover, SODIS requires clear water, does not remove the chemical contaminants from water, and it is not useful to treatment of large volumes of water (Meierhofer and Wegelin, 2002).

2.4.3 Factors affecting the efficiency of SODIS

The efficiency of SODIS is dependent on solar intensity, the level of chemical and microbial contamination. Bacterial decontamination rate by solar radiation is proportional to the

intensity of radiation and the temperature (Caslake *et al.*, 2004). The destructive wavelengths for the forms of microbial life are those near UV-A spectrum 320-400nm. When temperatures rise up to 50°C bactericidal activity is accelerated by a factor of two (2) due to the synergetic effect between radiation and temperature (Wegelin *et al.*, 1994). The chemical composition of water as well as suspended solid particles and faecal material protect the micro-organisms against radiation (Häder, 2007; WHO, 2015).

2.5 Genotoxic compounds in SODIS water

Genotoxicity is a property possessed by some substances that make them harmful to the genetic information contained in organisms. Genotoxins can be carcinogens, mutagens or teratogens (birth defect causing agents). Organic compounds such as aldehydes, phthalates and phenols in PET bottles may leach into SODIS water. PET contains phthalates (diesters of phthalic acid) which are used as plasticizers. They are loosely bound to plastics and therefore easily leach from the plastics into the water (Swan *et al., 2005*).

2.6. Synthesis of Polyethylene Terephthalate

Polyethylene terephthalate is a semi-crystalline polymer belonging to the family of polyesters well known as a durable packaging material for drinking water. In 2009, packaged water sales in PET accounted for 44% of the market volume for non-alcoholic drinks in Europe, with an average individual consumption of 105 litres per year (EFBW, 2011).

The synthesis of PET commences with the esterification of either terephthalic acid or dimethyl terephthalate with ethylene glycol, to give bis-(hydroxyethyl) terephthalate (BHET). The BHET is then polymerized up to about thirty repeat units (Awaja and Pavel, 2005). Inorder to achieve a degree of polymerization (DP) of about hundred repeat units, polycondensation is carried out with Sb, Ge or Ti-based catalyst at temperatures > 270°C and pressures > 50 Pa (Ravindranath and Mashelkar, 1986). To produce bottle-grade PET, the

degree of polymerization must be > 150 repeat units inorder to give bottle grade PET and this is normally accomplished through solid-state polymerization, a process that requires temperatures > 200°C, pressures > 100 Pa, and incubation times of at least 15 hours (Al-Ghatta *et al.*,1997). Thermal stability of PET depends on the type of co-monomers used for its production (Holland and Hay, 2002b) while for bottle-grade PET, co-polymerization with diethylene glycol (DEG) and isophthalic acid (IPA) is vital to minimize polymer thermal crystallization during the production of preforms and the blow-molding process. Both DEG and IPA reduce the size of spherulites and, as a result the final product comes out glassy and transparent (Holland and Hay, 2002a).

Polyethylene terephthalate is the plastic and phthalate is the additive (Enneking, 2006). PET is the main ingredient of the plastic bottle and may yield phthalates as chemicals that contribute to endocrine disrupting effects of water stored in a PET container (Sax, 2010).

2.6.1 The Manufacture of PET Bottles for Drinking Water

Amorphous Preforms obtained by processing PET granules are stretched by a blow-molding process carried out at 20°C to achieve bi-axially oriented bottles. Despite the good barrier properties of PET, packaging applications require even lower gas permeability hence blending of immiscible lamellar polyamide (PA) phases within the PET reduces the permeability of both O_2 and CO_2 by a factor of two or more. A hexanedioic acid polymer with 1,3-benzenedimethanamine (called MXD6) is mostly used as barrier material in PET bottles The barrier properties and the diffusion rate are directly affected by the degree of crystallinity and the orientation of polymer chains in PET bottles (FSA, 2007).

Additives such as plasticizers, antioxidants and colorants are added in small quantities while acetaldehyde (AA) scavengers are used for bottles intended for mineral water to minimize AA content of the melt-processed polyester. In most cases anthranilamide is the most preferred

AA scavenger because of its low cost, efficiency and ease of incorporation into PET (Rule et al., 2001).

2.7 Phthalates

Phthalates are dialkyl or alkyl aryl esters of 1, 2- benzenedicarboxylic acid (phthalic acid) with a general structure.

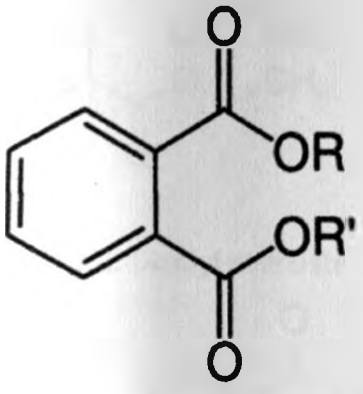

Figure 2.0: General Phthalate Structure

This general chemical structure represents an *o*-phthalate with R and R' being ester side chains which can be either linear aryl groups or alkyl groups with an aromatic ring. The two side chains can be identical, or they may differ, giving rise to different phthalates with different properties and consequently different biological activities. R and R' being the functional groups largely determine the properties of the respective phthalate. There are three possible positions for ester linkages but the most commonly used are based on the above 1, 2-benzenedicarboxylic structure (Kirby, 1972). Phthalates are made from alcohols and phthalic anhydride. The structures of the phthalate esters and the adipate of interest in this study are displayed below.

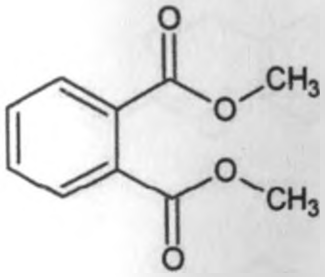

Figure 2.1: Dimethyl phthalate

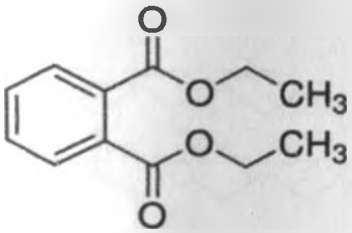

Figure 2.2: Diethyl phthalate

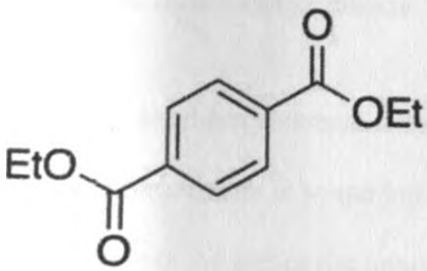

Figure 2.3: Di ethyl terephthalate

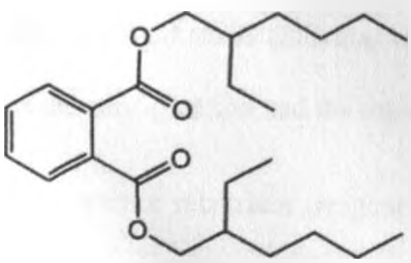

Figure 2.4: Bis-2-ethyl hexyl phthalate

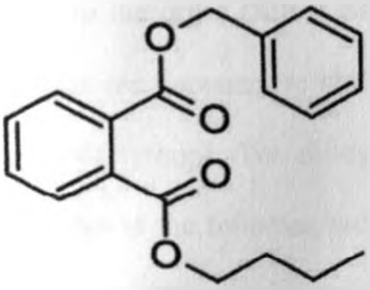

Figure 2.5: Benzyl butyl phthalate

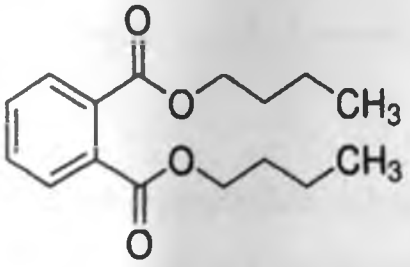

Figure 2.6: Di-*n*-butyl phthalate

DEHT (di-ethyl-hexyl-terephthalate) is a phthalate ester stoichiometrically equal to DEHP, i.e. the phthalate ester is bound to two ethylhexyl groups, but with a different spatial structure, because one of the carboxylic groups is placed differently on the benzyl ring (ECPI, 2009)

In their pure form phthalates are usually clear, neutral liquids, some with faint sweet odors and some with a faint yellow color (Cousins and Mackay 2000; USEPA, 2006). Phthalates also known as phthalic acid esters (PAEs) being water soluble are biologically active organic compounds that posses a lipophilic nature and easily accumulate in adipose tissues and infiltrate food stuffs (Sharman *et al.*, 1994; Jarosova, 2006). Their solubility largely depends on the configuration and the side chain length (Howard *et al.*, 1984).

They are not substrates (reagents) or precursors in the manufacture of PET but are additives not chemically bound to the plastic hence leach easily from PET bottles into contents. Consequently the potential for human exposure is significant (Enneking, 2006). The softening effects of the phthalates decrease with increasing length of the functional groups (Hoffmann,

1996) in the order DBP > BBP > DEHP > DINP >DIDP > DTDP. Consequently higher loadings are necessary to obtain a certain levels of softness for the phthalates with longer functional groups. The ability of these plasticisers to mix with the resins by processing decreases in the following order: BBP > DBP > DIHP > DEHP > DINP > DIDP > DTDP (Hoffman, 1996). Diethyl phthalate (DEP), a widely used plasticizer in a variety of consumer products including medical treatment tubings, cosmetic formulations as well as toiletries, is a colourless liquid with an aromatic odour, low volatility and a solubility of 1000mg/litre at 25°C.

The short chain phthalates such as dimethyl phthalate show solubilities of up to 4000 mg/L with long alkyl chain phthalates being virtually insoluble (< 1µg/L for C9 and longer). The log K_{ow} increases from 1.6 for dimethyl phthalate to more than 8 (for C9 and longer). The better solubility and thus the higher availability of the phthalates with short alkyl chains lead to their higher toxicity. Concurrently they are available for degrading microorganisms and hence not persistent. Those phthalates with higher molecular weights and low solubilities are strongly adsorbed to the soil and to suspended particulate matter in water and are not very accessible plasticizers' to biochemical processes leading to degradation (Staples *et al.*, 1997).

2.7.1 Mode of Action of Plasticisers

A plasticizer has solvent capabilities that enable it to penetrate the less strongly bound parts of the polymer. In the polymer, the plasticizer acts as a lubricant creating distance between the freely organized polymer chain parts, and shields the attraction forces between polar parts of the chain, and thereby weakens the attraction between the chain parts. This allows more free movement amongst the weakly bound chain parts making the material flexible. The plasticizer does not form chemical bonds with the polymer and may easily be separated from the

polymer matrix due to extraction by solvents, oils, water, surface rubbing, volatility, migration into adjacent media, or even by degradation mechanisms (Staples *et al.*, 1997).

2.7.2 Global Production of Phthalates

It is estimated that 11 billion pounds of phthalates are produced annually out of which about one million tonnes are produced from Western Europe. It is approximated that 1.8% of the total global production leaks into the environment (Hubert *et al.*, 1996). The annual global production of phthalates in the 1990s was approximately 4 million tonnes (Lin *et al.*, 2003) and out of the approximately one million tonnes of phthalates produced each year in Western Europe, approximately 900,000 tonnes are used to plasticize PVC. The most common are: di-(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DIDP) and diisononyl phthalate (DINP) (ECPI, 2003 a) representing more than 85% of the total volume of phthalate esters produced in Western Europe (Ecobilan, 2001). Of this amount di (2-ethylhexyl) phthalate accounts for 50% of all plasticizer usage (ECPI, 2003b).

DEHP and dioctyl phthalate production in 1994 was estimated to be 258 and 285 million pounds respectively while DIDP production was approximately 240 million pounds and 215 million pounds respectively (USEPA, 2006).

At EU level 341,000 DEHP tonnes were produced in 2007, out of which about 291,000 tonnes DEHP were used for manufacturing processes in the EU while the rest were exported. DEHP accounted for around 18% of all plasticizer usage in Western Europe. The consumption of DEHP has decreased markedly in the last decade.

Of the 341,000 tonnes produced in the EU in 2007, 187,000 tonnes were produced in Western Europe corresponding to 31% of the 1997 level of 595,000 t/y. In the early 1990s, DEHP represented about 51% of the total phthalate plasticiser market in Western Europe. In Denmark DEHP represented a major part of the phthalate use in the1990's and was replaced

by DINP around 2000. Currently DEHP seems to have been replaced by DINP or non-phthalate plasticisers in PVC products (Christensen *et al.*, 2007).

There has been a tremendous decrease in the total manufactured BBP tonnage in the EU over the last two decades. In the period 1994-1997, the total reported Western European manufacture of BBP was 45,000 tonnes/year whereas for 2004 a production volume of 19,500 tonnes was reported which by 2007 went below 18,000 tonnes (COWI, 2009a).

Significant part of the manufactured tonnage is exported to countries outside the EU however; the market for BBP has been decreasing over the last decade.

DBP, a very volatile fast fusing plasticiser popular for its use as a gelling aid in combination with other high molecular weight plastisers, has had its production decrease over the decades. In 1994 the production volume of DBP in the EU was 49,000 tonnes and in 1998 it was 26,000 tonnes, with an export of 8,000 tonnes. The total manufactured tonnage in 2007 in the EU was less than 10,000 tonnes. DBP represents less than 1% of the total produced phthalates in Europe (COWI, 2009b).

2.7.3 Uses of Phthalates

Phthalates acid esters have been used intensively in a variety of consumer products as plasticizers or additives to impart flexibility, durability, transparency and longevity. Their chemistry has it that the larger the phthalate molecule, the lower its volatility, extractability, softness and gelling temperature (Wilson, 1995). Consequently exploitation of their uses depends in part on their respective molecular weights.

When added to plastics, phthalates allow the long polyvinyl molecules to slide against one another through improved melt viscousity and production speeds of heated PVC. (Jarosova, 2006; Penalver *et al.*, 2000; Cai *et al.*, 2003). They are components of cosmetics, perfumes detergents, building products (sheeting, flooring, films), lubricating oils, PCB substitutes,

carriers in pesticide formulations and solvents (Orsi *et al.*,2006). In cosmetics they hold fragrance, reduce cracking of nail polish, the stiffness of hair sprays, moisturize the skin and make product penetration to the skin effective. The use has found ready market in manufacture of nutritional suppliments, gelling agents, glues, adhesives, modeling clays, sex toys in form of jelly rubber, shower curtains and eye shadows, enamel removers, nail extenders, bath soaps (Anonymous, 1985; Hawley, 1987; Kamrin and Mayor, 1991). These esters have also been used in the manufacture of umbrellas, soft shoes, raincoats, boots, automotive parts, notebooks, food containers and wrappers, intravenous storage bags, paints, printing inks, pharmaceutical coatings, liquid soap, fishing lures, soft drink and water bottles, pesticides formulations, alcohols and hand bags. Medical applications include blood transfusion bags and tubing, intravenous fluid bags and tubing, and other medical devices. Phthalates are also found in lubricating oils, solvents, and detergents (ECPI, 2010; Wittassek *et al.*, 2011; Sioen *et al.*, 2012).

Di-2-ethylhexyladipate blended with di-iso-octylphthalate is important in the manufacture of aircraft lubricants, food films, synthetic rubber, nitrocellulose and cellulose based lipsticks (Cadogan and Howick, 1992, 1996; Verschueren, 1996; National Toxicology Program, 1999).

While DMP and DEP are popularly used for cellulose-ester based plastics DBP is typically exploited for epoxy resins (Staples *et al.*, 1997). Because of its fast fusing property DBP is used as a gelling aid together with other higher molecular plasticisers in relatively low concentrations hence present in higher share of products. However, the market has decreased over decades. Dutch surveys of phthalates and other plasticisers in toys and childcare products demonstrate that 30% of 24 analysed products in 2004 contained DBP (FCPSA, 2008a). The share had decreased to 13% of the products in 2007 and 1% in 2008 (FCPSA, 2008b).

DEP is used in aspirin coatings, as diluents in polysulfide dental impression materials, intestinal tubings, dialysis tubings, as camphor substitute and in pharmaceutical packings (Verschueren, 1983; Anonymous, 1985; Hawley, 1987; USEPA, 1989; ATSDR, 1995).

2.7.4 Sources of Human and Environmental Exposure to Phthalates

The phthalates enter the environment primarily during production and manufacture (minor pathway) and by leaching, migration and volatilization (major pathway) during use and after disposal of the products.

These releases are mainly to soil and water as a result of leaching from landfills. From soils with low organic content phthalates may enter underground water (Mihovec-Grdić *et al.*, 2002). It is presumed that about 100 million tonnes of di-ethylhexylphthalate (DEHP) were present in the techno sphere at the end of the 1990s (Furtmann, 1994).

Phthalates are often found in water, soil, air, food products and in the human body (Castillo and Barcelo, 1998). Potential sources of phthalate acid esters (PAEs) to humans include drinking of contaminated water, eating of foods into which phthalates have leached from packing materials , consuming contaminated sea food, inhaling contaminated air or as a result of receiving medical treatment involving the use of medical materials made from polyvinyl chloride (PVC) softened with DEHP such as peritoneal dialysis and haemodialysis sets, infusion sets, PVC bags for storing bags and blood derivatives (WHO, 2003, Castillo and Barcelo, 1998; ATSDR,2002). Other sources include PVC parenteral nutrition tubes, children toys and products and repellents used in dielectric impregnation.

It has also been reported that phthalate esters migrate from plastic bottles and the cap into the contents of the bottle (Prepatpong and Kanchanamayoon, 2010). People who drink water containing DEHP higher than the recommended maximum level for a long time may have liver problems and increased risk to getting cancer (Holadova *et al.*, 2007).

Oral exposure is the main route for phthalates that are used as plasticisers such as DEHP while dermal and inhalative exposures are considered to be major routes for phthalates found in hygienic products such as soap, shampoo and conditioners (Latini, 2004; Klimisch *et al.*, 1992).

2.7.4.1 Phthalates in Food

PAEs can be found throughout in diets, including children's formulae and baby foods. Mortensen (2005) and Sorensen (2006) carried out studies to determine phthalate monoesters in human milk, consumer milk and infant milk and reported levels of MBP and MEHP in the ranges 0.6–3.9 µg/L and 5.6–9.9 µg/L respectively.

Phthalates have also been reported to leach easily into foods heated in plastic containers (Peterson and Breindahl, 2000; Sharman *et al.*, 1994; Guist, 1990.). DEHP and other phthalates have been reported in almost all analyzed milk, cream, cheese and butter samples in a study carried in UK, Spain and Norway (Sharman *et al.*, 1994; ATSDR, 2002).

Bosnir *et al.* (2003) found phthalates in food samples packed in plastics and attributed such presence to plastic residues from packaging or from the gloves that are used when packaging the food (Tsumura *et al.*, 2001a, 2001b, 2002).

In another study Castle *et al.* (1988) baked food samples that were packaged in card boxes with cellulose acetate windows containing 16-17% w/w DEP. On analysis the samples were reported to contain 1.7-4.5mg/kg DEP. In this study it was suggested that the phthalate may have volatized from the plastic windows to the food without direct contact or may have been adsorbed in the condensate on the window which may have then fallen back into the food. Based on these findings Kamrin and Mayor (1991) estimated a daily dietary exposure to DEP as 4mg/kg of cellulose acetate wrapped food.

Di-ethylphthalate was detected in pies, crackers and chocolate bars at 1.8 µg/g (average), 1.2µg/g -5.3µg/g as a migrant from pie carton windows, paperboard box, and aluminium foil respectively (Page and Lacroix, 1995). A similar study reported migration of phthalate esters from an aluminium foil-paper laminates to butter and margarine (Page and Lacroix, 1992).

Oysters obtained from a navigation canal in Louisiana, USA, and clams from the Chef Menteur and Rigolets tributaries of Lake Pontchartrain, Louisiana, were reported to contain 1100µg, 450µg, 340 µg diethyl phthalate/ kg wet weight respectively (Mc Fall *et al.*, 1985a).

2.7.4.2 Phthalates in Human Tissues

People may be exposed to phthalates through parenteral administration which occurs with the use of some medical devices. In a study carried out by (Christensen *et al.*, 1976) diethyl phthalate was found to be leached from polyvinyl chloride dialysis tubing containing aqueous electrolyte solution, human blood, or bovine plasma perfusates. The tubing was perfused with the aqueous electrolyte solution for 1 hour, resulting in a level of diethyl phthalate as 20 mg/L as determined by ultraviolet spectrometry although the levels dropped with extended perfusion time. When the tubing was perfused with either human blood or bovine plasma for 8 hours, infrared spectrometry showed diethyl phthalate levels 2-4 times greater than with water, suggesting that diethyl phthalate has greater solubility in lipid containing fluids than in inorganic solutions (Christensen *et al.*, 1976).

Although the relative contributions of exposures to total body burden at various ages are not established, studies have documented multiple exposure to multiple phthalates at all stages of life. This has been confirmed even in both animal and human amniotic fluids (The National academy of Sciences, 2008).

Infants in neonatal intensive care units (NICU) as well as patients receiving multiple treatments or transfusions through medical tubings containing PAEs get exposed to

phthalates. DEHP and its toxic metabolite monoethylhexylphthalate (MEHP) have been detected in serum of neonates following intravenous (I.V) fluid administration, extra corporeal membrane oxygenation (ECMO), mechanical ventilation, I.V feeding with nutrients and blood transfusions (Sjoberg *et al.*, 1985; Shneider *et al.*, 1991; Shea, 2003; Calafat *et al.*, 2004; Green *et al*; 2005). Studies carried by Blount *et al.* (2000) investigated levels of seven urinary phthalate metabolites in a human population and reported higher concentrations of monobutyl phthalate in women of reproductive age (20-40 years), a reproductive and developmental toxicant, than other age/gender groups.

DEP was detected in 42% of human adipose tissues taken from cadavers and surgical patients in various regions of the USA in 1982. Concentrations ranged from below the detection limit (0.20 µg/sample) to a maximum of 0.65 µg/g tissue wet weight (USEPA, 1986).

National Health and Examination Survey in the USA during 1988-1994 analysed presence of monoester metabolites of seven phthalates (monoethyl, monobenzyl, mono-2-ethylhexyl, monoisononyl, monooctyl, monobutyl and monocylohexyl), in urine of 289 adults after glucuronidase treatment and found monoethylphthalate at the highest concentration with a geometric mean level of 345 µg/ litre and a 95^{th} percentile of 3750 µg/litre. (Blount *et al.*, 2000).

In a similar study US Centre for Disease Control (2001), analysed urine of 1024 people ≥ 6 years of age and found out the 50^{th} and 90^{th} percentiles of urinary monoethylphthalate concentration to be 171 and 1160 µg/ litre, respectively.

As evidence of exposure phthalates esters and their metabolites various studies have detected and measured them in pregnant women (Adibi *et al.*, 2003), infants and children (Becker *et al.*,2004;Colon *et al.*, 2000; Calafat *et al.*, 2004).

2.7.4.3 Phthalates in Air

A study carried by Bornehag *et al.* (2004) analysed particulate dust in homes and reported presence of both BBP and DEHP in 38 out of 372 homes. In similar studies Oie *et al.* (1997) and Rudel, (2003) have detected phthalates in house dust and suggested that inhalation may be an important route of exposure. However, a study carried by Becker *et al.* (2004) found no significant association between metabolites in children urine and DEHP levels and house dust. The study findings of Bornehag *et al.* (2004) associated increased incidences of eczema and rhinitis in children with increased concentrations of BBP in house dust.

In a methodological pilot study Wallace *et al.* (1984) assessed exposure to 12 volatile organic compounds in 12 residents of New Jersey and USA. In this study DEP was detected in 1 out of 8 ambient air samples and 2 out of the exhaled breath samples.

Air emissions of di- (2-ethylhexyl) adipate from 148 industrial facilities amounted to about 315,000 kg in 1994 in the United States of America, according to the Toxics Release Inventory (USEPA, 1996).

Capon *et al.* (1996) in their study reported two cases of potential contact dermatitis in two women from the plastic of their computer mice which was known to contain phthalates. In this study one woman showed a positive reaction in a patch test with 5% DEP, and the other showed sensitization with 5% DMP. When the women covered their computer mice with a DEP free cover the lesions cleared.

Repeated dermal administration of DEP had no adverse effects on the histopathology of the spleen, lymph nodes thymus or thyroid/ brain weights in rats (up to 855mg/kg body weight) or mice up to 772 mg/kg after exposure for 2 years (NTP, 1995).

2.7.4.4 Phthalates in Drinking Water

Di-2-ethylhexyl adipate (DEHA) was found at microgram per litre levels in two of five samples of finished water from a water-treatment plant in the United States (USEPA, 1996; WHO, 1996). It was detected in drinking-water in New Orleans, Louisiana, at an average concentration of 0.10 µg/L but not in drinking-water in two smaller nearby cities (IARC, 1982). DEHA was also detected in the Delaware River at levels of 0.08–0.3 µg/L (Sheldon and Hites, 1979). It has also been identified in Europe as a trace level contaminant of the River Rhine (WHO, 1996) and in the Great Lakes of North America at levels of 0.01–7.0 µg/L (Hrudey *et al.*, 1976).

Sheldon and Hites (1979) also reported DEHA at levels of 2000 µg/L in a chemical plant source near the Delaware river, north of Philadelphia.

2.7.5 Occupational Exposure to Phthalates

Occupational exposure may occur in facilities that phthalates are manufactured or used particularly as plasticizers of PVC films through inhalation, mainly aerosol and in other materials used in food packaging such as adhesives, cellophane and hydroxyethyl cellulose films (WHO, 2003).

Exposure may also occur during the manufacture of rubber products, nonferrous wire, cosmetics, lubricants and hydraulic fluids (Opresko, 1984). Exposure concentrations of 0.25 mg/m^3 and 0.14 mg/m^3 just above the hot wire of a PVC film cutting machine have been reported in tests simulating normal operating conditions when the wire was operated at 182 °C and 104 °C, respectively (Van Houten *et al.*, 1974).

Cook (1980) estimated from test emission data that maximum di-(2-ethylhexyl)-adipate concentrations of 0.2 mg/m^3 in workroom air could be reached in hot wire operations.

The National Institute for Occupational Safety and Health reported non-detectable levels of di(2-ethylhexyl)adipate (less than 0.08 mg/m3) near a cool rod machine (operating

temperature of 190 °C) used to cut PVC film in a meat cutting and wrapping department of a grocery store (Daniels *et al.*, 1985).

2.7.6 Degradation of Phthalates

Environmental fate processes of phthalic acid esters are driven mainly by their hydrophobicities and ability to partition and adsorb to organic phases. Sorption to terrestrial soils plays an important role in reducing the mobility of these chemicals and may significantly delay their entry into groundwater and aquatic systems .The most significant environmental loss processes for phthalates occurs through biodegradation while abiotic processes, including volatilization, hydrolysis, and photolysis, are of minor environmental importance (CCME, 1993).

As diesters phthalates are hydrolysed in two steps forming the corresponding alcohol and phthalic acids which are further degraded either aerobically or anaerobically. Ultimate degradation involves degradation of the ester to carbon dioxide however; long chain phthalates indicate reduced degradability under both aerobic and anaerobic conditions (Liang et al., 2008). Temperature, nutrient content and accessibility to oxygen influence degradation rates. Phthalate esters are mainly set free by volatilization. In the atmosphere they are photo degraded with predicted half-lives of ca. 1 day while their photo degradation half life in water is much longer (Staples *et al.*, 1997). While abiotic hydrolysis under environmental conditions has been reported insignificant in acidic media, phthalates have been found to be so stable to an extent that even cleanup with concentrated sulphuric acid has been reported possible (Thurén and Södergren 1987).

DEHP has been reported to degrade in water, activated sludge and in soil and can be used as a sole carbon source by several microbes in pure culture studies (Richards and Shieh, 1986).

2.7.7 Toxicity of Phthalate Acid Esters and their Metabolites

Phthalate esters are classified as hormonally active agents (HAAs) or endocrine disruptors because they interfere with the endocrine system. Toxicological studies have linked some of these compounds to liver and kidney damage, and to possible testicular or reproductive-tract birth defect problems, characterizing them as endocrine disruptors.

In the body they are converted to toxic metabolites that easily react with biologically active substances and may impair vital functions of the body (Sharman *et al.*,1994; Jarosova 2006).

