ENVIRONMENTAL CONTAMINATION, DNA DAMAGE AND MECHANISM OF CYTOGENOTOXICITY INDUCED BY ELECTRONIC WASTES IN LAGOS, NIGERIA

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ABSTRACT

Indiscriminate disposal of electronic wastes (e-wastes) in Nigeria is on the increase. The release of hazardous substances from these wastes may have harmful consequences on the environment and public health. In Nigeria, there is dearth of information on environmental contamination and genotoxicity of e-wastes, and the mechanism of genetic damage. This study was designed to investigate the level of contaminants in soil, plant and well water; potential *in vivo* and *in vitro* cytogenotoxicity and mechanism of DNA damage induced by e-wastes in Lagos, Nigeria.

Dumpsites from two major electronic markets, Alaba International (AI) and Computer Village (CV), in Lagos were purposely selected. Sixteen USEPA priority Polyaromatic Hydrocarbons (PAHs), 28 WHO toxic Polychlorinated Biphenyls (PCBs), and 8 carcinogenic Polybrominated Diphenyl Ethers (PBDEs) were analysed in the dumpsites' Soils (AIS, CVS) collected at depth 0 – 10 cm, and Plant (*Amaranthus hybridus*; AIP, CVP) using gas chromatography-mass spectrophotometry. Lead, cadmium and copper concentrations in the soils, *A. hybridus* and Well Water (AIWW, CVWW) collected from the dumpsite vicinity were measured using atomic absorption spectrophotometry. Genotoxicity was carried out with the e-wastes Leachate (AIL, CVL) at different concentrations (0-50 %) and well water using the micronucleus and sperm morphology assays in mice, and *in vitro,* using the alkaline comet assay in human peripheral blood lymphocytes. The oxidative stress in mice exposed to AIL and AIWW was assessed using biochemical parameters [Catalase, reduced Glutathione (GSH), and Alanine Aminotransferase (ALT)] according to standard methods. The NIH/3T3 mouse fibroblast cell line exposed to AIL was used to assess cytotoxicity using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the cell cycle using flow cytometry technique. The Mitochondrial Membrane Potential (MMP) of the cell line was evaluated using JC-1 probe. Data were analysed using descriptive statistics and ANOVA at p=0.05.

The total PAHs (mg/kg), PCBs (µg/kg) and PBDEs were AIS=116.5, CVS=95.4, AIP=14.9, CVP=6.2; AIS=4063, CVS=3619, AIP=889, CVP=518 and AIS=34.7 mg/kg, CVS=13.3 mg/kg, AIP=31.1 µg/kg, CVP=11.5 µg/kg respectively. The metal (lead, cadmium and copper) concentrations (mg/L) respectively in the leachate (397.0-867.5, 12.1-18.0, 14.3-22.3), *A. hybridus* (12.8-23.3, 0.3-0.8, 1.0-1.6) and well water (0.1-0.4, 0.1-0.7, 0.001-0.7) were above

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NESREA and WHO limits. There was a concentration-dependent, significant induction of micronucleated polychromatic erythrocytes (AIL=9.3±0.9-24.5±1.3, CVL=9.0±0.8-23.0±0.8; AIWW=8.2±1.2-13.8±1.4, CVWW=8.0±0.4-12.3±0.8) and significant increase in sperm abnormalities (AIL=83.2±2.2-157.6±1.9, CVL=81.0±1.3-155.9±2.0; AIWW=67.9±1.9-88.1±1.4, CVWW=66.5±0.8-86.1±0.8) in mice. *In vitro,* the leachate induced significant increases in DNA damage (AIL=1.6±0.3-6.9±0.5; CVL=0.9±0.3-4.5±0.5) in human lymphocytes. There was a significant increase in GSH (AIL=8.7±0.1-14.2±0.1, AIWW=8.7±0.1-12.4±0.1 μm/g) and activities of Catalase (AIL=76.3±0.9-134.0±1.4, AIWW=78.0±0.8-96.0±0.8 μm/mg) and ALT (AIL=21.0±0.8-47.0±0.8, AIWW=20.0±0.8-36.8±0.5 U/mL) compared to the negative control. There was increased cell death (IC$_{50}$=30 %), significant disruption of MMP and induction of apoptosis evident in accumulation of sub/G1 (8.3-72.2 %) stage of the NIH/3T3 cell cycle.

Electronic wastes from Alaba international and Computer village market dumpsites contained organic and inorganic toxins that induced cytogenetic damage. Oxidative damage and apoptotic pathway could be the mechanisms of the cytogenotoxicity.

Keywords: Electronic wastes in Nigeria, DNA and oxidative damage, Organic and inorganic contaminants.

Word count: 475

DEDICATION

This is dedicated to those who are regularly exposed to electronic wastes either because of the need for ends-meat or out of compulsion. One day, we shall be free!!!. Also to the generation of our children and those yet unborn, who may reap the unpleasant consequences of our actions in allowing the flow of electronic wastes, and its subsequent indiscriminate disposal which has left our "world" uninhabitable, "we are sorry!!!".

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CERTIFICATION

I certify that this work was carried out by Okunola A. Alabi in the Cell Biology and Genetics Unit of the Department of Zoology University of Ibadan, Oyo State, Nigeria.

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ABBREVIATIONS

PAH   Polyaromatic hydrocarbons

PCB   Polychromatic biphenyls

PBDE Polybrominated diphenyl ethers

AO   Acridine orange

EB   Ethidium bromide

MN   Micronucleus

PCE   Polychromatic erythrocytes

NCE   Normochromatic erythrocytes

WEEE Waste Electrical and Electronic Equipment

CFC   Chlorofluorocarbon

POP   Persistent organic pollutants

OECD Organization for Economic Cooperation and Development

EPA   Environmental protection Agency

NCM   Nordic Council of Ministers

PWB   Printed wire boards

PVC   Polyvinyl chloride

PPO   Polyphenylene oxide

TBBPA Tetrabromobisphenol-A

BRF   Brominated flame retardants

EPR   Extended Producer Responsibility

RoHS Restriction of Certain Hazardous Substances

PIC   Prior informed consent

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CHAPTER ONE

Introduction

1.1 Background

The advent of electrical and electronic equipment (EEE) with new functions and design, stimulate consumers' purchasing desire towards the latest equipments. This leads to the rapid increase in the sales of new facilitated models of EEE in the world (LaCoursiere, 2005). In the EEE market, technological advances have prompted phenomenal growth in the past decade. With a flourishing economy and additional improvements in technology in the future, global growth of this market is expected to continue at respectable rates. This growth has brought on a worldwide consciousness regarding the environmental effects of materials used in this market (Laundry and Dawson, 2007). The rapid replacement of old models by latest advanced models causes short average lifespan of EEE and consequently leads to mass generation of waste electrical and electronic equipment (WEEE).

Electronic waste (e-waste) or WEEE is a waste type consisting of any broken or unwanted electrical or electronic appliances (Wong *et al*., 2007a). E-waste typically consists of a broad range of electrical and electronic products, including computers, mobile phones, televisions, refrigerators, ovens, vacuum cleaners, toasters, printers, faxes, fluorescent tubes and their components, such as printed circuit boards (National Safety Council, 1999). The generation of tremendous amount of bulky WEEE containing variety of hazardous substances is currently a major social problem and threat to the environment (Lee, 2005; Lee *et al*., 2007). For example in 2001, the quantity of electronic waste generated in the US was estimated around 2.26 million tones and these were mainly: (1) video products such as TVs, VCR decks, camcorders and TV/VCR combinations, (2) audio products including compact disk players, rack audio system and compact audio system and (3) information products like PC, computer monitors, telephones and fax machines. Accordingly, a tremendous amount of electrical wastes, viz electrical home appliances (EHAs) such as refrigerators, washing machines and air conditioners is generated every year (Lee *et al*., 2007). In the EU, total

electronic waste generation was found to be five million tones, simultaneously the average quantity of the generation of electronic waste was observed to be 14 kg per person in the year 2004 (Beck, 2004).

Disposal of e-waste is an emerging global environmental issue, as these wastes have become one of the fastest growing waste types in some parts of the world including the US and Europe (Global Futures Foundation, 2001; Schmidt, 2002). Complexity in the design and composition of electrical and electronic devices renders them undesirable for recycling and reuse for technical and economic reasons (Toffel and Horvath, 2004; Berkhunt and Hertin, 2004).

Despite of an enormous amount of e-waste being generated every year in the US and EU, their treatments are simply relying on incineration or landfill (Lee *et al*., 2007). Aside from landfilling, environmental organisations (Basel Action network, 2002; Brigden *et al*., 2005) have drawn attention to the large amount of e-waste that is being shipped from developed nations, particularly the US. Up to 80% of e-waste from the United States has seeped into Asia and Africa (Puckett *et al*., 2002; Schmidt, 2002, 2006; Johnson, 2006). There are some evidences that e-wastes which consumers turn in for recycling is actually shipped off to less-developed nations (for example, China, Nigeria) where disposal is haphazard at best and dangerous at worst (Schmidt, 2006). In many nations it is illegal to import e-wastes, but smugglers bring it in illegally for financial profit (Johnson, 2006). India, Pakistan, Nigeria and China, have now become prime destinations for the West's toxic waste (World report, 2005). Because Nigeria does not have any recycling process, the result is that many tons of e-waste material and repair residues are dumped in workshops, yards, roadsides, open fields, irrigation canals, riverbanks, ponds and rivers (Nnorom and Osibanjo, 2008a).

Hazardous chemicals can be released from e-waste through disposal and recycling process causing environmental pollution and adverse health effects. Studies have shown that e-waste contains potential harmful substances including metals such as: lead, tin, copper, silicon, carbon, iron, aluminium, cadmium and mercury (Kimberly *et al*., 2005; Jeffrey and Micheal, 2007; Wong *et al*., 2007a; Lee *et al*., 2007) in bulk; and germanium, gallium, barium, nickel,

tantalum, indium, vanadium, terbium, beryllium, gold, europium, titanium, ruthenium, cobalt, palladium, manganese, silver, antimony, bismuth, selenium, niobium, yttrium, rhodium, platinum, arsenic, lithium, boron and americium in trace amounts (Gonsebatt *et al*., 1995; Xinhui *et al*., 2007; Xia *et al*., 2007), and organic compounds such as polybrominated diphenyl ether (PBDEs), polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) (Ernst *et al*., 2003).

Several studies have reported soaring levels of toxic heavy metals and organic contaminants in samples of dusts, soil, river sediments, surface water and ground water in an e-waste recycling site (Puckett *et al*., 2002; Bridgen *et al*., 2005; Yu *et al*., 2006; Wong *et al*., 2007a). Derig *et al*. (2006) and Zhao *et al*. (2006) reported that large quantities of organic compounds generated during e-waste disposal are released into the surrounding area, resulting in high environmental levels of Persistent Organic Pollutants (POPs). Local residents are exposed to these toxic pollutants through inhalation, dermal exposure and oral intake (Bi *et al*., 2007). Human body burdens of PBDEs have increased markedly over the past several decades. Studies have shown that workers at electronic recycling plants have elevated serum levels of PBDEs (Sjodin *et al*., 1999; Thuresson *et al*., 2005). The calculated exposure of adults to dioxins through inhalation (68.9 and 126 pg TEQ/kg/day in the summer and winter respectively) were significantly higher than the World Health Organization recommended maximum of $1 - 4$pg TEQ/kg/day (Rhitu, 2007).

The effects of some of the constituents of these e-wastes have not been studied in details. However, Mueller (1994) reported that long-term use of the water heavily contaminated with e-waste for irrigation resulted in elevated trace loadings of the crop and paddy soils, potentially resulting in high levels of optic of toxic trace metals in rice and other edible food crops. In November 2003, a report by researchers at the Medical College, Shantou University, China, found that people employed in the incineration of circuit board and cleaning of plastics from used electrical goods in Guiyu had a high incidence of skin damage, headaches, vertigo, nausea, chronic gastritis, gastric ulcers and duodenal ulcers (Qui *et al*., 2004). Toxicology studies conducted with animals showed that BDE-209 (a form of PBDE) can

impact thyroid hormones and alter brain development (Kellyn, 2007).

Lagos in Nigeria is the second largest city in the world (BAN, 2005) and it is one of the major recipients of the large junk of imported e-waste in Africa. About 500 containers of Personal Computers (PCs) are offloaded monthly in Lagos, Nigeria, with an average of about 800 pieces per consignment. The two largest electrical and electronic markets in Lagos, where these imported goods are sent are: Computer Village, Ikeja, and Alaba International markets, Ojoo. Both areas were until 1999 a residential neighborhood. These two locations have both formal and informal dumpsites which are the final recipient of e-wastes. The e-wastes are routinely set ablaze to reduce the accumulating trash. It is however difficult to quantify the amount of e-waste dumped on such sites in Lagos because of lack of adequate records and use of informal dumpsites.

Despite the enormous amount of e-wastes indiscriminately dumped in the Nigeria environment, there is limited report on the environmental contamination, and none on human and animal body-burden of e-waste toxic constituents and the health effects on exposed individuals. There is therefore a need for a systematic assessment of the level of environmental contamination viz-a-viz the soil, plant and water of and in the vicinity of the e-waste dumping, and the potential genotoxic and mutagenic effects of human and animal exposure in the Nigeria environment.

1.2 Hypothesis of the study

Considering the rate and indiscriminate disposal of e-waste in these areas, the soils, plants and well waters in the environment may have been severely contaminated and the health of workers and residents may have been significantly affected.

1.3 Aim of the study

The aim of this study is to assess the level of contaminants in soils, edible plants and well water, the potential cytogenotoxicity and the mechanism of cytogenetic damage induced by e-wastes in Lagos, Nigeria.

The rationales for the study are:

(1) Nigeria is a major recipient of e-wastes from developed countries without any official recycling activity.

(2) There is limited information on the environmental contamination of e-wastes in Nigeria.

(3) There is no available information on mutagenicity and genotoxicity of e-waste in Nigeria.

(4) There is no information on the mechanism of cytogenotoxicity of e-wastes in the literature.

1.4 Objectives of the study

The specific objectives of this study are to:

1. assess the level of heavy metals, PAHs, PCBs and PBDEs contamination in soil and edible plant samples from e-waste dumpsites in the two electronic markets.

2. conduct physico-chemical and heavy metal analyses of raw and simulated leachates from e-waste dumpsites and well water sample from the vicinity of the two markets.

3. determine if the leachates (raw and simulated) from the two electronic markets can induce abnormally shaped spermatozoan, micronucleus in the bone marrow and reduction in sperm count in albino mice.

4. determine if the simulated leachates from the two markets can induce DNA damage in human peripheral blood lymphocytes and NIH/3T3 mouse fibroblast cell line.

5. determine if the well water from the two electronic markets can induce abnormally shaped spermatozoan, micronucleus in the bone marrow and reduction in sperm count in albino mice.

6. assess biomarkers (antioxidant enzymes) of oxidative stress in mice exposed to simulated leachate and well water from the two electronic markets so as to establish a possible mechanism of genotoxicity.

7. assess apoptotic markers in NIH/3T3 mouse fibroblast cell line exposed to simulated leachates from the two electronic markets as possible mechanism of cytogenotoxicity induced by e-wastes.

1.5 Importance of the study

The result of this study will help in assessing the contamination level of the environment within and around the EEE markets and the potential genotoxic effects of such contamination on the biological system. It will also serve as a baseline study in the elucidation of the possible mechanism of e-waste-induced cytogenotoxicity. This will be relevant as a working tool for campaigners against illegal importation of e-wastes from developed countries to developing nations. It will also help in public awareness of the people of the potential dangers of such wastes on both the environment and humans within the neighborhood. It is hoped that the findings will not only provide valuable information on the environmental quality of Computer Village and Alaba International markets, Lagos, but also present a scientific perspective of the environmental effects of e-wastes, so that further regulatory and scientific attention can be drawn to the issue.

CHAPTER TWO

Literature review

2.1 Definition of E-waste

There is no generally acceptable definition of e-wastes. Globally, WEEE/E-waste is the most commonly used terms for electronic wastes. Although there is no standard definition, a number of countries have come out with their own definitions, interpretation and usage of the term. No definition(s) of WEEE/E-waste was found in countries of Africa though e-waste initiatives are under implementation in South Africa.

The definition of e-waste as adopted by different countries has been documented by UNEP (2007a). In Argentina, E-waste has been stated as "discarded electronic and electrical equipment (EEE)" and "their wastes" and "end-of-life" batteries according to the publication of Decree 639 enacted in Buenos Aires. In Australia and Brazil, it is generally referred to as "end-of-life" or "discarded" electrical and electronic product and components. Environmental protection and Enhancement Act of Alberta, Canada (Electronics Designation Regulation A.R. 94/2004) defines "electronics" as all electrical and electronic equipment or devices whether intended for consumers, industrial or commercial use, and includes: television, computers, laptops and notebooks, including CPU, keyboards, mouse, cables and other components in the computer, computer printers including printers that have scanning or fax capabilities, or both, audio and video playback and recording systems, telephones and fax machines, cell phones or other wireless devices and electronic game equipment, but does not include electronic contained within and affixed to a motor vehicle (Figure 2.1). E-waste is referred to as "used consumer electric goods discarded by consumers" in "The law for recycling of specified kinds of home appliances" in Japan. This law covers TVs, refrigerators, washing machines and air conditioners. The New Zealand Import and Export (restriction) order (No2) 2004 includes photocopying equipment, and equipment containing mercury or lead batteries in its definition of e-waste. Act for Resource Recycling of Electrical and

Figure 2.1: Materials categorized as e-waste: (a) Phones: mobile, office, landlines and their accessories (b) Televisions and its accessories (c) Computers and its accessories (BAN, 2005).

Electronic Equipment and Vehicles of the republic of Korea however defines WEEE as electrical and electronic equipment or devices (including components and parts thereto) operated by electric currents and electromagnetic fields.

Solving the E-waste Problem (StEP, 2005) refers to e-waste as "the reverse supply chain which collect products no longer desire by a given consumer and refurbishes for other consumers, recycles, or otherwise processes waste", while Organization for Economic Cooperation and Development (OECD, 2001) defines it as "any appliance using an electric power supply that has reached its end-of-life". Basel Action Network on the other hand defines e-waste as "encompassing a broad and growing range of electronic devices ranging from large household devises such as refrigerators, air conditioners, cell phones, personal stereos, and consumer electronics to computers which have been discarded by their users". Basel Convention covers all discarded/disposed materials that possess hazardous characteristics as well as all wastes considered hazardous on a national basis.

The most widely accepted definition of WEEE/E-waste is the definition issued by the EU Directive. WEEE Directive (European Union, 2002) defines WEEE as "Electrical and electronic equipment which is waste including all components, sub-assemblies and consumables, which are part of the product at the time of discarding". It further defines "electrical and electronic equipment" (Directive 75/442/EEC) as "equipment which is dependent on electric current or electromagnetic fields in order to work properly and equipment for the generation, transfer and measurement of such current and field and designed for use with a voltage rating not exceeding 1000volts for alternating current and 1500 volts for direct current. Categories and list of products of electrical and electronic equipment covered by this Directive include:

- **Large household appliances**: large cooling appliances, refrigerators, freezers, other large appliances used for refrigeration, conservation and storage of food, washing machines, clothes dryers, dish washing machines, electric hot plates, microwaves, other large appliances used for cooking and other processing of food, electric heating appliances, electric radiators, other fanning, exhaust, ventilation and conditioning equipment.

- **Small household appliances**: vacuum cleaners, carpet sweepers, other appliances for cleaning, appliances used for sewing, knitting, weaving and other processing for textiles, iron and other appliances for ironing, mangling and other care of clothing, toasters, fryers, grinders, coffee machines and equipment for opening or sealing containers or packages, electric knives, appliances for hair-cutting, hair drying, tooth brushing, shaving, massage and other body care appliances, clocks, watches and equipment for the purpose of measuring, indicating or registering time scales.

- **IT and telecommunications equipment**: centralised data processing, mainframes, mini computers, printed units, personal computers (CPU, mouse, screen and keyboard included), laptop computer, notebook computers, notepad computers, printers, copying equipment, electrical and electronic typewriters, pocket and desk calculators and other products and equipment for the collection, storage, processing, presentation or communication of information by electric means, user terminals and systems, facsimile, telex, telephones, pay telephones, cordless telephones, cellular telephones, answering systems and other products and equipment of transmitting sound, images, or other information by telecommunications.

- **Consumer equipment**: radio sets, TV sets, video cameras, video recorders, Hi-fi recorders, audio amplifiers, musical instruments, other products or equipment for the purpose of recording or reproducing sound or image, including signals or other technologies for the distribution of sound and image than by telecommunications.

- **Lighting equipment**: luminaries for fluorescent lamps with the exception of luminaries in households, straight fluorescent lamps, compact fluorescent lamps, high intensity discharge lamps including pressure sodium lamps and metal lamps, low pressure sodium lamps, other lighting or equipment for the purpose of spreading or controlling light with the exception of filament bulbs.

- **Electrical and electronic tools (with the exception of large scale stationary industrial tools)**: drills, saws, sewing machines, equipment for turning, milling, sanding, grinding, sawing, cutting, shearing, drilling, making holes, punching, folding, bending or similar processing of wood, metals and other materials, tools for riveting, nailing or screening or removing rivets, nails, screws or similar uses, tools for wielding, soldering or similar use,

equipment for spraying, spreading, dispersing or other treatment of liquid or gaseous substances by other means, tools for mowing or other gardening activities.

- **Toys, leisure and sports equipment**: electric train or car racing sets, hand-held video game consoles, video games, computers for biking, driving, running, rowing etc. Sports equipment with electric or electronic components, coin slot machines.

- **Medical devices (with the exception of all implanted and infected products)**: radiotherapy equipment, cardiology, dialysis, pulmonary ventilators, nuclear medicine, laboratory equipment for *in-vitro* diagnosis, analyzers, freezers, fertilization tests, other appliances for detecting, preventing, monitoring, treating, alleviating illness, injury or disability.

- **Monitoring and control instruments**: smoke detector, heating regulators, thermostats, measuring, weighing or adjusting appliances for household or as laboratory equipment, other monitoring and control instruments used in industrial installations (e.g. in the control panels).

- **Automatic dispensers**: automatic dispensers for hot drinks, for hot or cold bottles or cans, for solid products, for money and all appliances which deliver automatically all kinds of products (Table 2.1).

Generally, there are three major pointers to understanding the definition of WEEE/E-waste. These are the definition of "electrical and electronic equipment", "loss of utility" and "way of disposal".

2.2 Sources, generation and transboundary movement of e-waste

Rapid technological change is a key feature of modern life. Technological advances allow each product generation to be smaller, smarter and cheaper than the last. With the demand for newer, more efficient and effective technology, older and outdated electronic items are becoming obsolete and are being discarded in significant amounts in various parts of the world. The gadgets and equipment so discarded ends up as electronic wastes or e-wastes. In computing for example, the average lifespan of a personal computer has shrunk from four or five years in the early 1990s to a mere two years today (EPA, 1998; National Safety Council, 1999). The pace of innovation together with consumer attitudes lead to many electronic products being disposed of well before the end of their working lives. The average working

Table 2.1: List of common Waste Electrical and Electronic Equipment (WEEE) items, including those normally considered as e-waste.

Item	Wt of Item (kg)	Typical life (year)
WEEE normally considered E-waste		
Computer[a]	25	3
Facsimile machine[b]	3	5
High-fidelity system[c]	10	10
Mobile telephone[c]	0.1	2
Electronic games[c]	3	5
Photocopier[b]	60	8
Radio[c]	2	10
Television[c]	30	5
Video recorder and DVD player[c]	5	5
WEEE not normally considered E-waste		
Air conditioning unit[b]	55	12
Dish washer[c]	50	10
Electric cooker[c]	60	10
Electric heaters[c]	5	20
Food mixer[c]	1	5
Freezer[c]	35	10
Iron[c]	1	10
Microwave[c]	15	7
Refrigerator[c]	35	10
Telephone[c]	1	5
Toaster[c]	1	5
Tumble dryer[c]	35	10
Vacuum cleaner[c]	10	10
Washing machine[c]	65	8

[a] (Betts, 2008). [b] (Robinson, 2009) [c] (Cobbing, 2008).

life of a mobile phone is seven years, but the average consumer changes their mobile every 11 months. This situation has resulted in making e-waste the fastest growing segment of the waste stream. The United Nations estimated that between 20 and 50 million tons of e-waste is discarded annually worldwide (Bridgen *et al.*, 2008).

2.2.1 Generation of e-waste

Most countries lack reliable data on e-waste generation because there is no standard definition of e-wastes, and the methods used to estimate e-waste generation are not compatible among countries. However, in the Unites States alone, nearly 250 million computers became obsolete between 2000 and 2005, while mobile phones are discarded at a rate of 130 million per year (National Safety Council, 2000). An average of 220 tons of computers and other e-wastes are dumped in landfills and incinerators every year in the US (National Safety Council, 2000; EPA, 2001).

In Europe, it was estimated that 6 million metric tons of e-waste was discarded in 1998 and will be doubled by 2010 (Cui and Forsberg, 2003; Darby and Obara, 2005). The volume of computer waste generated in South Korea in 2002 was estimated at 1.2 million, with a prediction of double (i.e 2.2 million) by 2005 (Oh *et al*., 2003). China generated about 1.6 million obsolete EEE in 2003 (Liu *et al*., 2006) with TV accounting for nearly half of the total, while Germany has a yearly electronic scrap waste stream of about 1.8 million. Approximately 300, 000 scrap PCs are generated each year in Taiwan with Austria generating about 85, 000 tons of e-scrap per year (Lee *et al*., 2000; Antrekowitsch *et al*., 2006). Switzerland and Denmark generated about 66, 042 and 118, 000 tons of e-waste respectively in 2005 while Thailand generated approximately 60, 000 tons of e-scrap in the same year. Norway discarded 144, 000 tons in 1995; Finland approximately 120, 000 tons in 2003 while France and Sweden generated 1,500,000 and 100, 000 tons respectively. Table 2.2 shows the e-waste generation in selected countries by EMPA project. Most African countries lack reliable data on the generation of e-waste.

The enormous volume of e-waste generated each year from the developed regions has posed

Table 2.2: E-waste generation in selected countries by EMPA project

Country	Total E-waste Generated (tonnes/year)	Categories of appliances counted as E-waste	Year
Canada	67 000	Computer equipment (computers, printers, etc) and consumer electronics (TVs)	2005
Germany	1 100 000	Office and telecommunications equipment, consumer entertainment electronics, large and small domestic appliances, refrigerators,fractions.	2005
Thailand	60 000	Refrigerators, air conditioners, televisions, washing machines, computers	2003
Switzerland	66 042	Office and telecommunications equipment, consumer entertainment electronics, large and small domestic appliances, refrigerators,fractions.	2003
USA	2 158 490	Video products, audio products, computers and telecommunicationsequipment	2000
UK	915 000	Office and telecommunications equipment, consumer entertainment electronics, large and small domestic appliances,refrigerators,fractions.	1998
Denmark	118 000	Electronic and electrical appliances including refrigerators	1997

(Source: Terazono *et al.*, 2006)

a serious challenge in sustainable waste management, not to mention the growing demand for electrical and electronic goods in some developing countries such as Nigeria. In Nigeria, rapid growth in Information Communication Technology (ICT) in the last decade with many Nigerians now having access to computer facilities at home, school, business centers, internet cafes and offices, as well as access to mobile telephones which has emerged as an integral and essential part of the culture and life of Nigerians is now playing a huge role in the development of Nigeria economy. This has led to an increase demand for computers, laptops and mobile phones as the phone subscription has increased from approximately 20, 000 in 1998 to about 27 million by the end of 2006 (Osibanjo and Nnorom, 2007). These advances in ICT depend to a large extent on second-hand/refurbished electrical and electronic equipment (EEE) such as personal computers (PCs), mobile phones and accessories. This has led to a substantial quantities of electronic waste locally generated.

2.2.2 Transboundary movement of e-waste

To deal with the generation of e-waste, effective management practices must be implemented throughout the entire life-cycle of the product: from design stage (cradle) to the end-of-life stage (grave), adopting solutions that are environmentally friendly, including practicing reuse and recycling. While some e-wastes are treated at its origin, some are illegally exported to other countries for recycling and disposal. In fact, recycling and disposal of e-waste are a phenomenon increasingly encountered in developing countries. The quantity of e-wastes on the planet has reached crisis level on a global scale, the bulk of which is being generated by the industrialised nations of the world (Azuka, 2009). Because the process of e-waste management is both expensive and labor-intensive, exportation of e-wastes to developing countries is one of the routes which the industrialised nations have taken in dealing with this menace so as to dodge the expenses of disposal and close public scrutiny within their borders, as less than 10% of discarded electronic products are currently being recycled (Ladou and Lovegrove, 2008).

It should be noted that this movement of e-waste from industrialised nations to developing nations is never always ex-gratia. As a matter of fact, there is a booming trade in used

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electronics, and by extension, e-waste. The export to developing countries is motivated entirely by economic forces (Azuka, 2009). The bulk of the e-waste generated by industrialised economies end up in developing countries after going through an export/import trail created by numerous players. Generators find it profitable because it saves cost and more often earns them income as they pass the used electronics to other persons to dispose off instead of incurring disposal cost, as well as provide them with the opportunity of complying with the legal/regulatory regions of the polity wherein they operate or dodging same. Middle men who could be called 'waste merchants' are participants as a result of the income derivable from the trade in used electronics and e-wastes and end purchasers are involved in the venture for the same reason as the middle man. Recycling companies in developed countries stripe e-waste of its most valuable parts, before shipping it to brokers in developing countries. The transboundary movement of e-wastes is often justified in the name of "recycling", a misleading phrase, for it is impossible to safely or effectively recycle many hazardous e-waste materials.

Limited information is available on the quantities of e-waste materials that move between countries. Approximately 10.2 million units of computers are exported annually from the US to developing countries including China, India, Pakistan, Nigeria and Ghana. The US e-waste recycling industry was reported to have once declared that about 80% of the e-waste they received was exported to Asia and 90% of which went to China (Hicks *et al*., 2005; Antrekowitsch *et al*., 2006). It is estimated that South Korea exports about 1.8 million used computers to China each year (Toxic Dispatch, 2004). In the UK, 160, 000 metric tons of secondary and waste electronic equipment were exported in 2003; 133, 000 tons of which was IT/telecom equipment. An estimated value of 110, 000 tons of this was declared exports and properly documented while 23,000 tons were undeclared or grey-market exports going to non-OECD countries. The Basel Convention estimates that from 1993-2001, the amount of waste travelling between countries more than quadrupled. Despite laws that expressly prohibit it, most of the international movement of hazardous waste is from the rich countries to poor ones.

In Africa, the city of Lagos in Nigeria is believed to be the representative of developments rapidly taking place in other cities of Africa, with rapid and massive growth in cell phones and computer technology. While no official figures exist, it is apparent that a very significant portion of this growth is fueled by the importation of second-hand equipment from rich developed countries. Report (BAN, 2005) showed that 500 (40-foot) containers of used computers; with each containing about 800 monitors or CPUs, which amounts to about 400, 000 second-hand or scrap units, come into the port of Lagos each month, imported primarily from Europe and North America (Figure 2.2).

This amounts to an annual importation of an estimated 5 million scrap units or 60, 000 metric tons containing up to 18, 000 tons of plastic materials. This is estimated to an importation of 15, 000-45, 000 tons of hazardous wastes containing about 1000-3600 tons of lead (Nnorom and Osibanjo, 2008a). While some of the imported materials to Lagos is fully functional and is directly re-used, or can be repaired, there is nevertheless a significant quantity of the imported computer equipment or parts, estimated by local experts to be between 25-75%, considered junks (BAN, 2005; Ladou and Lovegrove, 2008; Nnorom and Osibanjo, 2008a).

It is unmarketable due to either its lack of computing effectiveness, or due to the fact that it is un-economic to repair. Because most of the exports/imports are not pre-tested for functionality, it is not possible to know whether these exports are legally defined as hazardous wastes (i.e. requiring disposal whole or in part and being hazardous) under the Basel Convention. Once in Nigeria, they are put into the market and are bought as "tested" or "non-tested" electronics/electrical appliances (Hawari and Hassan, 2008).

E-waste is shipped to Africa under various guises. Amongst them is the movement to Africa and other third world nations subsequent to an agreement between the recipient and the generating country wherein the latter promises aids, money or the execution of a project within the territory of the recipient nation. This is the same ruse that is put to use in the movement of hazardous or toxic waste to Africa (Clapp, 2000). Governments of Africa countries have repeatedly entered into agreement with industrialised nations that will enable the latter stockpile and dump their waste which are often toxic, hazardous and

Figure 2.2: Transboundary movement of e-scrap from developed to developing nations under the disguise of digital gap. (BAN, 2005)

radioactive (Azuka, 2009). The economies of developing countries tend to be weak and unstable. They are often confronted with an urgent need to finance both domestic and international debt. In their desperate attempt to finance such debt, the governments of these countries are likely to offer their natural resources for sale at a discount (Ahmed, 2004; Kimani, 2009). In 1988 for example, the government of Guinea-Bissau, one of Africa's poorest nations, briefly agreed to accept over 15 million tons of toxic waste for 600 million dollars- four times its Gross National Product. The Minister for Trade and Tourism simply explained: "we need money" (African Business, 1988). In the same year, the government of Benin negotiated a bilateral deal with the French government to import radioactive and industrial waste in return for $1.6 million down payment and 30 years of economic assistance (adapted from http://www.american.edu/TED/benin.htm).

Also disheartening is the fact that the generators and waste merchants target illiterate and semi illiterate businessmen, offering them money so as to lure them into accepting the wastes they generate and deal in. For example, in 1987, a toxic waste trade between Italian and Nigeria businessmen took place in Koko, Nigeria. Italian businessmen shipped toxic waste of several Italian industries to Nigeria for storage in the backyard of a Nigeria businessman, who described them merely as miscellaneous construction materials. 18, 000 drums of hazardous waste were stored for approximately $100 a month. Months later, a scandal over the toxic waste was publicised after the barrels of waste began leaking into the surrounding area (adapted from http://www.american.edu/TED/NIGERIA.htm).

These used electronics, especially computers as well as their accessories and peripherals are also shipped to developing countries ostensibly for the purpose of helping to bridge the digital divide between the industrialised economies and the former. This is what they make the unsuspecting public within the country of generation believe or the charade which the generators and dealers in used electronics and e-waste put up when they seek to frustrate the provisions of municipal legislation within the country of generation requiring the recycling or disposal of e-waste in an environmental-friendly manner. It is indebatable that the trade in e-waste as well as its mechanics is fuelled by sheer economics (Azuka, 2009). This goes to

affirm the assertion that economic activity is both the cause of environmental harm in its diversified manifestations and paradoxically, the means by which resources can be generated that is capable of being put to use in addressing environmental harm.

African nations have become favored destinations for the export of e-waste emanating from US and the EU as the "cost of doing business" is minimized and profit is guaranteed (Green diary, 2007; Azuka, 2009). It is believed that the endemic poverty, high foreign debt burden, corruption, lack of environmental regulation or the existence of lax regulation coupled with inept enforcement mechanism as well as the pervasive lack of scientific/medical expertise and knowledge of the effect of e-waste on human health and its environmental impact has made Africa a target for e-waste merchants (Olawuyi, 2007). It is estimated that while it costs about $50 to recycle a personal computer in the US, unscrupulous importers pay no more than $15 a piece, which translate to a net gain of $35 for a US recycler. By extracting the usable parts and then dumping it at the backyard scrap-trading outfits, an importer can generate profit of about $10 per piece, thus giving birth to a win-win situation for all (Basu, 2008). This waste is shipped from ports in the countries of generation to the ports of countries that are prone to serious lack of regulation and are usually shipped with a sprinkling of a few functional electronic/electrical appliances.

2.3 Components and constituents of e-waste

Components which are assembled to produce "electrical and electronic equipment" are metals, motor/compressor, cooling, plastics, insulation, glass, LCD, rubber, wiring/electrical, concrete, transformer, magnetron, textile, circuit board, fluorescent lamp, incandescent lamp, heating element, thermostat, flame retardants/brominated flame retardants-containing plastic, batteries, CFC/HCFC/HFC/HC, external electrical cables, refractory ceramics fibers, radioactive substances and electrolyte capacitors (over L/D 25mm) (UNEP, 2007a).

Electronic goods are composed of hundreds of different materials both natural and man-made, many of which are toxic if processed improperly (National Safety Council, 2000; EPA, 2001). Composition of e-waste is very diverse and may contain more than 1000 different

substances which fall under "hazardous" and "non-hazardous" categories. Used electronics is a mix of bio-accumulative, non-degradable and carcinogenic lethal toxins. Broadly, it consists of ferrous and non-ferrous metals, plastics, glass, wood and plywood, printed circuit boards, concrete and ceramics, rubber and other items. Iron and steel constitutes about 50% of e-waste, followed by plastics (21%), non-ferrous metals (13%) and other constitutes. Non-ferrous metals consist of metals like copper, aluminum, and precious metals such as silver, gold, platinum, palladium, etc (UNEP, 2007a).

Electronic equipment is one of the largest known sources of heavy metals, toxic materials and organic pollutants in city waste. E-waste is known to contain dangerous chemical pollutants that are released into the atmosphere and underground water (Orisakwe and Frazzoli, 2010). The presence of elements like lead, mercury, arsenic, cadmium, selenium, hexavalent chromium, and flame retardants in e-waste and their components beyond the threshold quantities classifies them as hazardous waste (Nordic Council of Ministers (NCM), 1995; Five Winds International, 2001). These materials are found in different parts and component of used electronics (e-waste) in varied quantities as a result of the use to which they are put (Table 2.3). The composition of e-scrap depends strongly on the type and age of the scrap. For example, scrap from IT and telecommunication systems contain a higher amount of precious metals than scrap from household devices. In older devices, the content of noble metals is higher but also the content of hazardous substances than in newer devices (Antrekowitsch *et al*., 2006).

2.3.1 Metals

The use of trace elements in electronic equipments is to act as dopants. Dopants are chemical materials incorporated into a pure substance to alter its electricity conductivity (Ladou and Lovegrove, 2008). Trace elements, such as arsenic, antimony, phosphorus, gallium and indium are incorporated into the matrices of silicon-based chips. These elements are also used in the production of semi-conductors, such as gallium, arsenide or indium phosphide. The potential environmental release of toxic trace elements from semiconductor materials deposited in municipal incinerators or landfills resulting in unanticipated human exposures to

Table 2.3: The composition of a typical desktop Personal Computer (PC) weighing ∼60Ibs

Name	Content (% of total weight)	Weight of material in computer (Ibs)	Use/Contents
Plastics	22.9907	13.8	Includes organics, oxides other than silica
Lead	6.2988	3.8	Metal joining, radiation shield/CRT
Aluminium	14.1723	8.5	Structural, conductivity/housing, CRT, PWB, connectors
Germanium	0.0016	<0.1	Semiconductor/PWB
Gallium	0.0013	<0.1	Semiconductor/PWB
Iron	20.4712	12.3	Structural, magnetivity/(steel) housing, CRT, PWB
Tin	1.0078	0.6	Metal joining
Copper	6.9287	4.2	Conductivity/CRT, PWB, connectors
Barium	0.0315	<0.1	In vacuum tube/CRT
Nickel	0.8503	0,51	Structural, magnetivity/(steel) housing, CRT, PWB
Zinc	2.2046	1.32	Battery, phosphor emitter/PWB, CRT
Tantalum	0.0157	<0.1	Capacitors/PWB, power supply
Indium	0.0016	<0.1	Transistor, rectifiers/PWB
Vanadium	0.0002	<0.1	Red phosphor emitter/CRT
Beryllium	0.0157	<0.1	Thermal conductivity/PWB, connectors

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Gold	0.0016	<0.1	Connectivity, conductivity/PWB
Europium	0.0002	<0.1	Phosphor activator/PWB
Titanium	0.0157	<0.1	Pigment, alloying agent/(aluminium) housing
Ruthenium	0.0016	<0.1	Resistive circuit/PWB
Cobalt	0.0157	<0.1	Structural, magnetivity/(steel) housing, CRT, PWB
Palladium	0.0003	<0.1	Connectivity, conductivity/PWB
Manganese	0.0315	<0.1	Structural, magnetivity/(steel) housing, CRT, PWB
Silver	0.0189	<0.1	Conductivity/PWB, connectors
Antimony	0.0094	<0.1	Diodes/housing, PWB, CRT
Chromium	0.0063	<0.1	Decorative, hardener/(steel) housing
Cadmium	0.0094	<0.1	Battery, glu-green phosphor emitter/housing, PWB, CRT
Selenium	0.0016	0.00096	Rectifiers/PWB
Niobium	0.0002	<0.1	Welding allow/housing
Yttrium	0.0002	<0.1	Red phosphor emitter/CRT
Mercury	0.0022	<0.1	Batteries, switches/housing, PWB
Arsenic	0.0013	<0.1	Doping agents in transistors/PWB
Silica	24.8803	15	Glass, solid state devices/CRT, PWB

Adapted from Microelectronics and Computer Technology Corporation (1996)

these agents in the general population, is an important issue. Many of the agents used as dopants are highly toxic and in several cases are now indentified as known or probable human carcinogens. Indium, arsenide, indium phosphide, and aluminium gallium arsenide show clear evidence of carcinogenic potential (Tanaka, 2004; Liao *et al*., 2004; Chen, 2007).

Metallic elements present in e-waste include: lead, cadmium, mercury, antimony, arsenic, beryllium, nickel, chromium, cobalt, barium, selenium, zinc, yttrium, europium, gold, platinum, iron, aluminium, copper, tin, germanium, gallium, tantalum, indium, vanadium, ruthenium, tanialum, rhodium, americium, cobalt, palladium, manganese, silver, bismuth, niobium, silica, lithium, tritium, terbium etc (Nordic Council of Ministers (NCM), 1995; Five Winds International, 2001; Oh *et al*., 2003; Chen *et al*., 2004). The printed wire boards (also called circuit boards) found in most e-wastes, for example, may contain arsenic, cadmium, chromium, lead and mercury. Cathode ray-tubes (CRTs) in computer monitors and television may contain barium, cadmium, copper, lead, zinc, and several rare earth metals.

Lead is one heavy metal with known toxic property that is found in large amounts in many electronic devices (Nordic Council of Ministers (NCM), 1995). Typical printed wire boards (PWB) have been reported to contain approximately 50g of tin-lead solder/m^2 of PWB and approximately 0.7% of the total weight of a PWB (Electronic Industry Alliance, 2000). In CRTs, leaded glass provides shielding from X-rays generated during the picture projection process. Color CRTs contain 1.6-3.2kg of lead on average (Microelectronics and Computer Technology Corporation, 1996). It is estimated that about 70% of the heavy metals (including lead, cadmium, and mercury) found in landfills come from electronic equipment discards (Hawari and Hassan, 2008).

2.3.2 Plastics

WEEE contains a complex mix of materials including a range of different, often incompatible, polymer types. This complicates the task of recycling WEEE (Freegard *et al*., 2006; Nnorom and Osibanjo, 2008a). In general, about 8-12 different basic types of plastic are found in consumer electronics. Plastics make a significant contribution to the properties

of EEE offering a balance of properties that no other class of material can match (Dawson *et al*., 2004). Insulation, noise reduction, sealing, housing, interior structural parts, functional parts and interior electronic components are among the many uses of plastics in EEE (Brebu *et al*., 2004). The major resins in the electronic industry are high-impact polystyrene, HIPS (56wt.%), acrylonitrile butadiene styrene, ABS (20wt.%) and polyphenylene ether, PPE (11wt.%). The remaining 13wt% is made of other resins such as polyvinyl chloride (PVC), poly carbonate (PC) and polyphenylene oxide (PPO) (Kang and Schoenung, 2005).

PVC is a chlorinated plastics used in electronics products and for insulation on wires and cables. Chlorinated dioxins and furans are released when PVC is produced or disposed of by incineration (or simply by burning). These chemicals are highly persistent in the environment and many are toxic even in very low concentrations (Kimani, 2009). There is a great variation in the quantity and types of plastics in electronics, depending on the product and this varies from very small amount to more than half the material composition of some mobile phones (Fisher *et al*., 2004). In ICT and consumer equipment, for example, less than 30% of plastics are present while electronic toys may contain more than 70% plastics (Delgado *et al*., 2007). It has been estimated that out of about 60, 000 tons of second-hand EEE imported annually in Nigeria, there is as much as 18, 000 tons of plastics (Nnorom and Osibanjo, 2008a).

2.3.3 Organic compounds

A variety of organic compounds are also present in e-waste (National Safety Council, 2000; EPA, 2001). Until late 1970s, PCBs (polychlorinated biphenyls) were widely used in insulating fluids for electrical transformers and capacitors as well as flame-retardant plasticizers in PVC and other polymer applications (Silicon Valley Toxics Coalition, 2001; Electronic Waste Guide, 2010). Also present are the polychlorinated naphthalenes (PCNs); these are the precursors to PCBs, and share many of their properties (Silicon Valley Toxics Coalition, 2001). They are a large group of substances based on organic and inorganic halogens, phosphorus, nitrogen and minerals containing compound with strongly differing individual sets of properties. Flame retardants are used in electronic products to decrease the risk of fire, thereby increasing the fire resistance of the materials in which they are applied

(Nnorom and Osibanjo, 2008a). Brominated flame retardants are prevalent among other types of flame retardants because lower quantities of these compounds ensure the highest fire safety (Drohmann *et al*., 2004). They are a class of brominated chemicals commonly used in electronic products as a means for reducing flammability. In computers, they are used mainly in four applications: in printed circuit boards, in components such as connectors in plastic covers and in cables. They are also used in plastic covers of TV sets and in domestic kitchen appliances (Hawari and Hassan, 2008). Some brominated flame retardants, used in circuit boards and plastic casinos do not break down easily and build up in the environment. About 1000 tons of a brominated flame retardant called tetrabromobisphenol-A (TBBPA) was used to manufacture 674 million mobile phones in 2004. This chemical has been linked to neurotoxicity (Eliminate Toxic Trade, 2005). Brominated flame retardants (BFRs) contain up to 50-95wt% of bromine, and can be separated into aromatic, aliphatic and cyclo-aliphatics. BFRs are used preferentially because:

i. Of their number (about 75 diverse and different chemicals with various properties are available, though only about 30-40 are widely used in EEE).

ii. Of their efficiency in flame retardation.

iii. Of their universal applicability

iv. For some polymers, they are the only viable method of achieving the required flammability standards with some plastic resins

v. There is a lot of information on these compounds

vi. They can easily be recycled (De Boer, 2004; Dawson *et al*., 2004).

Three main classes of brominated flame retardants pose a hazard: polybrominated biphenyl (PBB), tetrabromobisphenol-A (TBBPA) and polybrominated diphenyl ethers (PBDEs). PBDEs are flame retardants that are mixed into plastics and components. There are no chemical bond between the PBDEs and the plastics and therefore they may leach from the surface of e-waste components into the environment.

Other organic constituents of electronic products as well as products of their low-temperature combustion include: chlorofluorocarbon (CFC), polycyclic aromatic hydrocarbons (PAHs), polyhalogenated aromatic hydrocarbons (PHAHs), polychlorinated dibenzo-p-dioxins

(PCDDs) and polychlorinated dibenzofurans (PCDFs) (Scheutz *et al*., 2004; Robinson, 2009).

2.4 E-waste management/legislation/policies

2.4.1 Management

Waste from electrical and electronic equipment (WEEE) is one of the priority streams in waste management because of its major challenges. It has in fact become an issue of concern to solid waste management professionals (Musson *et al*., 2000). Challenges faced by WEEE management are not only consequences of growing quantities of waste but also the complexity of WEEE. It is one of the most complex waste streams because of the wide variety of products from mechanical devices to highly integrated systems and the accelerating technological innovations (Yla-Mella *et al*., 2004). As a result of the variety of product models, size changes, compatibility issues, etc, the recovery of WEEE is very challenging (Kumar *et al*., 2005).

E-waste is a complex mixture of hazardous and non-hazardous waste, which consists of items of economic value. Therefore, it requires specialized segregation, collection, transportation, treatment and disposal. E-waste management systems have three major components:

1. Collection, sorting and transportation system.
2. E-waste treatment system.
3. E-waste disposal system.

E-waste collection system consists of producer/retailer take back system, municipal collection system and recyclers/dismantlers collection system. Since e-waste is hazardous in nature, it is collected, sorted, stored and transported under controlled conditions. Each of the agencies has its own e-waste collection and storage centers (UNEP, 2007b). The collection means will vary following distances, rural or urban patterns, and the size of collected appliances. An efficient e-waste collection and transportation system will ensure reuse, recycle and adequate e-waste management including avoiding damage or breaking components that contain hazardous substances. The major e-waste treatment techniques are decontamination and disassembly or repair followed by shredding of different fractions. E-waste fractions emitted after shredding go for metal recovery. The remaining fractions are

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disposed of either in landfills or incinerated.

2.4.1.1 Recycling

E-waste recycling involves the disassembly and destruction of the equipment to recover new materials (Cui and Zhang, 2008). Recycling can recover 95% of the useful materials from a computer and 45% of materials from cathode ray tube monitors (Ladou and Lovegrove, 2008). Mechanical separation of components is the first step in e-waste recycling. Components may be separated for reuse or metallurgical processing (He *et al*., 2006; Robinson, 2009). This process can be automated or carried out by hand. In poor countries, there is a risk that children may be employed to separate e-waste components (Ladou and Lovegrove, 2008). An open flame is often used to free components (Manomaivibool, 2009), which may result in exposure to volatilized contaminants (Robinson, 2009).

The journey from consumer to recycler is long and complex. It moves in a zigzag fashion. It involves players not only from the informal section (i.e. the recycling stream), but also players from the formal sector (i.e. manufacturers and retailers of electronic items). After securing e-waste from various sources, scrap dealers face the dilemma of deciding which electronics ought to be dismantled and which to be retained for resale. This dilemma arises because only a few models are in demand as second-hand products. Once the decision is made, the not-to-be-sold electronic components are taken to the storehouses for dismantling (Orisakwe and Frazzoli, 2010). The first step in the process of electronic disposal practices is the separation of each and every component. Only few working chips of higher value are sold, otherwise, the majority of electronic parts are broken up and sold off to different stakeholders for material recovery (Bandyopadhyay, 2008).

E-waste recycling has not been effective; this is because it presents difficulties for recycling due to the complexity of each item and the lack of viable recycling systems. Operations tend to be highly labor-intensive and therefore costly. This has resulted in an escalating global trade in obsolete, discarded e-waste collected in North America and Europe and sent to developing countries by waste brokers and so-called recyclers, where environmental

standards and working conditions are lower. As much as 80% of the e-waste collected for recycling in the US is not recycled domestically, but it is instead exported to developing countries (Hicks *et al*., 2005; Antrekowitsch *et al*., 2006). A pilot program conducted by the USEPA that collected scrap in a state in the US (San Jose, CA) estimated that it was 10 times cheaper to ship CRT monitors to China that it was to recycle them in the US (Roman and Puckett, 2002). Also, most e-waste is not recycled because e-waste items tend to go out with household waste and so receive no special treatment. This is often the case in developing countries, where separation of wastes before disposal is not strictly adhered to.

2.4.1.2 Crude recycling

Informal dismantling and recycling of e-waste, the so called "backyard activities" is emerging in developing countries. These crude recycling activities are taking place in Asia and Africa, aimed at material recovery from e-waste. Usually in these regions, e-scrap is mostly treated in "backyard operations" using open sky incineration, cyanide leaching and simple smelters to recover mainly copper, gold, and silver with comparatively low yield (BAN, 2002; 2005; Greenpeace International, 2005; Hageluken, 2006). Collection, manual dismantling, open burning to recover metals and open dumping of residual fractions are present in these countries. While in some countries these activities are performed by individuals (e.g. South Africa, Kenya, Morocco, Uganda, Senegal and Peru), countries like India and China reveal a large organised informal sector (UNEP, 2009). The centralised informal recycling locations are always found adjacent to the electronic and electric production centers. The incentive of reclaiming more material with lower cost becomes the main reason for the formation of these large-scale informal sectors in developing countries. These crude methods result in loss of resources, energy wastages and environmental pollution. Moreover, such "backyard recyclers" do not have waste water treatment facilities, exhaust/waste gas treatment and personal health protection equipment. There is a huge and mostly irreversible waste of resources and dangers posed to the environment and public health with such practices. This is because, players in these sectors do not know better practices or have no access to investment capital to finance even profitable improvements or implement safety measures (Roman and

Puckett, 2002; Liu *et al*., 2005). Serious adverse impacts on the environment and human health from e-waste recycling continue to occur today due to lack of regulation and enforcement.

At workshops in China, India, Bangladesh, Ghana and many other countries, lead solder and other metals are dissolved in open acid baths. Some e-wastes are burned in open fires to recover metals from plastics in which they are encased. The open burning, acid baths, and toxic dumping of e-waste introduce unconscionable levels of contaminants into fragile environment, and exposed the world's poorest people to a large number of toxic materials (Wong *et al*., 2007a; Li *et al*., 2007a). More disheartening is the fact that much of this work is carried out by children, using only rudimentary tools and little or no protective equipment/clothing (Figure 2.3). The public health and environmental costs of this recovery process are borne neither by the consumers nor by the manufacturers of these products. The cost are purposely shifted to the poor people least able to absorb them, and least likely to speak out against the unfairness of the trade (Ladou and Lovegrove, 2008).

While recycling reduces virgin material use, they do still require additional energy to be used to return them into manufactured products. Industries are also wary of reusing recycled materials/components because such items can have variable quality issues attached. The main challenges to effective recycling of e-waste include:

1. The laws/regulations that encourage e-waste recycling.
2. Accurate estimation of the quantities of e-waste that will be generated both in the short and long term.
3. Environmentally sound treatment in qualified facilities and
4. The success of the after-markets for materials recovered in recycling and recovery operations.

Despite these challenges, recycling appears to be the best substitute in e-waste management in-terms of technology and economic feasibility. In rich countries such as Japan, high tech recycling operations function well with little environmental impact (Aizawa *et al*., 2008). Modern techniques can recover high-Pb glass from discarded CRT with minimal

Figure 2.3: Scavengers of e-scraps, mostly children and teenagers (BAN, 2005).

environmental impact (Andreola *et al*., 2007). The efficacy of this process depends on investment. Given that only 10% of the electronic equipments are recycled, most e-waste is currently landfilled. As with other materials, leaching of many heavy metals such as Pb from e-waste is increased at low pH and in the presence of organic ligands. Studies have shown that compaction of e-waste, prior or during landfilling, can increase leaching through the disruption of circuit board components.

2.4.2 Legislation/Regulations on e-waste management

Throughout history, direct regulations through statutes and subordinate rules have been normal approach to pollution control and environmental protection. In the last two decades, there has been an increase in the number of environmental policies and legislations focusing on the product development process with a view to reducing the environmental impacts resulting from the products, throughout their entire lifecycle-from product design, manufacture, through to consumption and eventual end-of-life (EoL) management. Policies/laws/regulations related to e-waste management provide an institutional framework for their implementation. Countries in Europe and Japan have been the frontrunner in formulation of policies/laws/regulations for e-waste followed by their institutionalization and implementation.

2.4.2.1 EU Directives on e-waste management

These policies and legislations are almost all based on the principles of Extended Producer Responsibility (EPR). The EPR concept has become an established principle of environmental policy in many countries. EPR is a method of integrating sustainable development principles into international trade based on an international environmental law principle known as the "Polluter Pays Principle" (Kibert, 2004). EPR also called "Product Take Back" is the fundamental principle of WEEE Directive in EU, where producers are responsible for e-waste take back. The electronic industry must take responsibility for its products at the end of their useful lives. The policy instruments that lie under the EPR umbrella include different types of product fees and taxes such as advance recycling fees

(ARFs), product take-back mandates, virgin material taxes and combination of these instruments (Nnorom and Osibanjo, 2008a). This responsibility forms the basis for "take-back" legislation which is being implemented in the EU under the WEEE Directive which took effect in 2005. The WEEE Directive attempts to establish a new management programme that could have far reaching implications for product design and materials management.

The Directive encourages the design and production of electronic equipment to take into account and facilitate dismantling and recovery, in particular the reuse and recycling of electronic equipment, components and materials necessary to protect human health and the environment. The main objectives of this regulation include:

a. Reduction of waste arising from EoL EEE.

b. Improvement and maximisation of recycling, reuse and other forms of recovery for EoL EEE.

c. Minimisation of the impact upon the environment from the treatment and disposal of EoL EEE (Nnorom *et al*., 2007).

The WEEE Directive covers all electrical and electronic equipment with voltages up to 1000 AC and 1500 DC and will affect virtually all producers and manufacturers of EEE regardless of the company size (Widmer *et al*., 2005). The cost of treating historical waste is to be shared proportionately between producers on the markets when the costs arise. Producers must provide up-front financial guarantee to guard against costs arising from orphan WEEE. In addition, member states must take measures to minimize the disposal of WEEE by consumers as unsorted municipal waste.

Those European countries which are not part of EU either follow EU Directive or more stringent standards based on e-waste management. Majority of countries have regulations similar to WEEE Directives. Countries like Japan have regulations focused on "Reuse, Recycling and Recovery". Other countries like Canada and Australia are developing their systems based on the similar principles of "EPR" (UNEP, 2007b). Beside the WEEE Directive, a sister directive on Restriction of Certain Hazardous Substances (RoHS) was

enacted in February 2003. The RoHS directive has similar scope, although medical appliances and monitoring and control equipment are exempted. The RoHS directive prescribes that the use of certain substances be prohibited in most EEE applications. These substances include lead, mercury, cadmium, hexavalent chromium, polybrominated biphenyls (PBB) and polybrominated diphenyl ethers (PBDEs) (Walls, 2003). Both business-to-business and consumer products are covered. This directive, by banning the use of critical materials in electronic products sold in key world markets, may result in a significant change in the way products are designed for global sale.

It is anticipated that banning certain core substances from the manufacturing of electronic products will drive innovations and substitution. Research and technical assistance on lead-free electronics are developing and are widely acknowledged to be the result of EU Directives. The implementation of these two directives has raised awareness of issues surrounding the manufacture, use and disposal of EEE and together initiates measures to address generation of waste and encourage recycling. The European ICT industry has established process to successfully comply with the requirements of the two directives and there are indications that existing business initiatives are trying to extend these practices to the rest of the world. Unfortunately, the political process has taken its toll, and a gradual weakening of the original intent of RoHS has taken place. There are many exceptions to RoHS now including batteries and electronic equipment intended to protect national security or with a military purpose (Kimani, 2009).

2.4.2.2 UN (The Basel Convention) regulation on hazardous wastes

The foremost global initiative aimed at tackling the WEEE issue is the Basel Convention and Basel Ban. The Basel Convention is a multilateral agreement regulating the internal shipment of hazardous wastes. It began in 1987 following many decades of unregulated dumping of hazardous wastes in poor countries. Prior to this time, transboundary movements of hazardous waste were governed by the customary international law principle of "good neighborliness" or *sis utere tuo, ut alienum non laedas* as exemplified in the Trail Smelter Arbitration and reaffirmed in Principle 21 of the Declaration of the United Nations

Conference on the Human Environment (Trail Smelter Arbitration, 1941; Stockholm Declaration, 1972).

In June of 1987, following intense outrage expressed by developing countries, the Basel Convention was established. This led, in March 1989 to 118 nations signing the Basel Convention on the control of transboundary movement of hazardous waste and their disposal (UNEP, 2007b). The convention requires that participating nations reduce the transboundary shipment of hazardous wastes by minimising production, and by treating and disposal of the wastes as near to the source of production as is possible. The use of the word "control" in the Convention's title rather than 'prevention' or 'prohibition' was telling. During the negotiations leading up to the Basel Convention, the vast majority of nations made it clear that they wanted to ban waste trafficking entirely, particularly from developed to developing nations. Certain heavily industrialised countries however, most notably the US, fought to reject any such prohibition. Thus the Basel Convention became primarily an instrument to monitor the transboundary movements of hazardous waste rather than prevent it (Kimani, 2009).

With the exception of a ban on export to Antarctica, the Convention established only a weak control regime based on the principle of "prior informed consent (PIC)". Under such a regime, hazardous waste exports are not to take place unless the "competent authority" in the recipient country is notified in advance and gives written consent. The scheme requires the exporter/importer to seek and get the consent of the States through which the waste is to go through as well as that of the State of import before the actual movement of the hazardous waste. Furthermore, the Basel Convention enjoins parties to ensure that export/import of waste is carried out only where the state of export is lacking technical facilities and capacity to dispose of the waste in an environmentally sound and efficient manner or where the waste in question is raw material for recycling or recovery industries in the state of import (UNEP, 1989). The flaw in the foregoing is that the determination of whether an exporting state lacks the technical facility and capacity to dispose of e-waste and every other form of wastes is the preserve of the exporting state. This puts the exporting state in rather advantageous position

in terms of the waste (Azuka, 2009).

After ratification of the Convention in 1992, it became increasingly apparent that three key gaps or loopholes exist in the treaty (Lepawsky and McNabb, 2009). First, universally accepted definitions of hazardous waste were problematic because of their actual or potential contradiction of definitions used in the national laws of signatory countries. Second, the convention makes allowances for bilateral and multilateral agreements involving transboundary movements of hazardous waste between Basel signatories and non-signatories, so long as the provisions of those agreements are no less "environmentally sound" than those in the convention, yet, what constitutes environmentally sound remains ill defined. Third, is a provision in the original treaty that allowed transboundary movements of hazardous waste if the materials were to be reused or recovered through recycling, which led to the problem of some exporter's simply re-categorising waste materials for disposal as materials destined for recycling.