The United States Environmental Protection Agency (EPA) has listed 6 PAEs among the primary risk pollutants. These include; dimethyl-phthalate (DMP), di-ethylphthalate (DEP), di-*n*-butyl-phthalate, (DBP), di-2-ethylhexyphthalates (DEHP), di-*n*-octyl-phthalate; (DOP) and dibutyl benzyl phthalate, (BBP). Among them DEHP and DBP are the most frequently used. Metabolites of phthalates are biologically active compounds that exert developmental effects on organisms following exposure to phthalate esters. Mono-n-butylphthalate, (MBP), a metabolite of DnBP and mono-ethylhexylphthalate, (MEHP), a metabolite of DEHP has been reported to result in skeletal malformations and testicular development abnormalities just like BBP or DEHP (Ema *et al.*, 1992, 1994, 1996, Ema, 2003; Ema and Miyawaki 2001; Kessler *et al.*, 2004).

Metabolites of BBP are MBP and mono-n-benzylphthalate, (MBeP) While both MEHP and 2-ethylhexanoic acid (2-EHA) are metabolites of DEHP (Hines *et al.*, 2009; Meeker *et al.*, 2007).

Acute toxicity of phthalates is low. The LD_{50} for rats has been reported as 8-18g/kg and 31g/kg for DBP and DEHP respectively (Shibko and Blumethal 1973). In 1991 the European Food Commission set Tolerable Daily Intake (TDI) levels by humans to 25µg/kg per body weight per day for DEHP and 50µg/kg per body weight per day for DBP (Jarosova, 2006).

Phthalate esters and their metabolites have been reported to be developmental and reproductive toxicants affecting mainly male reproductive system and are suspected of disrupting the endocrine system that is responsible for sexual development and other physiological functions in both male and female.

In both *vitro* and *vivo* experiments involving live tissue cultures, hepatotoxicity, nephrotoxicity, teratogenicity, embryotoxicity and spermiotoxicity have been reported to be side effects associated with long term exposure to PAEs (Jarosova, 2006) hence characterised them as endocrine disruptors.

Swan *et al.*(2005) in their study provided evidence that maternal exposure to phthalates during pregnancy increased maternal urine concentrations of four metabolites (MEP, MBP, monobenzylphthalate(MBeP) and monoisobutylphthalate MiBP) and associated these with decreased anogenital distance (the distance between the anus and the base of the penis) in boys aged between 36 months to 2 years. To a large extent urinary phthalate do not represent the concentrations of oxidative metabolites in breast milk but instead reflect maternal exposure to the esters (Hines *et al.*, 2009).

Both BBP and DBP have MBP as their metabolite which has shown to be teratogenic in rats following maternal exposure during pregnancy leading to not only undescended testes, decreased anogenital distance, and decreased testes weight but also increase in both fetal death and fetal skeletal malformations (Ema *et al.*, 1992, 1996; Ema and Miyawaki, 2001; Saillenfait *et al.*, 2001; Saillenfait *et al.*, 2003; Tyl *et al.*, 2004). While studies of Liu *et al.* (2005) and Gray *et al.* (2006) included hypospadias (urethra on the underside of the penis) as one of the defects in male rat reproductive development following a prenatal exposure to BBP. Tyl *et al.* (2004) in addition reported delayed puberty in both sexes.

An Investigation on reproductive effects of butylbenzylphthalate (BBP) in pregnant and pseudo pregnant rats reported reduction of ovarian and uterine weights, progesterone levels and ovulatory follicles (Ema, 1998).

DBP has been reported to increase proliferative index in mammary terminal end buds and lobules as well as induce an increase in uterine weight/body weight ratio and decrease in vaginal opening in female rats when exposed to it on days 2-20 of life (Moral *et al.*, 2007). In another study Moral *et al.* (2011) reported that the same PAEs registered delayed of pubertal onset and increased proliferative index and modified morphology and expression profile in female rat mammary gland following prenatal exposure. DBP has been also reported to induce neoplastic transformation of human breast epithelial cell line (Fernandez and Russo, 2010; ATSDR, 2001).

In adult female rats, BBP exposure has resulted in incidence of mononuclear cell leukemia and liver disorder effects, including increased liver size (Nagao *et al.*, 2000; NTP-CERHR,2007).

Following increased mononuclear cell leukemia in female rats BBP has been classified as a possible human carcinogen (USEPA, 1986). However, WHO International Agency for Research on Cancer (IARC) classified the same phthalate as Not Classifiable as to carcinogenicity in 1999. IARC is reassessing the ester (USEPA, 2005).

Different phthalate esters (di-(2-ethylhexyl)phthalate, benzylbutylphthalate, di-isononyl-phthalate, di-octylterephthalate, dimethylphthalate and diethyl phthalate) were administered to different pregnant SD rats (3-16 rats per phthalate ester) at 750 mg/kg body weight per day from gestation day 14 to postnatal day 3 (Gray *et al.*, 2006).Twelve male off springs were examined for incidence of malformation, changes in body weights of dams or pups, effects on genital organs (testis, seminal vesicle, prostrate or epididymis, penis), liver, pituitary, or

adrenal gland weights and pubertal development. None of these effects were observed following treatment with DEP whereas with BBP and DEHP, induced shortened anogenital distances and decreased testis weights were noted. A cross-sectional study carried by Zhang (2006) reported reduction in human semen quality following phthalate exposure.

PET containers may yield endocrine disruptors under conditions of common use particularly with prolonged use/storage and elevated temperature. The disruptors could be one or more phthalates (Pennarum *et al.*, 2004).It has been suggested that prenatal exposure to anti androgenic phthalates may be associated with less male behaviour in boys and generally alternation of androgen- responsive brain development in humans (Swan, 2008).

In a study carried out by Montuori *et al.* (2008) on 71 commercial brands of bottled water in glass and PET, the concentrations of all phthalates in PET was 12 times higher whereas concentrations in glass bottles were below the limits of detections. The 50^{th} percentile for all phthalates in all samples was 1.3µg/l. The most abundant phthalates found were dibutylphtalate, di-isobutyl phthalate and diethyl-phthalate (DEP). However, this study did not measure the concentration of phthalates as a function of time. There is a possibility that the water was contaminated before bottling. Time is a dominant factor in governing the release of organic substances from PET (Wegelin *et al.*, 2001; Lilya, 2001; Nawrock *et al.*, 2002).

Farhoodi *et al.* (2008) involved the interaction of incubation time with storage temperature on the leaching of DEHP from PET bottles. Using a solution of 3% acetic acid as a food stimulant, they incubated the solution in PET for 120 days either at 25 ^{0}C or at 45°C. On day 0 no DEHP was detected but on the 25^{th} day the amount of DEHP in the solution at 25^{0}C was 1.2 mg/l, while that in the solution incubated at 45°C was 2.1 mg/l. On the 66^{th} day DEHP at

25°C was 1.4 mg/l whereas that at 45°C peaked at 2.5 mg/l. Biscardi *et al.* (2003) reported similar results after a 9-month incubation of water in PET bottled at room temperature.

Casajana and Lacorte (2003) investigated the effect of prolonged incubation on the concentration of various phthalates in water PET bottles compared with water from glass bottles. They found that three out of five brands showed measurable levels of di-(2-ethylhexyl) phthalate, DEHP, after 10 weeks of incubation with an average concentration of 0.134µg/l of DEHP. All the five brands showed measurable levels of DEP after 10 weeks of incubation with an average DEP concentration of 0.214µg/l.

Schmid *et al.* (2008) sought to determine whether solar disinfection (SODIS) of water promotes leaching of phthalates into water in PET bottles. After 17 hours of incubation in direct sunlight maximum concentrations of di-(2-ethylhexyl) adipate and DEHP were 0.046µg/l and 0.71µg/l respectively. Biscardi *et al.* (2003) used mineral water before bottling. They then filled PET bottles and glass bottles with mineral water both carbonated and uncarbonated. All bottles were stored at room temperature for 12 months of study. Samples were lyophilized with powder then shaken with acetone after which the acetone extracts were analyzed using GC/MS. No phthalates were detected in any sample for the first 8 months but at the beginning of the 9th month PET bottled non-carbonated water and 10th month for PET bottled carbonated water analyzed using GC/MS showed that DEHP increased from 0.4 to>3.0mg/l.

Biscardi *et al.* (2003) and Criado *et al.* (2005) identified di (2-ethyl-hexyl) phthalate (DEHP) and di- butylphthalate in water that was stored in PET bottles for several months. The study did not report any mutagenic or carcinogenic compound at critical concentrations. In a study to assess the leaching of DEHA and DEHP from PET bottles to water carried out in India, levels of DEHA ranging between 0.001 and 0.0256 µg/l were detected, while DEHP

concentrations varied from 0.006 to 0.532 µg/l (Senthil and Ligy, 2009). These are within the permissible limits in WHO guidelines for drinking water quality for DEHA and DEHP which are set at 80 µg/L and 8 µg/L respectively (WHO, 1993a ;WHO, 1993b).

Prepatpong and Kanchanamayoon (2010) carried out a study in Maha-Sarakham province in Thailand to characterize and quantify PAEs in drinking water stored in different brands of PET bottles and reported concentrations of DMP, DEP, DBP and DEHP as 0.16-0.53 mg L^{-1}, 0.54 mg L^{-1}, 0.13-0.33 mg L^{-1} and 0.28-0.49 mg L^{-1} respectively. The concentrations of DMP, DEP, and DBP were in safe levels while concentrations of DEHP in three samples were higher than the regulated maximum admissible concentrations. These studies agreed with the findings of another similar study that had been previously carried by Penalver *et al.* (2000) which too showed out the regulating maximum admissible concentrations at 6µg $L.^{-1}$

Prokupkova *et al.* (2002) reported that DBP and DEHP were in higher levels in mineral water than other samples while Nawrock *et al.* (2002) reported that room temperature, carbonyls.(formaldehyde, acetaldehyde and acetone) leached within 2-5 hrs from PET into water. Acetaldehyde concentrations were higher in newer bottles compared with bottles that were one month old.

Another possible source of formaldehyde can be as a result of natural organic matter during ozonation or chlorination. Formaldehyde has been proved to exhibit increased incidence of carcinomas of the nasal cavity in rats and mice (WHO, 2011). IARC has classified formaldehyde as a human carcinogen. A tolerable concentration of 2.6mg/L for ingested formaldehyde has been established based on a NOEL of 260 mg/L for histopathological effects in the oral and gastric mucosa of rats administered formaldehyde in their drinking water for 2 years using an uncertainty factor of 100 for interspecies and intra species variation.

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Evandri *et al.* (2000) reported genotoxic activity in mineral water samples after 8 weeks of storage both in the dark and in the light, using a plant-based genotoxic assay. They observed that effects were enhanced by sunlight exposure and slightly increased storage temperatures. However the study did not reveal any chemical compound (s) that could have been responsible for the identified effects.

Ubomba *et al.* (2010) in their studies also detected significant genotoxicity in both the dark and sunlight no-refill samples after 2 months. The same study reported that no further genotoxicity was observed after 2 months. No indicator organisms were added to sample bottles in this study to help monitor the levels of disinfection. There is a possibility that organic compounds from the destruction of microbial cell membranes and organs contribute to such gene toxicity.

Endocrine disrupting effects include increased adiposity and insulin resistance (Grun and Blumberg, 2009), decreased anogenital distance in male infants (Swan *et al.*, 2005), and decreased levels of sex hormones (Pan *et al.*, 2006; Hauser and Calafat, 2005; Sathyanarayana, 2008). These Reports also revealed that children and infants are vulnerable to toxic effects of phthalates.

Investigations at University of Missouri tested the effect of an unspecified brand of PET bottled water on proliferation of breast cancer cells and found out that PET bottled water triggered a 78% increase in the growth of breast cancer cells compared with control water. In this study, 1,200 breast cancer cells multiplied to 32,000 in 4 days when incubated in PET bottled water versus 18,000 for the control sample (Naidenko *et al.*, 2008). However, studies by Pinto and Reali (2009) did not prove that the endocrine disruptors leached from the PET bottle wall and suggested that they might have been present in the water before bottling.

For organic substances to leach from PET bottles into water, Wegelin *et al.* (2001) and Lilya (2001) found that time is a major determinant. The same was observed by Westerhoff *et al.*, (2008) in the case of antimony which is present in PET due to its use as a polymerization catalyst. Antimony from PET leaches under conditions not typically occurring in the SODIS process i.e. at 80°C for several days.

In one of the few human studies that have been carried out phthalates were investigated as a cause of precocious puberty in young Puerto Rican girls (Colon *et al.*, 2000). In this study, the serum levels of phthalates obtained from 41 girls with premature appearance of breast tissue were compared with 35 controls. Phthalate esters were detected in 68% of the cases and in the 17% of the controls and they were found significantly lower levels of phthalates than the cases. For the bis (2-ethylhexyl) phthalate (DEHP), the average concentration was 70 ppb in the controls compared to 450 ppb in the cases. Even if the study conclusions were limited due to small population size and the possibility of contaminated serum samples, the association between phthalates and premature thelarche is biologically plausible. Two studies present the first human data which demonstrate that phthalates are associated with increased DNA damage in sperm (Duty *et al.*, 2003a; Duty *et al.*, 2003b). A study carried by Duty *et al.* (2005) concluded that monobenzyl phthalate (MBzP) exposure was significantly associated with a 10% decrease in follicle stimulating hormones (FSH) concentration in adult men.

2.7.8 Exposure Limits/Toxicity Reference Values of Some Common Plasticisers

In order to assess the safety issues of food or water containing phthalates their intake can be Compared to the reference doses (RfD) defined by the USEPA. The RfD being an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

While the WHO standard concentrations for DEHA and DEHP in drinking water are set as 80 µg/L and 8 µg/L respectively their reported ranges are at 0.001-0.0256 µg/L and 0.006-

0.5320µg/L respectively (WHO 1993a, WHO, 1993b).The US Environmental Protection Agency (EPA) has set the Maximum Admissible Concentration (MAC) or the Maximum Contamination Level (MCL) of DEHP at 6 µg L^{-1} and the Threshold Limit Value (TLV) of DMP, DBP, and DEP as 5.0, 0.45, 0.55 and 5.0 mgL^{-1} respectively (US EPA, 1991).

Reference Dose (RfD) for Chronic Oral Exposure to Butyl benzyl phthalate (BBP) is set at 0.2mg/kg/day, based on liver-to-body weight and liver-to-brain weight ratios (USEPA, 1993) while that for dimethyl terephthalate (DMT) for Chronic Oral Exposure is 0.1mg/kg/day, based on chronic kidney inflammation in adult rats (USEPA,1995). This ester is known to significantly increase liver to body weight and liver to brain weight ratios in adults. The Minimal Risk Level (MRL) for Oral acute exposure to DMT is set at7 mg/kg/day, based on reproductive effects while the Oral intermediate exposure, remains 6 mg/kg/day, based on hepatic effects (ATSDR, 2006).

Reference Dose (RfD) for Chronic Oral Exposure to Dibutylphthalate (DBP) is 0.1 mg/kg/day, based on increased mortality in adult animals (USEPA,1990 ;USEPA,1993) While the Minimal Risk Level for Oral acute exposure of the phthalate is 0.5 mg/kg/day, based on developmental effect (ATSDR,2006). Di-*n*-octylphthalate (DnOP) has its Minimal Risk Level (MRL) for Oral acute exposure quoted as 3 mg/kg/day, while oral intermediate exposure value is considered as 0.4 mg/kg/day, based on hepatic effects (ATSDR,2006).

U.S. EPA Reference Dose (RfD) for Chronic Oral Exposure to DEP is 0.8 mg/kg/day based on decreased growth rate, food consumption and altered organ weights in adult rats (USEPA, 1993). Di-(2-ethylhexyl) phthalate has its Reference Dose (RfD) for chronic oral exposure set at 0.02 mg/kg/day, based on increased relative liver weight in adult animals (USEPA, 1991) and a cancer oral slope factor of 0.014 mg/kg/day, based on hepatocellular carcinoma and adenoma, using the linearized multistage procedure extra risk (USEPA,1991). The cancer drinking water risk for DEHP is 0.0000004 (µg/L) the maximum contaminant level(MCL)

and the maximum contaminant level goal (MCLG) are 0.006 mg/L and 0 mg/L respectively based on reproductive difficulties, liver problems, and increased risk of cancer in adult animals (USEPA, 2006).

ATSDR (2006) has set the Oral intermediate exposure to DEHP at 0.1 mg/kg/day, based on reproductive effects in adult animals while Oral chronic exposure is 0.06 mg/kg/day, based on reproductive effects in adult animals.

2.8 Adipates

Adipate plasticisers are part of aliphatic dibasic esters obtained when alcohols of similar chain length to those used in phthalate manufacture are esterified with adipic acid, rather than phthalic anhydride, to produce the family of esters.

For example, the most used adipate plasticiser di-2-ethylhexyl adipate (DEHA) also known as di-octyl adipate (DOA), is obtained from esterification of 2-ethylhexanol with adipic acid (ECPI, 2009; Krauskopf and Godwin, 2005). Adipic acid esters used in PVC applications improve low temperature performance relative to phthalates and give significantly lower plastisol viscosities in plastisol applications, due to the lower inherent viscosities of the plasticisers themselves. Adipates used are typically based on alcohols in the C8 to C10 range (ECPI, 2009). Typically, lower molecular weight alcohols are used with higher molecular weight acids, and vice versa, such that the total carbon content per molecule ranges between C18 and C26. This maintains the apolar/polar ratio required to provide PVC compatibility along with low temperature properties (Krauskopf and Godwin, 2005).

Relative to phthalates, adipates have higher volatilities and higher migration rates and as a result they are blended with phthalates to produce a compromise of properties (ECPI, 2009).

2.9 Mutagenicity Tests

Chemical data alone cannot show the integral impact of mixtures on living organisms. To overcome this limitation, bioassays are used to complement chemical analysis. Toxicity being a consequence of addition, synergism, antagonism and bio-activation can be shown directly by bioassays which minimize false positive or negative result and assist in human risk assessment (Lah *et al.*, 2005).

Most bacterial tester strains contain mutations which increase sensitivity to genotoxins. The *rfa* mutation for example causes a partial loss of cell wall and therefore increases permeability to larger molecules such as benzo[a]pyrene. The *uvrB* mutation of most Ames-tester strains deletes a gene coding for the DNA excision repair system and therefore hinders the repair of DNA damage. Since bacteria do not possess the metabolic capacity of eucaryotes, the tests are usually performed in the absence and the presence of S9 liver homogenate (supernatant of rat liver extract centrifuged at 9000 g). The Ames mutagenicity test is among the most popular assays employed to monitor toxicity levels of water.

2.9.1 Ames Mutagenicity Test

Ames test, also known as Salmonella assay is an *in vitro* test used as a screening test for evaluation of chemicals for mutagenicity (Maron *et al.*, 1983). The Ames assay, originally developed by Ames *et al.* (1973a) is well validated, widely used and allows comparison of results of investigators with the results of other researchers who commonly use it as the sole assay for testing genotoxicity/mutagenicity in water (Lah *et al.*, 2005a,b).

The test depends on concentration of the samples, necessitating the metabolic addition of activation homogenates system. However, the test is insensitive towards certain groups of compounds such as halogenated hydrocarbons and heavy metals (Haider *et al.*, 2002). Although the assay depends on the concentration of samples necessitating the metabolic addition of activation homogenates system, a greater amount of sample volume can be tested

without the need for concentration, thereby avoiding concentration methods that might otherwise change the original genotoxicity of the sample of interest (Monarca *et al.*, 1985; Stahl, 1991; Le Curieux *et al.*, 1996). Moreover, using unconcentrated samples for analysis of SODIS water gives a more accurate estimate of the genotoxic risk to which a SODIS user will be exposed since SODIS users do not concentrate their water before drinking (Ubomba *et al.*, 2010).

The assay is bacterial reverse mutation test that uses amino acid requiring strains of mutant *salmonella typhimurium* that have lost their ability to grow in the absence of histidine to detect potentially mutagenic substances .

Point mutation is carried in the histidine locus of the bacterium rendering it incapable of synthesizing one of the enzymes responsible for the production of the amino acid histidine. As a result the bacterium loses the ability to grow in ordinary nutrient medium lacking histidine not unless an external supply of histidine is provided. Reverse mutations caused by exposure to mutagenic compounds can reactivate their ability to synthesize histidine and thus can grow in the absence of histidine. Thus a mutagenic event is detected by the test compound restoring its ability to synthesize histidine in presence or absence of metabolic activation system causing spontaneous reversions that allow the bacterium to form a colony. The number of colonies at different concentrations of the test compound is compared with that of the negative controls and this indicates the degree of mutagenicity (Ames *et al.*, 1973a, 1973b, Maron and Ames, 1983). The most commonly used *Salmonella* strains in water screening are TA 98 and TA 100.

Different lesions are constructed on the histidine operon of different tester strains coupled with other mutations which increase their sensitivity to different mutagens. Both tester strains have *rfa* mutation which leads to a defective lipopolysaccharide (LPS) layer that coats the cell surface rendering the bacterium permeable to large (bulky) molecules.

The *uvrB* deletion mutation eliminates the accurate excision repair mechanism hence allowing more DNA lesions to be repaired by error prone DNA mechanism. The deletion through the biotin gene makes the bacteria biotin dependant. Hence strains carrying the uvrB mutation are deficient in excision repair of bulky lesions as measured by lack of survival following UV_{254} radiation

The plasmids PAQ1 and PKM101 confer tetracycline and ampicillin resistance respectively. While TA102 strain bears both plasmids, TA 98 and TA 100 tester strains bear only PKM101 which enhances chemical mutagenesis through increase in error prone recombination DNA repair pathway. The R factor plasmid carries the *muc*A and *muc*B genes to compensate for the weak mutagenic activities of the *umu* operon in *Salmonella*. Table 2.1 shows the genotypes of the Ames MPF™ *Salmonella typhimurium*.

Table 2.2: Genotypes of the Ames MPF™ *Salmonella typhimurium*

Strain	Reversion Event	Addition mutation	Plasmid
TA98	Frame shift	*rfa* mutation, *uvr*B deletion	pKM101
TA100	Base pair substitution	*rfa* mutation, *uvr*B deletion	pKM101
TA102	Base pair substitution	*rfa* mutation	pKM101, pAQ1
TA1535	Base pair substitution	*rfa* mutation, *uvr*B deletion	-
TA1537	Frame shift	*rfa* mutation ,*uvr*B deletion	-

These mutations affect the lipopolysaccharide component of the cell envelope and enable the strains to have permeability of bulky molecules.

Most genotoxic compounds in drinking water are nonvolatile, quite acidic and non-stable at high pH and rather polar. Among these compounds, the most abundant are the volatile

chlorinated and or brominated trihalomethanes, usually not easily recovered in drinking water extracts. Other natural or human activity related drinking water contaminants include halogenated derivatives of acetic acids, acetonitriles, ketones, phenolic compounds, arsenic, nitrates and bromates, which are responsible for cancer in rodents or in humans (Glaze *et al.*, 1993).

The halogenated hydroxyfuranones, although present at concentrations lower than 0.1 µg/l in drinking water, have been reported to be responsible for more than half of Ames test mutagenicity (Wright, 2002; Jansson, 1993) .The so called "Mutagen X" (MX), the most potent of these chlorohydroxyfuranones, has been identified as a very strong carcinogen in rats (Kumulainen *et al.*, 1997).

Although mutagenic potency can be detected in non concentrated samples of surface environmental fresh waters or waste water each contaminant is usually present in very low levels that make it difficult to detect. Therefore, extraction and concentration of such samples is necessary for reliable mutagenicity assessment. Liquid- liquid extraction using organic solvents provides quantitative information and is widely used (Takeshi *et al*., 2004).

Using Ames test in solid agar medium with *Salmonella typhimurium* (TA98 and TA100 strains) De fusco *et al.* (1990) found that some mutagenic activity occurred in mineral water stored for 1 month. Bottles exposed to sunlight recorded higher mutagenic activity than those that were in the dark. A similar study conducted by Monarca *et al.* (1994) revealed no mutagenic activity in mineral water after one month of storage. Biscardi *et al.* (2003) observed mutagenic behavior not only in samples without light exposure during mineral bottle storage but also from pipes supplying water for bottling process. None of these conditions resembled those experienced by PET bottles during SODIS use. However since SODIS users drink the SODIS water directly from the bottle this study therefore used the Ames

mutagenicity assay test using unconcentrated samples while the concentrated samples were used in chemical analysis.

2.10 Supernatant 9000g (S9)

This is a mammalian microsomal fraction of liver homogenate containing biotransformation enzymes usually obtained from male Sprague Dawley rats by pre- treating their livers with enzyme inducing agents such as arcolor 1254 (polychlorinated biphenyl mixture) Maron and Ames, (1983). S9 fraction is normally added to *in vitro* biotests to mimic mammalian liver xenobiotic metabolism hence vital in the assessing the performance of an assay (OSPAR Commission, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area- Kibera Slums, Nairobi, Kenya

The study on the mutagenicity of SODIS water was conducted in Nairobi city and the adjacent Nairobi metropolitan area. Nairobi is the capital city of Kenya that lies between $36^0$45"E to 37^0 05 E and 1^0 "S to 1^0 30" S. It is situated at mean latitude of 1700 m above sea level and covers an area of about 700 Km^2. The city serves the country as the centre for industrial, commercial and administrative activities. Nairobi dam was constructed in the late 1940's as source of fresh drinking water for the Nairobi city. The dam covers an area of about 356179 m^2 and has a volume of about 98,422 m^3 .The dam is shallow with an average depth of 2.76 m .The crest of the dam is approximately1680m above sea level whereas the inlet is 1700m.

Towards the lower part of the dam, the temperature and the wind velocity increase while the relative humidity decreases. The dam is heavily infested by water hyacinth and loss of water due to evapotranspiration is about 711,289m^3. There is plastic waste dumping and heavy silt deposition on the banks of the dam which has facilitated the reclamation of the dam for agriculture.

3.2 Glassware Cleaning

All glassware used for sampling, sample extraction, clean up and analysis was thoroughly cleaned by soaking in soapy detergent water for about 2-3 hrs followed by cleaning, rinsing with clean tap water, then distilled water and finally rinsed with acetone HPLC .The acetone rinsed glassware was then oven dried at 105°C overnight.

3.3 Chemicals

Phthalate analytical standard mix comprised of 500 ppm of each of the following plasticisers; dimethyl phthalate, diethyl phthalate di-*n*-butyl phthalate, benzyl butyl phthalate, bis -2-ethylhexyl adipate and bis -2-ethylhexyl phthalate).

Butyl benzoate was used as the internal standard. The phthalate ester mix and the internal standard were both of high purity (above 99%) and were obtained from Sigma Aldrich, USA. HPLC dichloromethane, acetone, hexane, isooctane, anhydrous sodium sulphate, sodium chloride, potassium chloride, magnesium chloride, glucose-6-phosphate, NADP, NaH_2PO_4, DMSO and silica gel were all obtained from Sigma Aldrich, USA.

Ames fluctuation kit; MPF^{TM} 98/100 Mutagenicity Assay kit that comprised of *Salmonella typhimurium* tester strains (TA100 and TA98), ampicillin, exposure, growth and reversion indicator media, and Aroclor 1254-induced rat liver (liver S9 fraction) was obtained from Xenometrix (Switzerland). The positive controls (2-nitrofluorene, 4-nitroquinone, *N*- oxide-2-aminoanthracene) were all obtained from Sigma Aldrich, USA.

3.4. Equipment and Apparatus Used

Analytical balance type A-160 from Fisher, Mettlor Toledo model OV-I60 was used for all weight measurements. Gallenkamp oven model OV 160 was used for glassware drying. A Gas chromatograph Hewlett Packard GC HP6890 Agilent series equipped with a mass spectrophotometer detector MSD 5972-2 Agilent Series (GC/MSD) was used for identification and quantification of the esters. UV/VIS spectrophotomer model 1700-SHIMADZU was used to analyse the nitrogen content in the samples at a wave length of 420 nm and determining the optical densities of the cultures used in the Ames mutagenicity assay at a wavelength 600 nm.

Environmental shaker, 37°C dry incubator, adjustable pipettes, 8 channel pipettor and sterile tips 50ml tubes with filter caps and spectrophotometer cuvettes were provided by Kenya Bureau of Standards (KEBS) while the 24 well plates and 384 micro titer plates were purchased from Sigma Aldrich,USA.

3.5 Sampling and Sample Treatment

A total of 5 different water samples were collected in triplicates using clean 2.5 liter amber coloured bottles capped with an aluminium foil. The blank was carried to the field and transported back to the laboratory together with other samples in cooler boxes accompanied with ice boxes. The samples had their conductivity, salinity and pH determined using ExStik II PH/Conductivity meter (Extech Instruments Corporation, USA) at the field. The water samples were collected from a municipal tap, a house, a dam and shallow unprotected well water in the Kibera slum. Double distilled water was used both as a blank and as a sample throughout the research.