Subsequently, the Ban Amendment to the Basel Convention (referred to as the Basel Ban) was adopted (UNEP, 1992). The Basel Ban seeks to strengthen the convention by prohibiting export of hazardous waste for any reason, from Organisation of Economic Cooperation and Development (OECD) member states to non-OECD states; essentially from wealthy to poorer countries. It provides robust incentive for countries desirous of reducing transboundary movements of hazardous wastes as well as creates a platform for the consolidation of policies geared towards treating and disposing of wastes as close as possible to their source of generation (Rummel-Bulska, 1994). Notwithstanding the fact that Basel Ban has been incorporated into the Basel Convention as Article 4(A), it has not yet entered into force (UNEP, 1989). The Ban is still under considerable attack from influential industrialised states, and many of the parties do not live up to the Ban's standards.

Having failed to achieve a global ban on hazardous waste trade from developed to developing countries; the latter were quick to pursue national and regional statutes toward this end (Kimani, 2009). Some of the agreements include:

- **The Lome IV Convention**: 70 nations of the African, Caribbean and Pacific nations (ACP), in December 1989, successfully concluded a waste trade ban with 15 member states of the EU, as part of their negotiations of the Lome IV Convention. The agreement prohibits the EU from exporting nuclear or hazardous wastes to the ACP states, while the ACP countries agreed to prohibit such waste imports from any country (Lome IV Convention, 1989).

- **The Bamako Convention**: The Bamako convention on the Ban on the import into Africa and the Control of Transboundary Movement and Management of Hazardous Wastes within Africa prohibits the import of any hazardous waste into the region. In January 1991, in Bamako, Mali, member states of the Organization of Africa Unity (OAU) adopted a treaty banning all forms of hazardous and nuclear waste imports to the African continent. The treaty also forbids import of products that have been banned for use in the country of manufacture. It came into force on 2 April, 1998, and by 2007, 29 of the independent countries that make up the African Union have signed while a total of 23 have ratified/acceded to the treaty (Azuka, 2009). In form, the Bamako Convention is similar to the Basel Convention. The major difference however, is that the Bamako Convention makes no exception with respect to the kind of waste that can be moved across state lines within Africa unlike the Basel Convention (Bamako Convention, 1991).

- **National Bans**: Many countries enacted unilateral hazardous waste import bans. Many have bans in law or policy as a result of the regional commitments. In 1986, three countries had committed to ban the import of hazardous wastes; by 1988, the figure had risen to 33 and by 1992 to 88. The total of import bans, with the adoption of the Waigani treaty, the Barcelona convention Waste Trade Protocol, and the Basel Ban was well over 100 by 1997 (Kimani, 2009).

2.5 E-waste management in developing countries

The management of e-waste in developing countries has been reviewed (Nnorom *et al*., 2007; Nnorom and Osibanjo, 2008a). Most developing countries including Nigeria have neither a well-established system for operation, storage, collection, transportation and disposal of

waste nor the effective enforcement of regulations relating to hazardous waste management (Mundada *et al*., 2004). They do not have legislation dealing specifically with e-waste and there is lax enforcement of existing laws dealing with general waste management. Formal recycling of e-waste using efficient technologies and state-of-the-art recycling facilities are rare. As a result, e-waste are managed through various low-end management alternatives such as disposal in open dumps, disposal with municipal waste at unlined landfills, backyard recycling and disposal into surface water bodies (Furter, 2004) (Figures 2.4 - 2.6). In the developing countries, there is a high level of reuse and repair activities for these imported devices. This returns some of the obsolete EEE to a second life. The unserviceable/unusable EEE or modules are usually disassembled (incomplete disassembly) for component retrieval for use in repair activities (Nnorom *et al*., 2007).

The present management activities are discussed below:

- **Repair and reuse activities**: While some of the imported electronics to the developing countries including Nigeria is fully functional and is directly reused, there is nevertheless a significant quantity of the imported electronic equipment or parts that is considered junk. As a result, there is high level of repair and reuse activities in Nigeria and most developing countries. BAN (2002) reported that there are about 3500 registered business involved in all manner of sales and repair of computers, phones, peripherals and software in Computer Village, Lagos, Nigeria; a hub of second-hand EEE in Nigeria.

- **Informal recycling**: crude recycling of e-waste is currently taking place in China, India and in some other countries in the Asia-Pacific axis (Nnorom and Osibanjo, 2008a). The primitive e-waste recycling procedures in developing countries include: dismantling of old electronic equipment with simple tools such as electric drill, cutter, hammer and screwdrivers into component parts such as monitor, hard drive, CD drive, wires, cables, circuit boards, transformer etc.

  - Large appliances such as circuit boards of computers are heater over coal fires to melt the solder and release valuable electronic components such as diodes, resistors and microchips.

  - Hand-held devices such as pagers, current circuit board of mobile phones etc are
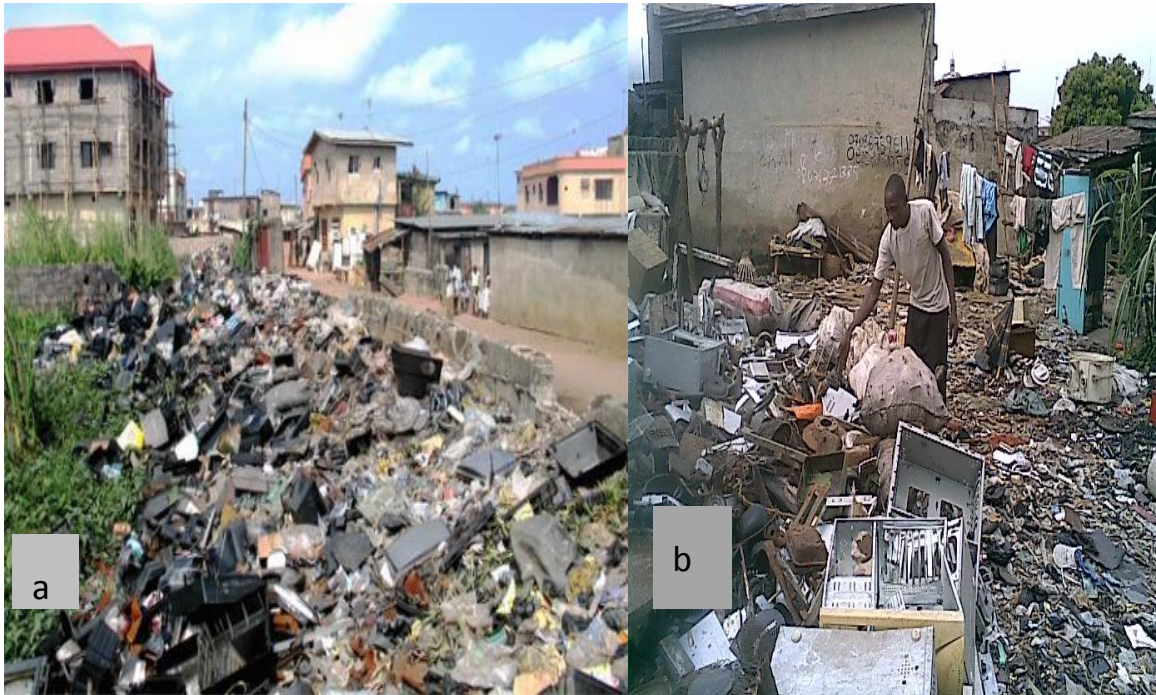
Figure 2.4: E-waste disposal methods in developing countries; a) informal waste dumpsites,

b) backyard dumps (BAN, 2005).

Figure 2.5: Open burning as a means of waste management in developing countries (BAN, 2005).

Figure 2.6: Disposal of e-waste with municipal solid waste (BAN, 2005)

- usually heated in open flame for the solder.
- Memory banks, chips or circuit boards which contain tiny amounts of gold and palladium are treated in acid bath with a medieval acid recipe-aqua regia, a highly corrosive and dangerous, fuming yellow or red solution.
- The rest materials with no economic value are set ablaze to reduce the volume.

These operations are usually carried out with little or no personal protection equipment or pollution control measures. These crude material recovery processes have resulted in environmental pollution while exposing millions of people to toxins in Asia. Beside the tremendous adverse effects on environmental and public health in this region, this is also a huge and mostly irreversible waste of resources (Hageluken, 2006). These activities have not yet caught-up in Africa, except South Africa where there is recycling activities for EoL WEEE. There is fear however, that if and when the crude material recovery technologies, currently wrecking havoc in some Asian countries are introduced into Africa, the environmental pollution from such activities in Asia may be a child's play (Nnorom *et al*., 2007).

- **Disposal with municipal solid waste**: Management of discarded electronics in the developing countries is taking place through traditional methods of municipal solid waste (MSW) management, namely landfilling and incineration (Osibanjo and Nnorom, 2007). Antrekowish *et al*. (2006) reported that up to 90% of e-scrap was landfilled in 2003 both in developing and developed nations. In most developing countries, e-waste is treated as the municipal waste, and no special attention is given to the activities related to its collection, handling and dismantling. Most of the activities related to its collection, handling and dismantling are mainly being performed by the organised or informal sectors lacking the technical and infrastructural abilities and knowledge about the serious implications of the e-waste handling and disposal on environment and human health. In Nigeria, obsolete electronic devices are usually stored for a while for perceived value (physical or emotional) before disposal with municipal waste. Because of the absence of a special framework for the separation, collection and management of e-waste in Nigeria, these electronic equipments are usually disposed with MSW at open dumps and into

surface waters. Osibanjo and Nnorom (2007) showed that there are no attempts at recovering materials from e-scrap using crude processes in major e-waste area in Nigeria, however, there are indications that waste collectors have also started collecting selected components of EEE, especially the printed wiring board for export, probably to Asia for recycling.

- **Open burning**: This is one of the commonest management of e-waste method in developing countries including Nigeria. Electronic markets such as the famous Computer Village and Alaba International markets, Lagos, Nigeria, have sites for the open burning of unusable electronic devices, replacement parts/modules and other wastes from repair and refurbishing activities. This method of management is extremely hazardous and has both health and environmental consequences. Wires and cables, as well as other components of EEE, including PWBs and plastic housing/enclosure are routinely burned in the open. This creates the potential for the release of heavy metals and persistent organic pollutants (POPs) such as PAHs, PCBs, PBDEs, dioxins and furans (Nnorom and Osibanjo, 2008a).

Several reasons have been given for the present low-end management of WEEE and/or existence of ineffective informal WEEE processing sector in the developing countries as reviewed by Nnorom *et al*. (2007). These include:

- The unwillingness of consumers to handout their EoL goods. This is because consumers view their waste as a resources and income-generating opportunity.
- There is general reluctance to pay for waste recycling and disposal services, particularly when consumers can make money by selling their old and broken appliances.
- There is a lack of awareness among consumers, collectors and recyclers of the potential hazards of WEEE.
- Lack of funds and investment to finance improvements in e-scrap recycling.
- Absence of ineffective take back programs for EoL WEEE.
- Absence of legislation dealing specifically with e-waste or ineffective/lax implementation of existing regulations on the transboundary movement of e-waste.

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2.6 Environmental and Public health effects of e-waste pollution

WEEE contains a myriad of toxic components and materials that can cause significant damage to the environment and human health if recycling and disposal is unregulated. Dumping of e-waste in any environment including Nigeria has negative health consequences such as leaching toxins into the soil, air and groundwater, which later enter into crops, animals and human body systems causing contamination and pollution. Medical experts have warned that exposure to these substances can cause damage to blood, nervous systems, DNA, immune systems, kidneys; and can lead to respiratory and skin disorders and lung cancers, and can interfere with regulatory hormones and brain development (Osuagwu and Ikerionwu, 2010). Various electrical and electronic devices have been confirmed hazardous using the toxicity characterization leaching procedure (TCLP) (Musson *et al*., 2000; Li *et al*., 2006). The actual operation of several end-of-life processes for e-waste such as landfills, incineration with MSW and mechanical recycling results in emission of heavy metals and organic pollutants to air, water and soil. Environmental and public health impact of e-waste has been documented (Robinson, 2009; Sepulveda *et al*., 2010). Table 2.4 shows the potential environmental contaminants arising from e-waste disposal or recycling

2.6.1 E-waste contamination of soil and terrestrial environment

Several studies have shown evidence of heavy metal pollution of soil from various electronic activities ranging from production, sales, repair, disposal and recycling processes. Soil have been heavily contaminated both with chemicals originating in electronic goods and those generated by the dangerous disposal of non-recyclable components. Pollutants have been found both at the sites directly involved in e-waste operations and in soils which were located at a considerable distance from the main processing area. Dumped electronics containing heavy metals and brominated and chlorinated flame retardants can affect the soil. The mobility of these substances towards the environmental compartments depends on diverse environmental parameters such as pH, organic matter content, temperature, adsorption-desorption process, complexertion, uptake by biota, degradation process, and the intrinsic chemical characteristics of the substance (Sauve *et al*., 2000; Georgopoulus *et al*., 2001; Hu,

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Table 2.4: Potential environmental contaminants arising from E-waste disposal or recycling

Contaminant	Relationship with e-waste	Typical E-waste concentration (mg/kg)	Annual global emission in E-waste (tons)
Polybrominated diphenyl ethers, polybrominated biphenyls, tetrabromobisphenol-A	Flame retardants		
Polychlorinated biphenyls	Condensers, transformers	14	280
Chlorofluorocarbon	Cooling units, insulation foam		
Polycyclic aromatic hydrocarbons	Production of combustion		
Polyhalogenated aromatic hydrocarbons	Product of low-temperature combustion		
Polychlorinated dibenzo-p-dioxins polychlorinated dibenzofurans	Product of low-temperature combustion of PVCs and other plastics		
Nonylphenol (NP)	Housing, casing, insulators		
Triphenyl phosphate (TPPs)	Casing of monitors		
Silica	Glass, solid state devices, CRT		
Americum (Am)	Smoke detectors		
Antimony	Flame retardants, plastics	1700	34,000
Arsenic (As)	Doping material for Si		
Barium (Ba)	Getters in cathode ray tubes (CRTs)		
Beryllium (Be)	Silicon-controlled rectifiers		

Cadmium (Cd)	Batteries, toners, plastics	180	3600
Chromium (Cr)	Data tapes and floppy discs	9900	198,000
Cobalt (Co)	Magnetivity, CRT, steel housing		
Copper (Cu)	Wiring	41,000	820,000
Gallium (Ga)	Semiconductors		
Indium (In)	LCD displays		
Lead (Pb)	Solder, CRTs, batteries	2900	58,000
Lithium (Li)	Batteries		
Mercury (Hg)	Fluorescent lamps, batteries, switches	0.68	13.6
Nickel (Ni)	Batteries	10,300	206,000
Selenium (Se)	Rectifiers		
Silver (Ag)	Wiring, switches		
Tin (Sn)	Solder	2400	48,000
Zinc (Zn)		5100	102,000
Rare earth elements	CRT screens		

Source: Azuka (2009) and Robinson (2009)

2002; Gouin and Harner, 2003; Qin *et al*., 2004; Sepulveda *et al*., 2010). Ionic and occasionally methylated heavy metals are particularly mobile and bioavailable (Dopp *et al*., 2004; Hirner, 2006). Lower brominated congeners of flame retardants such as PBDEs are particularly mobile while higher brominated congeners tend to bind to particles and exhibit lipophilic properties (Gouin and Harner, 2003). The release of PBDEs into the environment may occur through a variety of ways such as loss during manufacture of PBDEs or products containing PBDEs. The presence of PBDEs in indoor environment is assumed to occur due to their migration during product use (Talsness, 2008).

Soil within the area of electronic activities has been shown to contain higher amount/level of heavy metals whose origins are from the e-waste. Heavy metals not recovered during WEEE treatment and residual auxiliary substances like mercury and cyanide can leach through the soil after disposal of effluents and form inorganic and organic complexes within soils (Sepulveda *et al*., 2010). In some cases, certain metals were present at concentration over a hundred times higher than typical background levels for soil, including the highly toxic metal lead. Soil at an e-waste recycling site slum in Bangalore contained up to 39mg/kg Cd, 4.6mg/kg In, 957mg/kg Sn, 180mg/kg Sb, 49mg/kg Hg, 2850mg/kg Pb and 2.7mg/kg Bi (Ha *et al*., 2009). Similar study in Guiyu, a major recycling site in China, showed an elevated level of heavy metals compared to the Bangalore study (Wang *et al*., 2007). The Pb concentration of 3560-6450mg/kg dw has been reported in bottom ashes of WEEE recycling facilities in New Delhi (Bridgen *et al*., 2005), which was 254-461 times higher than the average content of Pb in bottom ash from three major power plants in and around New Delhi (Sushil and Batra, 2006).

Bridgen *et al*. (2008) showed that several chemical contaminants were found in the ash contaminated soil samples from open burning site at both Agbogboloshie and Korforidua e-waste sites in Ghana, where Pb, Cd and antimony were present at concentration over 100 times typical background levels for soils. Similar study by the authors in Nigeria reported a soaring level of heavy metals (especially Pb and Cd) over hundred times higher than the background level for soils. Numerous classes of organic compounds were also analysed in the

soil samples including many halogenated (chlorinated or brominated) chemicals. The authors concluded that many of the compounds identified are intentionally used in electronic devices.

Soils from a site where acid leaching was used to recover valuable metals, contained up to 4250ng/g PBDEs (Leung *et al*., 2007). There are elevated concentrations of PCBs, PAHs (Shen *et al*., 2009a) and PBDEs (Liu *et al*., 2008) in Chinese agricultural soils proximal to e-waste reprocessing sites. Luo *et al*. (2009a) reported PBDE concentrations of 191-9156ng/g (dry weight) in farmland soils 2km away from an e-waste recycling workshop. Soils from this region also contained polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), PCBs and PAHs at concentrations up to 100, 330 and 20, 000 ng/g respectively (Shen *et al*., 2009b). Elevated concentrations of PBDEs and PCBs in soils, plants and snails in Guiyu and the surrounding areas have also been documented (Liu *et al*., 2008). PBDEs are translocated from soils to plants. Leaves of bracken fern (*Pteridium aquilinum* L.), spider fern (*Pteris multifida* Poir), sorghum (*Sorghum bicolor* L.), Japanese dock (*Rumex japonicus* Huntt.) and Eastern daisy fleabane (*Erigerun annuus* L.) contained PBDEs at concentrations of 144, 116, 162, 278 and 326 ng/g (dry matter) respectively, when growing in soil containing 25, 479ng/g PBDEs (Yang *et al*., 2008). Although the bioaccumulation coefficients are small (<0.01), plant uptake might facilitate the entry of these contaminants into the food chain (Robinson, 2009).

Analysis of rice sample from another e-waste processing town in eastern China, Taizhou, had concentrations of Pb and Cd to be 2-4 times in excess of 0.2mg/kg, the maximum allowable concentrations of these elements in food stuffs in China (Fu *et al*., 2008). In the same town, Liang *et al*. (2008) measure elevated levels (up to 18ng/g) of PBDEs in chicken tissues and concluded that these toxins may pose a threat to humans and the ecosystem. Rice paddy soil adjacent to e-waste recycling area in Zhejiang province were shown to reduce the germination rate of rice, while micronuclei assay using *Vicia fabia* indicated that the contaminations in these soils promote DNA damage (Zhang and Min, 2009).

2.6.2 E-waste contamination of air

Many e-waste contaminants are spread into the air via dust. This is a major exposure pathway for humans through ingestion, inhalation and skin absorption (Mielke and Reagan, 1998). The fact that dust is a major source for PBDEs exposure has been confirmed by a recent analysis that incorporated PBDE exposure data from different media into a pharmacokinetic model indicating that 82% of the overall estimated intake of PBDEs is through house dust and that the youngest have a greater intake on a pro kilogram basis (Lorber, 2008). Air samples taken near Guiyu contained polychlorodibenzo-p-dioxins between 65 and 2765pg/m^3, the highest level of atmospheric dioxins ever reported (Li *et al*., 2007a). Σpenta BDEs in air samples in Guiyu were approximately two orders of magnitude higher than concentrations monitored in the urban areas of Hong Kong and Guangzhou (places which already have higher levels of these substances than other urban and rural areas around the world) (Deng *et al*., 2007); and approximately three orders of magnitude higher than in the air of semi-rural sites in Europe (Lee *et al*., 2004).

Combustion of e-waste containing flame retardants have resulted in concentrations of total PBDEs of up to 16,575 pg/m^3 in aerial samples near Guiyu, some 300 times than in nearby Hong Kong (Deng *et al*., 2007). Aerial contamination of PBDEs in the city of Guiyu exceeds 11, 000pg/m^3 during the daytime, dropping to under 5, 000pg/m^3 at night (Chen *et al*., 2009). Similarly, high aerial concentrations of particulate PAHs, Cr, Cu and Zn have been reported with the Pb concentration found to be higher than for many other cities in Asia (Deng *et al*., 2006).

The report of Wang *et al*. (2007) showed the presence of 22 PBDE congeners, with low brominated PBDEs, which are more toxic accounting for 94.6% of the ΣPBDE, which is more than 100 times higher than other published data. Higher concentrations of PCDD/Fs and PAHs were also reported. The authors attributed this high concentration in the air to e-waste open burning since PBDEs are released when plastics containing brominated flame retardants are heated (e.g. melting of polymers). PCDD/Fs and PBDD/Fs have been identified in the air around Guiyu at a range of 64.9-2365 pg/m^3 and 8.1-466 pg/m^3 respectively. These were the

highest documented values of these compounds in ambient air in the world and are attributed principally to WEEE dismantling activities (Li *et al*., 2007a). PCDD/Fs values reported in other regions range from non-detectable to 12pg of 1-TEQ/ m^3 (Lohmann and Jones, 1998; de Assuncao *et al*., 2005; Hassanin *et al*., 2006), while PBDD/Fs levels documented for Kyoto and Osaka, Japan ranged between 1.8-12.1 pg/m^3 and 4.2-17 pg/m^3 respectively (Watanabe *et al*., 1995; Hayakawa *et al*., 2004).

2.6.3 E-waste contamination of water, sediment and aquatic systems

E-waste contaminants can enter aquatic systems via leaching from dumpsites where processed or unprocessed e-waste may have been deposited. Similarly, the disposal of acid following hydrometallurgical processes into waters or into soils, as well as dissolution or setting of airborne contaminants can also result in the contamination of aquatic systems (Robinson, 2009). Wastewater from Guiyu (Puckett *et al*., 2002; Bridgen *et al*., 2005; Wong *et al*., 2007b) and India (Bridgen *et al*., 2005; Keller, 2006) were reported to have Pb concentrations ranging from 17-247 times higher than in other areas with no known electronic activities. Wang and Guo (2006) reported up to 0.4mg/l of Pb in river water downstream of a recycling plant in Guiyu, some 8 times higher than the local drinking water standard (0.05mg/l). In nearby Lianjing river, an elevated concentrations of Ag, Cr, Li, Mo, Sb, and Se were reported by Wong *et al*. (2007a). Sediment from lagoon adjacent to the disposal and burning areas in Agbogboloshie market in Ghana was found to contain an elevated level of Cu, Pb, Zn and Cd (Bridgen *et al*., 2008). Sediments collected in a cyanide leaching area (Keller, 2006), lianjing river (Bridgen *et al*., 2005) and near open burning sites (Puckett *et al*., 2002; Leung *et al*., 2006) in China contained heavy metals especially Pb in concentrations higher than other non-electronic activity sites.

Aside from heavy metals, several reports have shown the presence of organic compounds in higher concentrations in aquatic systems. Bioaccumulation of PBDEs have been reported in Nanyang river near Guiyu (Luo *et al*., 2007); in water snake caught in an aquatic ecosystem near an e-waste recycling plant (Wu *et al*., 2008); in water fowl (Luo *et al*., 2009b); peregrine falcons (Lindberg *et al*., 2004); Virginia fresh water fishes (Hale *et al*., 2001); wild whitefish

and farmed rainbow trout from Swiss lakes (Zennegg *et al*., 2003); and in fishes collected from rivers in Michigan and Illinois, USA (Rice *et al*., 2002). Higher concentrations of PCDD/Fs in Lianjing riverbank (Luksemburg *et al*., 2002) and Suzhou creek, China (Li *et al*., 2007b) and in sediments collected in Elbe river, Europe (Stachel *et al*., 2004) have been documented. The authors concluded that these higher concentrations were due to the prevalence of electronic activities in the areas.

2.6.4 Human health perspectives of e-waste pollution

A limited number of studies have investigated the levels of contaminants in the bodies of e-waste processing workers. By the nature of their work, these people are exposed regularly to a wide range of toxic substances in significant amount. There is also evidence to suggest that residents in the vicinity are also receiving elevated exposures. Although it has been difficult until recently, to clearly link environmental pollution with WEEE activities, however, published studies over the past few years clearly indicated a causal relationship between pollution levels and emissions from WEEE activities. According to Sepulveda *et al*. (2010), atmospheric pollution due to burning and dismantling activities seems to be the main cause of occupational and secondary exposure at WEEE recycling and other activities sites.

Atmospheric contamination by WEEE activities have been shown to cause bioaccumulation of toxic chemicals in the exposed humans, especially children. Eighty percent of children in Guiyu suffer from respiratory diseases and they are particularly vulnerable to Pb poisoning (Wasserman *et al*., 1998; Qui *et al*., 2004; Needleman, 2004; Jain and Hu, 2006). Significantly higher blood Pb (Huo *et al*., 2007; Li *et al*., 2008b) and Cd (Zheng *et al*., 2008) levels had been reported in children in Guiyu and its associated lower cognitive abilities than children from a nearby control town (Li *et al*., 2008b). Elevated levels of Cr in umbilical cord blood from infants in Guiyu, with positive correlation to DNA damage and mother's exposure to e-waste activities have been documented (Li *et al*., 2008a).

The adult human body burden of PBDEs has been measured in several countries. Human breast milk samples showed high concentrations of PBDEs in Sweden (Noren and Meironyte,

2000), Venice and Rome (Ingelido *et al*., 2007), Catolonia-Spain (Schumacher *et al*., 2007), Japan (Ohta *et al*., 2002), United States (She *et al*., 2002; Schecter *et al*., 2003) and Pacific Northwest (She *et al*., 2007). Aside human breast milk, elevated concentrations of PBDEs have also been reported in human adipose tissues (Johnson-Restrepo *et al*., 2005), liver tissues from stillborn fetuses (Schecter *et al*., 2007) and cord blood (Guvenius *et al*., 2003; Huo *et al*., 2007). The five congeners most prevalently found in human tissues are: BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154, which make up to approximately 90% of the total human burden (Talsness, 2008). Exceptionally high concentrations of BDE-209 have been found in Chinese electronic dismantling workers (Bi *et al*., 2007; Qu *et al*., 2007). Zhao *et al*. (2006) documented an elevated level of PCBs derived from e-waste in ground and surface waters, agricultural soils, rice, eggs, fish and ultimately humans. A similar study (Zhao *et al*., 2008) reported high concentrations of 58ng/g, 30ng/g, and 182ng/g of PBBs, PBDEs and PCBs respectively in human hair samples from an electronic activity area. A median concentration of total PBDEs in serum of WEEE dismantling workers, twice that of the control group has also been documented (Yuan *et al*., 2008). Reports by Bi *et al*. (2007) and Nouwen *et al*. (2001) showed the highest concentrations of PBDEs (especially BDE-209) and dioxins (total PCDD/Fs) respectively ever recorded in humans, in workers and residents of Guiyu.

Human health associations with PBDEs congener exposures have been documented. Herbstman *et al*. (2010) reported a prenatal exposure to PBDEs and its associated lowered levels of mental and physical development in children. Reduced fecundabilty (Harley *et al*., 2010), adverse birth outcome (Chao *et al*., 2006), decreased sperm concentration and testis size (Akutsu *et al*., 2008), attention problems and decrease fine manipulative abilities in school age children (Roze *et al*., 2009), diabetes and metabolic syndrome (Lim *et al*., 2008), cryptochidism and increased serum luteinising hormone (LH) (Main *et al*., 2007), adverse birth outcomes (e.g. stillbirth, low birth weight, premature delivery; Wu *et al*., 2008), decrease free androgen index (FAI), follicle-stimulating hormone (FSH), and LH with increase inhibin B, sex hormone–binding globulin (SHBG) and free T_4 in men (Meeker *et al*., 2009), increase SHBG, inhibin B and estradiol in infants (Meijer *et al*., 2008), and decrease

thyroid-stimulating hormone (TSH) and subclinical hyperthyroidism in pregnant women (Chevrier *et al*., 2010) are all documented human health effects associated with PBDEs exposure.

Consequential to atmospheric pollution with dioxins, human exposure in Guiyu has resulted in levels 15-56 times higher than the recommended maximum intake by WHO (Chatterjee, 2007). Human exposure to dioxins begins with atmospheric emissions, of which incineration releases the largest quantity (Beck *et al*., 1994). Studies (Schramm *et al*., 1992; Tirler *et al*., 2001; Nakao *et al*., 2002; 2005) have shown that dioxin levels in human hair reflect those in the atmosphere. Elevated levels of dioxin in human milk, hair and placenta has also been reported (Chan *et al*., 2007), an indication of sufficient intake levels of these dioxins by humans via air, water or food stuffs, to pose a serious health risk. Luksemberg *et al*. (2002) showed that the total PCDD/F concentrations in hair samples of residents near WEEE recycling facilities in Guiyu ranges from 16.4-25.6pg WHO-TEQ/g dw, and were similar to the PCDD/F value reported in hair samples from a very contaminated pentachlorophenol site in China (Luksemberg *et al*., 1997) and about 29-466 times higher than the PCDD/F level of exposed subjects to ambient air in Tsukuba and Ryugasaki, Japan (Miyabara *et al*., 2005).

E-waste recycling workers from villages in the Junhai county has recently been shown to have chromosomal aberrations at a rate some 20-fold higher than villagers not exposed to e-waste (Liu *et al*., 2009). The authors concluded that e-waste is a potential source of genetic mutation and may induce cytogenetic damage within the general population exposed to e-waste pollution. Table 2.5 shows the evidence from experimental and human studies, on mode(s) of action and main long term effects from chronic/repeated exposure to E-waste chemicals. No such reports exist in Africa, however, the environmental contamination found in Ghana and Nigeria, suggests that children and people working on or living near the e-waste sites could be exposed to toxic chemicals (BAN, 2005).

2.7 Current studies on e-waste in the Nigerian environment

In spite of the huge amount of e-scrap constantly flooding the African nations most especially

Table 2.5: Evidence from experimental and human studies, on mode(s) of action and main long term effects from chronic/repeated exposure to E-waste chemicals. Available safe dose (total daily, or weekly, dietary intake or upper level) are reported.