3.6 Determination of Physicochemical and Chemical Parameters of Samples

3.6.1 Determination of Conductivity

Calibration of the meter was done using the standards of conductivity 84 and 1413µS/cm conductivity standards. Distilled water was used to rinse the electrodes before determination of conductivity of a sample. Each time the electrodes were allowed to be immersed in a sample for a minute after which the reading was recorded.

3.6.2 Determination of Total Dissolved Solids and Salinity

TDS values were obtained by multiplying the conductivity value with a conversion ratio factor of 0.7 displayed on the meter once a reading had been taken. Salinity values were determined by multiplying the conductivity value with a fixed ratio of 0.5.

3.6.3 Determination of pH

Buffer solutions of pH 4, 7 and 10 were used to calibrate the meter. The pH reading of a sample was taken after thoroughly rinsing the electrodes with distilled water followed by immersion of the rinsed electrodes in the sample. For each sample, the tip of the electrode was placed in the water for a minute before a reading was recorded.

3.6.4 Determination of Temperature

Water temperature measurement was done in degree Celsius, using a digital thermometer by directly dipping the thermometer in the natural body of water. The temperatures were read and recorded to 1 decimal place.

3.6.5 Determination of Turbidity and Colour

Both turbidity and colour were obtained using the turbidity and colour meters respectively and results reported in 2 decimal places.

3.6.6 Determination of Total Suspended Solids (TSS)

Water samples were shaken thoroughly to homogenize and 100 ml was filtered through pre weighed Whatman filter paper No. 1 using a Buckner funnel fitted to a vacuum pump. The residue retained on the filter paper was dried to a constant weight in an oven at 105 ^{o}C. TSS was calculated as the weight of the residue per volume of the sample filtered and the results were expressed in mg/L.

$TSS = X_2 X_1 / V$ ..... **Equation (i)**

Where:

X_2= Weight of filter paper and dried residue in mg

X_1=Weight of filter paper in mg

V= Volume of sample in mL

3.6.7 Determination of Sulphate Ions in the Samples

A volume of 15 ml 2M hydrochloric acid was used to acidify 100 ml of neutralized sample in presence of methyl orange indicator after which the volume was topped up to 200 ml, boiled for five minutes while adding hot barium chloride in order to discourage co- precipitation. The sample was boiled further for 20 minutes, covered with an aluminium foil and allowed to cool overnight at room temperature. The weight of a clean cool sintered glass crucible that had been previously dried at 105˚C and kept in a desiccator was recorded as M_1.While fitted into a vacuum the precipitate from the test sample was filtered through sintered glass using gentle suction followed by thorough rinsing with cold water then hot water to ensure that all the chloride ions had dissolved. The filtrate was tested for chloride ions at intervals using silver nitrate after which the crucible was removed from the vacuum flask dried at 105 ˚C for 2 hours, transferred into a desiccator to cool and the crucible's new weight recorded as M_2. All other samples and the blank were taken through the same procedure and the concentration of the sulphate ions was calculated from the expression:

$[SO_4^{2-}] = (M \times 1000 \times 1000 \times 0.4116/V)$ mg/L.....**Equation (ii)**

Where V= the test portion volume in milliliters.

$M=M_2 . M_1 - M_0$

Where M_0 = Mass of blank in grammes, M_1=Mass of empty crucible, M_2= Mass of crucible + sample and 0.4116= the gravimetric factor (a constant)

3.6.8 Determination of Fluoride Ion Concentration

While on a calibration mode different standards of sodium fluoride (0.5ppm, 1.0ppm, 1.5ppm, 2.0ppm and 2.5ppm) were used to calibrate the fluoride ion selective machine. An equal volume of sample and total ionic strength buffer (mixture of 58 g NaCl, 57 ml glycerol acetic

acid in a liter solution buffered with NaOH to pH 5.4) was mixed thoroughly using magnetic stirer. The fluoride ion concentration was automatically displayed on the machine screen while on a reading mode.

3.6.9 Determination of Chemical Oxygen Demand (COD)

A volume of 50 ml water sample was poured into a 100 ml conical flask. Similarly 50 ml distilled water was transferred into another flask after which 5 ml $K_2Cr_2O_7$ (0.1 N, 3.67 g/L) solution was separately added to each flask. The flasks were incubated at 100°C for one hour while in a hot water bath and thereafter removed to cool for 10 minutes. Solutions of potassium iodide (5 ml) and concentrated sulphuric acid (10 ml) were both added into each flask and the resulting solutions thoroughly mixed. A burette was filled with 0.1 M sodium thiosulphate solution and a titration carried against each sample till the pale yellow colour disappeared. The volume of sodium thiosulphate used was recorded. One milliliter of starch was added into each flask turning the contents blue, and thereafter titration with sodium thiosulphate continued till the blue colour disappeared. The COD was calculated using the expression

$$COD\ (mg/L) = \frac{8 \times C \times V_B - V_A}{V_S} \quad \text{.....Equation (iii)}$$

Where,

C = Concentration of titrant (Mm/l)

V_A = Volume (ml) of titrant used for control

V_B = Volume (ml) of titrant used for water samples

V_S = Volume (ml) of water

3.6.10 Determination of Dissolved Oxygen (DO)

Equal volumes (2 ml) of manganese sulfate and alkaline iodine-azide solutions were separately pipetted and added in succession at the bottom of a glass bottle containing a

sample. A stopper was carefully placed on the bottle and contents shaken upside down for 8 minutes till a brown precipitate was noticed. On settling a volume of 2 ml concentrated sulphuric acid was added into the bottle and contents shaken thoroughly to encourage the dissolution of the brown precipitate. Fifty milliliters of this sample was transferred into a clean conical flask and titrated against 0.025 N sodium thiosulphate solutions in a burette until a yellow colour just appeared. Two drops of starch were added and the titration against sodium thiosulfate resumed until the blue colour obtained on adding starch just disappeared. The volume of sodium thiosulfate used in the titration was recorded and the amount of DO in mg/L was calculated using the expression,

DO mg/L = (8× 1000×N/V) v .....**Equation (iv)**

Where,

V = Volume of water samples used in titration

v = Volume of the titrant (sodium thiosulphate) used

N = Normality of the titrant

3.6.11 Determination of Dissolved Chloride Ions

A volume of 2 ml 5% potassium dichromate ($K_2Cr_2O_7$) was added into 50 ml of sample in a conical flask. A solution of 0.025 N silver nitrate was transferred into a burette and carefully titrated against each sample until a red colour just appeared. The concentration of chloride ions in the sample was calculated using the expression;

$[Cl^-]$ =Volume of $AgNO_3$ solution×35.5/Volume of water sample) mg/l .....**Equation (v)**

3.6.12 Determination of Iron Content

A stock solution of 10 mg/L ammonium ferrous sulphate was used to prepare calibration standards of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L, complexed with 2, 2-bipyridyl, (g/L) in 100ml mark volumetric flask. A mixture of 50 ml distilled water and 2 ml of 0.2N HCl was

transferred into a volumetric flask after which 40 ml of iron standard 0.2 mg/L was added. This was done for each standard separately after which 4 ml of hydroxyl ammonium chloride (the hydrochloric acid salt of hydroxylamine) solution was added followed by 5ml of ammonium acetate solution and 3 ml of 2, 2- bipyridyl solutions.

The contents of each flask were diluted to 100 ml, mixed thoroughly, their absorbances taken at a wavelength of 515 nm using the blank solution as the reference standard and a calibration chart prepared. A volume of 50 ml of each sample was treated the same way and the concentration of iron in mg/L was read from the graph. Results were reported to two decimal places.

3.6.13 Determination of Nitrogen Content

With the use of 100 ppm Sodium nitrate as the standard solution determination of nitrogen inform of nitrate ions was done using a UV/Vis spectrophotometer at a wavelength of 420 nm upon calibration. Results were recorded to two decimal places.

3.7 Microbiological Analysis of Samples

The microbiological analysis of water samples was achieved by employing the Multiple Tube Fermentation Techinique (MTFT) that consisted of a presumptive, confirmed and a complete test.

3.7.1 Presumptive Test for Coliform Group of Bacteria

Four grams of lactose broth were accurately weighed and dissolved in distilled water to make a liter solution after which 10ml of the resulting media was dispensed in 9 different test tubes for each sample. Triplicate volumes of 0.1 ml, 1 ml and 10 ml for each sample were dispensed in the test tubes with the 0.1 ml samples in tubes 1, 2 and 3 while the 1 ml samples in tubes 4, 5 and 6. Test tubes 7, 8 and 9 contained the 10 ml samples. All samples were incubated at

35°C for 24 hours and results for total coliform recorded using the Multiple Tube Techinique. Samples that tested positive to coliform group of bacteria were exposed to ultra violet (UV) light 6 for hours. Antibacterial effect of the solar light was scored by repeating the presumptive test for the already solar exposed samples. Those samples that gave positive results to coliform group of bacteria at this stage were taken for the confirmed test.

3.7.2 Confirmed Test for Coliform for Coliform Group of Bacteria

Accurately weighed 37.46 g eosine methylene blue (EMB) agar, was suspended in 1000 ml of distilled water and boiled to dissolve. The resulting solution was autoclaved at 121°C for 15 minutes, cooled to 45°C and shaken to oxidize methylene blue to restore its blue colour in order to suspend the precipitate which is an essential part. A sample that gave a positive result in the presumptive test was inoculated in EMB agar and incubated for 24 hours at 37°C and any that gave positive results to the confirmed test proceeded further for the complete test for coliforms.

3.7.3 The Complete Test for Coliform Group of Bacteria (Gram Staining)

Using a sterile loop single colonies were picked from separate EMB agar plates, inoculated into separate lactose broths and stroke on nutrient agar slants. The slants and agar plates were incubated at 37°C for 24 hours, gram reaction performed after growth and observations recorded.

Three drops of Crystal Violet (CV) stain was applied to a heat fixed smear of the bacterial culture on a slide. This was allowed to stand for 1 minute afterwhich it was washed gently with distilled water to remove the excess CV. The slide was then flooded with Gram's Iodine for 1 minute and rinsed gently with distilled water. Decolorization was done by applying an acetone-alcohol solution dropwise until the colour disappeared. The resulting Crystal Violet - Iodine complex was rinsed away using distilled water, after which, a counter stain; positively

charged Safranin was applied and allowed to stand for 1 minute. The Safranin flooded slide was allowed to stand for 1minute, rinsed with distilled water, air dried and while in an oil immersion viewed under microscope at a magnification of × 1000.

3.8 Exposure and Storage Conditions

A variety of new typical transparent PET bottles were filled with water samples, contents shaken for 20 seconds with the cap on to improve oxygen saturation after which they were each filled completely and recapped. The contents and the bottles were exposed to sunlight for a period of six hours to 90 days in a horizontal position on a roof of the chemistry laboratory. Water samples were harvested after 6 hours for the preliminary SODIS test and thereafter at 15 days, 30days, 60 days, and 90days intervals for chemical analysis (characterization and quantification of selected plasticisers) and Ames Mutagenicity assay. Solar intensities in the Nairobi region were monitored from the meteorological centre at Dagorretti on a daily basis. The control sample (double distilled water) was handled and analyzed just like any other sample throughout the study period.

3.9 Ames Mutagenicity Test

The Ames mutagenicity test is a version in liquid medium of the Ames test usually performed in agar plates. The mutagenicity test was performed as described by Legault *et al*. (1994) as described below;

3.9.1 *Salmonella typhimurium* Culture Preparation

A day prior to testing, frozen *Salmonella typhimurium* tester strains TA 98 and TA 100 in separate vials were removed from a - 80°C deep freezer and allowed to attain an ambient temperature for 5 minutes after which 200 µl of growth medium was added into each vial.

The semi solid pellets of TA100 and TA 98 *Salmonella* strains were mechanically dispersed using a sterile tip attached to a pipette to obtain a homogenous suspension of which 50 µl of each was dispensed in separate tubes containing 10 µl of 50 mg/ml ampicillin dissolved in a known volume of growth medium. The tubes then were incubated at 37˚C, 250 revolutions per minute (rpm) for 16 hours alongside a negative control that comprised of only pure growth medium. The cell cultures were maintained at room temperature during dosing and always refrigerated when not in use. The assay was strictly handled under a sterile hood.

3.9.2 Determination of Optical Densities (OD) of Cultures

Each of the 300 µl tester strain culture and negative control was transferred into separate cuvettes containing 2700 µl growth medium. After thorough mixing, contents of individual cuvettes were analyzed at a wavelength of 600 nm against a blank (pure growth medium). The actual optical densities were obtained by multiplying the spectrophotometer readings by constant factor 10. A culture was considered viable and fit for the assay if the actual OD_{600} was ≤ 2.0. An $O.D_{600}$ value less than or equal to 0.05 for the negative control meant that there was no contamination during culture preparation and Ames fluctuation test could proceed otherwise, if a value greater than 0.05 is read then the protocol should stop and fresh culture prepared.

3.9.3 Modification of Ames Mutagenicity Test using Induced Rat Liver

3.9.3.1 Preparation of S9 Mix

Stock solutions that served as components of the S9 induced rat liver mix comprised of 1.0M KCl, 0.25M $MgCl_2$ and 0.20M NaH_2PO_4 that were prepared using deionized water , autoclaved and stored at 4˚C. Others included filter sterilized sodium salts of 0.20M Glucose 6-phosphate and 0.04 M NADP kept at -20˚C till use. Just before use 6.7ml of 30 % S9 mix was prepared in a sterile hood by mixing known volumes of each component.

3.9.3.2 Preparation of Positive Controls for Samples and Exposure Working Solutions

Solutions of 100 µg/ml 2- nitrofluorene (2-NF) and 5µg/ml of 4- nitroquinoline- *N*-oxide (4-NQO) dissolved in dimethylsulphoxide (DMSO) served as positive controls for samples without S9 while a stock solution of 125µg/ml 2-aminoanthracene (2AA) in DMSO was used as the positive control for samples with S9 fraction. All dissolved positive control chemicals were always stored at -20°C when not in use. Both 10 X and 1 X exposure solutions were prepared as per the instructions in the kit provided by Xenometrix.

3.9.4 Dilution of Test Samples

Seven wells of a 24 well plate were appropriately oriented and labeled 0, 1, 2, 3, 4, 5 and 6 respectively. Wells 0 - 5 were added to 600 µl of sterile water while 1.2 ml of test sample was transferred to well 6. Using a sterile tip 600 µl of the test sample was transferred from 6 to 5. The same volume in consecutive series was transferred from well 5 to 4, 4 to 3, and 3 to 2 and lastly from well 2 to 1. Well 0 was treated as the zero- dose (negative control) for the assay and no test sample was transferred there.

For samples with S9, a mixture of 1.2 ml sterile water and 63 µl of 125 µg/ml 2AA in DMSO in a well labeled (+) served as a positive control while a similar well that comprised of 1.2 ml sterile water, 31.5 µl of both 5 µg/ml 4NQO and 100 µg/ml 2-NF in DMSO was used as the positive control for samples without the induced rat liver fraction.

3.9.5 Preparation of the 24 Well Exposure Plates

A volume of 15 µl sterile water was transferred to wells of plates without S9 after which 25 µl of 10× Exposure medium working solution was added to all wells. (TA98+/-S9, TA100+/-S9).

From the first column of the 24- well dilution plate (well 0-well 3) 185 µl aliquot was transferred to column 1,3 and 5 of the 24 exposure plate without S9 while a similar volume

was transferred from the second column of the dilution plate [4-6 and the(+)well] , to column 2,4 and 6 of the 24 exposure plate.

The transfer was repeated for the exposure plate with S9 using column 3 and 4 contents of the dilution plate followed by addition of 25 µl of TA98 culture to all wells of TA98 exposure plates or 25 µl uniform mixture of TA100 culture and 1X exposure medium to all wells of TA100 exposure plates.

Each well of S9 exposure plates was supplied with 15µl of S9 mix and contents mixed well by gentle tapping to ensure good mixing. Each 24-well exposure plate was then secured to the base of a 37˚C environmental shaker set at 250 rpm using a tape and incubated for 90 minutes and thereafter 2.8 ml indicator medium added to each well regardless of being with or without S9.

3.9.6 Transfer of Exposure Cultures to 384- Well Plates

Using an 8- channel pipettor 50µl aliquots were dispensed from wells of column 1and 2 of the 24 exposure plates into columns 1-12 and 13-24 of the 384 well plates respectively. An equal amount was similarly aliquoted from column 3 and 4 into the second 384 well plates while 50 µl aliquots from column 5 and 6 into the third 384 well plate each time different using sterile tips.

The different 384 well plates were carefully placed in sealable incubation plastic bags, the seal engaged to prevent evaporation after which they were placed in a 37˚C incubator for 2 consecutive days (48 hours).

3.9 7 Plate Scoring

The numbers of revertant yellow or partially yellow wells including those that had visible colonies at the bottom of the wells for both TA98 and TA100 with or without induced rat liver mix were manually scored and were compared with the zero dose (sterile water).

An increase in the number of revertant wells upon exposure to test sample relative to the zero dose controls indicated that a sample is mutagenic and consequently a sample was considered potentially mutagenic if the mutagenicity ratio (M.R) was ≥2.0.This ratio was obtained from the expression below

M.R= No. of revertant wells in sample / No. of revertant wells in the negative control……**Equation (vi)**

3.10 Chemical Analysis

3.10.1 Preparation of Analytical Standard Stock Solutions

All stock solutions for both the internal standard were prepared in HPLC isooctane. The amount of analytical standard required to prepare stock solutions was calculated using the expression $C = (M \times P) / V$ where C is required concentration, M is the mass of the standard to be prepared , P is the purity of the analytical standard taken as a fraction while V is the final volume of the stock solution to be prepared. One millitre of a ready to use 500ppm phthalate standard mixture (stock solution) that comprised of six analytes purchased directly from Sigma Aldrich was carefully transferred to a clean, dry and well labelled vial then sealed with polytetrafluoroethylene cork, and stored in a 2-4°c refrigerator after recording the mass of its contents.

Calibration stock solutions were obtained by diluting the stock solution based on the formula $C_1V_1=C_2V_2$ where C_1 and C_2 are concentrations of the stock solution and the standard to be prepared respectively while V_1 and V_2 are the volumes of the stock solution to be taken and the standard to be prepared. This was achieved by accurately weighing the required stock solution and diluting it with isooctane. Weight of an empty vial with its cap was taken, the required volume of stock solution added, the new weight recorded followed by topping up with isooctane to the required volume and the final weight taken so as to obtain solvent weight.

The entire vial content weights were recorded at the beginning and at the end of each day to monitor volatility of the solvent and appropriate keeper solvent additions made. A calibration curve for each of the analytes was developed and used in quantifying the plasticisers.

Exactly 10 mg of pure butyl benzoate were accurately measured in a dry clean 10 ml volumetric flask and then diluted with isooctane to a volume that gave a concentration of 1000 ppm standard stock solution for the internal standard for the use of method validation. The so obtained solution was carefully transferred to a labelled, clean and dry vial, sealed with polytetrafluoroethylene corks and stored in a 2-4°C refrigerator to serve as the main stock solution throughout the study. From this solution composite stock solutions of 2ppm, 30ppm, 50ppm and 100ppm for method validation were accurately prepared by taking appropriate known volumes of the solution and diluting them with respective known amounts of isooctane in volumetric flasks and then carefully transferring them to separate, well labelled, dry and clean vials, sealed with polytetrafluoroethylene corks and storing them in a 2-4°C refrigerator when not in use.

3.10 2 Method Validation

Prior to sample extraction method validation was achieved by subjecting distilled water to the entire analytical procedure required for phthalates and determining recoveries. Recoveries were determined by spiking 300µl of the phthalate standard mix into 500ml of distilled water.

3.10.3 Sample Extraction

Liquid - liquid partitioning was used to extract water samples with dichloromethane in triplicates. A volume of 500 ml of water was placed in 1 liter separating funnel and buffered to a pH of 7 using disodium hydrogen phosphate (Na_2HPO_4) buffer. 100 g sodium chloride was used in salting out organic compounds from aqueous phase to organic phase followed by addition of 60 ml dichloromethane. The mixture was shaken thoroughly with continuous release of pressure for 15 minutes and the layers left for 15 additional minutes to fully

separate. The bottom layer (organic phase) was collected slowly into an Erlenmeyer flask. The extraction of organics from each sample was repeated twice each time using 60 ml dichloromethane and drying each extract on anhydrous sodium sulphate (Na_2SO_4) while 2 ml of isooctane as the keeper solvent. The dichloromethane extracts were concentrated to 1 ml using a clean rotary evaporator after which they were transferred to clean dry and well labeled vials and then kept in a 2-4° C refrigerator.

3.10.4 Sample Clean up

Cleanup of sample concentrates was conducted using silica gel. A set of 6 chromatographic columns 25 cm × 1.5 cm diameter were each packed with 1cm Na_2SO_4 (previously activated overnight at 200°C) as the bottom layer, 10gm silica gel (previously activated overnight at 200°C) as the middle layer and 1cm of the baked out anhydrous Na_2SO_4 as the top layer. Prior to use, the columns were each successively eluted with 20 ml hexane, each time discarding the eluent. Each of the concentrated sample extracts from the 2-4°C refrigerators on attainment of room temperature was carefully and quantitatively transferred to an already preconditioned column by rinsing the respective vial four times with 1ml portions of hexane and then eluted with 180 ml of the same. The extract was then transferred into well labelled 250 ml clean dry round bottomed flasks that had been rinsed with 2 ml of hexane after which 2ml of isooctane were added to each eluent as the appropriate keeper solvent. The eluents were again concentrated to 1ml using a rotary evaporator and carefully transferred to pre-weighed clean, dry labelled vials.

3.10.5 Identification and Quantification of Plasticisers

Water samples were analyzed for 5 phthalate esters (DMP, DEP, BBP DBP and DEHP) and one non phthalate plasticiser, bis-2-ethylhexyl adipate. The 1 ml concentrates from the refrigerator were further concentrated to 0.15ml with a gentle stream of nitrogen and topped up to 0.20ml using 30 ppm butyl benzoate which served as the internal standard. Analysis was

achieved by injecting extracts to an Agilent 6890 series gas chromatograph equipped with a DB 5 mass spectrometer detector (30m×0.25mm×0.25microns) operating in a selective ion monitoring mode.

Identification of the analytes was achieved by comparing their individual retention times with those of the respective standards while quantification was based on external multilevel calibration curve of the standards catering for high, middle and lower bound limits .The vial levels (concentrations) of each plasticiser reference standard was plotted along the x-axis and the respective normalized peak response was plotted as y-axis. The response factor R^2 was considered as the constant of proportionality and the Cartesian equation for the resulting curve, $y = mx+c$, the relationships between the y-variables (normalized peak areas) and x-variables (concentrations) for individual PAEs reference calibration standards were obtained as illustrated in Figure 3.0

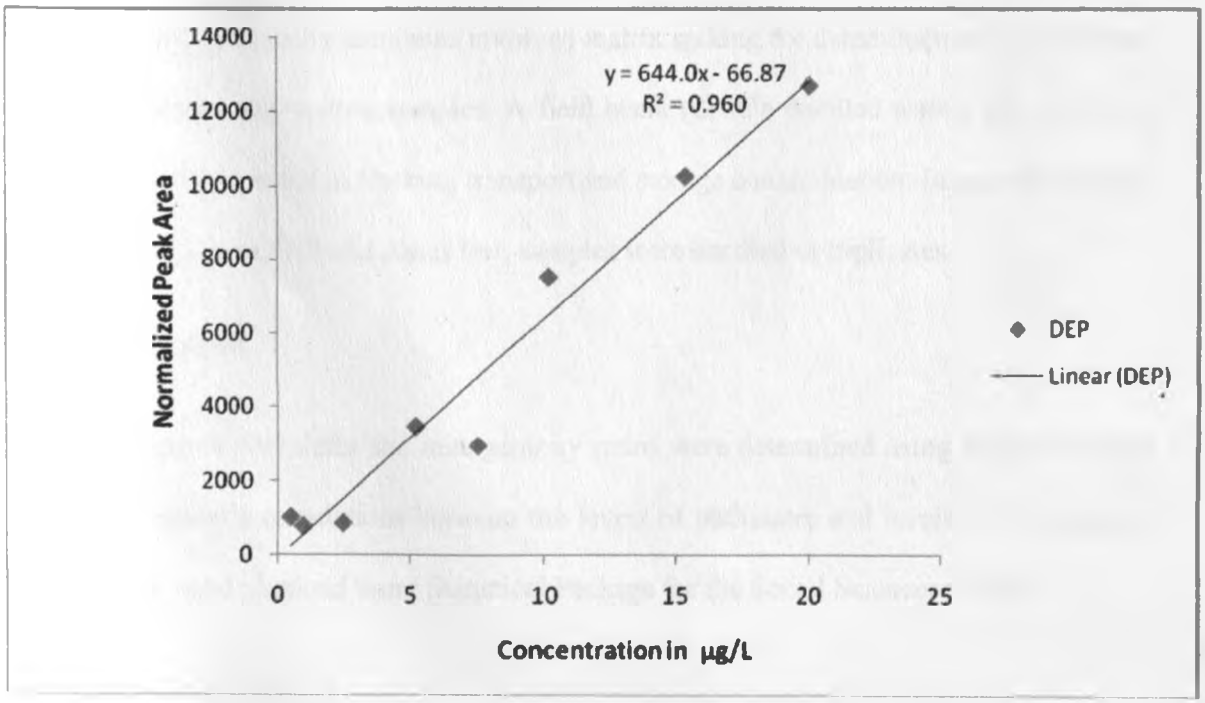

Figure 3.0: Calibration curve for diethyl phthalate

Where; c is the y intercept, m = gradient of the calibration curve, the unknown vial concentration= x-value in µg/L of a particular PAE which was then evaluated by equating to the corresponding normalized peak area obtained from the chromatogram

Given that the vial was 0.2 ml after concentration, the total amount of PAE in ng in the vial was extrapolated on the basis that the original water sample extracted was 0.5 L.

3.10.6 Working Conditions of Gas Chromatograph/Mass Spectrometer

The carrier gas was helium while the inlet temperature was set at 250°C. The injection mode was splitless while the column flow rate was 1 ml/minute. Starting oven temperature was 80° C. This was held for 2 minutes after which it was increased to 120°C within 5minutes at a rate of 8°C/minute. This temperature was held for 12 minutes then ramped up at 275°C at an increase rate of 8°C/minute.

3.11 Quality Control and Quality Assurance

Quality control and quality assurance involved matrix spiking for determination of recoveries, analysis of blanks and control samples. A field blank (double distilled water) was carried to the sampling site to assist in tracking transport and storage contamination. In microbiological, chemical analysis, SODIS and Ames test, samples were handled in triplicates.

3.12 Data Analysis

Concentrations of phthalates and mutagenicity ratios were determined using Microsoft excel while the Pearson's correlations between the levels of phthalates and levels of toxicities on various days were obtained using Statistical Package for the Social Sciences (SPSS).

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter summarises the findings of the entire study including the physicochemical parameters of the samples, microbiological status of samples, the effect of a six hour exposure to solar on sample quality, levels of toxicity of samples at various intervals and concentration of selected phthalate esters and DEHA in the samples at various days. Pearson's correlations between the toxicity levels of each sample and the analytes have also been discussed. Five samples were handled throughout in triplicates over a period of 90 days.

4.1. Microbiological Analysis of Samples

4.1.1 Presumptive Test for Coliform Group of Bacteria

In the presumptive test each sample showed positive reaction to coliform presence. Replicates that proved positive to coliform presence turned from purple to yellow with production of gas trapped in the durhum tubes. Figure 4.1 shows results of one of the 1ml replicates of the Kibera household water while Figure 4.2 and Figure 4.3 show results of the Municipal tap and Nairobi Dam water respectively.