Chemical dose	Mode(s) of action	Effects	Reference
PCDD/Fs	Significant bioaccumulation related to lipid solubility. Interaction with the AhR.	Reproductive and neurobehavioral development Immune development Carcinogenicity	TWI: 14pg WHO-TEQ/kgbw
PBDEs	Significant bioaccumulation related to lipid solubility. Interaction with thyroid hormones. BFR may activate Pathways related to nuclear receptors, as shown by the Expression of the CYP isoforms CYP1A1, CYP2B and CYP3A, representing of, respectively. Aryl-Hydrocarbon (AhR, dioxin receptor), Constitutive-Androstane and Pregnane-X receptors	Reproductive development Neurobehavioral development Thyroid function, Hormonal effect levels in animals start from *ca* 1mg/kgbw, but effects on spermatogenesis, suggesting hormonal causes have been observed at a low dose (60 µg/kgbw) of the PBDEs congener BDE-99	TDI: 0.15µg/kgbw
PCBs	Significant bioaccumulation related to lipid solubility. Congeners with different modes of action: DL PCBs are similar to PCDD/Fs (interaction with AhR), though generally less potent; NDL PCBs show different properties concerning toxicity and persistence: interference with the metabolism of	Both NDL and DL PCBs may exert a variety of toxicological effects, including carcinogenicity on multiple targets such as liver, thyroid, immune function, reproduction and neurobehavioral development. DL PCB may act as tumor promoters in tissues such as liver; different congeners may alter	TWI (DL PCB): 14pg WHO-TEQ/ kgbw

	thyroid and estrogens, oxidative stress	different pathways, such as the induction of oxidative stress and/or inhibition of apoptosis	
PAHs (high molecular weight)	Genotoxic damage	Carcinogenicity	
	Oxidative stress	Mutagenicity	
	Interaction with AhR	Teratogenicity	
Al	Interaction with Ca cell-cell communication	Skeletal development and metabolism, Neurotoxicity	
		Foetal toxicity	TWI: 1mg/kgbw
As	Oxidative stress	Skin alterations. Decreased nerve conduction	
	Interaction with glucorticoid receptor	Increased risk of diabetes and cancer (skin and other tissues)	
Cd	Oxidative stress	Kidney damage, renal toxicity, bone disease	0.14-0.26mg
	Interaction with essential elements as Ca and Se	(osteomalacia and osteoporosis). Possibly	per day
	Agonist of ERα	reproductive damage, and lung emphysema.	
Cu	Essential element[a], may be toxic at high dose levels	Liver damage	upper level: 5mg per day
Cr(VI)	Cytotoxicity	Carcinogenicity	
	Oxidative DNA damage, mRNA expression of	Reproductive functions	
	StAR, SF-1, 17β-HSD-1, 17β-HSD-2, FSHR, LHR	Endocrine function	
	ERα and ERβ		

	Hypothalamic-pituitary-gonadal axis	Ovotoxicity	
	Oxidative stress		
Fe	Essential element[a], may be toxic at high dose levels	Liver damage	not established
Hg	Interaction with sulphur aminoacids	Neurobehavioral development of children	
	Cell proliferation/differentiation/communication	(especially methymercury)	
	Interaction with Se, Methylmercury can bioaccumulate	Anemia, kidney damage, chronic neurotoxicity	
Pb	Interaction with sulphur aminoacids	Neurobehavioral development of children	
	Cell proliferation/differentiation/communication	Anemia, kidney damage, chronic neurotoxicity	
Se	Essential element[a], may be toxic at supranormal dose levels. Interaction with sulphur aminoacids	Hair loss, Nail brittleness, cardiovascular, renal and neurological abnormalities	300µg per day
Zn	Essential element[a], may be toxic at high dose levels. Impaired Cu metabolism	Increased risk of Cu deficiency (anemia, neurological abnormalities)	upper level: 25mg per day

[a]Essentiality or toxicity of chemical elements depends on chemical form, oxidation state and solubility.

Source: Frazzoli *et al*. (2010).

Nigeria, to date, only very few studies (mostly on management issues) have been documented on such an important issues as the pollution generated from crude e-waste processing, which is one of the major contributors of POPs to the environment. In the words of Frazzoli *et al*. (2010), "data are completely missing in the African scenario".

In Nigeria, the first documented study of e-waste and its potential effect was by Basel Action Network (BAN) in 2005. The study; "The digital dump-Exporting reuse and abuse to Africa", was a documentary of transboundary movement of e-waste into Nigeria. The study observed that an average of 500 containers enter Nigeria through the Lagos ports monthly, each containing about 800 monitors or CPUs, an indication of about 400, 000 second hand CPUs per month on the average and an annual importation of approximately 5 million scrap units or 60, 000 metric tons containing up to 18, 000 tons of plastic materials. The study further reported that between 25-75% of these transboundary e-waste exports are usually junk, which amounts to an importation of 15,000-45,000 tons of hazardous wastes containing about 1000-3600 tons of lead. There was also a similar study involving another non-profit organization called Greenpeace in 2009 (http://www.greenpeace.org/africa/en/bearingwitness/Following-the-e-waste-trail---Nigeria/). The organization followed a consignment of e-waste from USA to Lagos port, Nigeria. The study concluded that Nigeria, like other developing countries, with no capacity for environmental friendly recycling and technology, has become the dumping ground for West toxic wastes.

These studies were followed by few literatures on the material flow of e-waste into Nigeria; the present and possible management practices; and potential dangers of e-waste to the Nigerian environment (Schmidt, 2006; Osibanjo and Nnorom, 2007; Nnorom *et al*., 2007; Nnorom and Osibanjo, 2008a; Azuka 2009; Orisakwe and Frazzoli, 2010; Osuagwu and Ikerionwu, 2010). Only two studies exist in literature on the assessment of heavy metals from electronic components. Study by Nnorom and Osibanjo (2009) on heavy metal characterization of waste portable rechargeable batteries used in mobile phones, showed that the concentration of Co and Ni of the electrode of NiOH and Li-ion batteries are very high and far exceed the TTLC threshold limits used in the toxicity characterization of solid waste

by about 40 folds. A similar study on the toxicity characterization of waste mobile phone plastics, was the second documented e-waste component analysis in the Nigerian environment (Nnorom and Osibanjo, 2008b). The authors concluded that the level of Pb, Cd, Ni and As present in mobile phone plastics in the Nigerian environment may not be an immediate danger if appropriately managed. However, considering the large quantities being generated in Nigeria and the present low-end management practices in the country such as open burning, there appears to be a genuine concern over the potential for environmental pollution and toxicity to man and the ecology.

There is however an urgent need to study the causal relationship between the contamination levels and the various observed health implications on the human subjects occupationally and residentially exposed to these wastes. There is also a need for international collaboration in these studies as there are many limiting factors in Nigeria on such elaborate scientific studies. These limiting factors among other things include availability of modern equipment for air, soil, water, plants, animals and human analyses, as well as multidisciplinary approach to establish the causal effect of the observed health effects.

2.8 Future trends of e-waste generation

The global production of e-waste will change as economies grow and new technologies are developed. Electrical and electronic items have been known to be an integral part for the functioning of all but the most primitive economies, therefore, the total number of computers and other potential e-waste items is strongly correlated with the country's GDP in any given country (Robinson, 2009). For example, the report of the Basel Convention (2010)-'Recycling: from e-waste to resources' predicted that by 2020, e-waste from television will be 1.5 to 2 times higher in China and India while India e-waste from discarded refrigerators will double or triple (Robinson, 2009). Countries like Senegal and Uganda can expect e-waste flows from PCs alone to increase 4-8 fold by 2020. In South Africa and China, a prediction of e-waste from old computers by 2020 will jump by 200 to 400 percent from 2007 levels and by 500 percent in India.

The reports and predictions of Hischer *et al*. (2005) and Robinson (2009) show that there is

an exponential relationship between wealth and number of electronics, and that the richest billion people have 75% of all computers. This is an indication of faster increase in e-waste generation in richer countries than in poor countries for any given increase in GDP. For example, about 2.6% increase in GDP on the average in Europe between 2005 and 2008 recorded an annual growth of e-waste at a rate of 3-5% (Hischer *et al*., 2005).

Changes in technology will also affect the global mass of e-waste production. Short innovation cycles of hardware have led to a high turnover of devices (Robinson, 2009). The lifespan of central processing units in computers dropped from 4-6years in 1997 to 2years in 2005 (Babu *et al*., 2007). Advent of Liquid Crystal Displays (LCD) and more significantly, the increasing prevalence of laptop and notebook computers which weigh between 1 to 3 kg will significantly reduce the average mass of a discarded computer and hence the mass of computers in the e-waste stream (Micklethwait, 2009a; Robinson, 2009). E-waste growth from increasing wealth and shorter innovation cycles maybe offset by miniaturization and outsourcing of computer power, since in the case of netbooks, computing power and the associated potential e-waste production has been shifted from the end user to remote computing 'clouds', supported by warehouses of machines which may be located in another country (Micklethwait, 2009b; Robinson, 2009).

2.9 Recommended actions for effective management of e-waste

Recommended actions to cub the menace of e-waste have been documented (Hicks *et al*., 2005; Osibanjo and Nnorom, 2007; Hawari and Hassan, 2008). Some are targeted to create a wider awareness amongst the end-users, others are targeted to the individual nations and the international communities in enactment and subsequent strict enforcement of legislation and policies. These are as follows:

1. **Legislation for effective WEEE management**: as a result of the influx of transboundary e-waste and the low-end management practices in developing countries, there is an urgent need for introduction of legislation dealing specifically with e-waste in the developing countries. Established legislation such as EU Directives and draft legislation of National Development and Reform Commission

(NDRC), China, should be guidelines for such new legislations. Among other things, the following should be taken into cognizance:

a. WEEE collection, storage, recycling and/or disposal should be adequately funded.

b. Encouragement of WEEE recycling and disposal enterprises through legislative measures.

c. Development of best technology for WEEE management should be encouraged.

d. Have measures that will encourage the use and importation of EEE manufactured with non-toxic, non-hazardous substances and recyclable materials in accordance with the EU RoHS Directive.

e. Implementation of EPR mandating producers, importers/retailers the responsibility of collection, recycling and disposal of EoL EEE.

f. Introduction of standards and a certification system for second hand appliances as well as recycling and disposal enterprises in ensuring safety and environmentally friendly processing of WEEE.

2. The 3R initiative: the 3R initiative is one approach to dealing with the e-waste problem. It is summarize as follows:

a. Reduce: the amount of waste generated should be reduced and the use of toxic substances (such as Hg and Pb) should also be reduced or eliminated.

b.   Reuse: electronics which are still useable should be reused or parts of the items.

c. Recycle: the use of waste as a resource for the manufacture of other items.

The success of this initiative however depends on the organized collection and transportation of e-waste as well as the public recognition of hazards involved in e-waste.

3. Citizen's responsibility: The success of EPR depends on the full cooperation of the citizenry. Each citizen should be responsible for the type of EEE they purchase, whether it contains toxic substances or it is toxic-free, and the subsequent release of such items after he/she is done with it, to foster recycling by the appropriate bodies. The citizens also have to be educated not to dispose their WEEE with MSW or to store them in their attics or backyards.

2.10 Genetic toxicology

Human and animals (terrestrial and aquatic) are exposed to one form of contaminant or another; however, the level of exposure varies depending on the environment. Exposure could ultimately alter the health and well being, survival, sustenance and genetic make up of the organism. When the contaminants alter the genetic component, then it is referred to as genetic toxicant. In essence, genetic toxicology considers the adverse effects of toxicity on the material and mechanism of heredity. It helps to strike a balance between mutagenic and carcinogenic potential of a compound. Although over the years, it has focus on identification of hazardous chemicals/compounds but recently its application now covers the quantitative and even qualitative genetic risk assessment. The genotoxic risk of agents has been characterised for incidence of heritable diseases through germ cell mutations, or for risk of cancer via induction of somatic cell mutations (Cimino, 2006).

Genetic toxicology is a dynamic field that changes with demand for risk assessment of chemicals and wastes. Genetic toxicology gives improved understanding of basic cellular process and alterations that can affect the integrity of the genetic material and functions. Though the short-term analysis gives much insight into the mechanism of mutagenicity and likely the resultant carcinogenicity, analysis of mutagenicity in the sex cells contribute to better appreciation for a long term consequences of mutagenesis in human population. Improvement in both the quantitative and qualitative assessment of mutation in somatic cells and germ cells has enhanced assessment of risk of cancer process.

Genetic toxicological study is evolving to accommodate increasing quantity and multiplicity of constituents of waste. For example in most of the waste – domestic, municipal and industrial - the components are of multiple chemical characteristics and therefore it may be difficult to identify the exact agent responsible for toxicity. But, the modifications of the genotoxicological procedures allow for testing of multiple compounds, factoring in the possibility of synergistic, additive and antagonistic interactions.

The paradigm of genetic toxicology is moving towards (eco)toxicogenomics using techniques like comet assay to measure DNA damage by substances and microarray to assess the effect

of chemicals, wastes or toxicants on gene expression (Littieri, 2006). The microarray technique allows analysis of many genes simultaneously (Littieri, 2006; Iguchi *et al.,* 2006).

2.10.1 Genetic toxicology and cytotoxicity testing

Genetic toxicology testing is performed to determine the ability of substances to produce mutations or chromosome aberrations, or otherwise to induce DNA damage. Such testing has been central to the safety evaluation of chemicals since the late 1970s. Concern for induction of genetic damage began with concern for heritable gene and chromosomal germ cell mutations in the offspring of exposed individuals. However, with the accumulating evidence that mutagenesis was an early step in the development of a tumor, and that carcinogenic chemicals were mutagenic (Ames, 1971; Ames *et al*., 1973; McCann *et al*., 1975; Sugimura *et al*., 1976; Purchase *et al*., 1978), the emphasis of genetic toxicology testing switched from heritable mutations to carcinogenesis, and a positive test for *in vitro* or *in vivo* somatic cell mutations is considered presumptive evidence of carcinogenicity, and will often trigger a regulatory requirement for carcinogenicity testing.

The *in vitro* genetic toxicity assays currently used for regulatory approval of chemicals are the bacterial (*Salmonella*; *Escherichia coli*), mammalian cell mutagenicity (L5179Y mouse lymphoma cells; Chinese hamster ovary (CHO) cells), and/or mammalian cell chromosome damage (L5178Y; CHO or Chinese hamster lung (CHL) cells; human lymphocytes) assays. *In vivo* testing uses primarily the rodent bone marrow cell chromosome aberration or micronucleus (MN) assay. Substances that are positive in the *in vitro* tests are considered to be of most concern for inducing cancer or genetic mutations in rodents and, by extension, in humans, and often require further testing. These *in vitro* positives are then tested in rodents to determine if they have the capability of inducing genetic damage in the animal. *In vivo* genetic toxicity testing currently is also a prerequisite for identifying germ cell mutagens, that is, those that have the potential to mutate sperm or egg cells resulting in offspring either expressing or only carrying a mutant gene.

2.10.1.1 The micronucleus test

An alternative method for measuring gross chromosome damage is the MN test. In this procedure, after the cell completes mitosis, chromosome fragments resulting from breaks, or rearranged chromosomes, are not fully incorporated into the nuclei of the daughter cells, and appear as small secondary nuclei in the cell cytoplasm. Because MNs can be scored more easily than chromosome preparations, they are preferred by many for evaluating chromosome damage. In addition, because whole chromosomes are not incorporated into the main nuclei, this method can be used to easily score aneuploidy events, that is, the loss of a chromosome, although an additional staining procedure to identify MNs containing whole chromosomes is needed. One important difference between the traditional chromosome damage tests and the MN test is that, in the traditional test it is not necessary for the cells to complete mitosis and cell division before being scored for aberrations. However, in the MN test, in order for the MNs to form, it is necessary for the cell to complete mitosis and form intact daughter cells. One problem that arises from this process is that it is necessary to determine whether the cells being scored are those that have undergone a successful cell division (and therefore are able to express an MN if it exists) and those that, because of toxicity, were unable to enter mitosis and subsequently divide. To this end, a number of recent studies have been designed to compare the various methods for determining cell division and cytotoxicity (Fellows *et al*., 2008; Lorge *et al*., 2008).

Micronucleus assay explores the principle of *erythropoiesis* to detect the clastogenicity, aneugenicity and spindle poisoning potential of a substance. The genetic endpoint of this bioassay is the formation of *micronucleus* in the immature erythrocyte. Micronuclei which are also referred to as "Howell-Jolly-Bodies" are generally smooth, round or ellipse-shaped. (Krishna and Hayashi, 2000).

The process of erythropoiesis in the hematopoietic organs (bone marrow and spleen), involves proliferation and maturation of stem cells. Administration of a given substance during cell proliferation may cause chromosome damage and also act on the macromolecules related to the function of chromatid disjunction (e.g, tubulin) causing spindle dysfunction,

depending on the mechanism of action. These anomalies (a fragment or a whole chromosome) may lag behind in the cell during cell division and may not become integrated into the daughter nuclei, rather may eventually form micronuclei, which can be seen in the cytoplasm (Figure 2.7) (Krishna and Hayashi, 2000).

During maturation, when an erythroblast develops into polychromatic erythrocyte (PCE, young erythrocyte with basophile and stains bluish-purple with May-Gruenwald and Giemsa differential staining), the main nucleus is extruded; any micronucleus that remains behind in the otherwise enucleated cytoplasm. Recognition of micronuclei is enhanced because of the lack of the main nucleus. More over, the PCEs with time, lose RNA and contain primarily hemoglobin; thus, become normochromatic erythrocytes (NCE, mature erythrocytes). The NCEs have some distinguishing characteristic that differentiates it from PCEs. They are somewhat smaller than the PCE, acidophilic and stain pinkish-orange with May-Gruenwald and Giemsa differential staining).

The micronucleus bioassay has been recommended for both *in vitro* and *in vivo* routine toxicological assessment of chemicals and wastes (ICPEMC, 1983; Heddle *et al.*, 1983; DNH and W/DOE, 1988; Cimino, 2006). However important is the *in vitro* micronucleus assay, the *in vivo* procedure is of more relevance to assessing genotoxic potential to human putting into consideration: metabolism, pharmacokinetics and other biological activities. This bioassay has continued to evolve since its original description (Schmid, 1975).

The genotoxicity assay is assessed by an increase in the frequency of micronucleated PCEs (MNPCEs) in the test agent-treated animals which is indicative of an induce chromosome damage. However, the PCE-to-NCE ratio between test agent-treated animals and vehicle-control animals provide a cytotoxicity index (Krishna and Hayashi, 2000). Micronucleus assay can also be carried out on peripheral blood. Dobrzyńska and Gajewski (2000) evaluated the induction of micronuclei in mouse bone marrow by combined exposure of low doses of X-rays and acrylamide. Also, micronucleus assay has been used effectively in the evaluation of genetic damages involved in pathogenesis of some disease conditions in human (Aksoy *et al.,* 2006).

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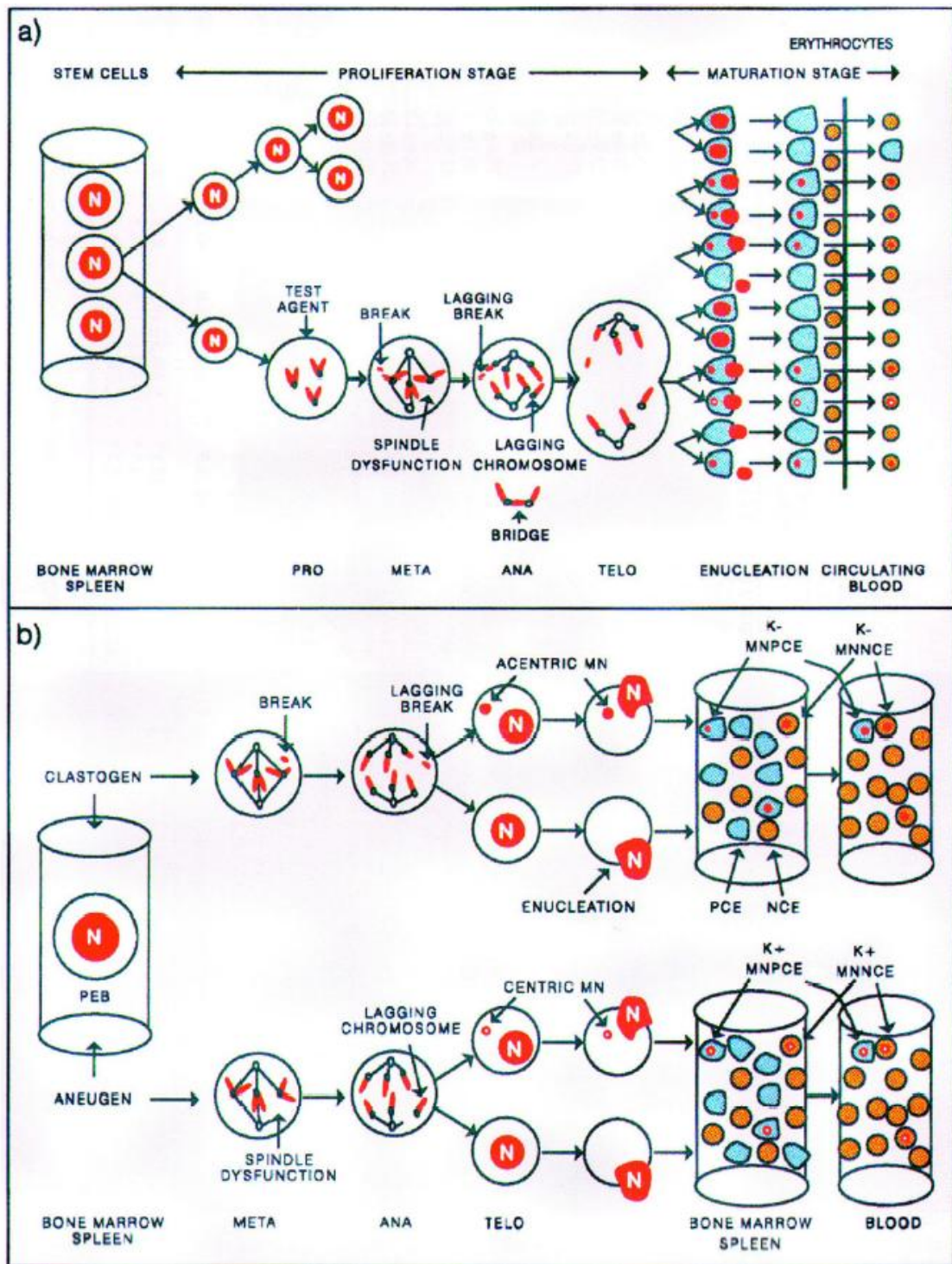
Figure 2.7: (a) The process of erythropoiesis in vivo; (b) the mechanism of micronucleus formation in the (PCEs) and (NCEs). Also, classification of kinetochore-positive (KC) and kinetochore-negative (K−) erythrocytes. N, nucleus; PEB, proerythroblast; MN, micronucleus. (Krishna and Hayashi, 2000)

2.10.1.1.1 Merits and demerits of micronucleus assay

Although there are shortcomings of micronucleus assay, including possibility of false-negative because of tissue specificity, inability to show the actual type of chromosome aberration induced by a clastogenic agent, it has some advantages over the chromosome aberration assay.

(i)   It has advantage of high turnover.

(ii)   It is technically simple and time and cost effective.

(iii)   The endpoint is more objective, easily recognised and amenable for automation.

(iv)   It detects both clastogens and aneugens.

(v)   It can be easily be integrated into general toxicological studies.

(vi)   Micronuclei can be observed throughout the cell cycle, and the problem of number of scorable cells is eliminated.

(vii)   A favourable karyotype of the sampling animal is not required.

(viii) The spontaneous background is low.

(ix)   Time of sampling is less critical because micronuclei formed during cell division persist through the next interphase.

2.10.1.2 Mouse sperm morphology test

For many years, semen analysis has been routinely performed to diagnose testicular damage and infertility in human and domestic animals. Though it is generally agreed that large reductions in sperm number or mobility or large increase in sperm with abnormal shapes are associated with reduced fertility (Amelar, 1966), it is not clear whether smaller changes are also associated with fertility changes. Sperm tests provide a direct measure of quality of sperm production in chemically treated animals. However, the genetic consequences of fertilisation by sperm affected by chemical exposure during spermatogenesis remain unclear; embryonic death or the transmission of genetic aberrations to live-born progeny, are also possibilities.

Studies evaluating the genetic consequences of chemically induced sperm changes have focused mainly on understanding the genetic basis of chemically shape abnormalities in mice.

A number of lines of evidence suggest that induced change in sperm morphology reflect genetic damages in the male germ cell. First, considerable evidence indicated that sperm shaping is polygenetically controlled by numerous autosomal and sex-linked genes including: T-locus alleles, factors on chromosome 17 etc. (Illison, 1969). Second, agents that show sperm shape abnormality in offspring of treated males also induce abnormalities in the exposed males (Topham, 1980).

Sperm tests have been conducted for several purposes. For the mouse sperm morphology, most exposures were to single purified agent selected to assess relationship with mutagenicity and carcinogenicity (Wyrobek *et al*., 1983). The sperm morphology test has also been used as a short-term bioassay for spermatotoxic effect of agents responsible for significant human exposures (e.g anesthetic gases). Studies have shown that sperm morphology test can also be used to evaluate commercial agents for which large potential human exposure may be involved at various phases of their manufacture (e.g anilines: Topham, 1980). The major uses of sperm test were to evaluate the ability of agents to modulate fertility (either by improving fertility or by acting as male contraceptive), to study side effects of drug therapy, and to develop animal model of chemically induced spermatogenic damage. Animal sperm tests (especially in studies where repeated sperm samples were taken before, during, and after exposure) have provide important data in dose dependence, spermatogenesis stage specificity as well as the reversibility of induced sperm effects (Wyrobek *et al*., 1983).

Wyrobek *et al*. (1983) critically evaluated the mouse sperm head morphology test and other test in non human mammals and reported that when the test germ cells are exposed *in vivo* such that a positive result in the test demonstrates an agent's ability to damaged spermatogenesis; the induction of abnormally shaped sperm in mice appears to be very sensitive to mammalian germ-cell mutagens, the test may therefore be a valuable tool for identifying germ-cell mutagens. In other words, the test is valuable tool in safety evaluation for assessing an agent's potential adverse effects on sperm production. Moreover, positive result in the mouse sperm morphology test appears to be highly specific for carcinogenicity although many carcinogens may produce negative responses in the test. Different strains of

mice may be insensitive to particular agents. It is quite possible that hybrid male are sensitive to mutagens/carcinogens because they carry heterozygous defects at a large number of alleles that are involved in the differentiation of spermatozoa, so any interference with differentiation of sperm by mutagens/carcinogens may be expressed as defects in sperm-head morphology. Furthermore, certain factors such as ischemia infection and changed in body temperature may cause false positive result or responses. However, careful study design and good animal husbandry should minimize the occurrence of these defects.

The mouse sperm morphology test is technically simple, inexpensive, easily quantitated and relatively rapid when compared with other *in vivo* short-term test. The test detects carcinogens of a variety of types including polycyclic hydrocarbons and alkylating agents (Wyrobek and Bruce, 1975). The test's protocol is adaptable to different species, dosage regimes, sampling times and routes of exposure and this versatility makes it a very useful animal model for human exposure. Consequently, far more compounds encompassing diverse classes of chemical and biological activities have been evaluated by the sperm morphology test than by any other sperm test in animals or man.

Advantages of sperm morphology test include:

1) Germ cells are exposed *in vivo*. A positive result demonstrates an agent's ability to damage spermatogenesis.

2) It is a valuable tool in safety evaluation for assessing an agent's potential adverse effect on sperm production.

3) The test is very sensitive to mammalian germ-cell mutagens and therefore identifies germ-cell mutagens.

4) The results are highly specific for carcinogenicity.

5) It is technically simple, inexpensive and relatively rapid (easily quantitated).

6) Adaptable to different species, dosage regimed, sampling times, routes of exposure etc.

7) Versatility makes it a useful animal model for human exposure.

Thus, the test may become a valuable tool for evaluating the human genetic hazard of

environmental and industrial chemical, since sperm abnormalities can be monitored in people (Mosuro, 1991).

2.10.1.3 Comet assay

A number of techniques for detecting DNA damage, as opposed to the biological effects (e.g., micronuclei, mutations, structural chromosomal aberrations) that result from DNA damage have been used to identify substances with genotoxic activity. Until recently, the most frequently used methods involved either the detection of DNA repair synthesis (so-called unscheduled DNA synthesis or UDS) in individual cells, or the detection of DNA SSB and ALS in pooled cell populations using the alkaline elution assay. The UDS technique is based on the replication of DNA during the excision repair of certain types of DNA lesions, as demonstrated by the incorporation of tritiated thymidine into the DNA repair sites. While providing information at the level of the individual cell, the technique is technically cumbersome, requires the use of radioactivity, and is limited in sensitivity. The alkaline elution assay ignores the critical importance of intercellular differences in DNA damage and requires relatively large numbers of cells. A more useful approach for assessing DNA damage is the single-cell gel (SCG) or Comet assay.

The terms "SCG" or "Comet" is used to identify the individual cell DNA migration patterns produced by this assay. O¨stling and Johanson (1984) were the first to develop a microgel electrophoresis technique for detecting DNA damage at the level of the single cell. In their technique, cells embedded in agarose were placed on a microscope slide, the cells were lysed by detergents and high salt, and the liberated DNA electrophoresed under neutral conditions. Cells with an increased frequency of DNA double-strand breaks (DSB) displayed increased migration of DNA toward the anode. The migrating DNA was quantitated by staining with ethidium bromide and by measuring the intensity of fluorescence at two fixed positions within the migration pattern using a microscope photometer. The neutral conditions used greatly limited the general utility of the assay.

Subsequently, Singh *et al*. (1988) introduced a microgel technique involving electrophoresis under alkaline (pH .13) conditions for detecting DNA damage in single cells. At this pH, increased DNA migration is associated with increased levels of frank SSB, SSB associated with incomplete excision repair sites, and ALS. Because almost all genotoxic agents induce orders of magnitude more SSB and/or ALS than DSB, this version of the assay offered greatly increased sensitivity for identifying genotoxic agents. Two years later, Olive *et al*. (1990a) introduced another alkaline version of this assay in which DNA is electrophoresed at a pH of 12.3. Since the introduction of the alkaline (pH 13) Comet assay in 1988, the breadth of applications and the number of investigators using this technique have increased almost exponentially. Compared with other genotoxicity assays, the advantages of the technique include:

(1) its demonstrated sensitivity for detecting low levels of DNA damage;

(2) the requirement for small numbers of cells per sample;

(3) flexibility;

(4) low costs;

(5) ease of application;

(6) the ability to conduct studies using relatively small amounts of a test substance; and

(7) the relatively short time period (a few days) needed to complete an experiment.

During the last decade, this assay has developed into a basic tool for use by investigators interested in research areas ranging from human and environmental biomonitoring to DNA repair processes to genetic toxicology. General reviews on this technique that have been published include Anderson *et al*. (1998), Rojas *et al*. (1999) and Speit and Hartmann (1999). Attractive uses of this assay in genetic toxicology include:

(1) as a potentially high-throughput screening assay;

(2) in mechanistic studies to distinguish between genotoxicity versus cytotoxicity induced chromosomal damage;

(3) in mechanistic in vivo studies to distinguish between genotoxic versus non-genotoxic carcinogens; and

(4) potentially, as part of a battery of *in vitro/in vivo* assays used for regulatory submissions. However, the SCG assay has yet to undergo appropriate multi-laboratory, international validation studies to demonstrate its inter-laboratory and intra-laboratory reproducibility and reliability and the adequacy of its performance against currently accepted methods (ICCVAM, 1997). As a test for genotoxicity, the Comet assay can be used to identify possible human mutagens and carcinogens (Anderson *et al*., 1998). However, a perfect correlation between chemicals positive in this test and carcinogenicity is not expected. The correlation would be expected to depend on chemical class and on the mechanism of carcinogenicity involved.

2.10.1.4 MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

The MTT colorimetric assay is an established method of determining viable cell number in proliferation and cytotoxicity studies. This assay is based on the cleavage of the yellow tetrazolium salt, MTT, to form a soluble blue formazan product by mitochondrial enzymes, and the amount of formazan produced is directly proportional to the number of living, not dead cells, present during MTT exposure. Since the MTT assay is rapid, convenient, and economical, it has become a very popular technique for quantification of viable cells in culture (Sylvester, 2011). Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past, however, MTT assay has proven to be one of the best. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve (Teodoro *et al*., 2011).

Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance (Bernardi, 1999).

The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves. The MTT method of cell determination is most useful when cultures are prepared in multiwell plates. For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells (Sylvester, 2011).

2.11 Biochemical tests for the assessment of oxidative damage

Many diseases (genetic diseases inclusive) are known to be caused by free radicals and oxidative stress within the biological system. Free radicals, also known as reactive oxygen species (ROS) are atoms or atomic groups that contain unpaired electrons. Since electrons have strong tendency to exist in a paired rather than an unpaired state, free radicals indiscriminately pick up electrons from other atoms, converting the other atoms into secondary free-radicals, and thus setting up a chain reaction that can cause substantial biological damage. Free radicals react with key organic substrates such as lipids, proteins and nucleic acids (especially DNA). Oxidation of these biomolecules can damage them, disturbing their normal functions and may contribute to a variety of disease state (OXIS International Inc., 2003). Oxidative stress on the other hand occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them using endogenous antioxidants. Oxidative stress is imposed on cells as a result of one of the following factors:

(i)   an increase in oxidant generation

(ii) decrease in antioxidant protection or

(iii) a failure to repair damages by oxidation.

Cell damage is induced by ROS (Nicholls and Budd, 2000) and so the generation of ROS in the system can be responsible for the cytotoxic and genotoxic effects observed in animals exposed to e-waste leachates.

The oxidative stress that ensues when the normal balance between the production of reactive oxygen species (ROS) and the antioxidant ability of the target cell is upset has been implicated in many diseases and ROS may interact with various critical cellular macromolecules, including DNA, to produce damage. Several different pathways by which oxidative DNA damage occurs have been proposed, including chemical modification of nucleotides. Fortunately, mammalian cells possess an efficient biological system to protect themselves from the damaging effects of ROS, which can be produced both endogenously and exogenously. Defence against xenobiotic toxicity, comprised of many kinds of antioxidants, is well characterized in mammals (Noguchi *et al.*, 2000). These are classified by function into four categories: preventive, that suppresses the formation of free radicals; radical scavenging, suppressing chain initiation and/or chain propagation reactions; repair; adaptation, with formation and transport of the appropriate antioxidant to the right site.

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition (Best, 2007). Antioxidants are notable for boosting the immune system because immune system cells in the bloodstream are so easily accessed by free radicals as well as by antioxidants. Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Superoxide dismutase, catalase, and glutathione reductase are among the most important antioxidant enzymes.

2.11.1 Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to

them by superoxide, the most common free radical in the body. The enzyme superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one oxygen. Superoxide dismutase helps the body use zinc, copper, and manganese. There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cells' cytoplasm, and Mn SOD protects their mitochondria from free radical damage. SOD acts as both an antioxidant and anti-inflammatory in the body, neutralising the free radicals that can lead to wrinkles and precancerous cell changes. The amount of this enzyme in the liver is a function of the presence of ROS in the same organ (Best, 2007).

2.11.2 Catalase

Catalase is one of the most potent catalysts known. The reactions it catalyses are crucial to life. Catalase catalyses conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. Catalase also uses hydrogen peroxide to oxidise toxins including Phenols, Formic Acid, Formaldehyde and Alcohols. Superoxide dismutase without catalase (CAT) or glutathione dismutase to remove hydrogen peroxide is of little value (Van Bladeren, 2000).

2.11.3 Glutathione dismutase (GSH)

The glutathione system (glutathione, glutathione peroxidase and glutathione reductase) is a key defense against hydrogen peroxide and other peroxides. Glutathione peroxidase 1 is part of the cellular antioxidant defence system by catalysing the reduction of hydrogen peroxide (and various organic hydroperoxides) to water using reduced glutathione as a co-substrate. GSH is the most abundant intracellular thiol-based antioxidant, prevalent in millimolar concentrations in all living aerobic cells, and plays an important role in the cellular defense cascade against oxidative injury (Armstrong, 1997; Van Bladeren, 2000; Hsu *et al*., 2002). It also serves to detoxify some endogenic and exogenic compounds with conjugation reactions catalyzed by glutathione S-transferases (Armstrong, 1997; Van Bladeren, 2000). GSH is a cofactor for glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide to

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water and oxygen, hence limiting the formation of hydroxyl radical, the highly toxic reactive oxygen species (Hsu *et al*., 2002).

2.11.4 Alanine aminotransferase (ALT)

An alanine aminotransferase (ALT) test measures the amount of this enzyme in the blood. ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT is measured to see if the liver is damaged or diseased. Low levels of ALT are normally found in the blood. But when the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels go up. Most increases in ALT levels are caused by liver damage. The ALT test is often done along with other tests that check for liver damage, including aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), and bilirubin. Both ALT and AST levels are reliable tests for liver damage (Hsu *et al*., 2002).

2.11.5 Aspartate aminotransferase (AST)

An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. AST formerly was called serum glutamic oxaloacetic transaminase (SGOT). Low levels of AST are normally found in the blood. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days. The AST test may be done at the same time as a test for alanine aminotransferase, or ALT. The ratio of AST to ALT sometimes can help determine whether the liver or another organ has been damaged. Both ALT and AST levels can test for liver damage (Van Bladeren, 2000).

2.12 Apoptotic studies

The term, "apoptosis" or "programmed cell death" defines a genetically encoded cell death program leading to specific biochemical and morphological alterations, which are distinct

from necrosis or accidental cell death (Pan *et al*., 2001; Galluzzi *et al*., 2007; Franco *et al*., 2009). Apoptosis is a well-controlled, tightly-regulated physiological process, in which the cells participate in self-destruction. A large body of evidence suggests that apoptosis is a central mechanism in embryogenesis and morphogenesis, immune system regulation, hematopoesis and control of normal tissue turnover (Vaux and Korsmeyer, 1999), but it has been also implicated in a variety of diseases (Arends and Wyllie, 1991). The morphological signs of apoptosis are cellular shrinkage, membrane blebbing, and nuclear condensation and fragmentation (Pennell and Lamb, 1997; Solomon *et al*., 1999; Vermes *et al*., 2000; Pan et al., 2001). Cell death is traditionally associated with necrosis, but there is evidence suggesting that some environmental pollutants are toxic and can trigger apoptosis (Robertson and Orrenius, 2000; Franco *et al*., 2009). It seems that low doses of toxicants preferentially induce pathways of active cell death and only very high doses lead to necrosis (Lennon *et al*., 1991; Gomez-Lechon *et al*., 2002). Failure of cells to undergo normal apoptotic cell death, or increased cell loss by apoptosis, may be involved in the pathogenesis of cancer, autoimmune disorders, neurodegenerative disorders, AIDS and myelodisplastic syndromes. Since apoptosis is involved in an ever-growing number of physiological and pathological processes, reliable methods for detecting cell death (apoptosis and necrosis) are essential. Several sensitive methods for detecting apoptosis have been developed, based on the different morphological or biochemical features of apoptosis and necrosis.

2.12.1 Mitochondrial membrane potential

Mitochondrial membrane potential is a key indicator of cell health and mitochondrial permeability transition which is an important step in the induction of cellular apoptosis. JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazo-lylcarbocyanine iodide), a lipophiliccationic dye, is widely used to detect mitochondrial de-polarization. It is a fluorescent dye that accumulates in mitochondrial and reports the membrane potential across the matrix membrane (Reers *et al*., 1991, 1995). Because this value is highly related to mitochondrial respiratory rate, JC-1 can be used as an indicator of mitochondrial activity. The evaluation of mitochondrial membrane potential of cells is crucial, since any disturbance can

dramatically change the maintenance of bio-energetic state of cells (Szabo *et al*., 2011), leading to deficiency in power generation and subsequent cell death (Bernardi, 1999; Teodoro *et al*., 2011).

2.12.2 Fluorescence microscopic analysis of cell death using Acridine orange/ethidium bromide (AO/EB) double staining

Acridine orange is taken up by both viable and nonviable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA) or red fluorescence if bound to single stranded nucleic acid (RNA). Ethidium bromide is taken up only by nonviable cells and emits red fluorescence by intercalation into DNA. There are four types of cells according to the fluorescence emission and the morphological aspect of chromatin condensation in the stained nuclei. (1) Viable cells have uniform bright green nuclei with organized structure (PMNCs also have orange cytoplasm). (2) Early apoptotic cells (which still have intact membranes but have started to undergo DNA cleavage) have green nuclei, but perinuclear chromatin condensation is visible as bright green patches or fragments. (3) Late apoptotic cells have orange to red nuclei with condensed or fragmented chromatin. (4) Necrotic cells have a uniformly orange to red nuclei with organized structure (Silva *et al*., 2012).

2.12.3 Cell cycle analysis using flow cytometry

Flow cytometry is adapted for analysis of various cellular components (nucleic acids, lipids, proteins, etc), organelles (lysosomes, mitochondria, etc) or functions (viability, enzymatic activities, etc). However, at the present time, many applications remain based on immunofluorescence or cellular DNA content studies. This technique has broadly contributed to improve knowledge on the cell cycle, usually only by taking DNA content into account. Studies are performed with DNA-specific dyes for which, considering staining conditions, emitted fluorescence is proportional to DNA content present in cells (Kerker *et al*., 1982). Mathematical algorithms and software adapted to analyse cell distribution histograms are then used to rapidly estimate cell repartition in the various cycle phases (Baish *et al*., 1975; Barlogie *et al*., 1976; Fox, 1980). However, these monoparametric analyses do not

discriminate cells in different metabolic compartments, but with the same DNA content. Multiparametric analyses can be used to improve cycle phase identification. They take into account DNA and other cellular components, such as RNA, total proteins (Darzynkiewicz and Kapuscinski, 1990; Darzynkiewicz *et al*., 1980; Shapiro, 1981), or surface antigens (Leong *et al*., 1984). They can also consider some events occurring during the cell cycle, like DNA synthesis (Dolbeare *et al*., 1985), cellular volume increase or chromatin conformation modifications (Darzynkiewicz and Kapuscinski, 1990). Moreover, kinetic studies can be accomplished to follow cell progression during each phase (Terry *et al*., 1991; Zaitterstrom *et al*., 1992). Cycle analysis by flow cytometry is interesting in fundamental research but also in the biomedical field. In pharmacology, it allows *in vitro* tests for new drugs, such as antitumoral factors (Ashihara *et al*., 1978; Kimmel and Traganos, 1985; Watson *et al*., 1987), in order to develop new treatments. In oncology, cell DNA content and their distribution in the various cycle phases can be used to detect pathological cells (Barlogie, 1984), to establish prognosis (Oljans and Tanke, 1986; Kallioniemi *et al*., 1988) or to monitor treatments (Baserga, 1984; Wijkstrom *et al*., 1984). Otherwise, flow cytometric analyses on plant cells concern generally their DNA content determination and their repartition in the various cell cycle phases (Bergounioux et al., 1988; Dolezel, 1991), and numerous studies deal with plant ploidy level determination (Brown *et al*., 1991). Cell DNA content studies have also contributed to improve information on testicular cells and sperm (Evenson *et al*., 1986), or on cell-virus interactions (Lehman *et al*., 1988). In recent years, flow cytometry has been used as a tool for the evaluation of cell cycle and cell death in cytogenotoxicity studies (Watanable *et al*., 2002). Flow cytometry involves the use of light scatter measurements as indicators of cell size and shape, whereas fluorescence intensity allows the evaluation of DNA content and quantification of nuclei in the cell cycle phases (Dolezel and Bartos, 2005; Dolezel *et al*., 2007). These parameters allow the evaluation of effects on the cell cycle and cell death. In addition to determining the relative cellular DNA content, flow cytometry also enables the identification of the cell distribution during the various phases of the cell cycle. Four distinct phases could be recognised in a proliferating cell population: the G1-, S- (DNA synthesis phase), G2- and M-phase (mitosis) (Dolezel *et al*., 2007). Diverse software containing

mathematical models that fit the DNA histogram of a singlet have been developed in order to calculate the percentages of cells occupying the different phases of the cell cycle.

CHAPTER THREE

Materials and methods

3.1 Sampling sites

The study sites are the main dumpsites of Alaba International and Computer Village markets in Lagos State (latitudes $6^{o}23´$N and $6^{o}41´$N and Longitude $2^{o}42´$E and $3^{o}42´$E), South-west, Nigeria (Figure 3.1). Lagos has a total landmass of about $8345km^2$, is physically small in size and is the most densely populated state in the country, with an estimated population of about 10 million inhabitants (Nigeria Population Commission, 2006). The Alaba International market and Computer village were residential neighborhoods only until late 1999 and early 2000; they contain hundreds of small-scale businesses that sell, repair, and service, ranging from fairly used EE, pirated software to brand new ones. Electronics in these markets are mostly used electronics imported from Europe, North America, and China (Basel Action Network, 2002). Workers in these markets are low-paid laborers who repair and resell these imported EEE. The markets have both formal and informal dumpsites where electronics which are not repairable are thrown and later set ablaze to reduce the volume.

3.2 Sample collection

3.2.1 Raw leachate and well water collection

Raw leachate was collected in May–July 2008 (rainy season) from different points at the edges (on the ground) of the dump sites where leachate flow out into the surrounding environment into clean 25 L plastic containers and immediately transported to the laboratory. The composite sample from each site was filtered using 15 cm filter paper (Whatman®, England), pH measured, and stored at 4°C until use. The raw leachates were labeled ARL and CRL for Alaba market raw leachate and Computer village market raw leachate respectively. Well water from 3 wells in each market in the vicinity of e-waste dumpsite was collected into a 25 L plastic container and immediately transported to the laboratory. The pH was measured and stored at 4°C until use. The well water were labeled AWW and CWW representing Alaba market well water and Computer village market well water respectively.
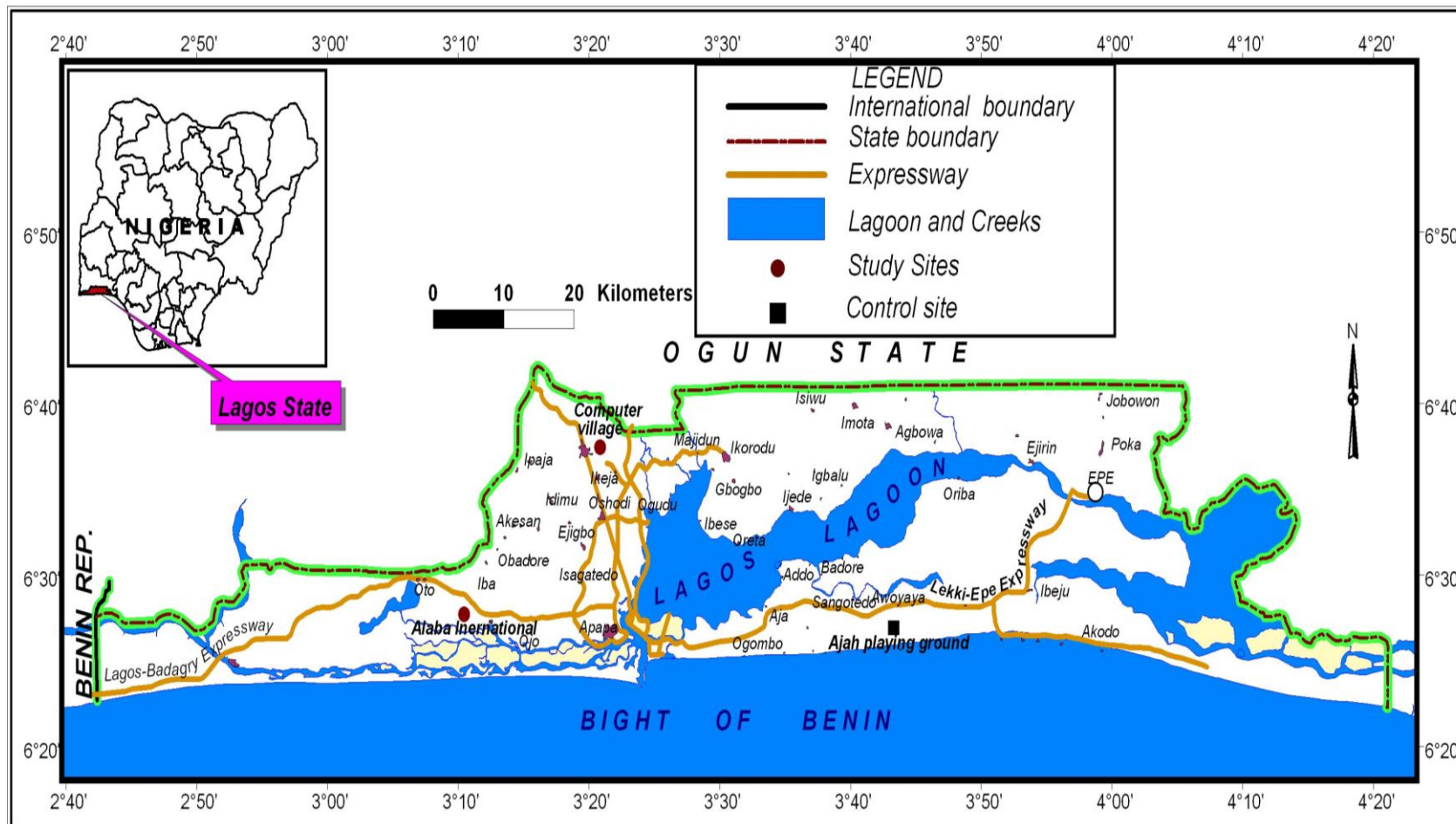
Figure 3.1: Map of Lagos state showing Alaba International and Computer Village electronic markets and Ajah playing ground

3.2.2 Soil collection and leachate simulation

Soil samples at a depth of between 0 and 10 cm from at least 10 different points on the dumpsite from each market were collected using stainless steel shovel in December 2007–February 2008 (dry season) into clean polyethylene bags and transported to the laboratory where the samples were pooled to form a composite sample for each study site. The soils were air-dried and subsequently ground to powder using mortal and pestle. Leachate simulation from these soils was carried out according to the American Society for Testing and Materials (ASTM) category-A extraction procedure as modified by Bakare *et al*. (2007). Soil sample from Ajah in Lagos state, a place with no history of electronic sales, dismantling or dumpsite, was collected and used as a control for the physico-chemical analysis. The leachates were designated Alaba-simulated leachate (ASL), Computer-simulated leachate (CSL) and Ajah-simulated leachate (AJL).

3.2.3 Plant collection

Edible plant samples: *Amaranthus hybridus,* identified in the Department of Agriculture, Babcock University, was collected from the study sites in Alaba International and Computer village markets. Plant samples were designated: Computer Village e-waste dumpsite plant (CMP) and Alaba e-waste dumpsite plant (AMP). The plants were rinsed with distilled water carefully to remove the dust on the surface, air dried for two (2) weeks and stored at -20°C until analysis.

3.3 Chemicals and reagents

Standard solutions of PBDEs (1614-LCS, 1668A-IS) and PCBs (1668a-LCS, 1668a-IS) were obtained from Cambridge Isotope Laboratories Inc., USA; PAHs (USEPA SW-846) were from Supelco (Bellfonte, USA). Silica gel (ICN silican 100–200 mesh) and basic aluminum (ICN 04574 Alumina B Super I) were purchased from ICN (Eschwege, Germany). RPMI-1640 (HyClone) was purchased from Thermo Scientific (Beijing, China). Dulbecco's phosphate-buffered saline (PBS; Ca2+ and Mg2+ -free) and lymphocyte isolation kit were purchased from Solarbio S & T Co. Ltd., Beijing, China. The comet assay kit was obtained

from KeyGEN (China). The cell culture media, fetal bovine serum, JC-1 probe (5,5′,6′6-tetrachloro-,1′,3,3′ tetraethylbenzymidazolcarbocianyne iodide) and DCFH-DA (2′,7′‑dichlorofluorescein diacetate) were purchased from Invitrogen (Carlsbad, CA, USA). The antibiotics penicillin/streptomycin and tripsin-EDTA were purchased from GIBCO (New York, NY, USA). Dimethyl sulfoxide (DMSO) was from Merck and all other reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

3.4 Sample analyses

3.4.1 Physico-chemical analysis

The leachates and well water were analysed for a number of standard physico-chemical properties, including chemical oxygen demand (COD), alkalinity, biochemical oxygen demand (BOD), total dissolved solids (TDS), chlorides, nitrates, ammonia, and phosphates according to the American Public Health Association (APHA, 1998).

Soil and plant samples were analysed for PAHs, PCBs, PBDEs and heavy metals.

3.4.2 Polyaromatic hydrocarbons

The method of Maskaoui and Hu (2009) was utilised to analyse PAHs. Briefly, 20 g of soil sample (10 g plant sample), 3 g copper granules, and 6 g sodium sulphate were placed into a conical flask. The mixture was spiked with 1 μg (10 μL) of the PAH internal standard (pyrene-dl0) and 50 mL dichloromethane was added before subjecting to a 30 min ultrasonication. The extract was concentrated to approximately 1 mL and cleaned in a silica gel column (4-mm i.d. x 90 mm). The column was then eluted by dichloromethane (3.5 mL). All the eluates were concentrated under a gentle stream of N_2 to about 100 μL. The concentrations of 16 United States Environmental Protection Agency (USEPA) priority PAHs were measured by GC/MS (Hewlett Packard Model HP6890 Series, DB5ms capillary column; HP5973 Mass Selective Detector, Palo Alto, CA, USA). For quality control, method blanks and a certified reference material (CRM 105-100, BNA/pesticide soil; Resource Technology Corporation, WY, USA) were used. The recovery obtained for individual PAHs ranged from 76 – 97 %.

3.4.3 Polybrominated diphenyl ethers

PBDE analysis was carried out according to the method of Yang *et al*. (2008). Briefly, 1 g of soil or plant sample was ground with 6 g anhydrous sodium sulfate into a free-flowing powder. An internal standard was added and the mixture was extracted with 200 mL of hexane/dichloromethane (1:1, v/v). The concentrated extracts were cleaned on a 15-mm i.d. column. The PBDE mixture was eluted with 70 mL of hexane:dichloromethane (1:1), and the final eluted volume was reduced under a gentle N_2 stream to 1 mL for soil and 50 μL for leaf samples. The concentrations of eight (8) congeners of primary interest by the USEPA (1996) were measured by GC/MS. For quality control, samples were measured in duplicate and procedural blanks were used. The recovery obtained for internal surrogate standard ranged from 82 – 98 %.

3.4.4 Polychlorinated biphenyls

The method of Chu *et al*. (1996) was used for the identification of PCBs. Briefly, 20 g soil sample (10 g plant samples) was thoroughly mixed and ground with 6 g anhydrous sodium sulfate to a flowing powder. Ultrasonic extraction was used to extract PCBs from the samples with 50 mL hexane/acetone (1:1 v/v) for 5 min. The extract was concentrated to approximately 3 mL by rotary evaporator. For the clean-up, the sample solution was shaken with concentrated H_2SO_4 in a test tube, after centrifugation, the acid layer was discarded. This treatment was repeated several times until the hexane layer was clean. The hexane layer was washed with 2% NaCl aqueous solution, dried with anhydrous sodium sulfate, and then concentrated to approximately 1 mL for column chromatographic clean-up. Alumina (Al_2O_3)-Ag^+ (10% silver nitrate) modified silica gel column chromatography was used in the second clean-up step. The first 120 mL hexane fraction was collected and concentrated to 0.2 mL for GC/MS analysis. The concentrations of 28 PCB congeners, which included twelve dioxin-like PCBs (PCB-77, -81, -114, -118, -105, -123,-156, -167,-126, -169, -189, -157) and seven indicator PCBs (PCB-28, -52, -101, -118, -138, -153, -180), were measured by GC/MS. The recovery rate of individual PCB congeners ranged from 87 - 97 %.

3.4.5 Heavy metals

Two grams of well-mixed respective soil sample was measured in 250 mL glass beakers and digested with 8 mL of aqua regia (a 3:1 mixture of concentrated HCl and HNO_3) on a sand bath for 2 hr. After evaporation to near dryness, the samples were dissolved with 10 mL of 2% nitric acid, filtered, and diluted to 50 mL with distilled water. Plant samples (0.5 g) were extracted with 10 ml (1:1) of concentrated HNO_3 and perchloric acid. This was later digested on a sand bath at 80°C for 2.5 hr. The mixture was filtered through Whatman No. 42 filter paper, and the filtrate was diluted to the desired volume with distilled water. The total concentrations of lead, cadmium, chromium, copper, iron, manganese, nickel, zinc and silver in the filtrate were determined by atomic absorption spectrophotometer (AAS ZEEnit 600/650, Germany). Quality assurance was guaranteed through double determinations and the use of blanks for correction of background and other sources of error.

3.5 Biological materials

Young male Swiss albino mice (*Mus musculus*, 6- and 10–11-weeks old) were obtained from the animal breeding unit of the Department of Physiology, University of Ibadan, Nigeria, and acclimatized for at least 2 weeks in a pathogen free, well-ventilated animal house of the Departments of: Zoology, University of Ibadan and Biosciences and Biotechnology, Babcock University, Ilisan Remo, Ogun State, Nigeria. Food (Ladokun pelleted feed®) and drinking water were supplied *ad libitum*. The mice were divided into two groups for each of the animal assays for each sample. Mice of 8 weeks of age were used for the micronucleus (MN) tests, while 12–14 weeks old mice were used for the sperm morphology and sperm count assays. Animals were cared for according to standard guidelines (CIOMS, 1985).

3.6 Cell culture

NIH/3T3 (mouse fibroblast) cell line was purchased from Banco de Células do Rio de Janeiro, Rio de Janeiro, Brazil. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 1.5 g/L sodium bicarbonate, 10 mM HEPES, pH 7.4, 100 U/mL penicillin G, 100 µg/mL streptomycin and 10% fetal calf serum at 37°C in a

humidified atmosphere consisting of 95% air and 5% CO_2. Cells were passaged by removing 90% of the supernatant and replacing it with fresh medium approximately twice a week and detachment using a 0.25% trypsin–EDTA solution. Cultures with 75–90 % confluence and greater than 95% of viable cells in trypan-blue exclusion tests were use for the experiments, and the cells were seeded the day prior to the addition of the leachate.

3.7 Micronucleus test

Five groups of mice (four mice per group, weight range of 22–30 g) per leachate sample were utilised in this assay. Each group corresponds to concentrations of 1, 5, 10, 25, and 50 % (v/v, leachate/distilled water) of each leachate sample as against the negative (distilled water) and positive (cyclophosphamide, 20 mg kg^{-1}bw, Roxane laboratories Inc, USA) controls. Each mouse per group was intraperitoneally (ip) administered 0.5 mL of each sample per day for 5 consecutive days.

Same number of animals per group was used for the well water. Five groups of 1, 2, 3, 4 and 5 weeks exposure was utilised in this assay. The animals were allowed to drink the well water in place of their normal water for the exposure periods. A negative control group which was given uncontaminated drinking water was utilised. Bone marrow preparation for MN assessment was according to Schmid (1976). Briefly, the animals were sacrificed by cervical dislocation. The femurs were removed and bone marrow flushed from the bones with Fetal Bovine Serum (Sigma Aldrich Cheme GmbH®, Germany). Cells were centrifuged at 1200g for 5 min, slides prepared and subsequently stained with May-Grunwald and Giemsa stains. At least 1000 cells per mouse were scored at x1000 for MN in polychromatic erythrocytes (MNPCE).

3.8 Sperm morphology assay

In this assay, same number and types of samples and controls as in the MN assay were utilised. Five mice were treated in each concentration and the 5-week exposure period was considered. This is because spermatogenesis in mouse takes about 34.5 days to complete (Bartke *et al*., 1974). For the leachate samples, an IP injection of 0.5 mL of each sample was

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administered to each mouse for 5 days after which it was left to complete the 35 days period. For the well water samples, mice were allowed to drink the well water for 1, 2, 3, 4 and 5 weeks for Groups 1-5 respectively. At the end of each exposure period, normal uncontaminated drinking water was restored till the end of the 35 days duration. At 5 weeks from the first injection/exposure, the mice were sacrificed by cervical dislocation and their caudal epididymes were surgically removed. Sperm smears were prepared from the epididymes as previously described (Wyrobek *et al*., 1983; Bakare *et al*., 2009). For each mouse, 1000 sperm cells were assessed for morphological abnormalities according to the criteria of Wyrobek and Bruce (1975).

3.9 Sperm counts

The caput epididymes in the testes of animals used in sperm morphology assay were surgically removed and minced in physiological saline. The counting of sperms was made from their suspension with the aid of RBC counting chamber of Neubauers' hematocytometer at 400X (Rastogi and Levin, 1987). Pooled sperm count from the mice in each group was expressed as mean sperm count per milliliter of suspension.

3.10 Leachate preparation for comet assay

Leachates (20%) were prepared from the homogenous mixtures of the respective soil samples according to standard procedure (ASTM, 1992; Bakare *et al*., 2007). Briefly, 200 g of each soil sample was added to 1,000 mL of distilled water (w/v) and mechanically shaken for 24 hr at $(29\pm1)^{\circ}C$. After shaking, the samples were allowed to settle for 30 min to sediment visible particles, and then were filtered with a 2 µm filter (Whatman®) to remove the suspended particles. Finally, each sample was centrifuged at 600 g for 15 min at room temperature, the supernatant was collected, and its pH measured.

3.11 Lymphocyte preparation and treatment

In this study, 2 mL of heparinised whole blood was collected by vein puncture from a healthy nonsmoking male volunteer with no recent exposure to radiation or drugs. For each

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experiment, 2 mL of RPMI-1640 was added to 2 mL of blood, layered over Solabio isolation fluid (3:1 mL), and centrifuged at 2000 rpm for 20 min. The media/isolation fluid interphase containing lymphocytes was removed and added to 5 mL RPMI-1640 medium. The suspension was then centrifuged at 1500 rpm for 10 min to pellet the lymphocytes, which were then re-suspended in RPMI 1640 at a concentration of $\sim 1 \times 10^6$ cells/mL. In a total volume of 1 mL, 50 µL of cell suspension was mixed with RPMI-1640 containing different concentrations of each leachate sample. The leachates were tested at final concentrations of 0.5, 1, 2, 5 and 7 %. The leachate-exposed cells were incubated for 3 hr at 37°C, together with samples serving as negative (RPMI-1640) and positive (hydrogen peroxide, 100 µM for 20 min) controls. After incubation, the lymphocytes were harvested by centrifugation at 2000 rpm for 5 min, and the cells were suspended in 100 µL of phosphate buffer saline (PBS). The viability of lymphocytes was determined by the Trypan blue dye-exclusion technique (Phillips, 1973) before conducting the comet assay.