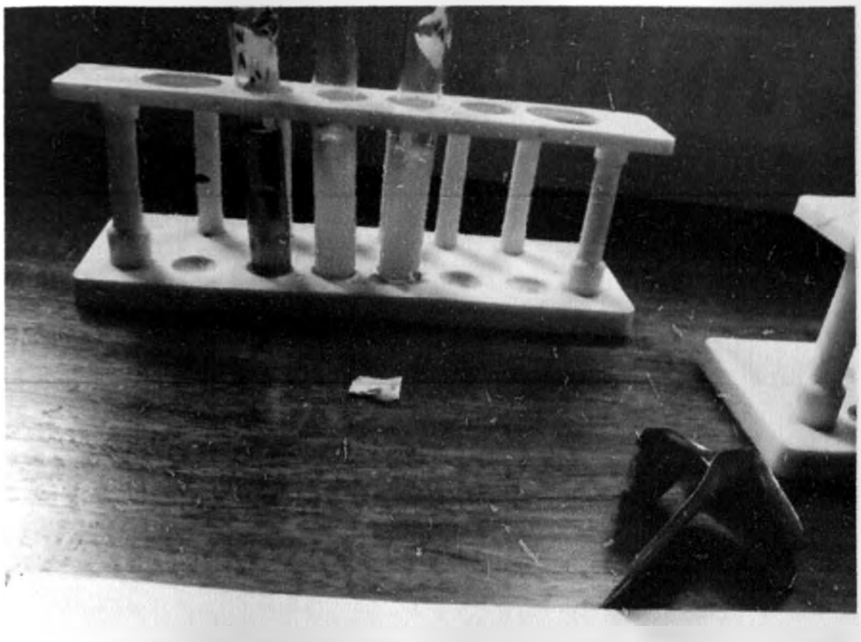

Figure 4.1; Kibera Household Water (1ml) at the presumptive stage indicating colour change from pink to yellow

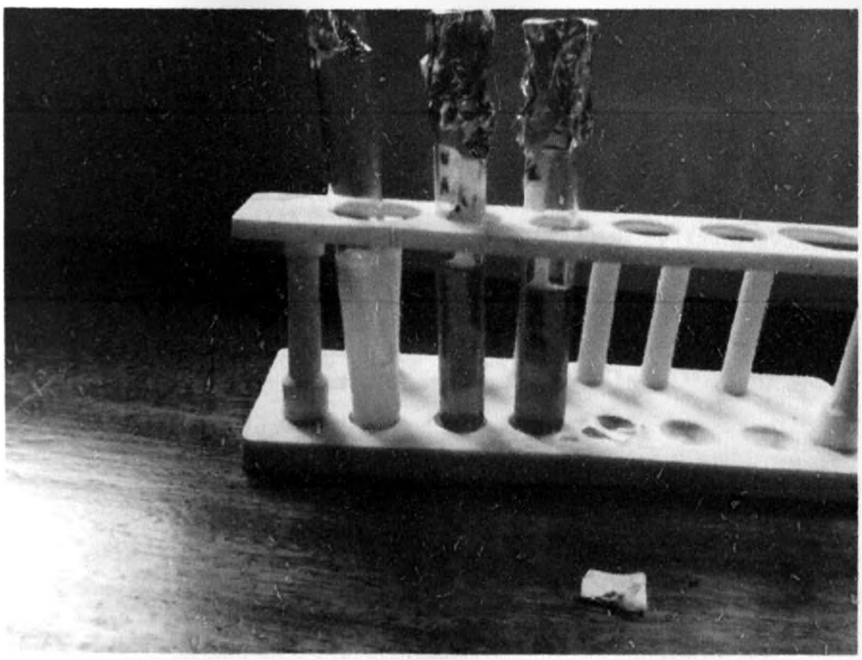

Figure4.2; Municipal tap water (1ml) at the presumptive stage indicating colour change from pink to yellow

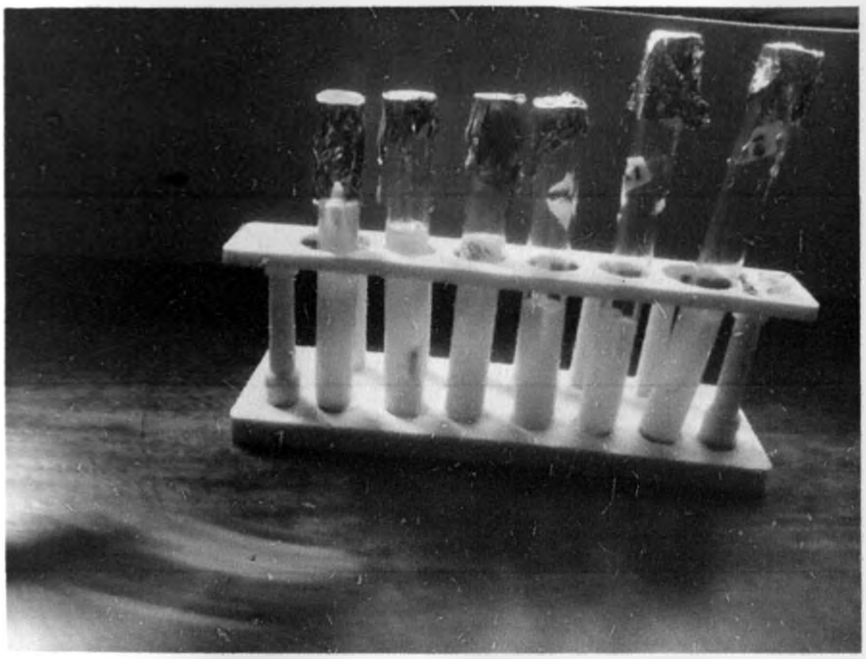

Figure 4.3; Nairobi Dam water (1ml and 0.1ml tubes) at the presumptive stage showing colour change from pink to yellow

Table 4.1 indicates that in the presumptive test the Most Probable Number (MNP) index counts per 100 ml of raw water samples using the multiple tube fermentation technique ranged between 7.00± 0.00 (in distilled water) and 2400.00± 0.00 in Nairobi dam water. The Undugu shallow well water recorded an average of 1966.67±750.56 MPN counts/100 ml while municipal tap and Kibera household water samples recorded an average of 313.33±127.02 MPN/100 ml and 886.67 ±369.50 MPN/100 ml.

Table 4.1: Average MPN Index/100ml of Water Samples N=3±S.D

Sample	Average MPN index/100ml of sample
Bottled distilled water	7.00±0.00
Municipal tap water	313.33±127.02
Kibera house water	886.67±369.50
Undugu shallow well water	1966.67±750.56
Nairobi Dam water	2400.00±0.00

The enumeration in all samples in the current study exceeded the standard limit set by the World Health Organization. Similar results were obtained by a study carried by Idowu *et al.* (2011) on well water in Sagamu, Nigeria. In their study all the wells were reported to have been grossly contaminated with bacteria pathogens such as *Escherichia coli* (72%) and *Salmonella typhi* (32.5%). The uncovered wells were more highly contaminated with bacteria pathogens than the covered well especially in the highly populated areas.

In comparison, pollution in the unprotected Undugu shallow well and the Nairobi dam samples was higher probably due to mismanagement of the local organic waste and poor sanitation in the slum. The dam had all sorts of wastes including human feaces which even made sampling a challenge. The MPN in both the well and dam water rendered it unfit not only for drinking but also in the preparation of food. Proximity of the well from the dam explains the reason as to why both samples qualify to be condemned for direct consumption.

4.1.2. Confirmed Test for Coliforms

On inoculating positive samples from the presumptive test in EMB and incubating them at 37°C for 24 hours small green metallic sheens and several pink colonies were noticed in all samples except for distilled water. The negative results for the distilled water at this stage implied that the contamination that was revealed at the presumptive stage was not due to feacal coliforms hence the sample did not proceed to the complete test. Figures 4.4 and 4.5 show the confirmatory stage results of the household and Undugu well replicates respectively.

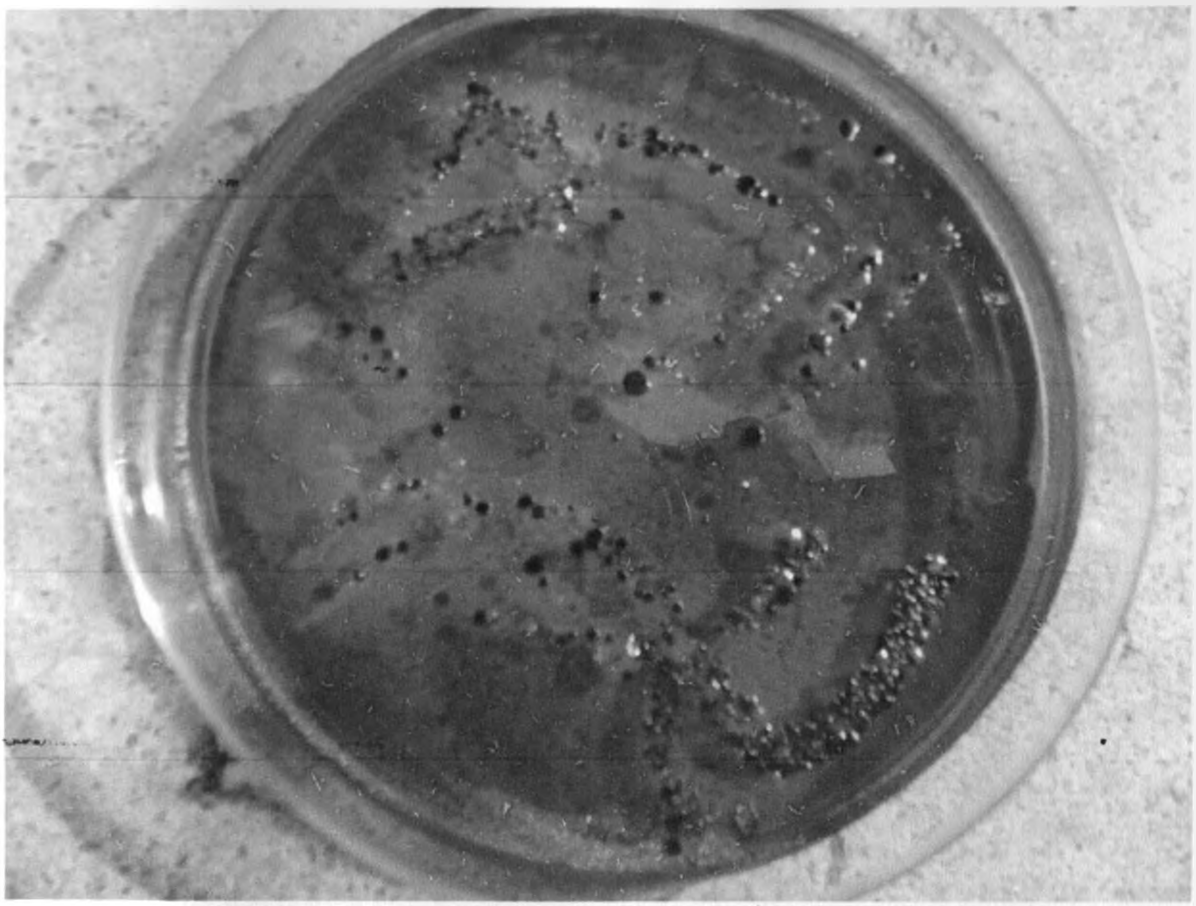

Figure 4.4: Kibera house water at the confirmatory stage; Green metallic sheens indicate presence of Coliform bacteria

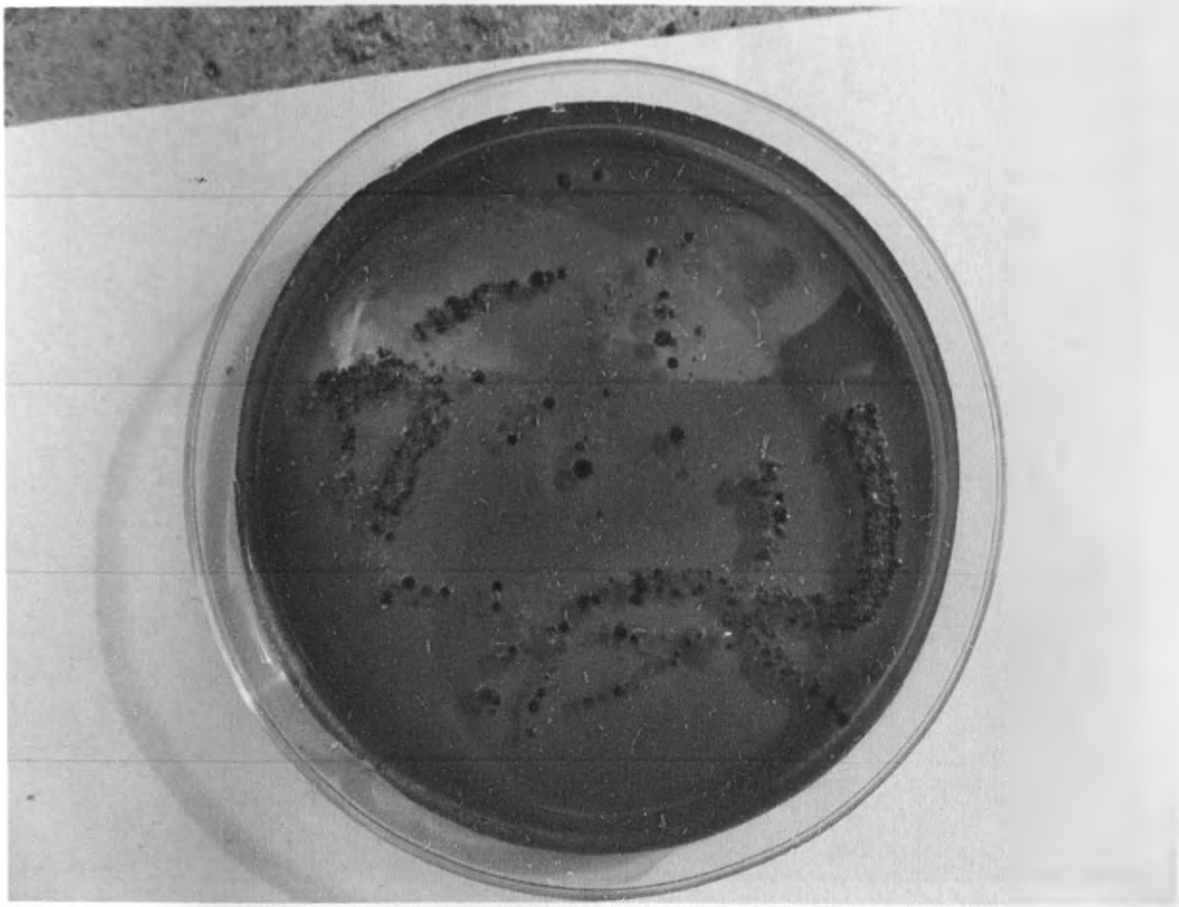

Figure 4.5:Undugu well water at the confirmatory stage ;Green metallic sheens indicate presence of Coliform bacteria

4.1.3 Completed Test for Coliform Bacteria

Gas production was noticed in the lactose broths in which inoculation of the single colonies picked from EMB agar plates had been done. On carrying out the gram stain procedure pink rods were observed under a light microscope at a magnification of ×1000. Figure 4.6 shows results of one of the replicates of Undugu well water at the completed test stage.

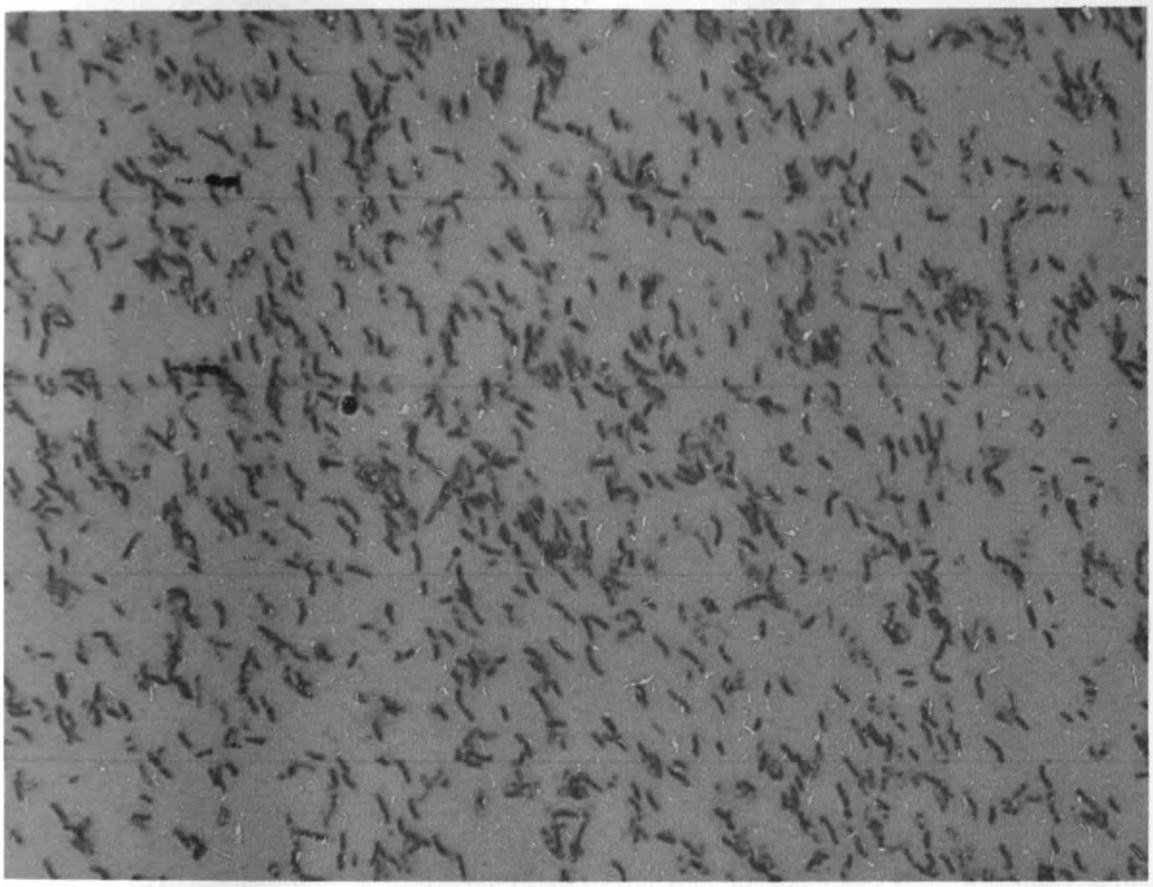

Figure 4.6: Gram negative Coliforms from Undugu well water (mg × 1000) at the completed bacterial test stage

The complete test confirmed presence of gram negative bacteria in municipal tap, Kibera household, Undugu shallow well water and Nairobi dam water. This was indicative of a positive complete test which meant that feacal coliforms were responsible for the contamination in all the mentioned samples. Over decades *E. coli* has been used as an indicator of faecal pollution in water because the bacterium has the advantage of not being capable of growing and multiplying in water except in warm and food laden waters. Therefore, its presence in water is indicator of recent faecal contamination (Akinluyi and Odeyemi, 1984).

Jensen *et al.* (2002) similarly reported presence of faecal coliforms in drinking water that had been stored for long periods. Monika *et al.* (2012) in their study confirmed presence of *E.Coli* in drinking water samples collected from different areas of Solan city in Himachal Pradesh,

India. Another study carried out by Abdelmonem *et al.* (2012) assessed microbioquality of drinking water in Al butala region in Sudan and reported different results in well water samples. Unlike this study all water samples taken from well source showed negative results to coliform test. However the same study revealed positive coliform test results similar to this study for water taken from public water stands. In their study coverless reservoirs were found to be the main probable sources of coliform group of bacteria. Apart from the careless handling of waste matter by Kibera slum residents, heavy rain may also have contributed in damping waste into the well and the dam. Scooping of water from wide mouthed storage containers in households using dirty utensils and hands was suspected to have contributed to the relatively high MPN/100 ml of the house sample. The exposed, old and poor quality plastic pipes that transport municipal water to Kibera easily burst, crack or break and hence easily allow faecal matter into the already treated water before it reaches the consumers.

In the slum, municipal water is sold at central places by vendors hence due to its scarcity, long queues at vendor sites or lack of finance sometimes compels some of the residents to fetch water directly from leaking municipal pipes which are in most cases either covered, surrounded by or immersed in raw human waste. In spite the fact that most strains of *Escherichia coli* and other coliforms are a collection of relatively harmless microorganisms that live in large numbers in the intestines of both cold-blooded animals and warm blooded such as man, some of them are pathogenic or opportunistic and can cause infections (Anatharanarayan and Paniker, 2005; Greenberg and Hunt, 1985). Results obtained from this study reveal that the water quality that reaches residents of Kibera is compromised.

4.2. SODIS Test

Figure 4.7 shows a section of the SODIS samples on a roof exposed to solar radiation.

Figure 4.7: A Section of the SODIS samples displayed on a roof

In this study *E.Coli* group of bacteria proved to be vulnerable to UV treatment since following a six hour exposure to solar radiation, 87.3% of the samples that had given positive reactions to coliform presence in the presumptive test turned negative to coliforms counts. This was an indication that solar disinfection (SODIS) is simply effective and affordable. All samples except Nairobi dam water replicates and one replicate of Undugu shallow well water turned *E.Coli* free. On that day solar intensity was 22.4mj/m^2. Related studies have similarly reported that all of the classically defined waterborne pathogenic bacteria have been found to be readily amenable to solar disinfection following 6 hour interaction under suitable field conditions (Wegelin *et al.*, 1994; Dejung et *al.*, 2007).

Sommer *et al.* (1997) and Sinton *et al.* (2002) reported that naturally occurring fecal coliforms

have much slow inactivation rates while light resistant sub populations of cultured E. coli have been reported to be easily inactivated than light-sensitive organisms in other studies (McGuigan *et al.*, 1994). Studies by Berney *et al.*, 2006 have shown that inactivation of *Escherichia coli* by solar disinfection is caused by disrupting a sequence of normal cellular functions in that ATP synthesis and efflux pump activity both cease shortly after the start of exposure followed by a gradual loss of membrane potential, reduction in glucose uptake, loss of culturability and permeability of the cytoplasmic membrane of the bacterial cells.

Inactivation of *E. coli* is dominated by DNA damage and largely depends on dissolved oxygen concentration (Reed, 1997), salt concentration (Moss and Smith, 1981), wavelength (Wegelin *et al.*, 1994; Webb and Brown, 1997), and post-irradiation growth conditions (Khaengraeng and Reed, 2005) while the route to cell death cell is dominated by the membrane damage (McGuigan *et al.*, 2012).

4.3. Levels of Physicochemical Parameters in Samples

Table 4.2 gives summarized baseline information on selected physiochemical and chemical parameters of the raw water samples. Nairobi dam water recorded the least concentration of dissolved oxygen 2.26±0.19 mg/L), but highest values of pH (8.12), conductivity 1512.67±1.53 µs, salinity 756.33±0.76 mg/L (ppm), TDS(1058.87±1.07 mg/L (ppm), TSS (216.54±4.22 mg/L), turbidity (5.89± 0.02NTU), temperature (25.67±0.46 ˚C) and COD (746±0.00 mg/L).

This was followed by the shallow well water which recorded conductivity, PH, salinity, TDS, DO, TSS, turbidity, color, temperature and chemical oxygen demand of 944.33±2.08µs, 7.91±0.20, 472.17±1.04 mg/L, 661.03±1.46 mg/L, 3.37±0.12 mg/L, 194±26.85.mg/L, 1.43±0.06 NTU, 16.59±1.86 colour units, 24.1±0.17˚C and 460.00±0.00 mg/L respectively.

Dissolved oxygen (DO) is a function of water temperature, amount of other substances dissolved in the water and atmospheric pressure. Dissolved oxygen concentrations in unpolluted waters normally range between 8 and 10 mg/L and concentrations below 5 mg/L adversely affect aquatic life (DFID, 1999; Rao, 2005). DO standard for drinking purpose is 6 mg/L whereas for sustaining fish and aquatic life is 4-5mg/L (Rao, 2005). The DO values for Nairobi dam water (3.37±0.12mg/L) and Undugu shallow well water (2.26±0.19 mg/L) from this study fell short of the recommended standards. This is an indicator that the two samples contained high concentrations of dissolved minerals such as nitrogen that result in excessive algae growth which upon decomposition consume dissolved oxygen and consequently lower its level. Furthermore, the dumping of solid waste at the banks of the dam may discharge pollutants into both the dam and the shallow unprotected well which elevates the biochemical oxygen loading increasing the oxygen demand which in turn suppresses the dissolved oxygen to below acceptable concentrations. The relatively low temperatures of the municipal tap water and Kibera household water explain the high levels of DO in the samples since high temperatures decrease oxygen solubility in water. DO concentration in hundred percent fresh water ranges from7.56 mg/L at 30°C to 14.62 mg/L at 0°C. In this study the Kibera household water and municipal tap water reported DO levels of 7.82 mg/L and 8.75mg/L respectively.

Conductivity, salinity and total dissolved solid concentrations of water are related. There is significant direct variation of these parameters with temperature, chemical oxygen demand, total nitrogen, sulphate ions and total suspended solids. These water quality parameters were not scored in distilled water but were at high levels in both the dam and well water followed by house water and tap water respectively. The implication here is that the dam and well water were heavily polluted and may have had high concentrations of inorganic dissolved ions such as fluoride, chloride, sulphate and probably nitrate. The high levels of TSS revealed in both the Nairobi dam water and the Undugu shallow well can serve as carriers of toxins.

Data obtained from a study carried by Gupta *et al.* (2009) in Kaithal city, Haryana (India) on physiochemical parameters in ground water similarly revealed considerable variations in the examined samples from different sources with respect to their chemical characteristics. Conductance was highest in water sample of Karnal By pass area and Sector 20 Area. The water sample from near old Anaj Mandi area revealed TDS in the order of 2900 mg/L while Samples from the Public Club area, Hospital area and Water Works area showed low level of TDS of the range less than 250 mg/L. Water quality parameters such as pH, electrical conductivity, TDS, chloride and ion concentration were reported to be within the maximum permissible limit prescribed by WHO and ICMR. However dissolved oxygen varied from 4.0 to 8.8 mg/L hence lower than the permissible level. In their study Gupta *et al.*(2009) Chloride ion concentration in water samples ranged from 35-875 mg/L while in this study the chloride ion levels ranged from 0.38±0.41 mg/.L to 27.02 mg/L±0.86.

The FEPA acceptable limit for conductivity in domestic water supply is 70 µs/cm (DWAF, 1996a). Except for the distilled water this limit was exceeded in all water samples hence the parameter does give concern and it implies that the water is unsuitable for direct domestic use. The high and varied conductivity values obtained in this study are similar to the findings of a previous study on the Keiskamma River water (Fatoki *et al.*, 2003). The turbidity values obtained in this study were within the WHO set standard of 5 NTU (WHO, 2004) except for Nairobi dam water which registered a turbidity value of 5.88±0.02.The distilled water, tap and household water registered no NTU hence met the FEPA guideline of 0 to 1 NTU for turbidities in water for domestic use (DWAF, 1998). The high turbidity level in Nairobi dam water can be associated with heavy microbiological contamination/suspended matter which hide the micro-organisms barring them from direct interaction with UV hence making it difficult to disinfect.

Water samples that register high turbidity even cause problems with water purification processes such as flocculation and filtration, which may consequently increase treatment cost (DWAF, 1998) .COD, a measure of the relative oxygen-depletion effect of a waste contaminant in terms of the actual amount of oxygen required by both potassium dichromate and concentrated sulphuric acid to breakdown both organic and inorganic matters specifically measures the oxygen demand of oxidizable pollutants in water samples hence serves as a determinant of the level organic matter and carbon. Reported COD levels for municipal tap water, Kibera household, Undugu shallow well and Nairobi dam water were 186.67±0.00 mg/L, 293.33±0.00 mg/L, and 460.00±0.00 mg/L and 746.00±00 mg/L respectively. This can be attributed to an increase in the addition of both organic and inorganic substance from the environment, as well as organic contaminants entering the systems. Findings of this study agree with the previous works of Ogunfowokan *et al.* (2005), Fatoki *et al.* (2003) and Morrison *et al.* (2001) who all reported varying significant COD levels in water samples. Similarly Assessment of physicochemical parameters in Ishasha River and Lake Edward, Albertine Rift Valley, East Africa revealed overall mean values of the parameters (pH, electrical conductivity, total dissolved solids, and water transparency) within safe limits of water quality standards during the study period in all sites (Mulongaibalu *et al.*,2014).

The concentration of iron in all environmental samples including the blank was below detection limits (BDL) while that of total nitrogen ranged from BDL in the blank to5.11±0.01 mg/L in the Nairobi dam water.(Acceptable ranges of total nitrogen are 2 to 6 mg/L). The dam water registered the highest fluoride ion concentration 1.13±0.03 followed by the shallow well water that recorded a concentration of 1.05±0.27 mg/L. The shallow well water registered the highest chloride ion concentration (27.02±0.86 mg/L) followed by the Nairobi dam water and the tap water that recorded a concentration of 13.84±0.43 mg/l and

5.13±0.08mg/L respectively. The house water and the distilled water recorded the lowest concentrations of 0.9±0.41 mg/L and 0.38 ±0.41 mg/L respectively.