3.12 Alkaline comet assay

The method of Bajpayee *et al*. (2005) was used with slight modification. Briefly, fully frosted slides were pre-coated with 1% normal-melting-point-agarose overnight, which formed the first/base layer. A mixture of 75 µL of 0.7% low-melting-point agarose (LMA) and 25 µL of lymphocyte suspension was applied as the second layer. Cover slips were immediately placed over the second layer, and the slides were chilled on ice for 10 min to solidify the agarose. The cover slips were removed and a third layer of 90 µL 0.5% LMA was applied, the cover slips replaced, and the agarose allowed to solidify over ice for 10 min. All samples were done in triplicate. The slides were immersed in cold alkaline lysis solution for 2 h at 4°C. After lysis, double-distilled water was used to rinse away excess salt. Then, slides were placed in chilled buffer for 20 min at room temperature in a horizontal electrophoresis tank pre-filled with cold alkaline electrophoresis buffer to loosen the tight double-helical structure of DNA for electrophoresis. Electrophoresis was then performed at 25 V, 300 mA for 20 min in electrophoresis buffer at 4°C. After electrophoresis, Tris buffer (0.4 M Tris, pH 7.5) was gently added drop-wise to neutralize excess alkali; the buffer was allowed to remain on the

surface of slides for 5 min. This neutralising procedure was repeated three times. The slides were then stained with 80 μL propidium iodide (2 μg/mL) for 10 min. All of the above procedures were performed in the dark to avoid additional DNA damage. The comets were viewed using a Nikon 90i fluorescence microscope, and images of 100 comets were collected for each concentration using a digital imaging system. Cells that overlapped were not counted. All the comet images were analysed using Comet Assay Software Project (CASP, Wroclaw University, Poland) and the tail length (TL), % tail DNA (%TDNA), and Olive tail moment (OTM) were recorded to describe DNA damage to lymphocytes.

3.13 MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

The cytotoxicity of the Alaba e-waste simulated leachate was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. This leachate sample was chosen because of its toxicity compared to raw leachate and Computer village leachates. Briefly, NIH/3T3 (1×10^6/well) was exposed to 10 μL/mL of the different concentrations (5 to 100 %) of the simulated leachate for 24 h. After incubation, cells were washed with fresh culture medium and 5 mg/mL of MTT was added, followed by incubation for 2 h at 37 °C. The purple precipitated formazan formed was dissolved in 100 μL of DMSO and the absorbance was measured spectrophotometrically (ELx800 Absorbance Microplate Reader, BioTek Instruments Inc., Winooski, VT, USA) at 540 nm using a micro-well system reader. The optical density of the control group (cells without the leachate) was considered equivalent to 100% viable cells, and cell viability was calculated as a percentage of the control. The IC_{50} (a concentration that produces 50% reduction in the viable cell number) was obtained using the program Prism 5.0. The IC_{50} was determined by nonlinear regression analysis between the logarithm of concentration and the normalized response (percentage of cell viability).

3.14 Biochemical tests for the assessment of oxidative damage

3.14.1 Animals and Treatment

Sixty male albino mice were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. They were divided into 5 animals per group. Group 1 (control A) received IP injection of distilled water for five consecutive days. Group 2 (control B) was allowed to drink tap water throughout the period of the experiment. Groups 3-7 received 1, 5, 10, 25 and 50 % concentrations of the simulated e-waste leachate, while groups 8-12 was allowed to drink well water for 1, 2, 3, 4 and 5 weeks respectively. The animals were sacrificed and the liver removed. The liver was weighed and homogenized in 4 volume of ice cold isotonic phosphate buffer, pH 7.4 and centrifuge at 10, 000 g for 15 minutes at 4^oC. The resultant supernatant fraction was used for the subsequent biochemical assays.

Reagents for the liver homogenization

Homogenizing buffer (0.1M Phosphate buffer, pH 7.4)

(a) 35.81 g of disodium hydrogen phosphate $Na_2HPO_4.12H_2O$ (BDH Chemical Ltd., England) was dissolved and made up to 1 liter with distilled water.

(b) 15.6 g of sodium dihydrogen phosphate $NaH_2PO_4.2H_2O$ (BDH Chemical Ltd., England) was dissolved and made up to 1 liter with distilled water.

3.14.2 Protein Determination

The Biuret method as described by Gornall *et al*. (1949) was used with slight modification. Potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions. The principle is because proteins form a blue-colored complex with cupric ions in an alkaline solution.

Reagents for protein determination

1. 0.9% NaCl (Normal saline): 0.9% of NaCl was dissolved in distilled water and made up to 100 mL with distilled water. This was stored at 4^oC.

2. 0.2M Sodium Hydroxide (NaOH): 8 g of NaOH dissolved in distilled water and the solution was made up to 1 liter.

3. Stock Bovine Serum Albumin (Stardard): 7.4 mg of BSA dissolved in 100 mL of 0.9% NaCl so that the final concentration gives 7.4 mg/100mL.

4. Biuret Reagent: 3 g of $CuSO_4.5H_2O$ and 5 g of Sodium potassium tatarate dissolved in 50 mL of 0.2M NaOH. 5 g of potassium iodide was added and the solution made up to a liter with 0.2M NaOH.

Procedure for protein determination

One milliliter of tissue homogenate was dissolved in 39 mL of 0.9% normal saline to give a 1 in 40 dilution. 3 mL of Biuret reagent was added to 2 mL of diluted sample. The mixture was incubated at room temperature for 30 minutes, after which the absorbance was read at 540 nm. The protein content of sample calculated as follows:

Protein (mg/100 mL) = OD of sample   X Concentration of Standard

OD of Standard

3.14.3 Determination of catalase activity

Catalase activity of the liver was determined according to the method of Sinha (1971). The principle is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured colorimetrically at 570 to 610 nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction was stopped at a particular time by the addition of dichromate acetate acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Reagents for the determination of catalase activity

1. 5% $K_2Cr_2O_7$: 5 g of potassium heptaoxochromate (VI) was dissolved in some distilled water and the solution was made up to 100 mL with the same.

2. 0.2M Hydrogen Peroxide (H_2O_2): 11.50 mL of 30% (w/w) H_2O_2 was diluted with distilled water in a volumetric flask and the solution was made up to 500 mL.

3. Dichromate/Acetic Acid solution: This reagent was prepared by mixing 5% solution of $K_2Cr_2O_7$ with glacial acetic acid (1:3 v/v).

4. 0.01M Phosphate Buffer, pH 7.0: 3.58 g of $Na_2HPO_4.12H_2O$ and 1.19 g of $NaH_2PO_4.2H_2O$ were dissolved in 900 mL of distilled water. Distilled water was then added to make up to 1 liter and the pH was adjusted to 7.0.

3.14.3.1 Determination of Hydrogen peroxide (H_2O_2) Standard Curve

Different amounts of H_2O_2 ranging from 10 to 80 µmoles were pipetted into test tubes and 2 mL of dichromate/acetate was added to each. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10 minutes in a boiling bath changed the color of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made up to 3 mL and the optical density measured with a spectrophotometer at 570 nm. The concentrations of the standard were plotted against absorbance (Table 3.1 and Figure 3.2).

3.14.3.2 Determination of Catalase Activity

One milliliter of the supernatant fraction of the tissue homogenate was mixed with 19 mL distilled water to give a 1:20 dilution. The assay mixture contained 4 mL of H_2O_2 solution (800 µmoles) and 5 mL of phosphate buffer, pH 7.0 in a 10 mL flat bottom flask. 1 mL of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1 mL portion of the reaction mixture was withdrawn and blown into 2 mL dichromate/acetic acid reagent at 60 seconds interval. The hydrogen peroxide content of the withdrawn sample was determined by the method described above (3.14.3.1).

Calculation of Result

The monomolecular velocity constant K for the decomposition of H_2O_2 by catalase was determine by using the equation for a first-order reaction. $K = 1/t \log S_o/S$

Where S_o = initial concentration of H_2O_2 and S = concentration of H_2O_2 at 1 min interval. The values of K were plotted against time in minutes and the velocity constant of catalase $K_{(o)}$ at 0 minute was determined by extrapolation. The catalase content of enzyme preparation was expressed in terms of catalase feiahigkeit or 'kat f' as:

$$\text{Kat f} = \underline{K_0}/\text{mg protein/mL}$$

Table 3.1: Protocol for the estimation of hydrogen peroxide

	H_2O_2 (mL)	Dichromate/acetic acid (mL)	Distilled Water (mL)	H_2O_2 conc. (µmoles)	Absorbance (570 nm)
1	0.05	2	0.95	10	0.094
2	0.10	2	0.90	20	0.194
3	0.15	2	0.85	30	0.264
4	0.20	2	0.80	40	0.359
5	0.25	2	0.75	50	0.440
6	0.30	2	0.70	60	0.530
7	0.35	2	0.65	70	0.640
8	0.40	2	0.60	80	0.720
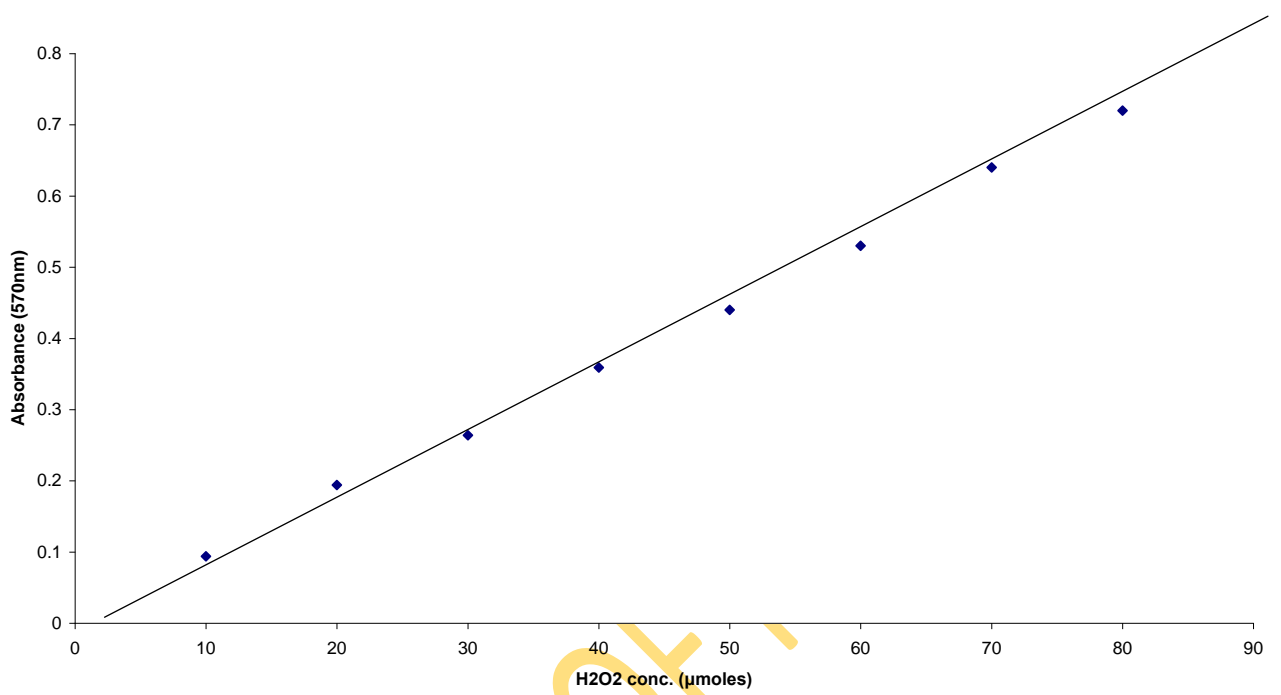
Figure 3.2: Catalase Activity - Standard curve for the estimation of hydrogen peroxide

3.14.4 Determination of Super Oxide Dismuatse (SOD) Activity

The levels of total SOD activity in the liver homogenates were determined by the method of Misra and Fridovich (1972). The ability of super oxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Super oxide anion (O_2) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per super oxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical involving super oxide radical and hence could be inhibited by SOD.

Reagents for SOD activity

(a) 0.3mM Epinephrine: 0.01 g of epinephrine (Sigma Chemical Co, London) was dissolved in 17 mL of distilled water.

(b) 0.05M Carbonate Buffer (pH 10.2): 14.32 g of $Na_2CO_3.10H_2O$ and 4.20 g of $NaHCO_3$ were dissolved in distilled water and made up to 1000 mL with distilled water and the pH adjusted to 10.2.

Procedure for the determination of SOD activity

Tissue homogenate (0.1 mL) was diluted in 0.9 mL distilled water to make a 1:10 dilution of the homogenate. An aliquot of 0.2 mL of the diluted tissue supernatant was added to 2.5 mL of 0.05M carbonate buffer, (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 mL of carbonate buffer, 0.3 mL of epinephrine and 0.2 mL of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Calculation of Result

Increase in absorbance per minute = $\dfrac{A_3 - A_0}{2.5}$

Where A_0 = absorbance at time, t = 0; A_3 = absorbance at time, t = 150 seconds

%inhibition = $100 - (100 \text{ X Increase in absorbance for substrate})$

(Increase in absorbance of blank)

1 unit of SOD activity is given as the amount of SOD necessary to cause 50% inhibition of the oxidation of epinephrine.

Blank = reference cuvette

3.14.5 Determination of Reduced Glutathione (GSH)

The total sulphydryl groups, proteins-bound sulphydryl groups and free sulphydryl groups (such as reduced glutathione) in biological samples can be determined using Ellman's reagent (DTNB). The level of reduced glutathione in the lever homogenates was determined by the method of Jollow *et al*. (1974). The reduced form of glutathione (GSH) in most instances is the bulk of cellular non-protein sulphydryl groups. This method is based upon the development of relatively stable yellow complex formed when Ellman's reagent (5´, 5´ - dithiobis –[2-nitrobenzoic acid] react with free sulphydryl compounds (such as reduced glutathione). The chromophoric product, 2-nitro5-thiobenzioc acid, resulting from the reaction of Ellman's reagent with reduced glutathione possesses a molar absorption at 412 nm. The absorbance at 412 nm is proportional to the reduced glutathione content.

$$GSH + R - S - S - R´ \longrightarrow R - S^- + GSS^-$$

$R - S^-$ = Yellow complex

Reagents for the determination of GSH

(a) Glutathione Working Standard: 0.04 g of GSH (Sigma Chemical Co., London) was dissolved in 100 mL of 0.1M phosphate buffer, pH 7.4 and the stored at 4 ^{0}C.

(b) 0.1M Phosphate Buffer, pH 7.4:

(i) 35.81 g of disodium hydrogen phosphate $Na_2HPO_4.12H_2O$ was dissolved and made up to 1 liter with distilled water.

(ii) 15.6 g of sodium dihydrogen phosphate $NaH_2PO_4.2H_2O$ was dissolved and made up to 1 liter with distilled water.

0.1M phosphate buffer was prepared by adding 4 volumes of solution (i) to 1 volume of solution (ii) and the pH of the resulting solution adjusted to 7.4 using a pH meter.

(c) Ellman's Reagent (DTNB)

0.04 g of DTNB (Sigma Chemical Co, London) was dissolved in100 mL of 0.1M
Phosphate buffer, pH 7.4. DTNB reagent is stable for at least 3 weeks when stored at
4^oC.

(d) Precipitating Reagent: 4% sulphosalicyclic acid

4% sulphosalicyclic acid ($C_7H_6O_6S.2H_2O$: mol. wt. 254.22) (BDH Chemical Ltd,
England) was prepared by dissolving 4 g of sulphosalicyclic acid in 100 mL of
distilled water. This reagent is stable for approximately three weeks at 4^oC.

3.14.5.1 Procedure for Reduced Glutathione (GSH) Calibration Curve

Serial dilutions of the stock GSH solution were made using the phosphate buffer for dilution
(to a total volume of 0.5 mL). 4.5mL of Ellman's reagent was then added to each solution and
the absorbance of the yellow solution that developed was read at 412 nm within five minutes
of color development. Absorbance was then plotted against reduced glutathione concentration
(Table 3.2 and Figure 3.3).

3.14.6 Blood Collection and Measurement of serum AST and ALT in treated mice

After the last day of exposure, the mice were fasted overnight for 14 hours. Blood samples
for serum ALT and AST were obtained by cardiac puncture under diethyl ether anaesthesia
(Sigma Chemical Co., St. Louis, U.S.A.). The blood samples were collected into plain sample
bottles and centrifuged at 3000 rev/min. for 30 minutes to separate sera. Serum AST, and ALT
were all assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.).
Transaminases activities were measured as kinetic reaction using IFCC method. The
absorbance of reaction was determined at 546 nm by spectrophotometer.

3.14.7 Lipid peroxidation

Lipid peroxidation in the liver tissue was evaluated by measuring total malonaldehyde
(MDA) with the BIOXYTECH MDA-586 assay kit (Oxis Research, Portland, OR). Briefly,
liver tissues were homogenized in the presence of 5mM butylated hydroxytoluene. Extracts

Table 3.2: Protocol for Reduced Glutathione (GSH) Calibration Curve

	GSH (mL)	Phosphate Buffer (mL)	Ellman's Reagent (mL)	GSH Conc. (g/mL)	Absorbance (412 nm)
1	0.00	0.50	4.50	0.00	0.00
2	0.02	0.48	4.50	8.00	0.06
3	0.05	0.45	4.50	20.00	0.16
4	0.10	0.40	4.50	40.00	0.30
5	0.20	0.30	4.50	80.00	0.62
6	0.30	0.20	4.50	120.00	0.94
7	0.40	0.10	4.50	160.00	1.25
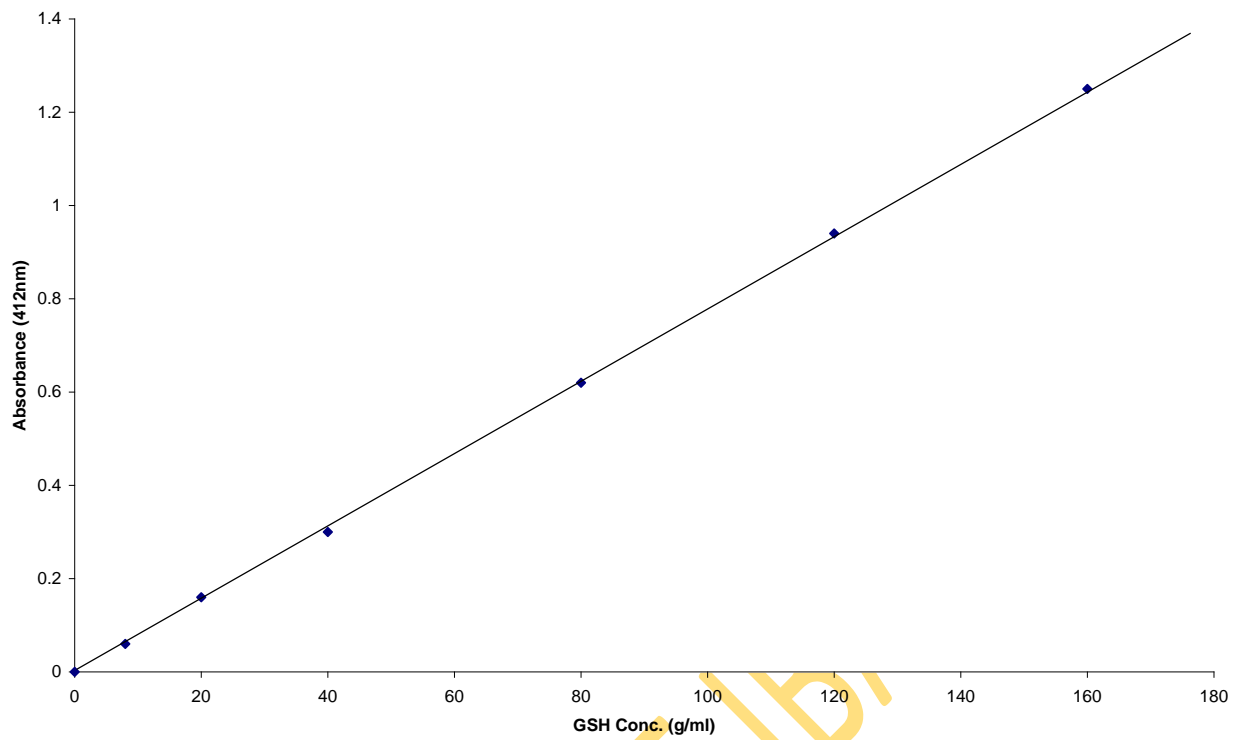
Figure 3.3: Reduced Glutathione (GSH) Calibration Curve

were centrifuged at 10,000 g for 10 minutes at 4^0C, and 0.1 N HCl and 500 mM butylated hydroxytoluene (pH 1–2) was added to the supernatant and incubated for 80 minutes at 60^0C to hydrolyze Schiff base adducts of MDA. The samples were centrifuged at 3,000 g for 10 minutes at 4^0C, and the supernatant was used to determine concentrations of total MDA.

3.15 Determination of mitochondrial membrane potential of the NIH/3T3 treated cells

The possible effect of e-waste leachate in distrupting mitochondrial membrane potential was evaluated using the lipophilic cationic fluorescent probe JC-1. NIH/3T3 cells were seeded at 5×10^5 cells/well in 12-well dishes 24 h prior to exposure to 10 μL/mL of 10, 20 and 40 % concentrations of the Alaba simulated leachate, and was then incubated for 24 h at 37 °C (5% CO_2). After incubation, the supernatant containing the leachate was removed and JC-1 (10 μg/mL) was added, followed by incubation for 20 min at 37 °C (5% CO_2). The visual evaluation of the cells was carried out under a fluorescence microscope (Nikon Eclipse TS100 inverted microscope, Nikon Instruments Inc., Melville, NY, USA). Cells were then harvested by trypsinisation, washed twice with phosphate buffer saline (PBS) and finally resuspended in 500 µL of PBS. A total volume of 100 µL was used to measure the fluorescence by a spectrofluorimeter (Perkin‑Elmer LS55, Boston, MA, USA). JC-1 was excited at 595 nm, the red emission fluorescence was detected at 560 nm and the green fluorescence was detected at 485 nm (Cossarizza *et al*., 1993). The red/green fluorescence ratio for each sample was calculated and normalised as a percentage of the untreated control (100%). The changes in mitochondrial potential were indicated as a decrease in the red and green fluorescence intensity ratio. FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone) (1 µM), a chemical electron transport uncoupler, was used as a positive control.

3.16 *In vitro* determination of reactive oxygen species generation using DCFH-DA probe

Intracellular reactive species formation was evaluated using 20,70-dichlorofluorescein diacetate (DCFH-DA), which is oxidised to dichlorofluorescein (DCF) in the presence of ROS (Sauer *et al*., 2003). The assay was carried out according to the method adapted by

Cordova *et al.* (2011) with modification for leachate's exposure period. NIH/3T3 cells were seeded in 24-well plates (1.5 x 10^4cells/well) 24 h prior to exposure to the different concentrations of the Alaba simulated leachate and incubated for 24 h at 37 °C (5% CO_2). After incubation, the supernatant containing the leachate was removed and cells were stained with 10 μM of DCFH-DA and incubated in the dark for 30 min at 37 °C. Cells were later harvested and washed four times with PBS. A total volume of 100 μL was used to measure the fluorescence by a spectrofluorimeter. The evaluation was performed by the quantification of green fluorescence in the spectrofluorimeter (Perkin Elmer LS55, Boston, MA, USA) with wavelengths of excitation/emission of 480/520 nm, respectively (Sauer *et al.*, 2003). The results obtained as fluorescence units were expressed as mean ± SD of ROS, compared with non-treated cells and normalised to total protein content. H_2O_2 (2000 μM) was used as the positive control.

3.17 Cell cycle analysis

To analyse the cell cycle of the cells treated with the Alaba simulated leachate, flow cytometry was used according to the method of Yang *et al.* (2007). Briefly, NIH/3T3 cells (4×10^5/well) were incubated for 24 h prior to the exposure to 100 μL/mL of 10, 20 and 40 % concentrations of the leachate for 24 h at 37 °C (5% CO_2), in 12-well plates. After incubation, cells were harvested and centrifuged for 10 min at 400 g at room temperature. Subsequently, cells were washed with PBS and centrifuged as above. The supernatant was discarded and 200 μL of cold 70% ethanol was added followed by incubation at 4 °C for 30 min. After incubation, 1 mL of the mixture of PBS and (2%) bovine serum albumin (BSA) was added and centrifuged for 10 min at 400 g. The supernatant was removed and 500 μL of RNase (100 μg/mL) in lysis buffer (0.1% Triton‑X in PBS) was added. The cells were stained with 4 μL of propidium iodide (PI) (20 μg/mL). DNA content was analysed using the FACSCanto flow cytometry equipment (Becton Dickinson, San Diego, CA, USA). The cell population in each phase of the cell cycle was determined using WinMDI 2.9 software.

In order to analyze the relationship between ROS generation and cell cycle alterations, cells were treated with 1ml of 1mM N-acetylcysteine (NAC), an inhibitor of ROS generation, an

hour before exposure to the different concentrations of the leachate.

3.18 Acridine orange/ethidium bromide (AO/EB) double staining assay for the determination of necrotic and apoptotic cells

Acridine orange/ethidium bromide (AO/EB) double staining was utilized for the determination of apoptotic and necrotic cells. Briefly, NIH/3T3 cells (4×10^5/well) were seeded for 24 h prior to the exposure to the leachate for another 24 h at 37 °C (5% CO_2), in 12-well plates. The supernatant was removed after incubation and replaced by PBS. 5 μL of the dye mixture (Acridine orange and ethidium bromide; 1:2.25) was added and the plates observed under the fluorescence microscope (Nikon Eclipse TS100 inverted microscope, Nikon Instruments Inc., Melville, NY, USA). The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei). In each experiment, more than 300 cells/concentration were counted.

3.19 Statistical analysis

The SPSS® 15.0 and Microsoft® Excel 2003 statistical package were used for data analysis. Data obtained were expressed as % frequency and mean ± standard deviation in each assay. Percentage PCE, NCE, MNPCE, and MNNCE were calculated and the ratio of PCE to NCE was also recorded. Significance at the different dose-level of each assay was tested by using the Dunnett t-test. Differences between the negative control-group and individual dose-groups were analysed at the 0.05 and 0.01 probability levels. The significance of differences among the mean sperm counts of different groups was determined by Student's t-test. Pearson correlation (r) was used to show the strength of association between the results of the different assays. Analysis of variance (ANOVA) was performed on all experimental data, and means were compared using SPSS version 15.0 software. Results of the comet assay were expressed as mean ± S.E. of the triplicates for each leachate sample. The mean values of %TDNA, OTM, and TL at each concentration of each leachate sample were compared with the negative control using one-way ANOVA followed by Dunnett's multiple comparison test

at a p<0.05 level of significance. Correlation coefficients also were calculated to analyse concentration-response relationships. Results of the mitochondrion membrane potential, cell cycle analysis, and *in vitro* ROS were presented as mean ± SD of triplicates from three independent experiments. Multiple comparisons were made by one-way analysis of variance (ANOVA), followed by Dunnett's post hoc tests, to compare against a single reference group. A value of p < 0.05 was considered to be significant.

CHAPTER FOUR

Results

4.1 Physico-chemical and heavy metal analyses

Tables 4.1, 4.2 and 4.3 show the physico-chemical and heavy metal parameters analysed in the e-waste leachates, well water, plant samples and the control. The pH values were within the limit set by National Environmental Standards and Regulation Enforcement Agency (NESREA, 2007) and USEPA (2009). The values for COD, BOD and TDS were higher than the allowable levels in drinking water. The COD was at least doubled the USEPA allowable level in the leachate samples and more than five times the NESREA allowable limit. The levels of BOD and TDS were in the same trend with COD. The values obtained for Pb, Cd, Cr, Fe, Mn, Ni, Zn and Ag in the leachates were higher compared with control, and higher than the maximum allowable levels in drinking water (NESREA, 2007; USEPA, 2009). Pb and Cu were the most abundant metals in the leachate samples. Pb has the highest concentration in the leachate samples while As has the least. The table further shows that there is a higher heavy metal contamination in the leachates from Alaba International market than the leachates from Computer village market. In the water samples, heavy metals were present in various concentrations with the concentration of Pb higher than the allowable standard by both NESREA (2007) and USEPA (2009) in the two markets. The concentration of Cd was higher than allowable standard by USEPA only in the well water from Alaba International market although within the range of allowable standard by NESREA, while the Cd concentration in well water from Computer village market is within the allowable standard by both NESREA and USEPA. Pb and Cu were the most predominant contaminants among the heavy metals in the plant samples. All the heavy metals except Fe, Ni and Ag were present at high concentrations in the plant samples from both markets.

4.2 Polyaromatic hydrocarbons

The total and individual concentrations of the 16 USEPA priority PAHs in the soil and plant samples from the two major e-waste markets in Lagos, Nigeria are shown in Table 4.4.

Soil: Total PAH concentrations in the soil were 95.37 and 116.49 mg/kg for Computer village

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Table 4.1: Physico-chemical characteristics of raw and simulated leachates and well water from e-waste dump sites at Alaba International and Computer village markets, Lagos, Nigeria.

Parameters*	ARL	ASL	AWW	CRL	CSL	CWW	AJL	NESREA[a]	USEPA[b]
pH	7.00	6.81	7.11	6.91	6.98	7.06	7.2	6.5-8.5	6.5-8.5
COD	703	920.04	471	680	753.2	456	440.01	250	410
BOD	322	580	146	306	448	168	146.12	30	250
TDS	760	680	390	690	615.7	348	389	1200	500
Salinity	0.4	0.2	-	0.3	0.4	-	0.1	-	-
Alkalinity	372	62	-	262	98	-	97.23	20	-
Chlorides	200	212	139	168	231	152	193.26	250	250
Ammonia	2.20	1.6	-	1.76	1.41	0.01	0.14	0.5	0.03
Phosphates	1.04	2.3	1.8	2.1	3.6	1.21	1.1	5.0	5
Nitrates	0.12	1.10	1.1	0.62	1.22	0.8	0.3	10	10

*Units of the parameters are in mg/L except for salinity in parts per thousand and pH which has no unit.

COD = Chemical Oxygen Demand, BOD = Biochemical Oxygen Demand,

TDS= Total Dissolved Solid

[a]National Environmental Standards and Regulation Enforcement Agency (2007) Permissible limits for drinking water.

[b]USEPA- US Environmental Protection Agency (http://water.epa.gov/drink/contaminants/index.cfm#List). Accessed on 23/06/2010.

ND- Not detected

ASL – Alaba simulated leachate; ARL – Alaba raw leachate; AWW- Alaba well water; CSL – Computer village simulated leachate; CRL – Computer village raw leachate; CWW- Computer village well water

Table 4.2: Heavy metals characteristics of raw and simulated leachates and well water from e-waste dumpsites at Alaba International and Computer village markets, Lagos, Nigeria.