Table 4.2: Levels of Selected Physicochemical and Chemical Parameters of Water Samples N=3±S.D

Parameter	PB	PT	PH	PW	PD
conductivity(µs)	0±0.00	88.93±0.57	94.47±1.36	944.33±2.08	1512.67±1.53
PH	7.00±0.01	6.97 ±0.01	6.87±0.29	7.91±0.02	8.12±0.015
temp(°C)	23.40±0.10	23.47±0.15	23.40±0.10	24.10±0.17	25.67±0.46
DO(mg/L)	9.90±0.02	8.75±0.31	7.62±0.02	3.37±0.12	2.26±0.19
TDS(mg/L)	0.00±0.00	62.25±0.40	66.13±0.95	661.03±1.46	1058.86±1.07
salinity	0.00±0.00	44.47±0.03	47.23±0.68	472.17±1.04	756.33±0.76
TSS(mg/L)	2.18±1.86	181.40±2.08	113.30±60.36	194.00±26.85	216.54±4.22
Colour units	0.00±0.00	0.00±0.00	0.00±0.00	16.59±1.86	161.67±0.58
Turbidity(NTU)	0.00±0.00	0.00±0.00	0.00±0.00	1.43±0.06	5.89±0.02
COD(mg/L)	0.00±0.00	186.67±0.00	293.33±0.00	460.±0.00	746.±0.00
Sulphate ions(mg/L)	0.14±0.24	26.07±8.33	18.93±8.63	43.36±0.63	34.85±16.01
Iron content (mg/L)	ND	ND	ND	ND	ND
fluoride ions(mg/L)	0.47±0.01	0.16±0.12	0.22±0.03	1.05±0.27	1.13±0.01
Chloride ion(mg/L)	0.38±0.41	5.13±0.08	0.90±0.41	27.02±0.86	13.84±0.43
total nitrogen mg/L	ND	1.93±0.00	1.98±0.01	5.03±0.01	5.11±0.01

PB: Bottled Distilled water ***PH****: Kibera house water* ***PW****: Undugu Shallow Well water* ***PT:*** *municipal tap water* ***PD****:* Nairobi *Dam water.* ***ND****: Not detected*

4.4 Mutagenicity/Toxicity Levels in Samples

To determine levels of toxicity in the various SODIS samples Ames mutagenicity assay for aqua samples was employed. At the onset of the protocol, the optical densities of the tester strains; TA98 and TA100 were determined at a wavelength of 600nm.They were found to be within the acceptable limits (≥ 0.2 for cultures and < 0.05 for negative control) as shown in the table 4.3.

Table 4.3: Optical Densities of Tester strains Used

Tester Strain	Absorbance(A)	Optical Density(A×10)
TA98	0.32	3.12
TA100	0.30	3.00
Negative Control(DMSO)	0.002	0.02

Within two days (48 hours) of incubation at 37°C cells that had undergone reversion to histidine prototrophy grew into either yellow or pale yellow colonies. The number of wells containing revertant colonies for each dose was compared to the zero dose (sterile water) control and a sample that revealed a mutagenicity ratio (M.R) ≥ 2.0 was termed potentially mutagenic.

The bottled distilled water gave negative results to the Ames mutagenicity test throughout the study. Mutagenicity was below detection limits on day zero in bottled distilled water samples without the S9 liver fraction while those samples that had bottled distilled served with metabolic activation recorded a M.R of 1.00 each .The highest M.R value(1.93 ± 0.02) for bottled distilled samples was reported on day 60 using tester strain TA98 with metabolic activation (S9). This suggests that as much as PAEs may be contributing to toxicity of water stored in PET bottles there could be other pollutants or chemicals that top up the effect.

With or without metabolic activation, municipal tap water in Kibera recorded the lowest M.R value (0.92 ± 0.12) on day zero. This sample turned potentially mutagenic on day 60 of study

when it recorded M.Rs of 2.5±0.59 and 2.83±0.94 using tester strain TA98 without and with S9 respectively. However, day 90 results indicated that the toxicity had dropped to 1.88±0.18 and1.88±0.88 respectively rendering the sample non toxic.

A similar trend was noted using tester strain TA100 with or without S9 for the Kibera house water that recorded potential toxicity on day 60 (2.84±0.02 and or2.88±0.47) but had its toxicity drop to less than 2.0 (1.8±0.00 and or 1.92±0.12) respectively on day 90 rendering it non toxic. This suggests that both the Kibera household water and municipal tap water had either the same or different pollutant(s) which may have contributed to the potential mutagenicity of the water at day 60. Since both water samples originate from the Nairobi water company the chemical was suspected to be chlorine. This is a clear indication that both tester strains are sensitive to mutagenicity and that time is an influential factor in detecting toxicity.

In all samples levels of toxicity dropped from day 60 to day 90 except in Municipal tap water of Kibera using tester strain TA100 without S9 in which toxicity ratio increased from 3.61±0.08 at day 60 to3.75±0.35 at day 90.This could suggest that, to a large extent the chemicals in water contained in the Plastic bottles could be undergoing chemical changes that have them get converted to less toxic intermediates.

Throughout the study period both the Undugu shallow well water and Nairobi dam water recorded M.Rs $\geq$ 2.0 with the highest value for toxicity level for Nairobi Dam water (3.63±0.53) recorded on day 60 using TA98 with S9 while its lowest value of toxicity (2.40±2.80) recorded on day 90 using TA98 without S9.Undugu shallow well water registered the overall highest level of toxicity (4.6±0.88) on day 30 using TA100 with S9.The lowest level of toxicity (2.13±1.24) for this sample was recorded on day zero using TA98 without S9.

In this study samples with induced rat liver fraction (S9) generally gave higher M.R values than the corresponding samples without S9 and TA100 recorded better sensitivity to the Ames mutagenicity assay than TA98 in that in most samples it gave a higher number of revertant wells to the histidine prototrophy. A similar study carried by Park *et al.* (2000) on determination of the mutagenicity of drinking water from three Korean cities using the Ames assay with the help of TA98 and TA100 tester strains reported that all samples were mutagenic in TA98 without S9 mix and in TA100 with and without S9 mix. Unlike this study their study revealed highest number of revertant wells per plate in the absence of S9 mix Furthermore, unlike this study their study used concentrated samples. This difference in sensitivity of the two tester strains to chemicals is in agreement with findings of Jurado *et al.* (1993).

Although samples with induced rat liver fraction (S9) generally gave higher M.R values than the corresponding samples without S9 results of the present study show that all water samples except the bottled distilled water were mutagenic to both S. *typhimurium* TA98 S.and *typhimurium* TA100 and with or without S9-mix hence the use of S9 mix with TA98 or TA100 tester strains does not favour mutagen concentration. This is in agreement to the findings of Mouna *et al.* (2015) who showed that Mutagenic and genotoxic effects of Guelma's urban wastewater, Algeria in the Ames test was independent of the use of S9 metabolic activation. Similarly, studies carried by Hartmann *et al.* (1999) and Gupta *et al.* (2009) revealed that genotoxicity of hospital wastewater was independent of the use of induced rat liver metabolic activation. In similar study White and Rasmussen (1998) reported that the putative genotoxins in both surface waters and municipal wastewaters are primarily direct acting. S9 addition does not enhance the response.

Table 4.4 reports the mutagenicity ratio in each sample using *salmonella typhimurium* tester strains TA98 and TA100 with and without metabolic activation at days 0 (zero), 15,30, 60 and

90.Values greater or equal to 2.0 imply that a sample is potentially mutagenic at a particular day hence unfit for consumption.

Table 4.4: Mutagenicity Ratio (levels) in SODIS Samples using TA98 and TA100 Tester strain with and without metabolic Activation N=3 ±S.D

Sample/strain	Day 0	Day 15	Day 30	Day 60	Day 90
PB-S998	ND	0.5±0.00	0.83±0.24	01.86±0.04	1.33±0.00
PB+S998	ND	0.5±0.00	1.67±0.00	1.93±0.02	1.67±0.00
PB-S9100	ND	1±0.00	1.58±0.12	1.19±0.04	1.44±0.16
PB+S9100	0.25±0.35	1±0.00	1.33±0.00	1.75±0.00	1.88±0.17
PT-S998	0.92±0.12	1.08±0.12	1.5±0.23	2.5±0.59	1.88±0.18
PT+S998	0.92±0.12	1.33±0.23	1.67±0.24	2.83±0.94	1.88±0.88
PT-S9100	1.08±0.11	1.41±0.12	1.58±0.12	3.61±0.08	3.75±0.35
PT+S9100	1.08±O.11	1.75±0.12	1.91±0.12	3.83±0.07	3.75±0.35
PH-S998	0.88±0.18	1.87±0.18	1.93±0.10	2.5±0.10	2.4±2.8
PH+S998	0.96±0.12	1.92±0.12	1.93±0.45	2.92±0.12	2.6±0.18
PH-S9100	1.75±0.71	1.83±0.24	1.83±0.00	2.84±0.02	1.8±0.00
PH+S9100	1.88±0.53	1.88±0.40	1.88±0.18	2.88±0.47	1.92±0.12
PW-S998	2.13±1.24	3.13±2.29	3.5±2.12	4.38±1.24	3.125±2.3
PW+S998	2.38±1.6	3.25±2.12	4.68±1.31	4.5±0.71	3.00±2.12
PW-S9100	2.63±1.59	4.13±1.6	4.5±1.76	3.38±1.6	2.25±1.06
PW+S9100	2.75±1.76	4.38±1.94	4.69±0.88	4.0±1.06	2.88±0.88
PD-S998	2.75±0.71	3.25±1.06	3.17±0.75	2.88±0.18	2.4±2.8
PD+S998	2.88±0.88	3.3±0.99	3.38±0.53	3.63±0.53	2.38±1.6
PD-S9100	2.92±0.59	2.67±0.47	3.04±0.29	2.75±0.35	2.5±0.1
PD+S9100	3.0±0.94	2.83±0.23	3.08±0.12	3.0±0.71	2.63±1.59

PB: Bottled distilled water: municipal tap water ***pH****: Kibera house water* ***PW****: Undugu shallow well water* ***PD****: Nairobi dam water* ***–S998****:TA98 without Induced Rat Liver,* ***+S998:*** *TA98 with Induced rat Liver,* ***-S9100:*** *TA100 without Induced rat liver,* ***+S9100:*** *TA100 with induced rat Liver.*

Mutagenicity ratio of samples ranged from non-detectable levels up to 4.69±0.88 in Undugu well water at day 30 using tester strain TA100 with metabolic activation. The variation in mutagenicity was attributed to use of water samples from different sources. A similar trend in mutagenicity was observed in a study carried out using different PET bottles and different types of mineral water (Monarca *et al.*, (1994).

A study carried by Evandri *et al.*, (2000) showed genotoxic/mutagenic activity in water samples after 8 weeks of storage both in the dark and in the light, using a plant-based genotoxic assay. This study similarly detected significant toxicity in municipal tap water and Kibera household water that had been stored in PET bottles at day 60 at analysis (8.57 weeks). TA98 with or without metabolic activation revealed reduced M.R values at day 90 which rendered the municipal tap water non mutagenic but maintained potential mutagenicity of the household water. Use of TA100 reported maintained the potential mutagenicity of the tap water but gave values that did not qualify house water mutagenic at day 90. Ubomba *et al.* (2010) also detected significant genotoxicity in both the dark and sunlight non-refill SODIS samples after 2 months. The study did not detect any genotoxicity beyond that period. Similarly in this study after day 60 about 75% of the SODIS samples recorded a drop in M.R values with 40% samples being non mutagenic. This is an indication that whichever pollutants could be responsible for mutagenicity, they may be undergoing chemical changes such as photo degradation which consequently affect their concentrations in SODIS water hence the significant drop in toxicity. Similarly studies carried out by Nawrock *et al.* (2002) and Wegelin *et al.* (2001) reported that time is a dominant factor in governing the release of organic substances from PET. However, contrary to their report this study had 50% of the samples that were potentially mutagenic at 2 months remain mutagenic after 2 months of exposure (at day 90).

4.5 Chemical Analysis of Water Samples

4.5.1 Recovery, Identification and Quantification of PAEs

The mean recovery for all the Phthalates ranged between 81.70±12.47 to 106.57±28.42%. This is within the acceptable range hence results were not corrected. Identification of PAEs was done by comparing chromatographic retention times of individual PAEs in the standard phthalate mixture that contained analytes of interest. Quantitative analysis was done based on external standard method using multilevel calibration curve covering different PAEs. An internal standard was applied to all samples and to standard mix of various concentrations for normalization purposes due to detector fluctuations. A plot was then obtained for each PAE standard in different concentrations. The amount of PAEs and the signal obtained from the MS gave linear relationships with correlation coefficients ranging from 0.90 to 0.96 as shown in Table 4.5.1.

Table 4.5.1: Correlation Coefficients for Selected Phthalates

Analyte	Correlation Coefficient
Dimethyl Phthalate	0.92
Diethyl Phthalate	0.96
Pentachlorophenol	0.91
Di –*n*- butyl phthalate	0.90
Benzyl butyl phthalate	0.91
Bis-2-ethylhexyl adipate	0.90
Bis-2-ethylhexyl phthalate	0.91

All analytes of interest (dimethyl phthalate, dibutyl phthalate, benzylbutyl phthalate, diethyl phthalate, bis-2-ethylhexyl phthalate and bis-2-ethylhexyl adipate) were detected in all samples as well as in the blank.

4.5.2 Levels of Bis-2-ethylhexyl Adipate (DEHA) in Samples

Concentrations of DEHA ranged from 0.24 µg/L±0.0.00 in dam water on day 15 to 152. 97 µg/L in Kibera house water at day 60 as it is indicated in table 4.5.2.

Table 4.5.2 Levels of Bis -2-ethylhexyl adipate in SODIS Water Samples (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	0.67±0.34	1.61±0.71	1.75±0.00	0.81±0.18	0.24±0.00
Day 15	2.48±0.00	0.39±0.23	2.35±0.00	3.69±0.00	6.22±5.78
Day 30	4.82±3.18	0.80±0.00	4.88±0.00	8.82±0.00	3.16±2.18
Day 60	1.28±0.00	38.54±11.18	152.97±0.00	91.57±0.00	0.55±0.19
Day 90	7.23±0.00	21.27±0.00	89.31±41.43	38.00±0.00	45.70±17.50

PB: Bottled Distilled water ***PT****: Municipal tap water* ***PH****: Kibera house water* ***PW****: Undugu shallow well water* ***PD****: Nairobi Dam water*

A similar study carried by Serodio and Nogueira (2006) found DEHA at average concentrations of 0.15 mg/L in bottled water slightly higher than in tap water (0.09 mg/L). Considering the Maximum Contaminant Level (0.4 mg/L) set by EPA, (1998) the current study reported levels of this analyte within acceptable limits. However at day 60 when levels were highest in house water the sample had turned potentially mutagenic and levels of bis-2-ethylhexyl adipate were higher than the recommended WHO guideline value (80 µg/L) in drinking water WHO,1996).This implies that the concentrations of this PAE may have contributed to the reported toxicity which rendered the sample unfit for drinking.

4.5.3 Levels of Benzyl Butyl Phthalate (BBP)

From table 4.5.3 house water recorded the highest BBP concentration (28.27±0.00 µg/L) on day zero of exposure while the highest concentration of BBP in Nairobi dam water (5.88±0.00 µg/L) was recorded on the 15th day of exposure. As reflected in the same table the concentration of this plasticizer in distilled varied from BDL to 10.55±0.00 µg/L on the 30th

day of exposure. Similarly tap water recorded the highest concentration of this analyte on the 30th day of analysis.

Table 4.5.3: Levels of Benzyl butyl phthalate in SODIS Water Samples (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	BDL	2.57±0.00	28.27±0.00	5.08±0.00	2.20±1.65
Day 15	BDL	BDL	0.52±0.00	0.43±0.00	5.88±0.00
Day 30	10.55±0.00	10.66±0.00	3.01±0.84	0.05±0.03	0.29±0.13
Day 60	1.81±0.00	1.66±0.66	2.63±0.00	5.91±0.36	2.05±1.75
Day 90	3.57±0.00	0.58±0.08	1.01±0.99	2.80±0.00	0.32±0.07

***PB**: Bottled distilled water PT: Municipal tap water **PH**: Kibera house water **PW**: Undugu well shallow water **PD**: Nairobi Dam water*

These concentrations are much lower than the DWEL value (0.7 mg/L) set by EPA. There is no set MCL for BBP in drinking water but the reference dose is quoted at 0.2 mg/kg/day (EPA, 2012).The revealed levels of this analyte in this study were considered safe.

4.5.4 Levels of Di-*n*-butyl Phthalate (DBP)

As shown in Table 4.5.4 the highest concentration of di-*n*-butyl phthalate (61.08 µg/L ± 13.32) was recorded in the distilled water on the 30th day of analysis. There was a tremendous increase in the concentration of DBP leaching into the bottle contents from day zero (2.13 µg/L) ± 0.00) through day 15 (50.00 µg/L ± 0.00) up to day 30 (61.08 µg/L ± 13.32), a trend that was similarly noticed with house water. In all analysed samples there was a drop in levels of DBP in samples that had been exposed to U.V from the 30th day of exposure to the 90th day except in Nairobi dam water in which DBP levels fluctuated throughout the study period.

Table 4.5.4: Levels of Di-*n*- butyl phthalate in SODIS Water Samples (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	2.13±0.00	4.14±0.00	3.37±0.00	11.98±1.31	2.23±0.63
Day15	50.00±10.56	BDL	4.02±0.00	2.86±0.00	14.89±0.00
Day30	61.08±13.32	11.80±0.32	6.71±3.10	3.15±1.90	0.380.00
Day60	14.40±0.00	1.64±0.00	BDL	2.37±0.00	5.23±0.00
Day90	BDL	1.25±0.00	BDL	1.14±0.83	8.68±0.00

***PB**: Bottled distilled water: **PT** Municipal tap water **PH**: Kibera house water **PW**: Undugu shallow well water **PD**: Nairobi Dam water*

The Threshold Limit Value for DBP was set at 0.45 mg/L hence the reported range (from BDL to 61.08 µg± 13.32) for this phthalate is considered to be within acceptable limits. Similarly a study carried by Prepatpong and Kanchanamayoon (2010) reported Safe DBP levels (0.13-0.33 mg L^{-1}) in drinking water.

4.5.5 Levels of Diethyl Phthalate (DEP)

Table 4.5.5 shows that levels of DEP varied from BDL to 1.69±0.00 µg/L in the bottled distilled water at day 15. There was a significant decrease in levels of this phthalate from day 60 to day 90 in all SODIS water samples. Revealed levels from this study are not alarming since the TLV is 0.55 mg/L while the DWEL set value is 4.0 mg/L (USEPA, 1991).

Table 4.5.5: Levels of Diethyl phthalate in SODIS water Samples (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	0.14±0.00	BDL	BDL	0.07±0.04	0.89±0.00
Day 15	1.69±0.00	BDL	0.17±0.00	BDL	0.96±1.01
Day 30	0.17±0.07	0.12±0.00	BDL	0.09±0.00	0.38±0.18
Day 60	0.27±0.15	0.78±0.11	0.55±0.00	1.64±0.05	0.30±0.23
Day 90	0.10±0.00	0.34± 0.23	0.09±0.02	0.30±0.06	0.10±0.06

***PB**: Bottled distilled water PT: Municipal tap water **PH**: Kibera house water **PW**: Undugu shallow well water **PD**: Nairobi Dam water*

Similarly safe levels of DEP (0.54 mg/L) were reported in previous studies (Prepatpong and Kanchanamayoon, 2010).These findings were in agreement with the report from a similar study carried by Penalver *et al.*, (2000), Casajuana and Lacorte (2003) confirmed presence of this phthalate ester at an average concentration of 0.214 µg/L in water that had been stored in PET bottles for a period of 10 weeks at a temperature of 30°C. Currently there is no recorded MCL for this PAE in drinking water.

4.5.6 Levels of Bis-2-ethylhexyl Phthalate

Levels of bis-2-ethylhexyl phthalate residues ranged between BDL in distilled water and 27.20±3.08 µg/L in the dam water at day 15. Except for dam and well water all other samples had a drop in levels of this analyte between day 60 and day 90. As it is evident from Table 4.5.6 generally levels of this phthalate ester in all SODIS water samples fluctuated throughout the study period.

Table 4.5.6: Levels of Bis-2-ethylhexyl phthalate in SODIS water Samples (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	1.54±0.49	5.46±0.00	8.72±0.00	4.61±0.05	1.44±0.60
Day 15	9.22±4.84	0.16±0.03	2.98±0.00	11.47±5.31	27.20±3.08
Day 30	2.51±1.37	0.84±0.64	0.90±0.0.15	1.02±0.21	5.21±4.40
Day 60	3.40±1.81	6.66±0.23	5.65±1.58	2.28±0.00	1.13±0.85
Day 90	BDL	1.68±0.22	3.24±0.51	2.60±2.00	3.85±0.95

PB: Bottled Distilled Water: PT Municipal tap water ***pH****: Kibera house water* ***PW****: Undugu shallow well water* ***PD****: Nairobi Dam water.*

Prepatpong and Kanchanamayoon (2010) reported DEHP levels in drinking water within the range 0.28-0.49 mg/L. These concentrations were higher than the Maximum Admissible concentration (MAC) of the phthalate which is quoted at 6 µg/L (USEPA, 1991).

A study carried by Schmid *et al.* (2008) revealed DEHP at average levels of 0.36 ± 0.21 µg/L following a 17 hour exposure of water contained in PET water to sunlight while Leivadara *et al.* (2008) in the process of determining organic compounds in bottled mineral waters that had been exposed to Solar radiation for a period of 3 months quantified DEHP at levels less than 0.02 µg/L. In this study average concentration for this analyte in bottled distilled water is reported at 0.19 µg/L. In other studies Biscardi *et al.* (2003) and Criado *et al.* (2005) identified di (2-ethyl-hexyl) phthalate (DEHP) and di- butyl phthalate(DBP) in water that was stored in PET bottles for several months. Their studies did not report any mutagenic or carcinogenic compound at critical concentrations. Similarly, this study does not report any analyte at critical levels.

4.5.7 Levels of Dimethyl Phthalate (DMP)

Levels of this plasticizer in SODIS water samples were reported mostly in dam water on day 30, day 60 and day 90 as 0.24±0.00 µg/L, 0.18±0.00 µg/L and 0.42±0.00 µg/L respectively as indicated in table 4.5.7. Otherwise concentrations of DMP were below detection limits in all other samples in all days (76%) except in the well water of day zero, tap water of the 60th day and house water of day 90 in which concentrations of DMP were found to be 0.18 µg/L ±0.07, 0.20±0.13 0.16 µg/L ±0.12, and 0.03±0.00 µg/L and respectively.

Table 4.5.7: Levels of Dimethyl phthalate in SODIS water Sample (µg/L±S.D, N=3)

Day/Sample	PB	PT	PH	PW	PD
Day 0	BDL	BDL	BDL	0.18±0.07	BDL
Day 15	BDL	BDL	BDL	BDL	BDL
Day 30	BDL	BDL	BDL	BDL	0.24±0.00
Day 60	BDL	0.20±0.13	BDL	BDL	0.18±0.00
Day 90	BDL	BDL	0.03±0.00	BDL	0.42±0.00

PB: Bottled Distilled water: PT: Municipal tap water ***PH****: Kibera house water* ***PW****: Undugu shallow well water* ***PD****: Nairobi Dam water*

with cellulose acetate windows containing 16-17% w/w DEP. On analysis the samples were

The TLV for DMP is 5.0mg/L hence this study reported levels were acceptable. Similarly Prepatpong and Kanyanchamayoon (2010) in their study reported DMP at safe levels (0.16-0.53 mg/L) while another study on presence and release of phthalic acid esters in drinking and other endocrine disrupting compounds in water reported DMP at a mean concentration of 0.214 µg/L.

In this study Phthalates of interest were detected both in the samples and the procedural blank. Similarly a study carried by Castillo and Barcelo, (1998) reported presence of PAEs in the blank. This was attributed to leakages from laboratory plastics, solvents and polymeric sorbents from the preconcentration techniques (Castillo and Barcelo, 1998).

According to the results PAEs and DEHA are present in SODIS water and are therefore ingested daily by consumers. This confirms that drinking water stored in PET bottles is a major source of human exposure to plasticizers.

Figures 4.8.1 4.8.2, 4.8.3, 4.8.4 and 4.8.5 report the levels of all the analytes and pentachlorophenol in distilled water, Municipal tap water, Kibera house water, Undugu shallow well water and Nairobi dam water respectively. Pentachlorophenol although not a plasticiser, was a component of the standard mix for purposes of monitoring the efficiency of extraction. PCP (CAS No.87-86-5) is primarily used in treating wood from fungal growth hence contamination of drinking water easily occurs during use. Log homes treated with PCP can also be a potential source (WHO, 2011).

The figures reveal that there was a drop in levels of PCP from day 60 to day 90 in all samples except for well water in which the increase from the 30^{th} day (BDL) to the 90^{th}day (0.51±0.81 µg/L) was remarkable.

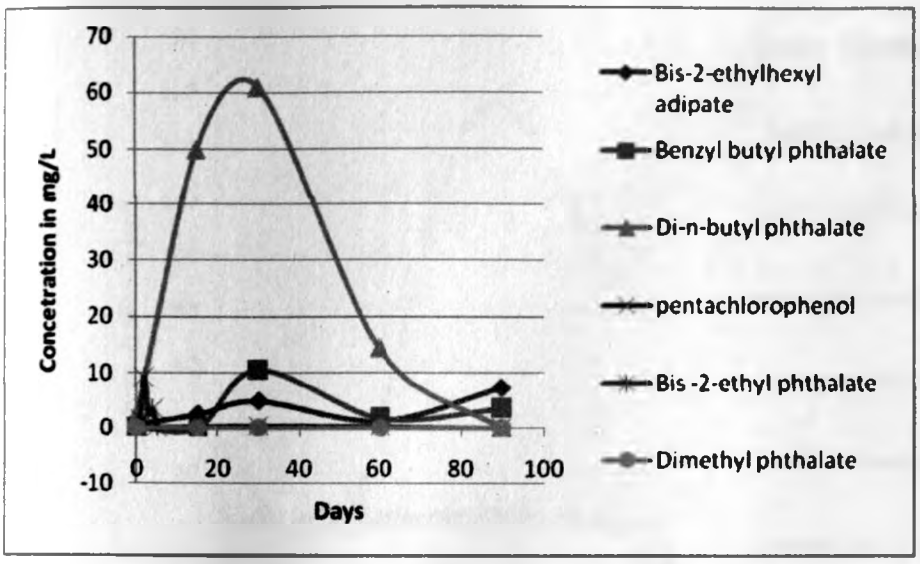

Figure 4 8.1: Levels of Different PAEs in' SODIS' Distilled water

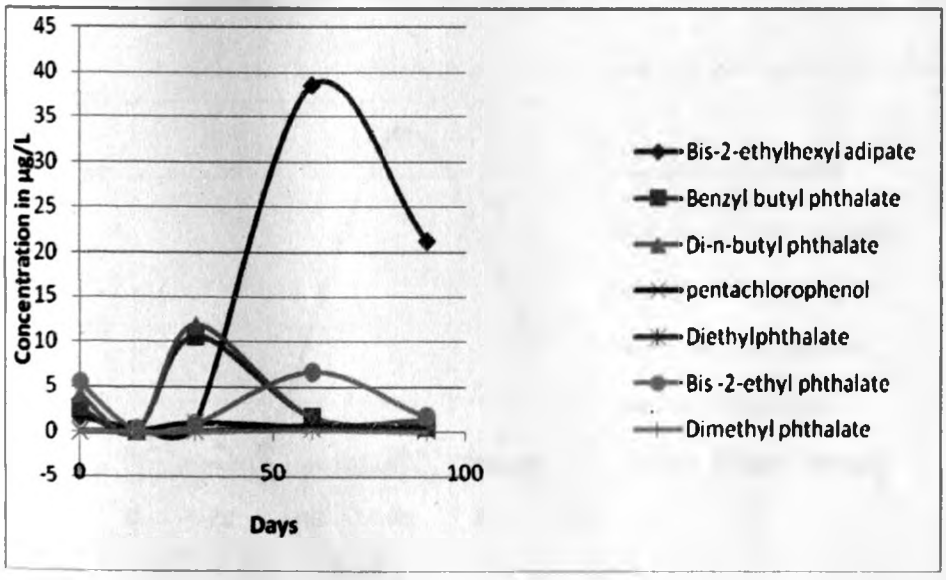

Figure 4.8.2; Levels of Different PAEs in' SODIS' Municipal tap water

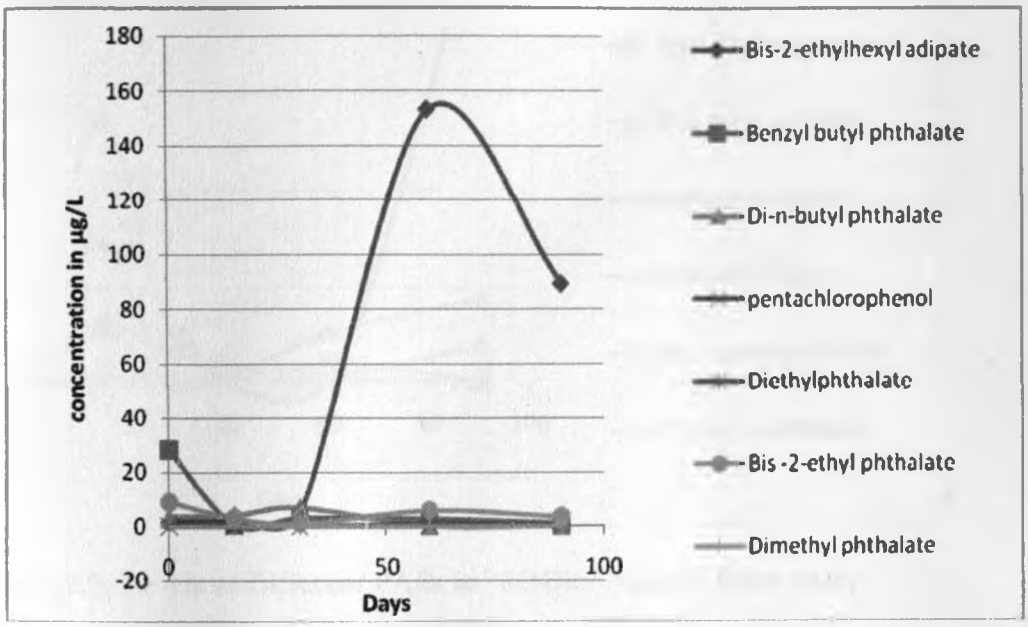

Figure 4.8.3: Levels of Different PAEs in SODIS Kibera House Water

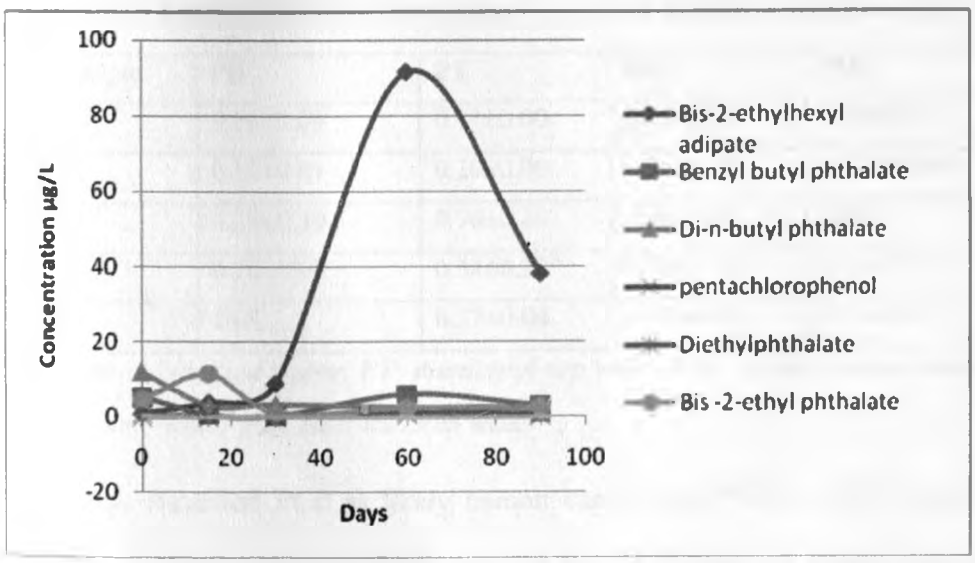

Figure 4.8.4: Levels of Different PAEs in' SODIS' Undugu well water

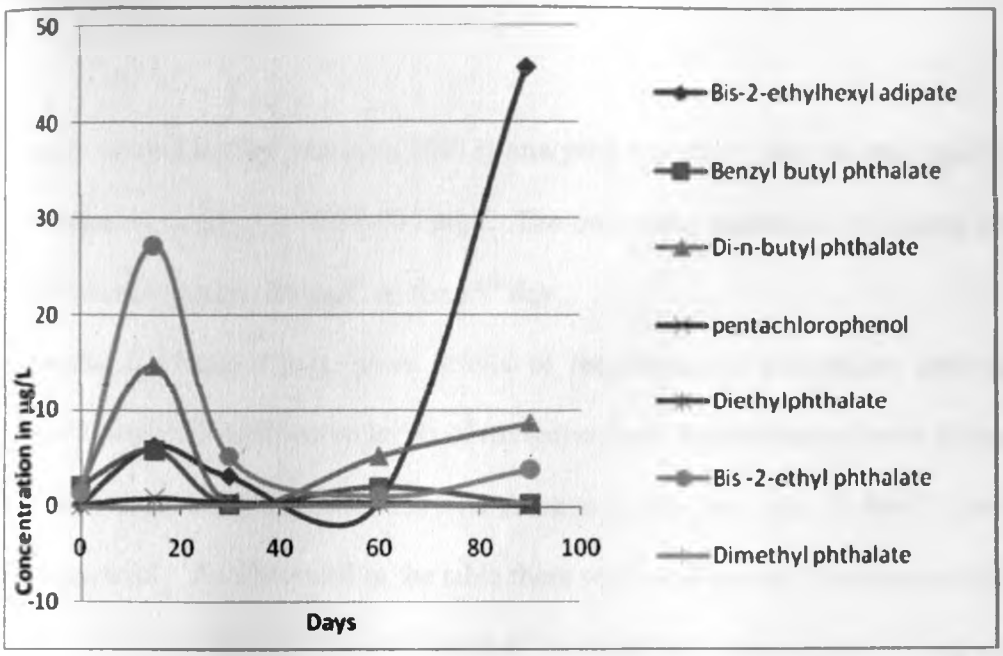

Figure: 4.8.5: Levels of Different PAEs in' SODIS' Nairobi Dam water

Table 4.5.8 reports the concentrations of PCP in all water samples

Table 4.5.8: Levels of Pentachlorophenol (µg/L) in SODIS water Samples

Day/Sample	PB	PT	PH	PW	PD
Day 0	0.06±0.00	0.11±0.00	0.25±0.00	0.54±0.05	0.03±0.01
Day 15	0.19±0.01	0.10±0.00	0.26±0.18	0.17±0.0.00	0.92±0.28
Day 30	0.29±0.10	0.90±0.19	0.03±0.00	BDL	0.14±0.10
Day 60	0.20±0.07	0.54±0.23	0.53±0.07	0.23±0.00	0.16±0.07
Day 90	BDL	0.37±0.04	0.11±0.02	0.51±0.81	0.15±0.00

PB: Bottled Distilled water: PT: municipal tap water ***PH:*** *Kibera house water* ***PW:*** *Undugu shallow well water* ***PD:*** *Nairobi Dam water*

IARC has classified PCP as likely human carcinogen (WHO, 2011).The WHO guideline value for this contaminant in drinking water is set at 0.009 µg/L (WHO, 2011) While the US Environmental Protection Agency (USEPA) maximum contaminant level (MCL) in drinking water is 0.001 mg/L. In this study levels of this likely carcinogen in house and tap water ranged from 0.03±0.00-0.90±0.19 µg/L which generally exceeded the WHO acceptable limits.

A study carried out by Lee *et al* (2013) analysed tap water samples and reported PCP at a concentration range of 0.002-0.007 mg/L .The dam water registered the highest concentration of this phenol (0.92±0.28 µg/L on the 15th day.

Appendix II (Table 4.6.7), gives details of the Pearson's correlations between levels of pentachlorophenol and scored levels of toxicities in all samples using tester strains TA98 and TA100 with or without induced rat liver fraction at day zero, day 15, day30 ,day 60 and day 90 respectively. As illustrated in the table there were no Pearson's correlations between levels of pentachlorophenol and toxicity levels of Undugu well water at day 60 using tester strain TA98 metabolic activation or TA100 without activation. However, the highest revealed Pearson's correlation (0.99) was between this water contaminant of interest and tap water at day zero using tester strain TA100 without S9. This implies that the chemical was directly responsible for the scored toxicity of tap water on day zero. Pearson's correlations reported at day 30 were all negative except for distilled water at day zero using TA98 without S9, Nairobi Dam (0.92) and Undugu well water enriched with tester strain TA100. The highest correlations at day 60 (0.75) and day 90(0.73) were registered with toxicity of Undugu well water using tester strains TA100 with S9 and TA98 without S9 respectively.

Table 4.5.9 summarizes the average daily intake of different plasticizers in the non-refill SODIS samples in micrograms per kilogram per day.

Table 4.5.9: Average daily intake of different PAEs in the non-refill SODIS samples (µg/kg/day)

Sample/Analyte	DEHA	BBP	DBP	DEP	PCP	DEHP	DMP
PB	0.18	0.12	0.95	0.02	0.01	0.12	0.00
PT	0.70	0.11	0.14	0.01	0.01	0.11	0.00
PH	2.79	0.26	0.10	0.01	0.01	0.16	0.00
PW	1.59	0.11	0.16	0.02	0.01	0.16	0.00
PD	0.62	0.08	0.23	0.02	0.02	0.29	0.01

Assuming a daily water consumption rate of 2 L and an average body weight of 60 kg for adults, the average daily intake of bis -2-ethylhexyl adipate by way of drinking SODIS water from the municipal tap or household SODIS water in Kibera was calculated to be 1.04 µg/kg/d or 4.19 µg/kg/d respectively Corresponding values for the Undugu unprotected shallow well and Nairobi dam water were reported as 2.38 µg/kg/d and0.93 µg/kg/d respectively. It is suspected that since the well and dam may have had extra pollutants that may were lacking in the house water or tap water these chemicals may have reacted with leached PAEs to give metabolites products that could not be detected in the investigation. Consequently distilled water gave the lowest value (0.27 µg/kg/d).

Similarly, average daily intake of benzyl butyl phthalate was found to be 0.12 µg/kg/d, 0.11 µg/kg/d, 0.26 µg/kg/d, 0.11 µg/kg/d and 0.08 µg/kg/d for distilled water, municipal tap, household, well and dam non-refill SODIS samples respectively. All these values are within acceptable limits since the reference dose as it can be noted from health advisories released by EPA in the appendix XI is set at 0.2 mg/kg/day.

There is no available data related to acceptable reference dose for DMP in drinking water. However, table 4.6.7 shows that when values are rounded off to 2 decimal places, the average daily intake of this PAE was almost negligible except in dam water in which it was approximately 0.01 µg/kg/day. Therefore in almost all days DMP was not a threat to SODIS water consumers. The BDL values in most samples in all days can easily be used to explain that the PET bottle manufacturers (Safe Park Kenya Limited) may be using minimal or no DMP as a plasticizer. In this study concentrations of bis-2-ethylhexyl phthalate (DEHP) residues ranged between BDL and 27.20±3.08 µg/L while estimated daily intake levels ranged from 0.11µg/kg/day to 0.29 µg/kg/day which were considered quite lower than the RfD of 20 µg/kg/d released by the EPA (2012). Similarly a study carried out by Yu Liu *et al.* (2013) also reported DEHP levels lower than the reference dose.

4.6. Correlations between Levels of Selected Plasticisers and Levels of Toxicity of SODIS Water Samples Using Tester Strains TA98 and TA100

Correlations were obtained using Pearson's Correlations from SPSS tool to establish probable relationships between the concentrations of the plasticisers in SODIS samples and levels of toxicity of the same samples on days 0 (zero), day 15, day 30, day 60 and day 90 using tester strains TA98 and TA100 both with metabolic activation and without. Numerical values ranged from -1.00 to 1.00 which is a linear relationship between two sets of data. In this analysis the strength of the linear correlation of the variables is determined by the numerical value of the correlation coefficients such that a correlation coefficient of 0 (zero) means that there is no relationship between the considered sets of data while when a numerical value less than 0.5 is recorded the relationship between the sets is considered weak.

A correlation coefficient that is greater than 0.5 is considered strong and significant whether negative or positive. The positive values imply direct proportionality of variables while the negative values indicate existence of inverse variation relationships. Results in appendix II (table 4.6.1) give details of the correlations that prevail between the levels of bis-2-ethylhexyl adipate and the levels of toxicity in bottled distilled water, municipal tap water in Kibera, Kibera house water, Undugu well water and Nairobi dam water using tester strains TA98 and TA100 with and without metabolic activation at day zero, day 15 day 30, day 60 and day 90 respectively.

Tables 4.6.2, 4.6.3, 4.6.4 4.6.5 and 4.6.6, all in appendix II give details of similar correlations but use benzyl butyl phthalate (BBP), di-*n*-butyl phthalate (DBP), Diethyl phthalate (DEP), Bis-2-ethylhexyl phthalate (DEHP) and Dimethyl phthalate (DMP) respectively. The correlations recorded are either positive or negative and either significant or insignificant as shown in the quoted tables.

4.6.1 Correlations between Levels of Bis- 2-ethylhexyl Adipate (DEHA) and Levels of Toxicity of SODIS Samples

Appendix II (Table 4.6.1) indicates that the level of bis- 2-ethylhexyl adipate had no correlation with levels of toxicity of any water samples using both tester strains TA98 and TA100 with or without induced rat liver fraction (S9) from day zero up to and including day 30. However recorded Pearson's correlations between this phthalate and all samples on both day 60 and day 90 were all positive except with Nairobi Dam water at day 90 using tester strain TA100 with metabolic activation. The highest significant Pearson's correlation (0.99) was scored at day 90 between levels of this analyte and levels of toxicity of Kibera household water using TA100 without induced rat liver fraction.

The highest Pearson's correlations at day 60 (0.85) was between bis-2-ethyl hexyl adipate and the toxicity levels of the samples was recorded using Undugu Well Water with tester strain TA100 without metabolic activation respectively. Pearson's' correlations between concentrations of bis-2- ethyl hexyl adipate and level of toxicity of the tap water with and without S9 revealed the highest correlation (0.97) at day 90 using tester strain TA100. These correlations reveal that the levels of this phthalates contributed to the toxicities of the samples quoted in the respective days. From the table it is clear that the level of toxicity of distilled water had highest correlation (0.76) with this analyte at day 90 using both strains (TA100 and TA98) with metabolic activation.

4.6.2 Correlations between Levels of Benzyl butyl phthalate (BBP) and Levels of Toxicity of SODIS Samples

Appendix II (Table 4.6.2) indicates that day 30 registered the most significant negative correlations (-0.97) between BBP and toxicity of Municipal tap water using TA98 with and without S9. The most positive significant correlations (0.88, 0.85 and 0.83) were revealed at day 60 with Kibera household water using tester strain TA100 without S9, Municipal tap water using tester strain TA100 with S9 and Nairobi Dam water using TA98 without S9 respectively. However, there was no Pearson correlation between levels of benzyl butyl phthalate and the reported level of toxicity of Nairobi Dam Water at day 30 using tester strain TA100 with metabolic activation.

This implies that BBP contributed to the levels of toxicity reported on day 60 for Kibera house water using TA100 S9, Municipal tap water using TA100 with S9 and Nairobi water using TA98 without S9 but did not contribute to the levels of toxicity reported in Nairobi dam water at day 30 using TA100 without S9

4.6.3 Correlations between Levels of Di-*n*-butyl Phthalate (DBP) and Levels of Toxicity of SODIS Samples

Appendix II (Table 4.6.3) gives details of the Pearson's correlations between levels of di-n-butyl phthalate and scored levels of toxicities in all samples using tester strains TA98 and TA100 with or without induced rat liver fraction at day zero, day 15, day 30 ,day 60 and day 90 respectively.

The most significant correlations (0.93) were recorded between levels of this PAE and toxicities of Undugu well water at day zero using tester strain TA98 without metabolic activation. This is interpreted to mean that DBP played a major role in the reported toxicity in this sample on day zero and that leakage of phthalates cannot be held responsible for the same. The Pearson's correlations between levels of this analyte and toxicity of Municipal tap

water and Kibera house water using TA100 without S9 were 0.91 and 0.88 respectively on day zero.

Toxicity of Nairobi dam water did not reveal any correlation with levels of DBP at day 90 using tester strain TA100 with metabolic activation. This implies that reported M.R of Nairobi dam water at day 90 did not have anything to do with reported levels of DBP in the sample on that day.

Other significant and positive correlations (0.85 and 0.80) were reported at day zero between levels this analyte and toxicity levels of Municipal tap water and Kibera household water using TA100 with S9 Toxicity level of Undugu well water scored a correlation of 0.69 with di-n- butyl phthalate using TA98 with S9 at day zero.

4.6.4 Correlations between Levels of Diethyl Phthalate (DEP) and Levels of Toxicity of SODIS Samples

Appendix II (Table 4.6.4) gives details of the Pearson's correlations between levels of Diethyl phthalate and levels of toxicities in all water samples using tester strains TA98 and TA100 with or without induced rat liver fraction (S9) at day zero, day 15, day 30, day 60 and day 90 respectively. The table clearly indicates that levels of this PAE had no correlation with house water at day 30 and day 90 using tester strain TA100 without metabolic activation. However the level of toxicity of the same water had a significant correlation (0.72) with the ester at day 60 when the water was reported to be potentially mutagenic. This is a clear indication that the revealed toxicity levels were partly due to DEP. At day 15 all correlations were negative with the most significant (-0.85) recorded with Undugu well water using tester strain TA100 with metabolic activation. The highest positive and significant correlations (0.85) between this ester and with toxicity levels was revealed at day 60 with Undugu well water using TA98 without metabolic activation. With distilled water highest correlations(0.78) were registered at day zero using tester strain TA98 without metabolic activation of induced rat liver (S9)

whereas with Nairobi dam water highest correlations(0.81) were at day 90 using TA100 with S9. No Pearson correlation was scored between this phthalate and level of toxicity in Nairobi dam at day one. This means that as much as Nairobi dam water was potentially mutagenic as from day zero DEP cannot be held responsible for its high toxicity levels.

4.6.5 Correlations between Levels of Bi-2-ethylhexyl Phthalate (DEHP) and Levels of Toxicity of SODIS Samples

The appendix II (Table 4.6.5) gives details of the Pearson's correlations between levels of bis-2- ethylhexyl phthalate and scored levels of toxicities in all samples using tester strains TA98 and TA100 with or without induced rat liver fraction (S9) at day zero, day 15, day 30 ,day 60 and day 90, respectively. As it can be noted in the table the levels of bis-2- ethylhexyl phthalate scored no correlations with levels of toxicity of house water at day 90 and Nairobi dam water at day 60 using tester strain TA100 without S9. The most significant positive correlation (0.78) was revealed at day 15 with distilled water using TA98 with induced rat liver fraction and at day 30 with the same sample using the same strain but without the rat liver fraction. This implies that *salmonella typhimurium* bacteria responded well to the Ames mutagenicity assay with or without metabolic activation. It can also be concluded that the reported levels of toxicity in distilled water at day 15 were largely contributed by levels of bis-2- ethylhexyl phthalate that prevailed.

The Pearson's correlations between bis-2- ethylhexyl phthalate and scored levels of toxicities in all samples using both tester strains TA98 and TA100 with or without metabolic activation at day zero were insignificant except with Undugu shallow well water using TA 100 without S9 which was scored as 0.56. This is an implication that levels of bis-2- ethylhexyl phthalate did not have much influence on the toxicity levels of water samples.

The most significant Pearson's correlations (-0.84) between house water and concentrations of bis-2- ethyl hexyl phthalate was recorded at day 60 using TA 98 with metabolic activation.

This means that mutagenic ratio and levels of house water on this day varied inversely The municipal tap water had 0.54 as the highest correlation with this phthalate ester at day 90 using tester strain TA98 with S9 while for Nairobi dam water scored correlations were insignificant except- 0.51 recorded at 60 using TA98 without metabolic activation meaning that this phthalate had little influence in toxicity levels scored in the sample.

4.6.6 Correlations between Levels of Dimethyl Phthalate (DMP) and Levels of Toxicity of SODIS Samples

Appendix II (Table 4.6.6) gives details of the Pearson's correlations between levels of Dimethyl phthalate (DMP) and levels of toxicities in all samples using tester strains TA98 and TA100 with or without induced rat liver fraction (S9) at day zero, day 15, day 30, day 60 and day 90, respectively. From the table (Table 4.6.6) it can be noted that in more than 75% of the results reveal levels of this phthalate below detection limits. Consequently recorded levels of toxicity in Nairobi Dam water at day 60 and day 90 using TA100 with metabolic activation had no significant correlations with the concentrations of DMP in the sample.

However the most significant Pearson's correlation (0.93) was recorded between levels of this ester and the toxicity of Undugu well water at day zero using tester strain TA98 without metabolic activation. Therefore concentrations of DMP had a direct influence on toxicity level of Undugu well water on day zero before any phthalate leaked into SODIS water. This3 implies that the Undugu well water was already chemically polluted.

CHAPTER FIVE

CONCLUNSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study detected and quantified all analytes of interest (bis-2-ethylhexyl adipate, benzyl butyl phthalate, di-*n*-butyl phthalate, diethyl phthalate, bis-2-ethylhexylphthalate and dimethyl phthalate) that were present in and that leached into the water samples from in polyethylene terephthalate bottles. Therefore, it was concluded that water stored in plastic bottles contributes to consumer exposure to endocrine disrupting chemicals. Levels of these plasticizers fluctuated throughout the study period. Generally assuming a daily water consumption rate of 2 liters and an average body weight of 60 kg for adults, the average daily intake of plasticizers in this study were found to be lower than reference doses released by EPA hence considered safe in isolation .They ranged between 0.18-2.79 µg/kg/day),0.08-0.26 µg/kg/day) ,0.10-0.95 µg/kg/day), 0.01-0.02 µg/kg/day), 0.11-0.16 µg/kg/day) and 0.00-0.01 µg/kg/day for DEHA, BBP, DBP, DEP DEHP and DMP respectively. However, a portion of detected quantities of these plasticizers is believed to have come from background pollution in the analytical procedure and the environment itself rather than entirely from the PET bottle.

Using the Multiple Tube Fermentation Technique feacal coliforms were confirmed present in four out of five water samples. Upon a six hour exposure of samples to solar radiation an 87.3% antibacterial effect was scored for contaminated replicates hence SODIS was confirmed a suitable affordable water treatment method.

Municipal tap water and Kibera household water are not microbiologically safe for drinking before treatment but on exposure to SODIS these samples are safe. Nairobi dam water was

5.2 Recommendations

5.2.1 Research Recommendations

- Extraction of phthalate esters and DEHA from SODIS water using SPE, SPME and stir bar sorptive extraction (SBSE) techniques and other bioassays such as the comet assay to investigate mutagenicity in order to compare results is highly recommended.

- Research on toxicity of SODIS water should include quantification of Phthalic acid (PhA) the main degradation product of diesters in samples.

- *Salmonella typhimurium* may also be substituted with *E.coli* strains (either as wp2 *uvrA or* wp2 [pKM101/ or as a combination [*"E.coli Combo"*]) *in* the Ames mutagenicity assay while TA98 and TA100 can be substituted with TA1535 and TA1537 to compare results. These tester strains are normally used to detect mutagens that are not easily detected by TA 98 and TA 100 (Prival *et al.*, (1998).

- Since phthalates are considered to be carcinogenic a study on cancer patients in Kenya should be carried out to establish the co-relationship between plasticizers and their metabolites, in urine or their blood and grades of the cancers. This will assist policy makers in regulating the quantity and or type of plasticizers to be used in the manufacture of plastics locally or imported to the country alongside the disposal and management of unused or waste solvents in the phthalate industries.

- Due to differences in enzyme activities in substance specificity and ion regulation between species use of isolated human hepatocytes in which all of the metabolic pathways function is recommended in place of the induced S9 rat liver fraction which only accounts for phase I enzymes.

- Sustained monitoring of toxicity levels of drinking water stored in PET bottles between the 30th and the 60thday using automated or robot scoring is recommended to establish the exact day beyond which the bottle is unsafe to use.

5.2.2 Policy Recommendations

- .Consumer awareness should be given priority so as to encourage people to cut down plastic use instead go for materials such as stainless or glass hence minimize health risks that may be associated. This will reduce not only the cancer burden but also endocrine related disorders.

- The government should ensure that analysis of the type, quality and quantity of plasticizers in the PET bottles from different manufactures in Kenya is routinely carried out by the Kenya Bureau of Standards or any equivalent body and that all plastic waste is recycled or handled with caution to minimize exit of plasticizer residues into environmental waters. Continuous sustained analysis of PET packed consumer products including SODIS water, mineral water, beverages and drugs should be carried to provide estimated average levels of PAEs daily intake hence assist in risk assessment associated with plasticizer intake.

- Heavy research on the Toxicity of Nairobi Dam should be done and correlated with levels of all possible contaminants both individually and cummulatively inorder to establish the root cause of the problem since the water is used directly by the Kibera residents and indirectly by non residents since it is even used to irrigate the green vegetables and yams which are consumed in the surrounding environs.

- Urgently a standard landfill should be built to serve Kibera residents appropriately. This will facilitate the recovery and protection of the Nairobi dam and the Mbagathi River which are abused as dumping sites by residents.
- The shallow unprotected well in Undugu Primary School should be condemned by the Ministry of Health which should also to ensure that both the school and the larger community do not use its water for consumption or irrigation.