Parameters*	ARL	ASL	AWW	CRL	CSL	CWW	AJL	NESREA[a]	USEPA[b]
Lead	672.9	867.52	0.41	397	428	0.11	0.03	0.05	0.00
Cadmium	12.14	18.04	0.007	14.21	15.07	0.002	0.26	0.005	0.005
Chromium	4.00	4.41	0.002	6.11	8.00	-	0.1	0.1	0.100
Copper	14.32	22.31	0.001	16.19	18.43	0.72	-	0.05	1.30
Iron	6.41	8.65	0.02	3.21	4.16	0.03	0.06	0.03	0.300
Manganese	1.35	2.1	-	1.10	1.91	0.43	1.1	0.1	0.050
Nickel	2.41	1.91	-	0.98	1.31	-	.	0.01	-
Zinc	15.0	21.2	-	12.98	15.38	1.16	4.82	1.5	5
Silver	0.82	1.10	0.002	-	0.62	-	-	0.01	0.10

*Units of the parameters are in mg/L

[a]National Environmental Standards and Regulation Enforcement Agency (2007) Permissible limits for drinking water.

[b]USEPA- US Environmental Protection Agency (http://water.epa.gov/drink/contaminants/index.cfm#List). Accessed on 23/06/2010.

ND- Not detected

ASL – Alaba simulated leachate; ARL – Alaba raw leachate; AWW- Alaba well water; CSL – Computer village simulated leachate; CRL – Computer village raw leachate; CWW- Computer village well water

Table 4.3: Heavy metals characteristics of *Amaranthus hybridus* from e-waste dumpsites at Alaba International and Computer village markets, Lagos, Nigeria.

Parameters*	AMP	CMP	AJP
Pb	23.3	12.8	-
Cd	0.82	0.26	-
Cr	0.004	0.001	-
Cu	1.62	1.02	-
Fe	-	-	0.001
Mn	0.02	0.001	0.001
Ni	-	-	-
Zn	0.04	0.01	-
As	-	-	-

*Units of the parameters are in mg/L

AMP – Alaba market plants; CMP- Computer village market plants; AJP- Ajah plants

Table 4.4: Concentration of PAHs in soils and plants collected from e-waste dump sites in Lagos, Nigeria (mg/kg dry wt).

Congeners	Soil			Plant	
	CMS	AMS	AJS	CMP	AMP
Naphthalene	3.34	3.01	0.001	0.20	0.30
Σ2 rings	3.34	3.01	0.001	0.20	0.30
Acenaphthylene	0.089	0.24	ND	0.06	0.08
Acenaphthene	4.86	13.50	ND	0.85	1.69
Fluorene	2.03	26.41	0.002	0.63	0.97
Anthracene	ND	ND	ND	ND	ND
Phenanthrene	79.64	68.37	ND	4.25	11.05
Σ3 rings	86.62	108.52	0.002	5.79	13.79
Fluoranthene	0.30	1.01	0.001	0.02	0.04
Pyrene	4.88	3.49	ND	0.05	0.09
Benzo(a)anthracene	0.01	0.06	0.001	0.001	0.001
Chrysene	0.02	0.11	ND	0.01	0.08
Σ4 rings	5.21	4.67	0.002	0.08	0.22
Benzo(b)fluoranthene	0.03	ND	ND	0.01	ND
Benzo(k)fluoranthene	0.02	0.03	ND	0.01	0.02
Benzo(a)pyrene	0.07	0.10	ND	0.01	ND
Dibenz(a,h)anthracene	0.06	0.03	ND	0.03	0.02
Σ5 rings	0.18	0.16	-	0.06	0.04
Indeno(1,2,3)pyrene	0.002	0.07	ND	0.001	0.002
Benzo(g,h,i)perylene	0.02	0.06	ND	0.02	0.61
Σ6 rings	0.02	0.13	-	0.02	0.61
Total PAH	95.37	116.49	0.005	6.15	14.95
LMW PAHs	89.96	111.53	0.003	5.99	14.09
HMW PAHs		5.41	4.96	0.16	0.86
0.002				0.07	0.12
[a]Σ 7 Carcinogenic PAHs	0.27	0.4	-		
Dutch standard for					
unpolluted soil		20-50μg/kg			

ND-not detected (or value <0.001), LMW-low molecular weight, HMW-high molecular

weight.

CMS-Computer Village market dumpsite soil; AMS-Alaba International market dumpsite soil; AJS-Ajah soil; CMP-Computer Village market plants; AMP-Alaba International market plants.

[a]USEPA, United States Environmental Protection Agency B2 classification: chrysene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene.

and Alaba market dumpsites respectively against 0.005 mg/kg for the control. The highest concentration (116.49 mg/kg) was at the e-waste dumpsites in Alaba International market. Only the soil samples from Alaba market dumpsites contained all the seven USEPA carcinogenic PAHs (chrysene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene and dibenz(a,h)anthracene). Low molecular weight (LMW; specifically 3 rings) PAHs were predominant in soil samples from both markets accounting for 94.33 - 95.74 % of the total PAHs in the samples (Figure 4.1).

To estimate the source of the pollution in these areas, four molecular indices (LMW/HMW, fluoranthene/pyrene, benzo(a)anthracene/chrysene, and benzo(a)pyrene/benzo(g,h.i)perylene) were employed. The LMW/HMW ratio theory is based on the fact that petrogenic contamination was characterized by the predominance of the lower molecular weight PAHs (tri- and tetra-aromatics) while higher molecular weight PAHs dominate in pyrolytic PAH contamination (Muel and Saguem, 1985). The LMW/HMW ratio was between 17 and 23 (i.e higher than 1) in samples from both markets, an indication of petrogenic sources (Table 4.5). Fluoranthene to pyrene ratio above one typically suggests pyrolytic origin, and a value below one is typical of a petrogenic source. The result of this study showed that the ratios for both markets are ≤1. Another isomer ratio is benzo(a)anthracene/chrysene, where values >0.9 are associated with pyrolytic origin (Gschwend and Hites, 1981). Quantitation shows that the ratio of benzo(a)anthracene to chrysene in the study sites in both markets is between 0.5-0.6. On the other hand, a ratio of benzo(a)pyrene/benzo(g,h.i) perylene in the range of 1–5 may suggest contamination through wood and coal burning (Maher and Aislabie, 1992). Table 4.5 shows that the two samples are contaminated with wood and coal products burning.

Plants: Total PAH concentrations in plants were 6.15 and 14.95 mg/kg for Computer village and Alaba International market plants respectively. The higher concentration was recorded in plants from Alaba International market dumpsites. Three-ring compounds dominated the concentration profile in all the samples (Figure 4.2). The concentrations of the 7 USEPA carcinogenic PAHs in plants were between 0.07 (CMP) and 0.12 (AMP) µg/kg and accounted

Table 4.5: Molecular indices for soils and plants from e-waste dump sites in Lagos, Nigeria.

Sites	Fluor/Pyr	BaA/Chr	LMW/HMW	BaP/BghiP
Soil				
CMS	0.06	0.5	17	4
AMS	0.29	0.6	23	2
Plants				
CMP	0.4	0.1	37	0.5
AMP	0.4	0.01	16	-

CMS-Computer Village market dumpsite soil; AMS-Alaba International market dumpsite soil; AJS-Ajah soil; CMP-Computer Village market plants; AMP-Alaba International market plants.
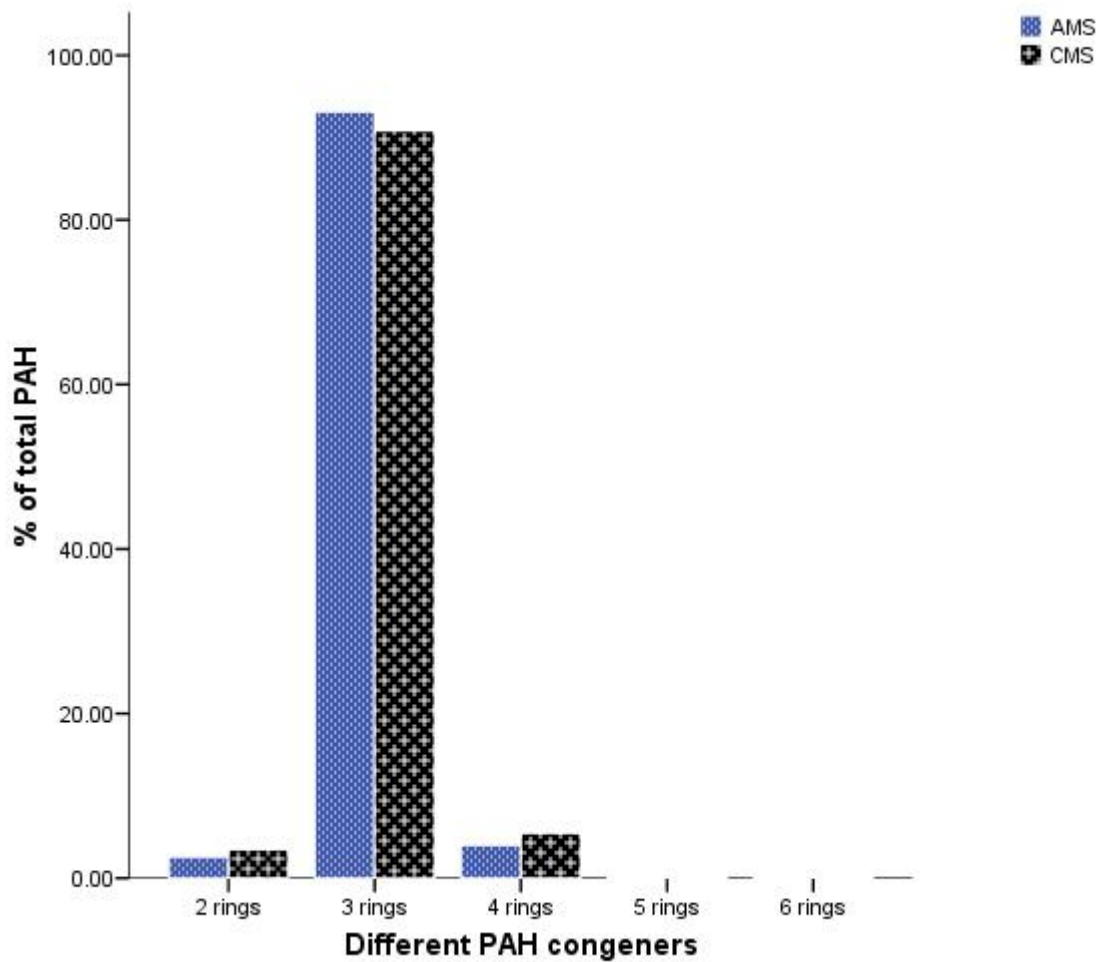
Figure 4.1: The percentage distribution of 2- to 6-ring PAH congeners to the total PAH in soil samples collected from e-waste dumpsites in Lagos, Nigeria.

CMS - Computer Village market soil; AMS - Alaba International market soil.
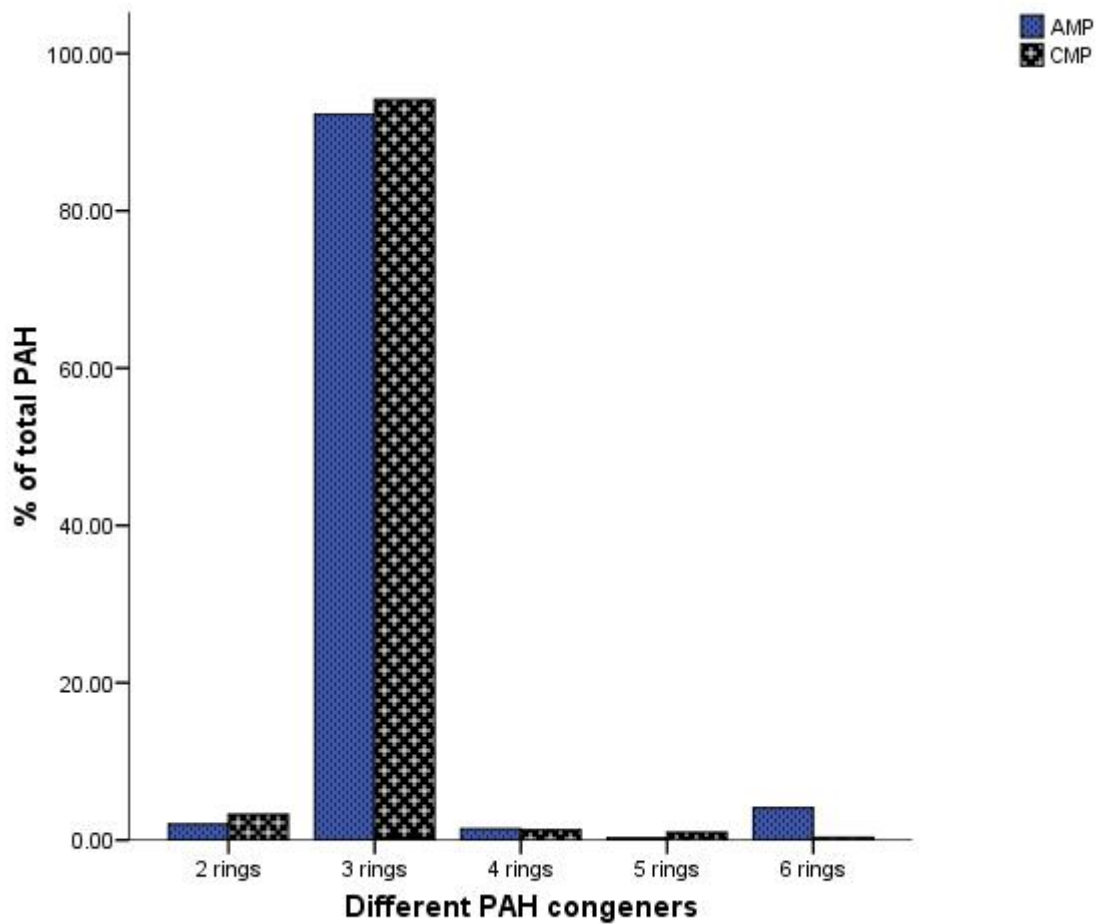
Figure 4.2: The percentage distribution of 2- to 6-ring PAH congeners to the total PAH in

plant samples collected from e-waste dumpsites in Lagos, Nigeria.

CMP - Computer Village market plants; AMP - Alaba International market plants

for 1 and 0.8 % of the total PAH concentrations respectively. Diben(z)anthrene was the most prevalent carcinogenic PAH, while anthracene was not detected in any of the samples. This is also consistent with the soil sample analysis from where the plants were derived in the two markets. The LMW/HMW were 16 and 37 (i.e. >1) for AMP and CMP respectively, indicating a petrogenic source (Table 4.5).

The ratio of fluoranthene to pyrene on the other hand was less than 1 (0.4) in all plant samples, suggestive of a petrogenic origin. The value of the benzo(a)anthracene/chrysene ratio, which ranged from 0.01 to 0.1 in the plants from the two markets.

4.3 Polybrominated diphenyl ethers

The concentrations of eight PBDEs in the soil and plant samples are shown in Table 4.6.

Soil: All the eight PBDE congeners were present in the soil samples, with total concentrations of 13.33 (CMS) and 34.74 (AMS) mg/kg. The major PBDE congeners in the soil samples were BDE-99 and BDE-209. BDE-209 was the main contaminant in Alaba International market dumpsites while BDE-99 was the main contaminant in Computer village market dumpsites. The concentration of $\Sigma penta$-BDE is the most predominant in the samples from both markets, with values of 4.54 (CMS) and 10.04 (AMS) mg/kg.

Plants: The total concentrations of PBDE congeners in the plant samples were generally lower than the values obtained in the soil samples. The total PBDEs varied from 11.51 (CMP) to 31.07 (AMP) µg/kg. Similar to soil, the major PBDE congener in the plant samples was BDE-209, with percentages of 49.09 and 61.47 for CMP and AMP respectively. The percentage distribution of the Σtri-BDE to Σhepta-BDEs and BDE-209 in soil and plant samples is illustrated in Figures 4.3 and 4.4 respectively. The general patterns of percentage of the sum of tri- to hexa-BDE distribution in plant samples were similar to those in the soil samples.

4.4 Polychlorinated biphenyls

Table 4.7 shows the concentrations of PCB congeners found in soil and plant samples.

Table 4.6: Concentrations of PBDEs in soils and plants collected from e-waste dump sites in Lagos, Nigeria.

Congeners	Soils (mg/kg dry wt)			Plants (µg/kg dry wt)	
	CMS	AMS	AJS	CMP	AMP
BDE 28	0.27	1.71	0.001	0.23	0.58
Σ*tri*-BDE	0.27	1.71	0.001	0.23	0.58
BDE 47	1.58	5.47	ND	1.30	2.53
Σ*tetra*-BDE	1.58	5.47	ND	1.30	2.53
BDE 100	2.21	4.89	ND	1.46	2.92
BDE 99	2.33	5.15	ND	1.53	3.08
Σ*penta*-BDE	4.54	10.04	ND	2.99	6.00
BDE 154	1.39	2.44	0.001	0.54	1.25
BDE 153	2.21	2.53	ND	0.55	1.29
Σ*hexa*-BDE	3.60	4.97	0.001	1.09	2.54
BDE 183	1.52	2.75	ND	0.25	0.32
Σ*hepta*-BDE	1.52	2.75	ND	0.25	0.32
BDE 209	1.82	9.80	ND	5.65	19.10
Σ*total*-BDE	13.33	34.74	0.002	11.51	31.07
%total BDE-209	13.65	28.21	-	49.09	61.47
Australian standard	0-5				

ND-not detected (or value <0.001).

CMS-Computer Village market dumpsite soil; AMS-Alaba International market dumpsite soil; AJS-Ajah soil; CMP-Computer Village market plants; AMP-Alaba International market plants.

Figure 4.3: The percentage distribution of the sum of the tri-BDEs to the sum of deca-BDEs in soil samples collected from e-waste dumpsites in Lagos, Nigeria.

CMS - Computer Village market soil; AMS - Alaba International market soil.
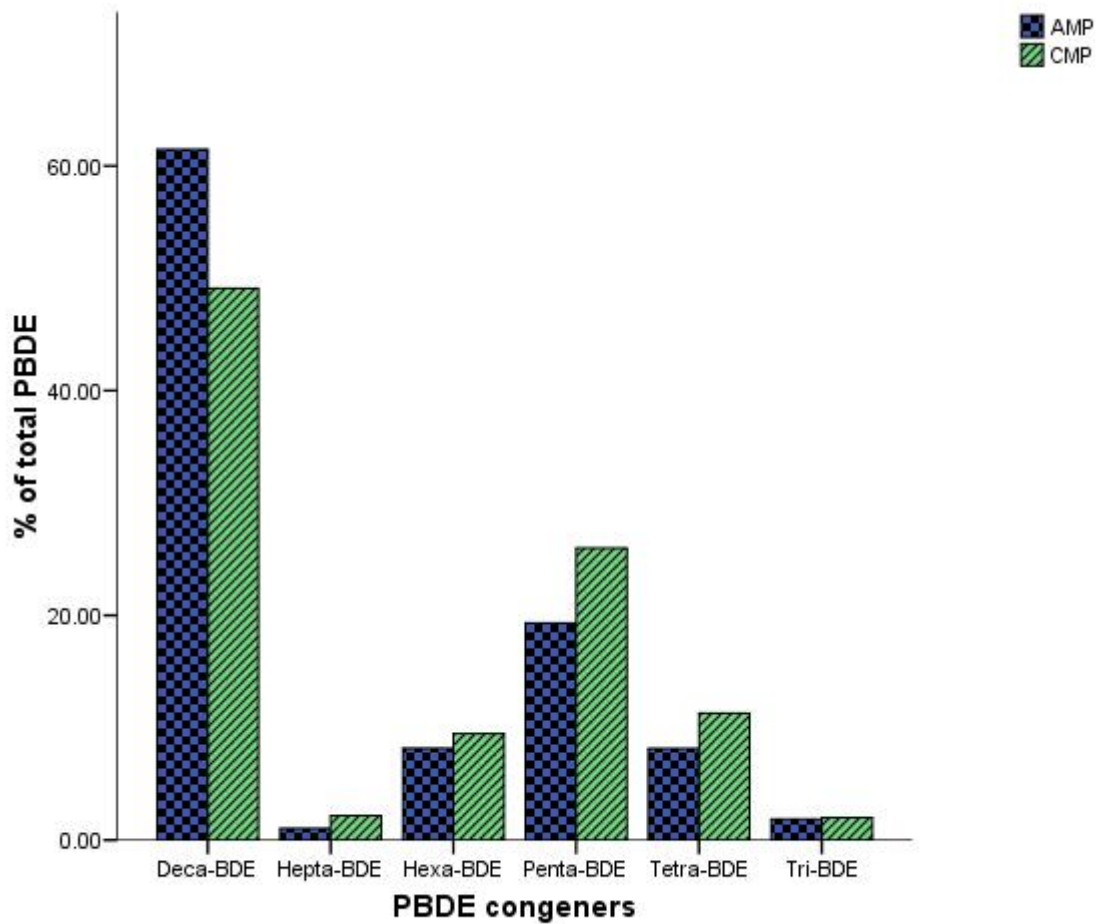
Figure 4.4: The percentage distribution of the sum of the tri-BDEs to the sum of hepta-BDEs and BDE-209 in plant samples collected from e-waste dumpsites in Lagos, Nigeria.

CMP - Computer Village market plants; AMP - Alaba International market plants

Table 4.7: Concentrations of PCBs in soils and plants collected from e-waste dump sites in Lagos, Nigeria (μg/kg dry wt)

Congeners	Soils			Plants	
	CMS	AMP	AJS	CMP	AMP
CB 8	189	726	0.1	46	59
Σdi-PCB	189	726	0.1	46	59
CB 18	640	116	0.02	53	56
CB 28	302	833	ND	14	28
Σtri-PCB	942	949	0.02	67	84
CB 52	369	740	0.01	73	91
CB 44	249	479	ND	29	39
CB 66	247	276	ND	7	60
CB 101	164	83	0.03	2	8
CB 77	59	19	ND	12	46
CB 81	24	20	ND	6	46
Σtetra-PCB	1112	1617	0.04	129	290
CB 114	78	63	ND	8	15
CB 118	61	48	ND	6	12
CB 123	39	7	0.01	12	36
CB 105	356	124	ND	42	67
CB 126	65	17	0.001	8	12
Σpenta-PCB	599	259	0.011	76	142
CB 153	176	83	0.001	17	36
CB 138	90	100	ND	4	35
CB 128	130	64	ND	9	59
CB 167	58	28	ND	4	26
CB 156	13	5	ND	3	2
CB 157	29	6	0.01	3	12
CB 169	ND	30	ND	14	ND
Σhexa-PCB	496	316	0.011	54	170
CB 170	73	26	ND	10	9
CB 180	105	47	ND	4	2
CB 187	ND	61	ND	13	ND

CB 189	2	1	ND	8	22
$\Sigma hepta$-PCB	180	135	-	35	33
CB 195	62	44	ND	45	49
$\Sigma octa$-PCB	62	44	-	45	49
CB 206	20	3	ND	55	51
$\Sigma nona$-PCB	20	3	-	55	51
CB 209	19	14	ND	11	11
$\Sigma deca$-PCB	19	14	-	11	11
[a]ΣindicatorPCBs	1267	1934	0.041	120	212
Total PCBs	3619	4063	0.182	518	889

ND-not detected (or value <0.001).

CMS-Computer Village market dumpsite soil; AMS-Alaba International market dumpsite soil; AJS-Ajah soil; CMP-Computer Village market plants; AMP-Alaba International market plants.

[a]Total indicator PCBs = sum of concentrations of PCB-28, -52, -101, -118, -138, -153, -180.

Soil: Total PCB concentrations were 3619 and 4063 µg/kg for CMS and AMS respectively. The sum of indicator PCBs ranged from 1267 (CMS) to 1934 (AMS) µg/kg, which accounted for 28.5 − 47 % of the total PCB concentration in the soil samples. Generally, the indicator PCBs most predominant were CB-28 and CB-52, with the concentration pattern for the indicator PCBs being similar in both samples. The total WHO-toxic PCBs in the samples ranged from 368 (AMS) to 784 (CMS) µg/kg. The most predominant WHO-toxic PCBs were CB-105, 114, and 118 in both samples. In general, very similar profiles of PCB congeners (Figure 4.5), pattern of indicator PCBs, and WHO-toxic PCBs were observed at both locations with tetra-PCBs being the predominant contaminant.

Plants: Total PCBs ranged from 518 (CMP) to 889 (AMP) µg/kg and the pattern of total PCBs was similar to the results obtained in the soil samples. The total indicator PCB concentration ranged from 120 (CMP) to 212 (AMP) µg/kg. The value obtained for total WHO-toxic PCBs was between 22% (CMP) to 30% (AMP) of the total PCBs (Figure 4.6). The plant samples from the two markets have a similar PCB congener pattern with the soil samples from where they were derived.

Pearson correlation analysis of the heavy metals and the organics in soil showed that there was a significant strong positive correlation (r=0.888, p<0.001) between PAHs and PBDEs, a strong positive correlation between PAHs and PCBs (r=0.754), and a moderate positive correlation between PAHs and heavy metals (r=0.418). A positive correlation was also observed between PCBs and PBDEs (r=0.695). In the plant samples, PAHs and PBDEs showed a moderately strong positive correlation (r=0.678) while a moderate and weak negative correlations were observed between PAHs and PCBs (r=-0.531), and PCBs and heavy metals (r=-0.195) respectively.

4.5 MN assay

E-waste leachates

Figure 4.7 shows the results of MNPCE observed in the bone marrow of mice exposed to the leachate samples. Compared to negative control, the results showed a significant increase in
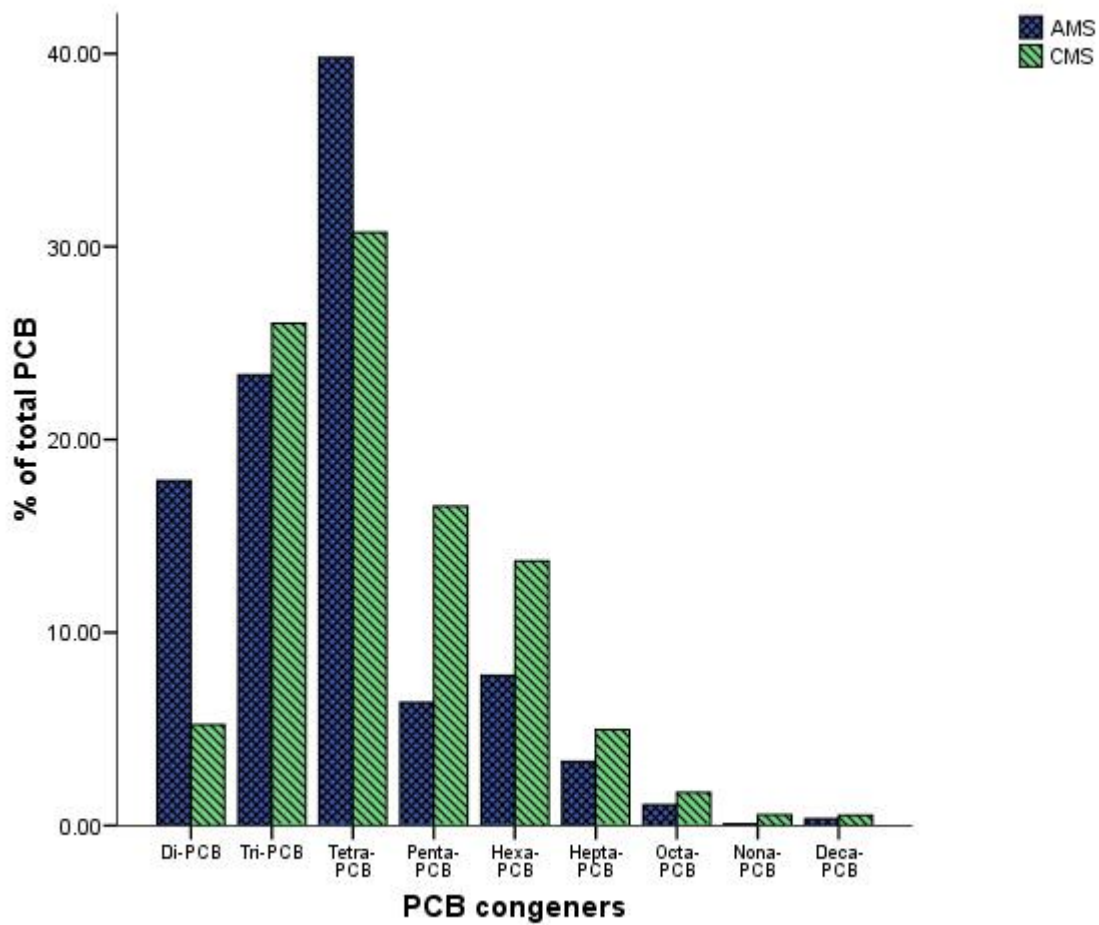
Figure 4.5: The percentage distribution of the sum of the di-PCBs to the sum of deca-PCBs

in soil samples collected from e-waste dumpsites in Lagos, Nigeria.