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APPENDICES

Appendix I: Levels of PAEs in Different Water Samples

Table 4.5.10: Levels of PAEs and DEHA in Kibera House Water (µg/L) ±S.D, N=3 BDL=Below Detection Limit

PAE/day No.	Day 0(zero)	Day15	Day 30	Day60	Day90
Butyl benzyl Phthalate	28.27±0.00	0.52±0.00	3.01±0.84	2.63±0.00	1.01±0.99
Di ethyl phthalate	BDL	0.17±0.00	BDL	0.55±0.00	0.09±0.02
Bis-2-ethyl phthalate	8.72±0.00	2.98±0.00	0.90±0.15	5.65±1.58	3.24±0.51
Bis(2-ethylhexyl) Adipate	1.75±0.00	2.35±0.00	4.88±0.00	152.97±0.00	89.31±41.43
Di-n-butyl phthalate	3.37±0.00	4.02±0.00	6.71±3.10	BDL	BDL
Dimethyl phthalate	BDL	BDL	BDL	BDL	0.03±0.00
Pentachlorophenol	0.25±0.00	0.26±0.18	0.03±0.00	0.53±0.07	0.11±0.02

Table 4.5.11: Levels of PAEs and DEHA in Distilled Water (µg/L)±S.D, N=3 BDL=Below Detection Limit

PAE/day No.	Day 0(zero)	Day15	Day 30	Day60	Day90
Butyl benzyl Phthalate	BDL	BDL	10.55±0.00	1.28±0.00	3.57±0.00
Diethyl phthalate	0.14±0.00	1.69±0.000	0.17±0.07	0.27±0.15	0.10±0.00
Bis-2-ethyl phthalate	1.54±0.49	9.22±4.84	2.51±1.37	3.40±1.81	BDL
Bis(2-ethyl hexyl) Adipate	0.67±0.34	2.48±0.00	4.82±3.18	1.28±0.00	7.23±0.00
Di-n-butyl phthalate	2.13±0.00	50.00±10.56	61.08±13.32	14.40±0.00	BDL
Dimethyl phthalate	BDL	BDL	BDL	BDL	BDL
Pentachlorophenol	0.06±0.00	0.19±0.02	0.29±0.10	0.20±0.07	BDL

Table 4.5.12: Levels of PAEs and DEHA in Nairobi Dam Water (µg/L)±S.D, N=3 BDL=Below Detection Limit)

PAE/day No.	Day 0(zero)	Day15	Day 30	Day60	Day90
Butyl benzyl Phthalate	2.20±1.65	5.88±0.00	0.29±0.13	2.05±1.75	0.32±0.007
Diethyl phthalate	0.88±0.00	0.38±0.18	0.38±0.18	0.30±0.23	0.10±0.06
Bis-2-ethyl phthalate	1.44±0.60	27.20±3.08	5.21±4.40	1.13±0.85	3.85±0.95
Bis(2-ethyl hexyl) Adipate	0.24±0.00	6.22±5.78	3.16±2.18	0.55±0.19	45.70±17.50
Di-n-butyl phthalate	2.23±0.63	14.89±0.00	0.38±0.00	5.23±0.00	8.68±0.00
Dimethyl phthalate	BDL	BDL	0.24±0.00	0.180.00	0.42±00
Pentachlorophenol	0.23±0.34	0.92±0.28	0.49±0.62	0.46±0.52	0.18±0.05

Table 4.5.13: Levels of PAEs and DEHA in Undugu Shallow Well Water (µg/L) ±S.D, N=3 BDL=Below Detection Limit

PAE/day No.	Day 0(zero)	Day15	Day 30	Day60	Day90
Butyl benzyl Phthalate	5.08±0.00	0.43±0.00	0.052±0.03	5.91±0.36	2.80±0.00
Diethyl phthalate	0.07±0.04	BDL	0.09±0.00	1.164±0.05	0.30±0.06
Bis-2-ethyl phthalate	4.61±0.05	11.47±5.31	1.02±0.21	2.28±0.00	2.60±2.00
Bis(2-ethyl hexyl) Adipate	0.81±0.18	3.69±0.00	8.82±0.00	91.57±0.00	38.00±0.00
Di-n-butyl phthalate	11.98±1.31	2.86±0.00	3.15±1.90	2.37±0.00	1.14±0.83
Dimethyl phthalate	0.18±0.07	BDL	BDL	BDL	BDL
Pentachlorophenol	0.54±0.05	0.17±0.00	BDL	0.23±0.00	0.51±0.18

Table 4.5.14: Levels of PAEs and DEHA in Kibera Municipal Tap Water (µg/L)±S.D, N=3
BDL=Below Detection Limit

PAE/Day	Day 0(zero)	Day15	Day 30	Day60	Day90
Butyl benzyl Phthalate	2.57.±0.00	BDL	10.66±0.00	1.61±0.66	0.58±0.08
Diethyl phthalate	BDL	BDL	0.12±0.00	0.78±0.11	0.34±0.23
Bis-2-ethyl phthalate	5.46±0.00	0.16±0.03	0.84±0.64	6.66±0.23	1.68±0.22
Bis(2-ethyl hexyl) Adipate	1.61±0.71	0.39±0.23	0.80±0.00	38.54±11.18	21.27±0.00
Di-n-butyl phthalate	4.14±0.00	BDL	11.80±0.32	1.64±0.00	1.25±0.00
Dimethyl phthalate	BDL	BDL	BDL	0.20±0.13	BDL
Pentachlorophenol	0.11±0.00	0.10±0.00	0.90±0.18	0.54±0.23	0.37±0.04

Appendix II: Correlations between Levels of Selected Plasticisers and Levels of Toxicity of SODIS Samples

Table 4.6.1: Correlations between levels of bis-2-ethylhexyl adipate (DEHA) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		DEHA Level day zero	DEHA Level at day15	DEHA Level at day30	DEHA Level at day 60	DEHA Level at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	.	.	.	.22	.59
	Sig. (2-tailed)	.	.	.	.72	.29
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	.	.	.	.37	.86
	Sig. (2-tailed)	.	.	.	.54	.07
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	.	.	.	.44	.84
	Sig. (2-tailed)	.	.	.	.46	.08
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	.	.	.	.85	.96
	Sig. (2-tailed)	.	.	.	.07	.01
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.	.	.	.72	.91
	Sig. (2-tailed)	.	.	.	.17	.03
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	.	.	.	.33	.76
	Sig. (2-tailed)	.	.	.	.59	.13
toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.	.	.	.27	.74
	Sig. (2-tailed)	.	.	.	.66	.15
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	.	.	.	.62	.96
	Sig. (2-tailed)	.	.	.	.26	.01
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	.	.	.	.63	.84
	Sig. (2-tailed)	.	.	.	.25	.07
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.	.	.	.58	.82
	Sig. (2-tailed)	.	.	.	.31	.09
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.	.	.	.32	.70
	Sig. (2-tailed)	.	.	.	.61	.19
toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	.	.	.	.79	.97
	Sig. (2-tailed)	.	.	.	.11	.01
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	.	.	.	.72	.99
	Sig. (2-tailed)	.	.	.	.17	.00
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.	.	.	.39	.78
	Sig. (2-tailed)	.	.	.	.52	.12
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.	.	.	.48	.15
	Sig. (2-tailed)	.	.	.	.41	.81
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.	.	.	.39	.76
	Sig. (2-tailed)	.	.	.	.52	.13
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	.	.	.	.72	.97
	Sig. (2-tailed)	.	.	.	.17	.01
toxicity of kibera house water [TA100 with S9]	Pearson Correlation	.	.	.	.69	.94
	Sig. (2-tailed)	.	.	.	.20	.02
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.	.	.	.34	.01
	Sig. (2-tailed)	.	.	.	.57	.99
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	.	.	.	.07	-.49
	Sig. (2-tailed)	.	.	.	.92	.41

Table 4.6.2: Correlations between levels of Benzyl butyl phthalate (BBP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		BBP level at day 0	BBP level at day 15	BBP level at day 30	BBP level at day 60	BBP level at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	-.17	.78	-.84	.29	-.69
	Sig. (2-tailed)	.79	.12	.08	.63	.20
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	-.03	.58	-.97	.58	-.54
	Sig. (2-tailed)	.97	.31	.01	.31	.35
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	-.08	.58	-.94	.58	-.63
	Sig. (2-tailed)	.89	.30	.02	.30	.25
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	-.13	.06	-.74	.93	-.52
	Sig. (2-tailed)	.83	.93	.16	.02	.37
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.17	.08	-.85	.83	-.68
	Sig. (2-tailed)	.78	.90	.07	.08	.20
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	-.12	.69	-.93	.48	-.61
	Sig. (2-tailed)	.84	.20	.02	.42	.28
toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.05	.66	-.97	.44	-.66
	Sig. (2-tailed)	.94	.23	.01	.46	.23
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	-.17	.38	-.89	.79	-.46
	Sig. (2-tailed)	.79	.53	.05	.11	.44
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	-.10	.40	-.86	.69	-.75
	Sig. (2-tailed)	.87	.50	.06	.20	.15
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.28	.20	-.90	.69	-.78
	Sig. (2-tailed)	.65	.75	.04	.20	.12
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.00	.66	-.92	.42	-.75
	Sig. (2-tailed)	1.00	.23	.02	.48	.15
toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	-.02	.08	-.81	.91	-.54
	Sig. (2-tailed)	.97	.90	.10	.03	.35
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	-.13	.22	-.84	.88	-.44
	Sig. (2-tailed)	.84	.72	.08	.05	.46
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.38	.31	-.96	.57	-.72
	Sig. (2-tailed)	.53	.61	.01	.32	.17
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	-.28	.20	-.17	.20	-.80
	Sig. (2-tailed)	.66	.75	.78	.75	.12
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.15	.51	-.96	.51	-.80
	Sig. (2-tailed)	.82	.38	.01	.38	.13
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	-.07	.24	-.87	.85	-.56
	Sig. (2-tailed)	.91	.70	.06	.07	.32
Level of toxicity of kibera house water [TA100 with S9]	Pearson Correlation	-.23	.36	-.84	.80	-.54
	Sig. (2-tailed)	.71	.55	.08	.10	.35
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.07	.06	-.15	.06	-.88
	Sig. (2-tailed)	.91	.92	.81	.92	.05
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	-.37	.00	.48	-.32	-.32
	Sig. (2-tailed)	.54	1.00	.42	.60	.60

Table 4.6.3: Correlations between levels of Di-n-butyl phthalate (DBP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		Level of DBP at day 0	Level of DBP at day 15	Level of DBP at day 30	Level of DBP at day 60	Level of DBP at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	.29	-.51	-.77	-.69	.78
	Sig. (2-tailed)	.63	.38	.13	.20	.12
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	.58	-.40	-.63	-.54	.58
	Sig. (2-tailed)	.31	.50	.25	.35	.31
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	.58	-.50	-.73	-.63	.58
	Sig. (2-tailed)	.30	.39	.17	.25	.30
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	.93	-.53	-.60	-.52	.06
	Sig. (2-tailed)	.02	.36	.28	.37	.93
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.83	-.68	-.74	-.68	.08
	Sig. (2-tailed)	.08	.21	.15	.21	.90
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	.48	-.45	-.70	-.61	.69
	Sig. (2-tailed)	.42	.45	.19	.28	.20
toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.44	-.51	-.74	-.66	.66
	Sig. (2-tailed)	.46	.38	.15	.23	.22
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	.79	-.38	-.56	-.46	.38
	Sig. (2-tailed)	.12	.53	.32	.44	.53
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	.69	-.67	-.82	-.75	.40
	Sig. (2-tailed)	.20	.22	.09	.15	.50
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.69	-.76	-.84	-.78	.20
	Sig. (2-tailed)	.20	.14	.09	.12	.75
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.42	-.61	-.83	-.75	.66
	Sig. (2-tailed)	.48	.28	.09	.15	.23
Level of toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	.91	-.53	-.62	-.54	.08
	Sig. (2-tailed)	.03	.35	.27	.35	.90
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	.88	-.40	-.54	-.44	.22
	Sig. (2-tailed)	.05	.51	.35	.46	.72
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.57	-.67	-.78	-.72	.31
	Sig. (2-tailed)	.32	.22	.12	.17	.61
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.20	-.76	-.78	-.78	.20
	Sig. (2-tailed)	.75	.14	.12	.12	.75
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.51	-.66	-.84	-.77	.51
	Sig. (2-tailed)	.38	.22	.08	.13	.38
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	.85	-.52	-.65	-.56	.24
	Sig. (2-tailed)	.07	.37	.23	.32	.70
toxicity of kibera house water [TA100 with S9]	Pearson Correlation	.80	-.46	-.63	-.54	.36
	Sig. (2-tailed)	.10	.44	.25	.35	.56
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.06	-.89	-.83	-.88	.06
	Sig. (2-tailed)	.92	.04	.08	.05	.92
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	-.32	-.33	-.26	-.32	.00
	Sig. (2-tailed)	.60	.60	.67	.60	1.00

Table 4.6.4: Correlations between levels of Diethyl phthalate (DEP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		DEP level at day 0	DEP level at day 15	DEP level at day 30	DEP level at day 60	DEP level at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	.78	-.28	.44	.19	-.05
	Sig. (2-tailed)	.12	.65	.46	.76	.94
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	.58	-.23	.31	.39	-.25
	Sig. (2-tailed)	.31	.71	.61	.52	.69
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	.58	-.33	.27	.44	-.10
	Sig. (2-tailed)	.30	.59	.66	.46	.88
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	.06	-.52	-.20	.85	.23
	Sig. (2-tailed)	.93	.36	.74	.07	.71
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.08	-.64	-.26	.80	.11
	Sig. (2-tailed)	.90	.24	.67	.10	.86
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	.69	-.25	.39	.31	-.15
	Sig. (2-tailed)	.20	.69	.52	.61	.81
toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.66	-.30	.33	.30	-.22
	Sig. (2-tailed)	.23	.63	.59	.63	.72
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	.38	-.28	.15	.61	-.06
	Sig. (2-tailed)	.53	.64	.81	.28	.92
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	.40	-.55	.03	.63	.17
	Sig. (2-tailed)	.50	.33	.96	.25	.78
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.20	-.67	-.20	.68	.05
	Sig. (2-tailed)	.75	.22	.75	.21	.94
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.66	-.40	.28	.33	-.07
	Sig. (2-tailed)	.23	.51	.65	.59	.91
toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	.08	-.52	-.19	.82	.12
	Sig. (2-tailed)	.90	.37	.76	.09	.84
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	.22	-.35	.00	.72	.00
	Sig. (2-tailed)	.72	.57	1.00	.17	1.00
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.31	-.53	-.05	.51	-.18
	Sig. (2-tailed)	.61	.36	.93	.38	.77
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.20	-.72	-.20	.41	.78
	Sig. (2-tailed)	.75	.17	.75	.49	.12
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.51	-.49	.13	.44	-.09
	Sig. (2-tailed)	.38	.41	.84	.46	.89
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	.24	-.46	-.04	.73	.06
	Sig. (2-tailed)	.70	.44	.95	.16	.92
toxicity of kibera house water [TA100 with S9]	Pearson Correlation	.36	-.38	.09	.67	.09
	Sig. (2-tailed)	.56	.53	.89	.23	.89
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.06	-.85	-.38	.37	.72
	Sig. (2-tailed)	.92	.07	.53	.55	.17
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	.00	-.37	-.16	-.04	.81
	Sig. (2-tailed)	1.00	.54	.80	.94	.10

Table 4.6.5: Correlations between levels of bis-2-ethylhexyl phthalate (DEHP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		Level of DEHP at day 0	Level of DEHP at day 15	Level of DEHP at day 30	Level of DEHP at day 60	Level of DEHP at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	-.24	.76	.78	-.66	.48
	Sig. (2-tailed)	.70	.13	.12	.23	.41
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	-.26	.74	.58	-.80	.40
	Sig. (2-tailed)	.67	.15	.31	.11	.51
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	-.22	.71	.58	-.76	.36
	Sig. (2-tailed)	.73	.18	.30	.14	.56
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	-.08	.31	.06	-.65	-.14
	Sig. (2-tailed)	.90	.61	.93	.24	.82
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.16	.26	.08	-.51	.12
	Sig. (2-tailed)	.80	.67	.90	.39	.84
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	-.28	.78	.69	-.77	.42
	Sig. (2-tailed)	.64	.12	.20	.13	.48
toxicity of municipal tap water in kibera[TA98 with S9]	Pearson Correlation	-.15	.73	.66	-.69	.54
	Sig. (2-tailed)	.81	.16	.23	.20	.35
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	-.30	.62	.38	-.84	.10
	Sig. (2-tailed)	.62	.26	.53	.08	.87
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	-.06	.51	.40	-.64	.19
	Sig. (2-tailed)	.92	.39	.50	.24	.76
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.24	.30	.20	-.44	.32
	Sig. (2-tailed)	.70	.62	.75	.46	.60
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	-.10	.68	.66	-.63	.50
	Sig. (2-tailed)	.88	.21	.23	.26	.39
toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	-.04	.34	.08	-.65	-.03
	Sig. (2-tailed)	.95	.58	.90	.24	.96
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	-.23	.50	.22	-.78	.00
	Sig. (2-tailed)	.72	.39	.72	.12	1.00
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.20	.41	.31	-.46	.51
	Sig. (2-tailed)	.75	.50	.61	.43	.39
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.24	-.01	.20	.00	-.08
	Sig. (2-tailed)	.70	.98	.75	1.00	.90
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.03	.57	.51	-.58	.49
	Sig. (2-tailed)	.96	.32	.38	.31	.40
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	-.12	.47	.24	-.72	.07
	Sig. (2-tailed)	.84	.42	.70	.17	.92
toxicity of kibera house water [TA100 with S9]	Pearson Correlation	-.26	.57	.36	-.80	.04
	Sig. (2-tailed)	.67	.31	.56	.11	.95
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.56	-.22	.06	.28	.10
	Sig. (2-tailed)	.33	.72	.92	.65	.87
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	.22	-.33	.00	.43	-.26
	Sig. (2-tailed)	.72	.59	1.00	.47	.67

Table 4.6.6: Correlations between levels of Dimethyl phthalate (DMP) and levels of toxicity of SODIS samples using tester strains TA100 and TA98 (N=3)

		Level of DMP at day 0	Level of DMP at day 15	Level of DMP at day 30	Level of DMP at day 60	Level of DMP at day 90
Level of toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	.29	.	.78	.38	.78
	Sig. (2-tailed)	.63	.	.12	.52	.12
Level of toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	.58	.	.58	-.06	.58
	Sig. (2-tailed)	.30	.	.30	.92	.31
Level of toxicity of kibera house water [TA98 without S9]	Pearson Correlation	.58	.	.58	.07	.58
	Sig. (2-tailed)	.30	.	.30	.91	.30
Level of toxicity of undugu well water [TA98 without S9]	Pearson Correlation	.93	.	.06	-.17	.06
	Sig. (2-tailed)	.02	.	.93	.79	.93
level of toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.83	.	.08	-.22	.08
	Sig. (2-tailed)	.08	.	.90	.73	.90
Level of toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	.48	.	.69	.15	.69
	Sig. (2-tailed)	.42	.	.20	.81	.20
Level of toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.44	.	.66	.08	.66
	Sig. (2-tailed)	.46	.	.23	.90	.23
Level of toxicity of kibera house water [TA98 with S9]	Pearson Correlation	.79	.	.38	-.14	.38
	Sig. (2-tailed)	.11	.	.53	.82	.53
Level of toxicity of undugu well water [TA98 with S9]	Pearson Correlation	.69	.	.40	.13	.40
	Sig. (2-tailed)	.20	.	.50	.83	.50
level of toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.69	.	.20	-.12	.20
	Sig. (2-tailed)	.20	.	.75	.84	.75
Level of toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.42	.	.66	.22	.66
	Sig. (2-tailed)	.48	.	.23	.72	.23
Level of toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	.91	.	.08	-.24	.08
	Sig. (2-tailed)	.03	.	.90	.70	.90
Level of toxicity of kibera house water [TA100 without S9]	Pearson Correlation	.90	.	.22	-.24	.22
	Sig. (2-tailed)	.05	.	.72	.70	.72
Level of toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.57	.	.31	-.20	.31
	Sig. (2-tailed)	.32	.	.61	.75	.61
Level of toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.20	.	.20	.76	.20
	Sig. (2-tailed)	.75	.	.75	.14	.75
Level of toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.51	.	.51	.06	.51
	Sig. (2-tailed)	.38	.	.38	.92	.38
Level of toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	.85	.	.24	-.15	.24
	Sig. (2-tailed)	.07	.	.70	.81	.70
Level of toxicity of kibera house water [TA100 with S9]	Pearson Correlation	.80	.	.36	-.02	.36
	Sig. (2-tailed)	.10	.	.56	.97	.56
Level of toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.06	.	.06	.66	.06
	Sig. (2-tailed)	.92	.	.92	.22	.92
Level of toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	-.32	.	.00	.87	.00
	Sig. (2-tailed)	.60	.	1.00	.06	1.00

Table 4.6.7: Correlations between levels of Pentachlorophenol (PCP) and levels of toxicity in SODIS samples using tester strains TA100 and TA98 (N=3)

		PCP Level at day 0	PCP Level at day 15	PCP Level at day 30	PCP Level at day 60	PCP Level at day 90
toxicity of bottled distilled water [TA98 without S9]	Pearson Correlation	0.54	0.72	0.01	0.23	0.26
	Sig. (2-tailed)	.3	.17	.99	.70	.67
toxicity of municipal tap water in kibera [TA98 without S9]	Pearson Correlation	.77	.54	-.42	-.16	.26
	Sig. (2-tailed)	.13	.35	.49	.80	.68
toxicity of kibera house water [TA98 without S9]	Pearson Correlation	.78	.52	-.29	-.06	.37
	Sig. (2-tailed)	.12	.37	.63	.93	.54
toxicity of undugu well water [TA98 without S9]	Pearson Correlation	.98	-.03	-.40	-.35	.73
	Sig. (2-tailed)	.00	.97	.51	.57	.16
toxicity of Nairobi Dam water [TA98 without S9]	Pearson Correlation	.98	.01	-.52	-.18	.64
	Sig. (2-tailed)	.01	.99	.37	.77	.25
toxicity of bottled distilled water [TA98 with S9]	Pearson Correlation	.69	.64	-.22	.00	.28
	Sig. (2-tailed)	.20	.25	.72	1.00	.65
toxicity of municipal tap water in kibera [TA98 with S9]	Pearson Correlation	.69	.62	-.31	.05	.23
	Sig. (2-tailed)	.20	.27	.61	.93	.71
toxicity of kibera house water [TA98 with S9]	Pearson Correlation	.89	.31	-.43	-.35	.46
	Sig. (2-tailed)	.04	.61	.47	.57	.43
toxicity of undugu well water [TA98 with S9]	Pearson Correlation	.87	.31	-.21	.00	.62
	Sig. (2-tailed)	.06	.60	.73	1.00	.27
toxicity of Nairobi Dam water [TA98 with S9]	Pearson Correlation	.90	.14	-.48	.00	.55
	Sig. (2-tailed)	.04	.83	.42	1.00	.34
toxicity of bottled distilled water [TA100 without S9]	Pearson Correlation	.68	.60	-.18	.17	.33
	Sig. (2-tailed)	.21	.29	.78	.79	.59
	N	5	5	5	5	5
toxicity of municipal tap water in kibera [TA100 without S9]	Pearson Correlation	.99	.01	-.49	-.35	.66
	Sig. (2-tailed)	.00	.99	.40	.57	.23
toxicity of kibera house water [TA100 without S9]	Pearson Correlation	.95	.15	-.49	-.42	.54
	Sig. (2-tailed)	.02	.81	.40	.48	.35
toxicity of undugu well water [TA100 without S9]	Pearson Correlation	.82	.28	-.57	.00	.33
	Sig. (2-tailed)	.09	.65	.32	1.00	.59
toxicity of Nairobi Dam water [TA100 without S9]	Pearson Correlation	.33	.08	.57	.59	.75
	Sig. (2-tailed)	.59	.90	.32	.30	.15
toxicity of bottled distilled water [TA100 with S9]	Pearson Correlation	.77	.46	-.34	.10	.37
	Sig. (2-tailed)	.13	.44	.58	.87	.54
toxicity of municipal tap water in kibera [TA100 with S9]	Pearson Correlation	.96	.17	-.44	-.29	.59
	Sig. (2-tailed)	.01	.79	.46	.64	.30
toxicity of kibera house water [TA100 with S9]	Pearson Correlation	.90	.28	-.31	-.27	.58
	Sig. (2-tailed)	.04	.65	.61	.67	.31
toxicity of undugu well water [TA100 with S9]	Pearson Correlation	.26	-.03	.46	.75	.66
	Sig. (2-tailed)	.68	.96	.44	.15	.23
toxicity of Nairobi Dam water [TA100 with S9]	Pearson Correlation	-.32	-.08	.92	.69	.40
	Sig. (2-tailed)	.60	.90	.03	.19	.51

Table 4.6.8: Correlations between TA 98 and TA100 *Salmonella typhimurium* tester strains, N=3

		PB98	PB_98	PB100	PB_100	PT98	PT_98	PT100	PT_100
PB98	Pearson Correlation	1	.91	.68	.92	.98**	.97	.91	.95
	Sig. (2-tailed)		.03	.20	.03	.00	.01	.03	.01
PB-S98	Pearson Correlation	.91*	1	.86	.93*	.88*	.86	.77	.82
	Sig. (2-tailed)	.03		.06	.02	.05	.06	.13	.09
PB+S100	Pearson Correlation	.68	.86	1	.87	.58	.59	.53	.61
	Sig. (2-tailed)	.20	.06		.06	.31	.30	.36	.28
PB-S100	Pearson Correlation	.92*	.93*	.87	1	.85	.83	.88	.92*
	Sig. (2-tailed)	.03	.02	.06		.07	.09	.05	.03
PT+B98	Pearson Correlation	.98**	.88*	.58	.85	1	.98*	.90*	.93*
	Sig. (2-tailed)	.00	.05	.31	.07		.00	.04	.02
pT-S98	Pearson Correlation	.97**	.86	.59	.83	.98**	1	.83	.88
	Sig. (2-tailed)	.01	.06	.30	.09	.00		.08	.05
PT+S100	Pearson Correlation	.91*	.77	.53	.88	.90*	.83	1	.99**
	Sig. (2-tailed)	.03	.13	.36	.05	.04	.08		.00
PT-S100	Pearson Correlation	.95*	.82	.61	.92*	.93*	.88	.99**	1
	Sig. (2-tailed)	.01	.09	.28	.03	.02	.05	.00	
PH+S98	Pearson Correlation	.93*	.89*	.86	.98**	.85	.85	.84	.90*
	Sig. (2-tailed)	.02	.05	.07	.00	.07	.07	.08	.04
PH-S98	Pearson Correlation	.98**	.87	.76	.96*	.91*	.92*	.89*	.94*
	Sig. (2-tailed)	.01	.05	.14	.01	.03	.03	.04	.02
PH+S100	Pearson Correlation	.76	.54	.19	.47	.82	.88*	.58	.62
	Sig. (2-tailed)	.13	.34	.76	.42	.09	.05	.30	.26
PH-S100	Pearson Correlation	.76	.53	.15	.46	.83	.88	.61	.64
	Sig. (2-tailed)	.14	.36	.81	.44	.08	.05	.28	.25
PW+S98	Pearson Correlation	.89*	.84	.71	.78	.86	.93*	.62	.71
	Sig. (2-tailed)	.05	.08	.18	.12	.06	.02	.26	.18
PW-S98	Pearson Correlation	.62	.77	.71	.56	.61	.69	.26	.36
	Sig. (2-tailed)	.27	.13	.18	.32	.28	.20	.67	.55
PW+S100	Pearson Correlation	-.07	.11	.38	-.02	-.13	.03	-.45	-.33
	Sig. (2-tailed)	.91	.86	.53	.97	.84	.97	.45	.59
PW-S100	Pearson Correlation	.18	.32	.54	.22	.11	.27	-.20	-.07
	Sig. (2-tailed)	.77	.60	.34	.73	.86	.67	.75	.90
PD+S98	Pearson Correlation	-.26	-.16	.10	-.27	-.31	-.14	-.61	-.50
	Sig. (2-tailed)	.67	.79	.88	.66	.62	.83	.27	.39
PD-S98	Pearson Correlation	.21	.18	.13	.00	.24	.40	-.18	-.08
	Sig. (2-tailed)	.74	.77	.83	1.00	.70	.51	.77	.90
PD+S100	Pearson Correlation	-.41	-.17	-.22	-.51	-.32	-.26	-.65	-.63
	Sig. (2-tailed)	.49	.78	.72	.38	.61	.67	.24	.26
PD-S100	Pearson Correlation	-.18	-.06	-.22	-.40	-.07	.01	-.48	-.44
	Sig. (2-tailed)	.77	.92	.73	.51	.91	.99	.41	.46

PB: *Bottled distilled water,* ***PT:*** *Municipal Tap Water,* ***PH:*** *Kibera house Water,* ***PW:*** *Undugu Shallow Well Water,* ***PD:*** *Nairobi Dam Water-**S98**:TA98 without Induced Rat Liver, +**S98**: TA98 with Induced rat Liver,-**S100**: TA100 without Induced rat liver, +**S100**: TA100 with induced rat Liver*

Appendix III: (Courtesy of Xenometrix 2012)

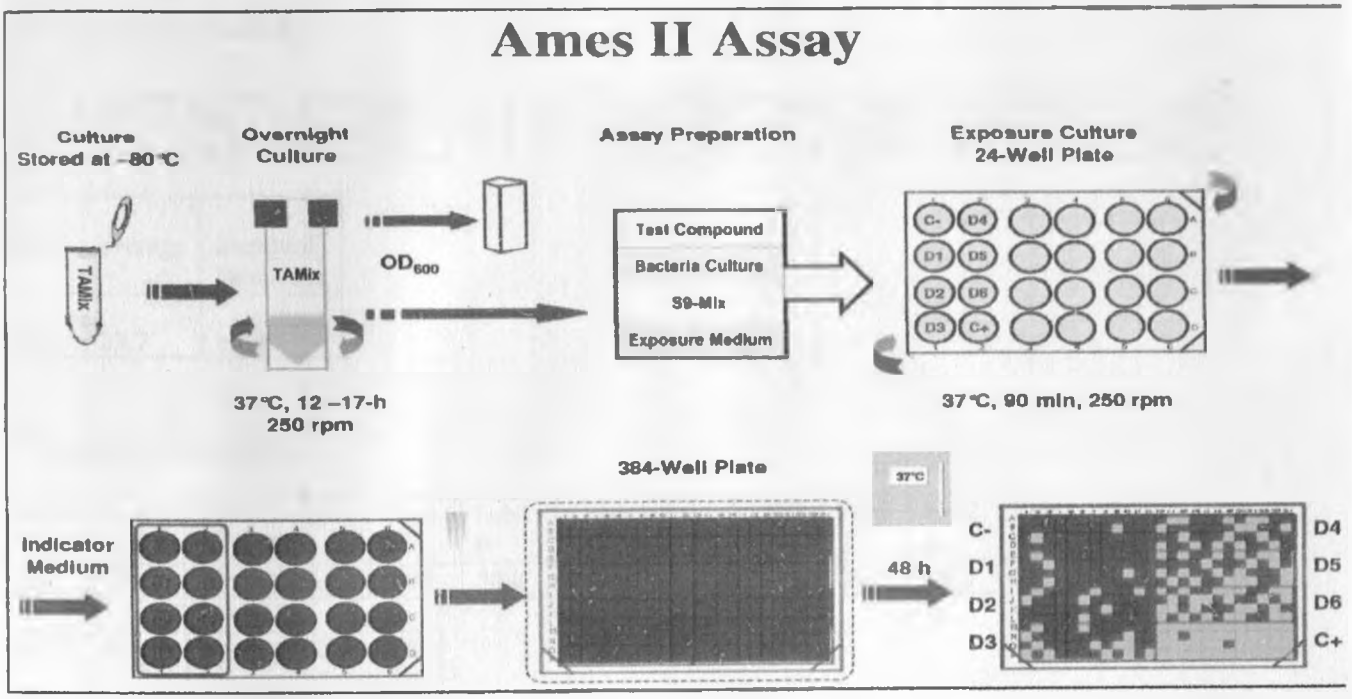


Figure 4.13: Major steps in the Ames mutagenicity test

Appendix IV: Daily Radiations (mj/m2) during the study Period

1st Interval (Day0-15 S)

Jan30	Jan31	Feb1	Feb2	Feb3	Feb4	Feb5	Feb6	Feb7	Feb8	Feb9	Feb10	Feb11	Feb12
22.4	**31.2**	**30.7**	**30.0**	**31.3**	**25.7**	**25.7**	**31.0**	**30.7**	**29.8**	**30.9**	**30.0**	**30.0**	**30.4**

Feb13	average	Standard Deviation
30.4	**29.7**	**±2.4**

2nd Interval (Day16-30)

Feb14	Feb15	Feb16	Feb17	Feb18	Feb19	Feb20	Feb21	Feb22	Feb23	Feb24	Feb25	Feb26
31.2	**30.2**	**23.2**	**26.8**	**23.9**	**30.0**	**31.1**	**30.8**	**31.0**	**26.2**	**30.2**	**29.5**	**18.6**

Feb27	Feb28	average	Standard Deviation
21.8	**25.6**	**27.35**	**±4.00**

3rd Interval (Day31-Day60)

Feb29	Mar01	Mar 2	Mar 3	Mar 4	Mar 5	Mar 6	Mar 7	Mar 8	Mar 9	Mar10	Mar11	Mar12
29.2	**18.9**	**21.8**	**24.9**	**30.5**	**21.6**	**24.2**	**30.0**	**29.0**	**31.1**	**31.1**	**31.0**	**30.4**

Mar13	Mar14	Mar15	Mar16	Mar17	Mar18	Mar 19	Mar20	Mar21	Mar22	Mar23	Mar24
31.0	**29.8**	**29.1**	**28.6**	**28.**	**26.5**	**26.0**	**25.0**	**28.5**	**28.5**	**23.6**	**28.3**

Mar25	Mar26	Mar27	Mar28	Mar29	average	Standard Deviation
27.8	**30.4**	**29.3**	**29.9**	**28.5**	**27.75**	**±3.17**

4th Interval (Day61-Day90)

Mar30	Mar31	Apr1	Apr2	Apr3	Apr4	Apr5	Apr6	Apr7	Apr8	Apr10	Apr11	Apr12	Apr13	Apr14
29.0	**25.70**	**28.2**	**12.8**	**18.8**	**14.5**	**8.8**	**8.9**	**19.6**	**14.2**	**22.8**	**26.0**	**31.0**	**13.30**	**21.90**

Apr15	Apr16	Apr17	Apr21	Apr22	Apr23	Apr24	Apr25	Apr26	Apr27
12.4	**19.4**	**17.9**	**22.5**	**22.5**	**11.4**	**13.4**	**19.9**	**21.4**	**11.0**

Apr28	average	Standard Deviation
20.4	**19.27**	**±6.02**

Summary of Solar Radiations during the Study Period.

Day Interval	Average Radiation(mj/$m^{2)}$	Std Dev.
0-15	29.7	±2.43
16-30	27.35	±4.00
31-60	27.75	±3.17
61-90	19.27	± 6.02

Appendix V: Drinking Water Standards and Health Advisories for some chemicals (USEPA 2012)

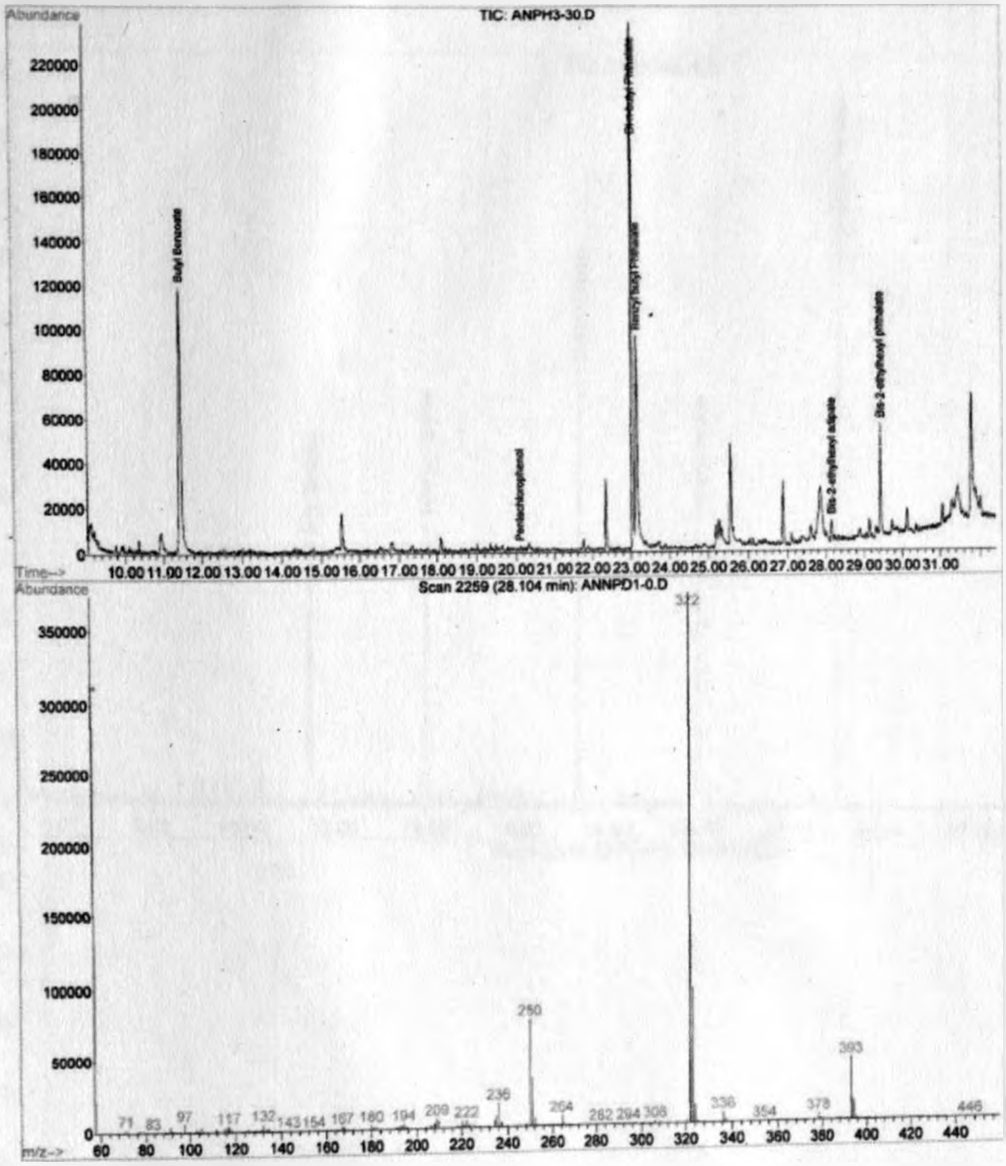
Chemical/ CAS No.	MCLG mg/L	MC mg/L	HA-one day mg/L	HA-Ten day mg/L	RfD mg/kg/day	DWEL mg/L	Lifetime mg/L	Mg/L at 10^{-4} cancer risk	Cancer description
BBP 85-68-7	-	-	-	-	0.2	7.0	-	-	C
DBP 84-74-2	-	-	-	-	0.1	4.0	-	-	D
DEP 84-66-2	-	-	-	-	0.8	30	-	-	D
DEHA 103-23-1	0.4	0.4	20	20	0.6	20	0.4	3	C
DEHP 117-81-7	Zero	0.006	-	-	0.02	0.7	-	0.3	B_2
DMP 131-11-3	-	-	-	-	-	-	-	-	D
PCP 87-86-5	Zero	0.001	1.0	0.3	0.005	0.2	0.04	0.009	L

(USEPA2012) Drinking Water Standards and Health Advisories

A-Human carcinogen
B-Probable human carcinogen
B_1-indicates limited human evidence
B_2-indicates sufficient evidence in animals and inadequate or no evidence in humans
C-Possible human carcinogen
D-Not classifiable as to human carcinogenicity
E-Evidence of noncarcinogenicity for humans
L-Likely to be a human carcinogen

Appendix VI: Phthalate esters Spectra for SODIS Kibera House Water at Day 30

```
ile        : C:\HPCHEM\1\DATA\ANPH3-30.D
perator    : Victor
cquired    : 28 Jun 2012  13:54      using AcqMethod THAL30MB
nstrument :     GC/MS Ins
ample Name: Pthalate Sample Day 30
isc Info   :
ial Number: 6
```



Appendix VII: Spectra for 7.5ppm phthalate Standard Mix

```
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Operator    : Victor
Acquired    :  2 Jun 2012   15:28      using AcqMethod THAL30MB
Instrument :    GC/MS Ins
Sample Name: Calibration standard 4
Misc Info   :
Vial Number: 3
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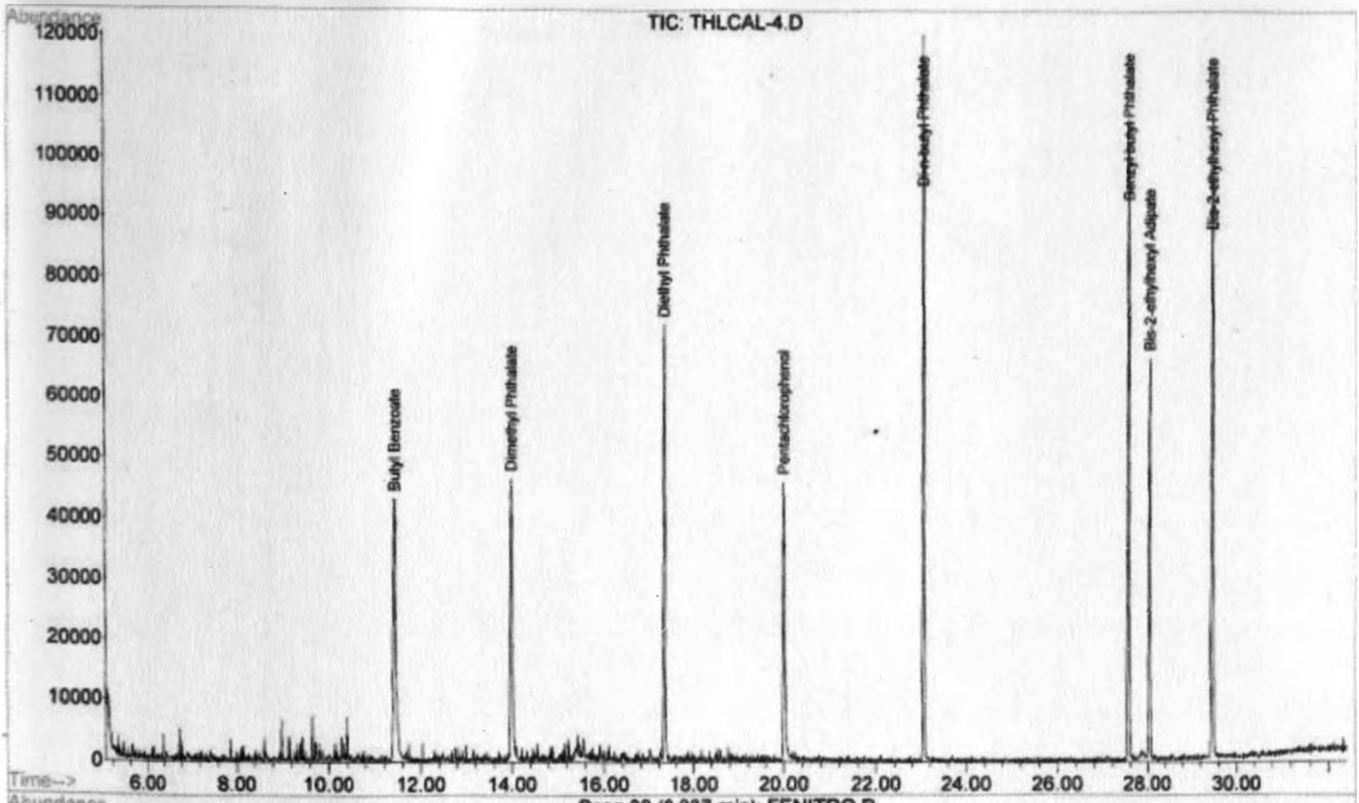
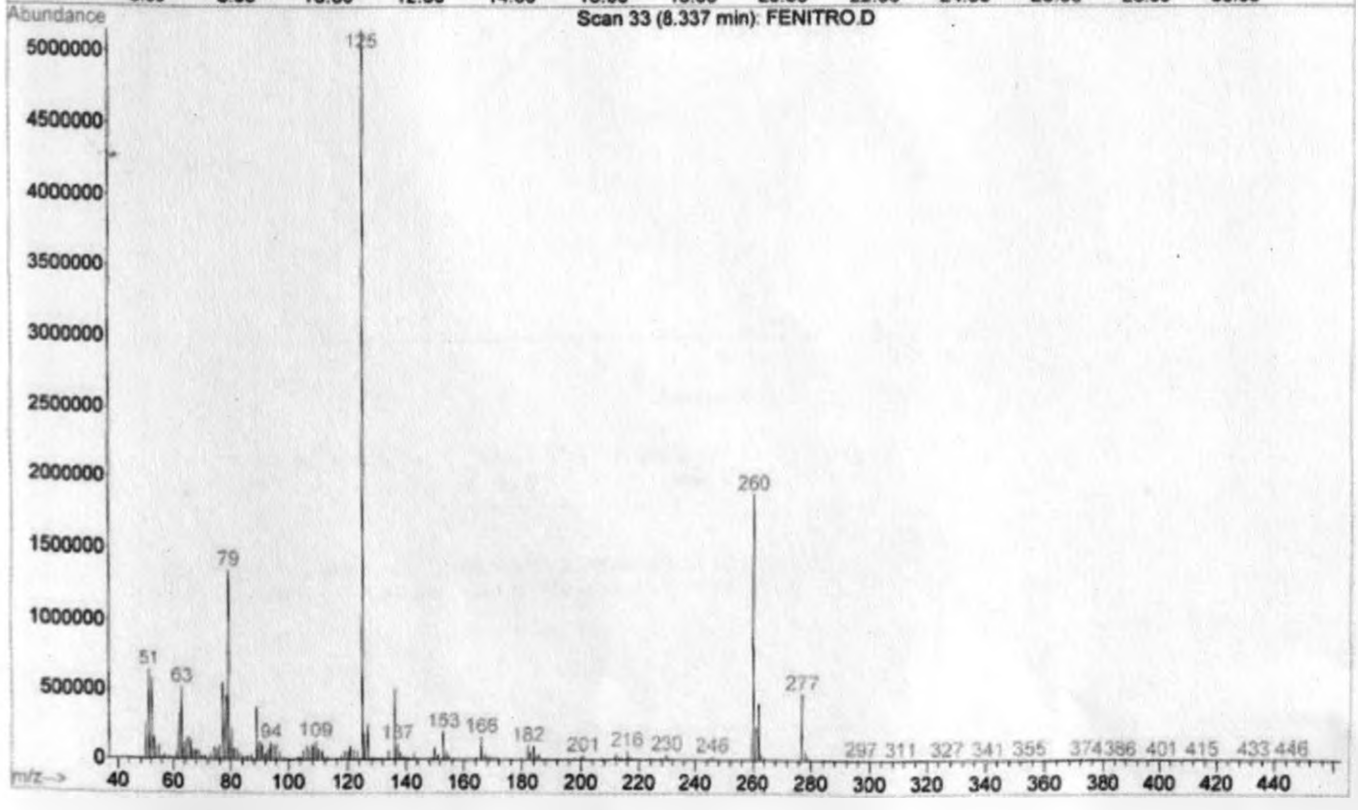
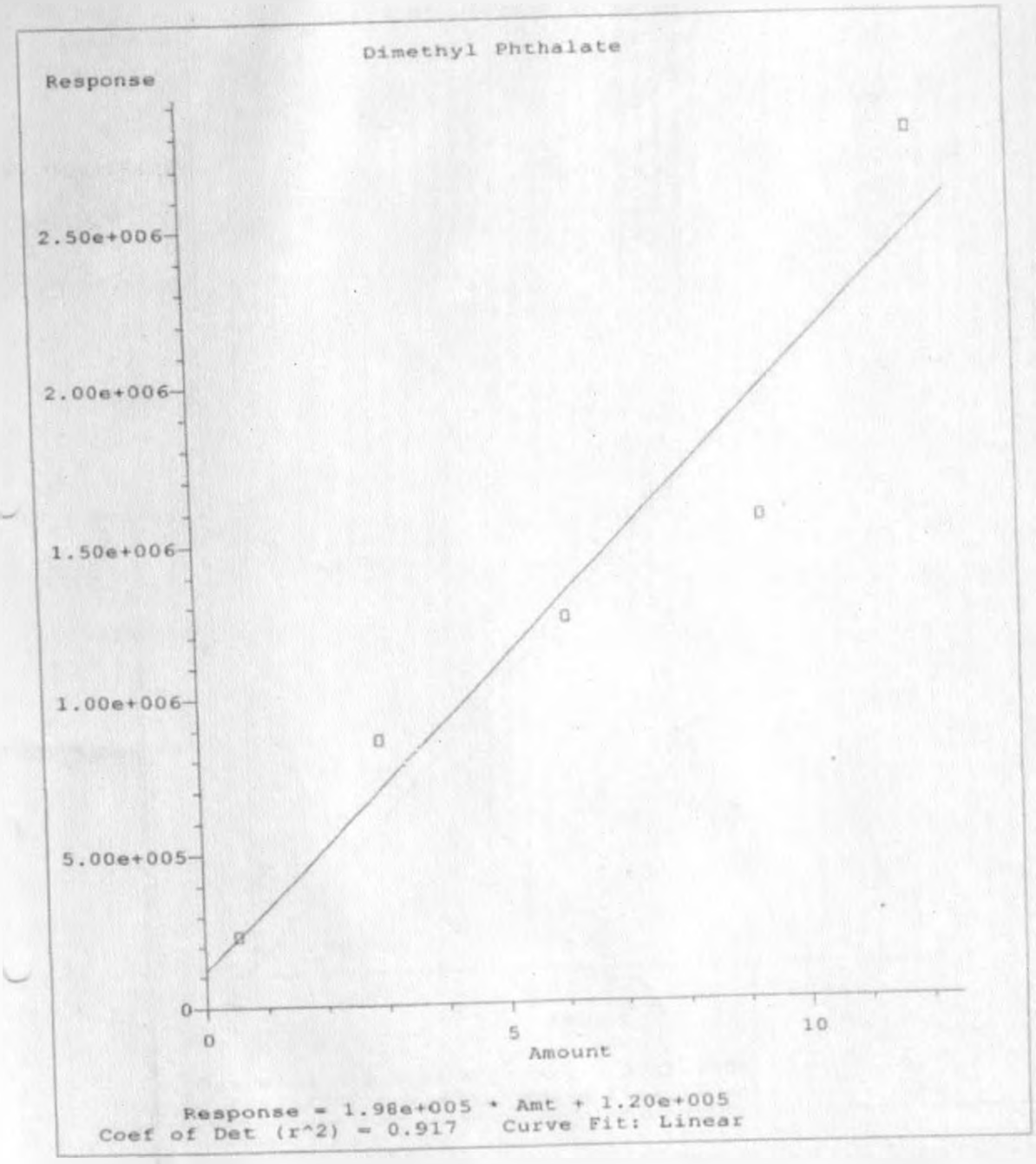


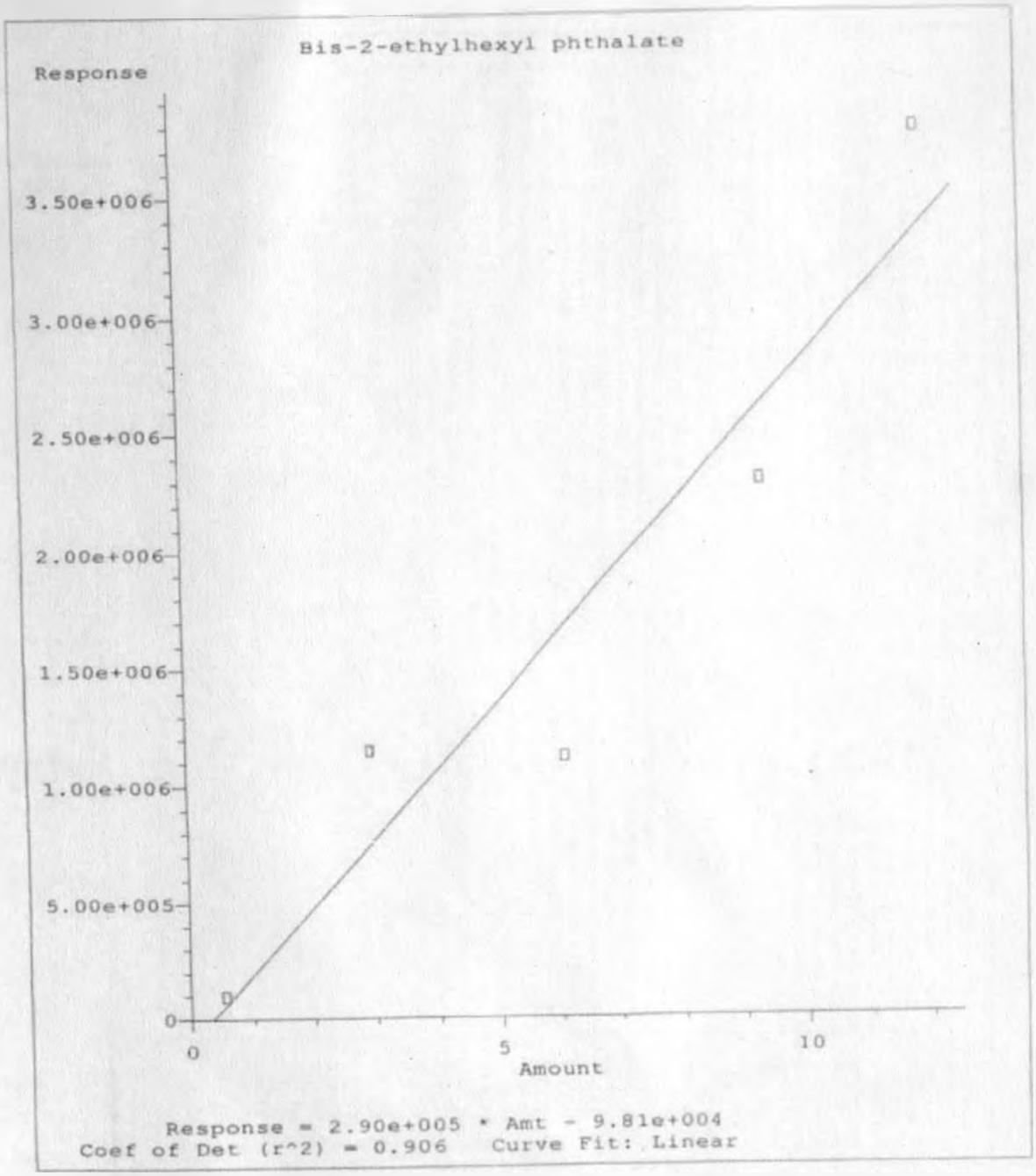


Appendix VIII: Calibration Curve for Dimethyl Phthalate



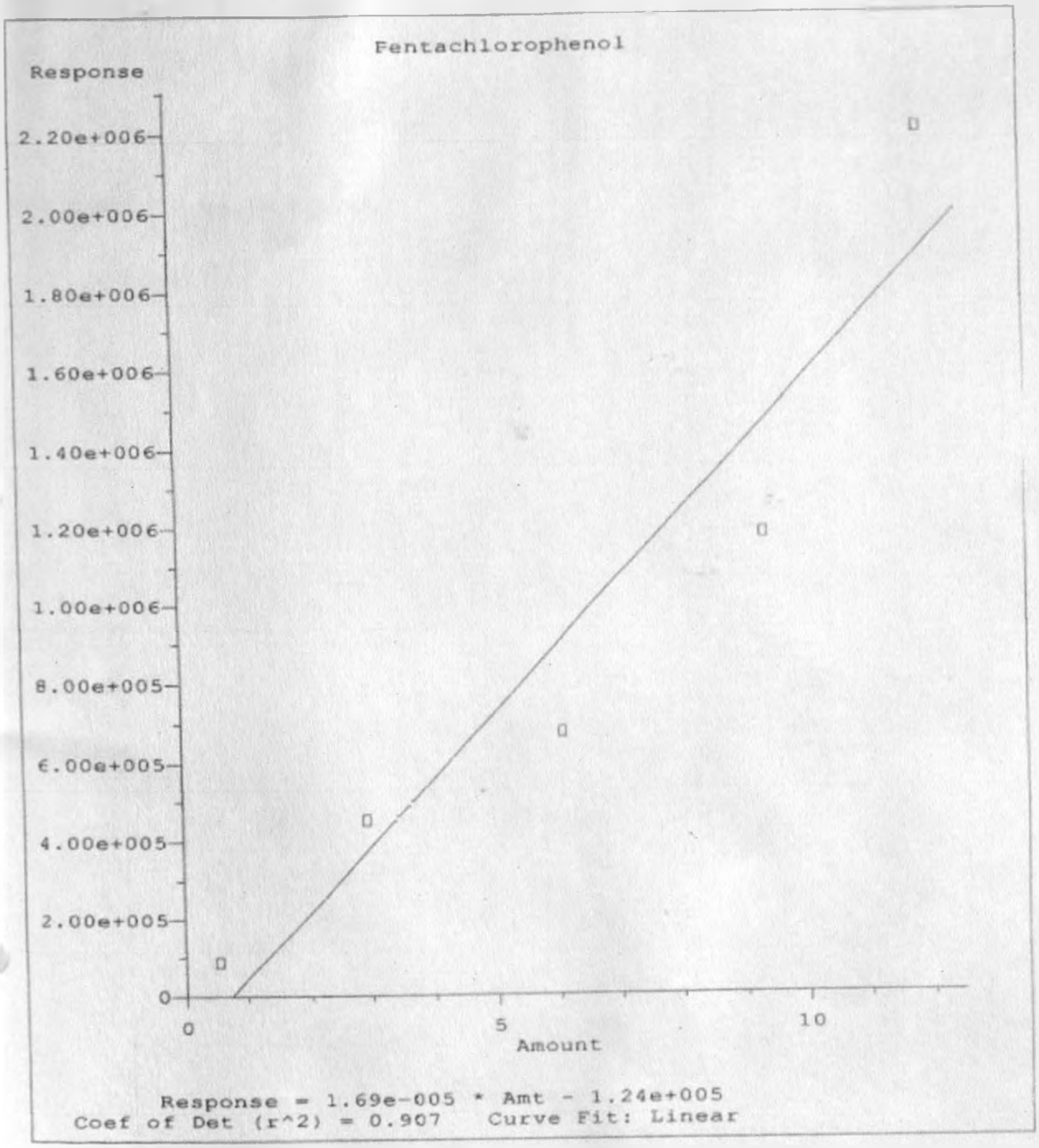
Method Name: C:\HPCHEM\1\METHODS\THAL30M8.M
Calibration Table Last Updated: Sat May 11 03:56:55 2013

Appendix IX: Calibration Curve for Bis -2-ethylhexyl phthalate



Method Name: C:\HPCHEM\1\METHODS\THAL30M8.M
Calibration Table Last Updated: Sat May 11 03:56:55 2013

Appendix X: Calibration Curve for Pentachlorophenol



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Calibration Table Last Updated: Sat May 11 03:56:55 2013

Appendix XI:

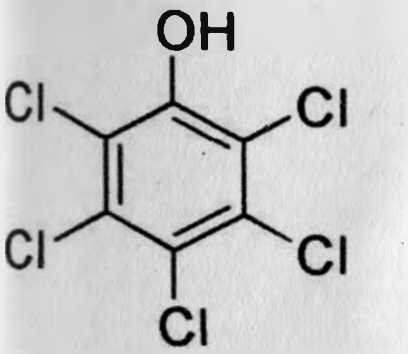

Figure 4.14: Pentachlorophenol