CMS - Computer Village market soil; AMS - Alaba International market soil.
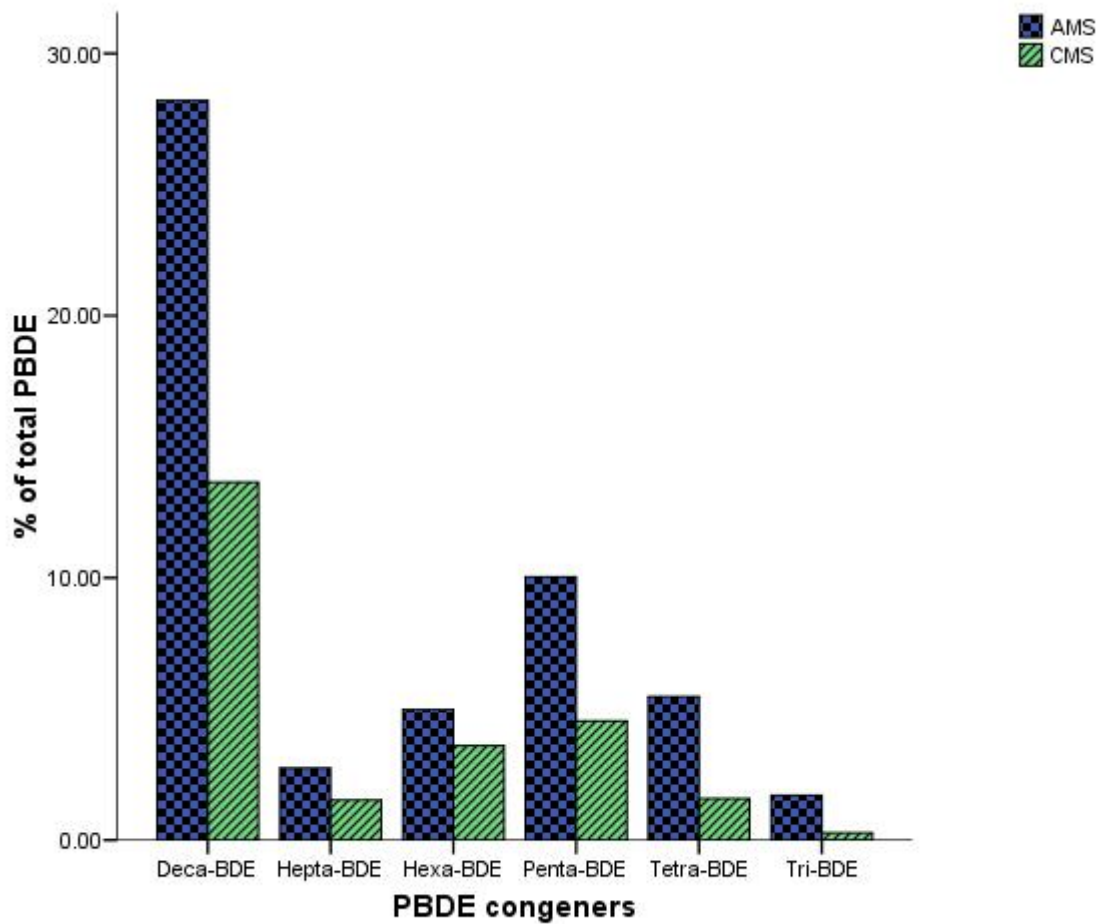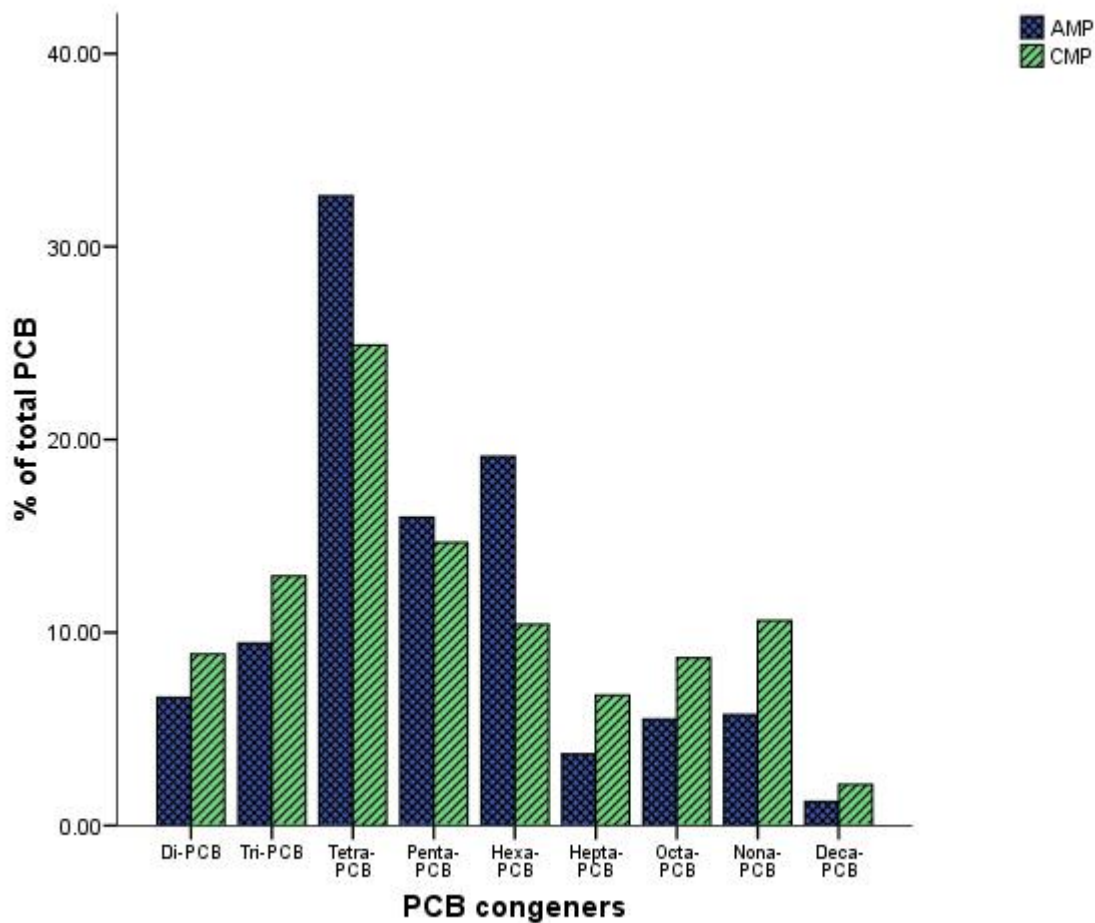
Figure 4.6: The percentage distribution of the sum of the di-PCBs to the sum of deca-PCBs

in plant samples collected from e-waste dumpsites in Lagos, Nigeria.

CMP - Computer Village market plants; AMP - Alaba International market plants

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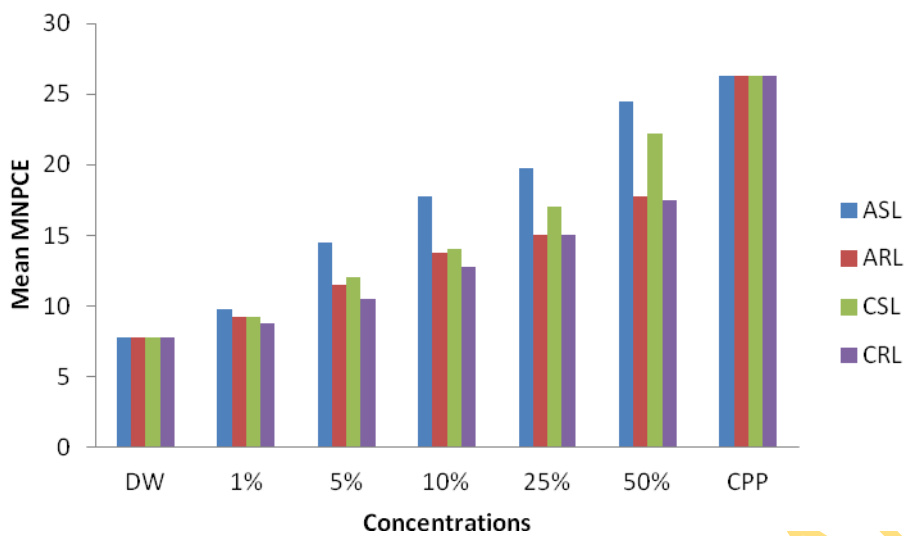
Figure 4.7: Mean of MNPCE induced in the bone marrow of mice by different concentrations of leachates from e-waste dumpsite at Alaba International and Computer Village markets.

ASL-Alaba simulated leachate; ARL-Alaba raw leachate; CSL-Computer village simulated leachate; CRL-Computer village raw leachate; DW –distilled water, CPP – cyclophosphamide (20 mgkg^{-1} bw).

MNPCE at tested concentrations except at the 1% concentration in all the samples, as the values were two-fold higher than negative control (Table 4.8). There was a dose-dependent significant rise in NCE (i.e. PCE : NCE) and MNNCE at all concentrations except at the 1% concentration compared to the negative control (Figure 4.8). The simulated leachates induced more MN in both PCE and NCE of exposed mice than the raw samples.

Well water

Figure 4.9 shows the results of MNPCE observed in the bone marrow of mice exposed to the well water samples. Compared to negative control, the results showed a significant increase in MNPCE at 4 and 5 weeks exposure in the Alaba market well water and 5 week exposure in Computer Vilage market well water (Table 4.8). There was an exposure-duration dependent significant rise in NCE (i.e. PCE : NCE) and MNNCE at all exposure levels compared to the negative control (Figure 4.10).

The well water from Alaba market induced more MN in both PCE and NCE of exposed mice than the Computer Village market well water. Figure 4.11 shows the PCE and MNPCE observed in the exposed animal. Cyclophosphamide induced a significant MN as positive control.

4.6 Sperm morphology assay

Leachates

The test samples induced significant concentration-dependent abnormal sperm cells at all concentrations except at 1% compared to the negative control (Table 4.9). The frequencies of abnormal sperm cells in the negative and positive controls were 2.88% and 16.46%, respectively. The tested concentrations of 1%, 5%, 10%, 25%, and 50% of ASL and ARL induced 3.18%, 4.74%, 9.66%, 11.02%, and 11.76%; 2.98%, 4.60%, 9.38%, 10.92%, and 11.58% abnormalities, respectively (Tables 4.10); while CSL and CRL at same concentrations induced 2.96%, 4.62%, 9.56%, 10.88%, 11.64%, and 2.82%, 4.48%, 9.26%, 10.78%, and 11.46% abnormalities, respectively (Tables 4.11). Folded sperm cells have the highest occurrence while double tail sperm cells have the least occurrence in both samples (Tables 4.12 to 4.15). The simulated leachates induced more abnormal sperm cells than the

Table 4.8: The mean±SD of Micronucleated Polychromatic Erythrocytes induced in mice exposed to raw and simulated leachates and well water from e-waste dump sites at Alaba International and Computer village markets, Lagos, Nigeria.

Conc (%)	Mean ± SD			
	ASL	ARL	CSL	CRL
Distilled water	8.25±0.96	9.75±0.5	8.0±0.82	7.5±1.29
1	9.75±0.96	9.25±0.96	9.5±0.58	9.0±0.82
5	14.5±1.29*	11.5±2.15*	13.5±1.29*	11.0±0.82*
10	17.75±1.71*	13.75±1.26*	17.0±0.82*	13.0±1.41*
25	19.75±0.46*	15.0±1.63*	18.5±1.29*	14.5±1.29*
50	24.5±1.29*	17.75±2.99*	23.0±0.82*	17.25±0.96*
Cyclophosphamide (20mg/kgbw)	26.0±1.16*	25.0±0.59*	25.5±0.58*	24.51±1.00*
Week(s)	AWW		CWW	
Distilled water	7.98±0.96		7.98±0.96	
1	8.16±1.17		7.99±0.39	
2	8.6±0.21		8.9±1.51	
3	10.01±2.01		9.6±1.00	
4	11.11±0.74*		10.71±0.61	
5	13.81±1.41*		12.32±0.82*	
Cyclophosphamide (20mg/kgbw)	24.44±1.00*		24.44±1.00*	

*significant at $p < 0.05$

ASL − Alaba simulated leachate; ARL − Alaba raw leachate; AWW- Alaba well water; CSL − Computer village simulated leachate; CRL − Computer village raw leachate; CWW- Computer village well water.
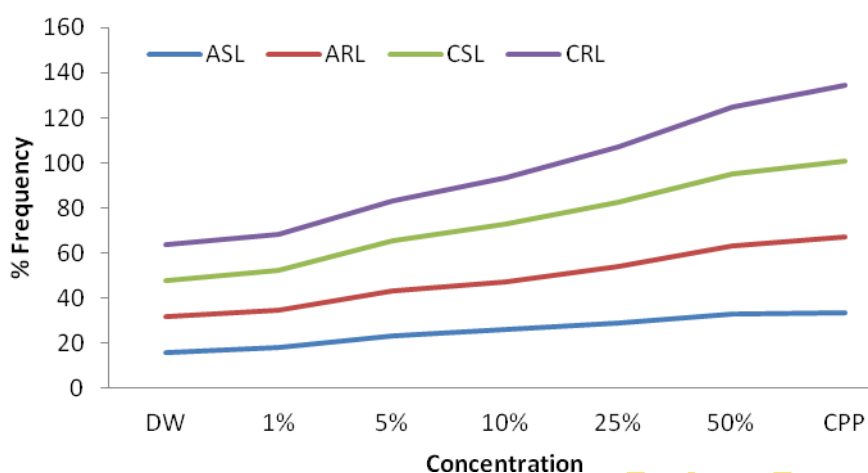
Figure 4.8: The percentage NCE induced in the bone marrow of mice by different concentrations of from e-waste dumpsite at Alaba International and Computer Village markets.

ASL-Alaba simulated leachate; ARL-Alaba raw leachate; CSL-Computer village simulated leachate; CRL-Computer village raw leachate; DW –distilled water, CPP – cyclophosphamide (20 mgkg^{-1} bw).
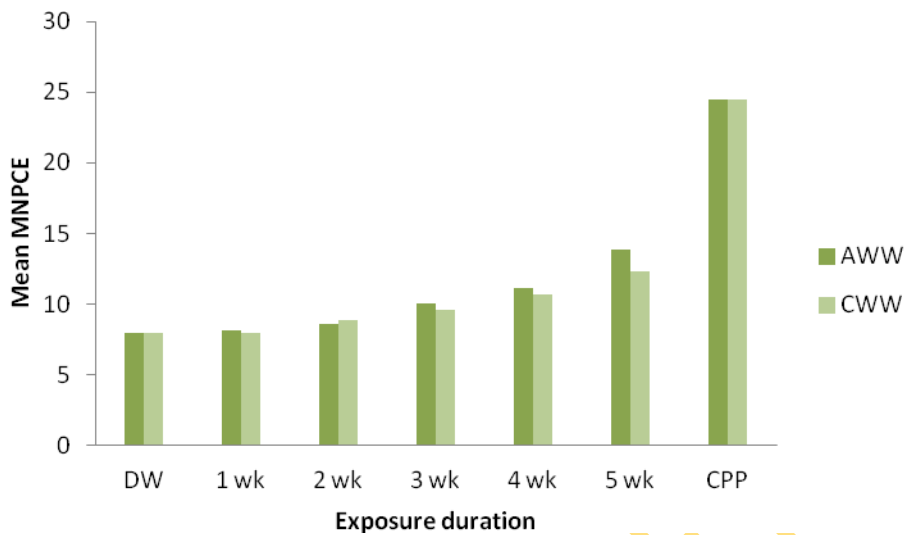
Figure 4.9: Mean of MNPCE induced in the bone marrow of mice by well water from e-waste dumpsite.

AWW- Alaba International well water; CWW-Computer Village well water.

DW –distilled water, CPP – cyclophosphamide (20 mgkg^{-1} bw).
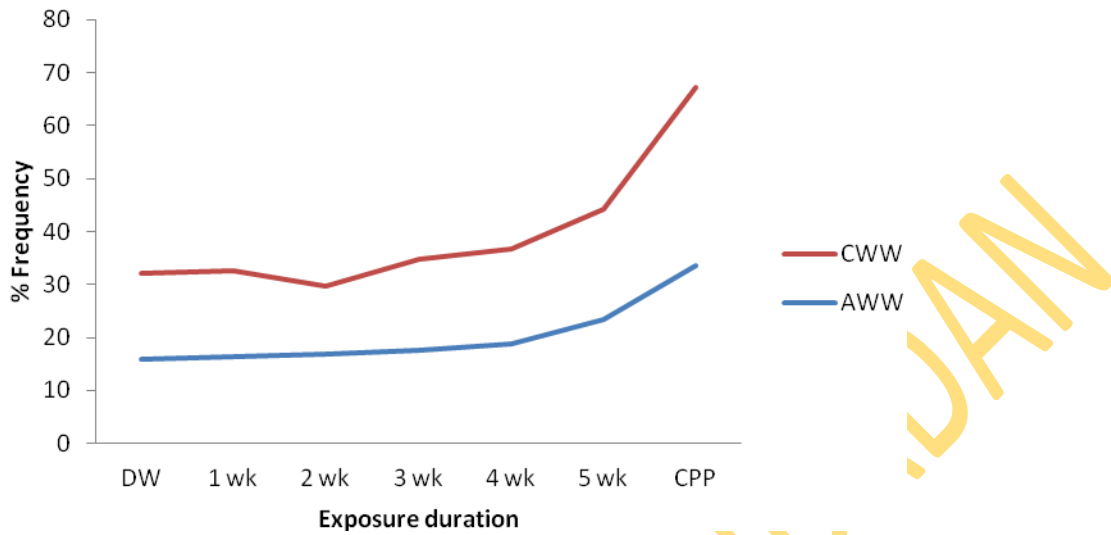
Figure 4.10: The percentage NCE induced in the bone marrow of mice by well water from e-waste dumpsites at Alaba International and Computer Village markets.

AWW- Alaba International well water; CWW-Computer Village well water.

DW –distilled water, CPP – cyclophosphamide (20 mgkg^{-1} bw).
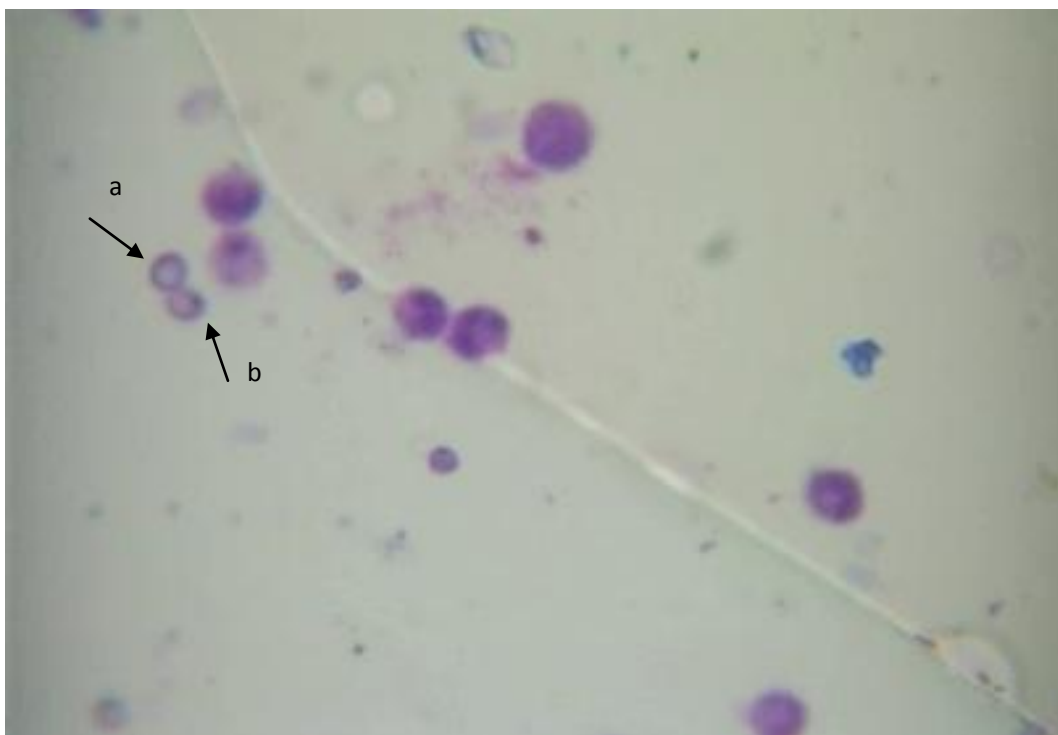
Figure 4.11: Normal PCE (a) and MNPCE (b) observed in bone marrow of mice exposed to the e-waste leachates and well water (May Grunwald and giemsa stain, Magnification x100).

Table 4.9: The mean±SD of abnormal sperm cells induced in mice exposed to raw and simulated leachates and well water from e-waste dump sites at Alaba International and Computer village markets, Lagos, Nigeria.

Conc (%)	Mean ± SD			
	ASL	ARL	CSL	CRL
Distilled water	68.8±4.97	67.89±1.00	68.3±2.28	66.6±1.92
1	83.8±3.03	83.2±2.17	83.41±2.13	81.01±1.30
5	86.0±3.81*	87.4±0.89*	87.01±1.58*	86.0±1.00*
10	136.6±2.30*	133.8±1.92*	135.4±2.21*	133.2±1.95*
25	149.2±5.45*	150.2±1.92*	147.88±0.80*	146.17±3.01*
50	157.6±1.95*	155.8±2.28*	155.92±2.03*	153.42±2.53*
Cyclophosphamide (20mg/kgbw)	204.6±1.95*	203.1±0.89*	205.12±2.30*	204.81±1.00*
Week(s)	AWW		CWW	
Distilled water	66.34±1.00		66.34±1.00	
1	67.92±1.97		66.5±0.76	
2	68.6±0.61		66.9±4.15	
3	68.76±2.50		68.8±1.00	
4	74.21±2.04		71.1±1.61	
5	88.06±1.41*		86.1±0.82*	
Cyclophosphamide (20mg/kgbw)	201.81±1.00*		201.81±1.00*	

*Statistically significant at $p < 0.05$

ASL − Alaba simulated leachate; ARL − Alaba raw leachate; AWW- Alaba well water; CSL − Computer village simulated leachate; CRL − Computer village raw leachate; CWW- Computer village well water.

Table 4.10: Summary of sperm-head aberrations induced by ASL and ARL from e-waste dumpsite in Alaba International market after 5 weeks of exposure.

Concentrations	No of animals used	Total number of cells counted	Number of abnormal sperms		% frequency of abnormality	
			ASL	ARL	ASL	ARL
Distilled water	5	5000	144	144	2.88	2.88
1%	5	5000	159	149	3.18	2.98
5%	5	5000	237	230	4.74*	4.60*
10%	5	5000	483	469	9.66*	9.38*
25%	5	5000	551	546	11.02*	10.92*
50%	5	5000	588	579	11.76*	11.58*
Cyclophospha mide (20mg/Kgbwt)	5	5000	823	823	16.46*	16.46*

*significant at p<0.05; ASL – Alaba simulated leachate; ARL – Alaba raw leachate;

Table 4.11: Summary of sperm-head aberrations induced by CSL and CRL from e-waste dumpsite in Computer village market after 5 weeks of exposure.

Concentrations	No of animals used	Total number of cells counted	Number of abnormal sperms		% frequency of abnormality	
			CSL	CRL	CSL	CRL
Distilled water	5	5000	139	139	2.78	2.78
1%	5	5000	148	141	2.96	2.82
5%	5	5000	231	224	4.62*	4.48*
10%	5	5000	478	463	9.56*	9.26*
25%	5	5000	544	539	10.88*	10.78*
50%	5	5000	582	573	11.64*	11.46*
Cyclophosphamide (20mg/Kgbwt)	5	5000	818	818	16.36*	16.36*

*significant at $p < 0.05$; CSL – Computer village simulated leachate; CRL – Computer village raw leachate.

Table 4.12: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by different concentrations of ASL in mice after 5 week exposure.

ASL	Hook at wrong angle	Banana shape	No hook	Folded	Nubbed hook	Amorphous head	Tail at wrong angle	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	13	11	21	41	16	31	7	2	-	2	-	144
1%	18	8	18	46	11	38	8	4	4	2	2	159
5%	26	18	32	52	23	50	12	3	10	4	7	237
10%	54	38	57	95	48	85	37	10	30	23	5	483
25%	59	43	62	118	50	93	41	30	30	18	6	551
50%	69	40	62	128	58	110	44	25	35	10	5	588
TOTAL	226	147	231	439	190	376	142	72	109	57	25	2014
% occurrence	11.22	7.30	11.47	21.80	9.43	18.67	7.05	3.58	5.41	2.83	1.24	
Cyclophosphamide	99	58	84	158	82	140	60	42	58	27	10	823

Table 4.13: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by different concentrations of ARL in mice after 5 weeks exposure.

ARL	Hook at wrong angle	Banana shape	No hook	Folded	Nubbed hook	Amorphous head	Tail at wrong angle	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	13	11	21	41	16	31	7	2	-	2	-	144
1%	14	8	24	45	14	33	8	-	1	2	-	149
5%	20	22	30	59	24	43	18	10	4	-	-	230
10%	52	40	54	89	43	74	36	32	24	20	5	469
25%	62	53	68	99	50	84	46	28	32	15	8	546
50%	67	58	73	104	55	89	49	26	34	15	6	579
TOTAL	215	181	249	396	186	323	157	96	95	52	19	1969
% occurrence	10.92	9.19	12.65	20.11	9.45	16.40	7.97	4.88	4.82	2.64	0.97	
Cyclophosphamide	99	58	84	158	82	140	60	42	58	27	10	823

Table 4.14: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by different concentrations of CSL in mice after 5 weeks exposure.

CSL	Hook at wrong angle	Banana shape	No hook	Folded	Nubbed hook	Amorphous head	Tail at wrong angle	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	15	11	21	31	16	31	8	2	3	1	-	139
1%	19	14	23	36	12	30	10	-	4	-	-	148
5%	34	24	30	48	20	42	16	10	6	1	-	231
10%	69	44	59	70	50	72	46	30	26	7	5	478
25%	82	51	68	93	62	86	52	15	26	3	5	544
50%	95	59	74	100	60	90	71	10	20	-	2	582
TOTAL	299	192	254	347	204	320	195	65	82	11	12	1981
% occurrence	15.09	9.69	12.82	17.52	10.30	16.15	9.84	3.28	4.14	0.56	0.61	
Cyclophosphamide	127	82	101	130	86	120	89	15	55	9	3	818

Table 4.15: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by different concentrations of CRL in mice after 5 weeks exposure.

CRL	Hook at wrong angle	Banana shape	No hook	Folded	Nubbed hook	Amorph ous head	Tail at wrong	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	15	11	21	31	16	31	8	2	3	1	-	139
1%	16	5	27	32	10	37	10	-	4	-	-	141
5%	26	15	42	40	22	47	13	5	10	4	-	224
10%	56	35	72	82	42	77	42	25	30	2	-	463
25%	71	40	86	96	48	83	50	27	36	-	2	539
50%	92	39	106	110	40	83	58	7	30	6	2	573
TOTAL	261	134	333	360	162	327	173	64	106	12	4	1936
% occurrence	13.48	6.92	17.20	18.60	8.37	16.89	8.94	3.31	5.48	0.62	0.21	
Cyclophosp hamide	127	82	101	130	86	120	89	15	55	9	3	818

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raw leachates. Intra-concentration comparison of the concentration in each sample was not significant.

Well water

The well water samples induced an increase, exposure-duration dependent, abnormal sperm cells at all concentrations compared to the negative control (Table 4.16). The frequencies of abnormal sperm cells in the negative and positive controls were 2.84% and 17.56%, respectively. The tested exposure duration of 1, 2, 3, 4, and 5 weeks of AWW and CWW induced 2.88%, 3.06%, 3.46%, 4.88%, and 6.84%; 2.82%, 3.00%, 3.32%, 4.58%, and 5.46% abnormalities, respectively. The percentage increase in abnormal sperm cells was significant (p<0.05) at 4 and 5 weeks exposure duration only for both samples. Just like the leachate samples, folded sperm cells have the highest occurrence while double tail sperm cells have the least occurrence in the well water (Tables 4.17 and 4.18). The Alaba market well water induced more abnormal sperm cells than the Computer Village well water. Intra-concentration comparison of the concentration in each sample was not significant. Figures 4.12- 4.17 show the different sperm abnormalities observed.

4.7 Sperm count

Leachates

Table 4.19 shows the result of the mean sperm count in mice exposed to raw and simulated leachates from both Alaba International and Computer Village markets. The negative control showed mean sperm count ranging from 17.201×10^6 - 17.961×10^6 mL^{-1} while the positive control showed a reduction in mean sperm count in the range of 8.631×10^6 - 8.982×10^6 mL^{-1}. The mean sperm count significantly (p<0.05) decreased in a concentration-dependent manner at all concentrations except at 1% concentration in ASL, ARL and CSL, and 1 and 5 % concentrations in CRL (Table 4.19). The decrease in mean sperm count was significant with respect to inter-concentration variations but not significant with respect to inter-animal variations.

Table 4.16: Summary of sperm-head aberrations induced by AWW and CWW from the neighborhood of Alaba International and Computer village markets after 5 weeks of exposure.

Exposure duration (weeks)	No of animals used	Total number of cells counted	Number of abnormal sperms		% frequency of abnormality	
			AWW	CWW	AWW	CWW
Distilled water	5	5000	142	142	2.84	2.84
1	5	5000	144	141	2.88	2.82
2	5	5000	153	150	3.06	3.00
3	5	5000	173	166	3.46	3.32
4	5	5000	244	229	4.88*	4.58*
5	5	5000	342	273	6.84*	5.46*
Cyclophospha mide (20mg/Kgbwt)	5	5000	878	878	17.56*	17.56*

*significant at $p < 0.05$; AWW- Alaba well water; CWW- Computer village well water.

Table 4.17: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by AWW in mice after 1-5 weeks exposure duration.

AWW	Hook at wrong	Banana shape	No hook	Folded	Nubbed hook	Amorphous head	Tail at wrong angle	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	15	11	21	31	16	31	8	2	6	1	-	142
1 week	18	8	20	33	19	33	9	-	4	-	-	144
2 weeks	22	10	20	30	15	40	10	1	4	1	-	153
3 weeks	32	12	22	33	11	42	10	5	5	1	-	173
4 weeks	45	19	28	44	20	56	18	3	10	1	-	244
5 weeks	50	29	45	51	36	68	22	17	17	5	2	342
TOTAL	167	78	135	191	101	239	69	26	40	8	2	1056
% occurrence	15.81	7.39	12.78	18.09	9.56	22.63	6.53	2.46	3.79	0.76	0.19	
Cyclophosphamide	100	92	111	140	96	167	89	15	55	9	3	878

Table 4.18: Summary of the frequency and percentage occurrence of the different types of abnormal sperm cells induced by CWW in mice after 1-5 weeks exposure duration.

CWW	Hook at wrong angle	Banana shape	No hook	Folded	Nubbed hook	Amorphous head	Tail at wrong angle	Short tail	Pin head	Thin tail	Double tail	Total
Distil water	15	11	21	31	16	31	8	2	6	1	-	142
1 week	20	9	22	30	19	32	7	-	4	-	-	141
2 weeks	23	7	21	33	16	35	7	1	7	1	-	150
3 weeks	25	8	24	32	18	37	8	4	9	1	-	166
4 weeks	39	18	33	45	25	41	11	3	12	2	-	229
5 weeks	44	23	38	50	30	46	16	8	16	2	-	273
TOTAL	151	65	138	190	108	191	49	16	48	6	0	
% occurrence	15.70	6.76	14.35	19.75	11.23	19.86	5.09	1.66	4.99	0.62	0	
Cyclophosphamide	100	92	111	140	96	167	89	15	55	9	3	878

Figure 4.12: Normal sperm cell. A normal sperm have both head and the tail. The tail is well attached and the hook on the head is well curved-out. The tail is usually long enough for locomotion of the whole sperm (Eosin-Y stain, Magnification x100).
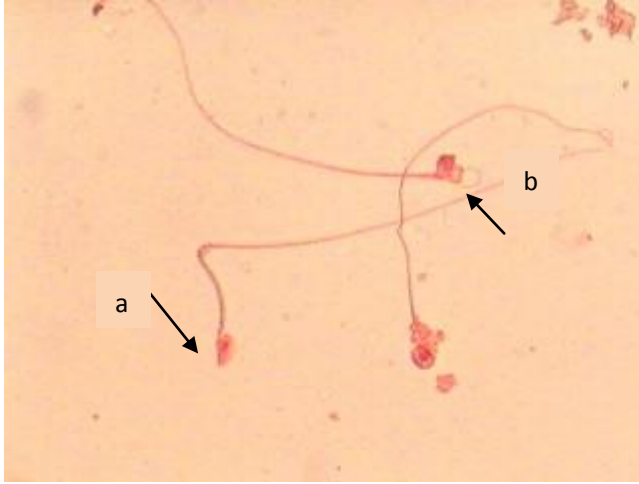
Figure 4.13: (a) Sperm cells with no hook. There is complete absence of hook in this type of aberration. (b) Sperm cell with amorphous head. The head of these sperms are not defined. It is different from that of the normal sperm, which has both base and hook (Eosin-Y stain, Magnification x100).
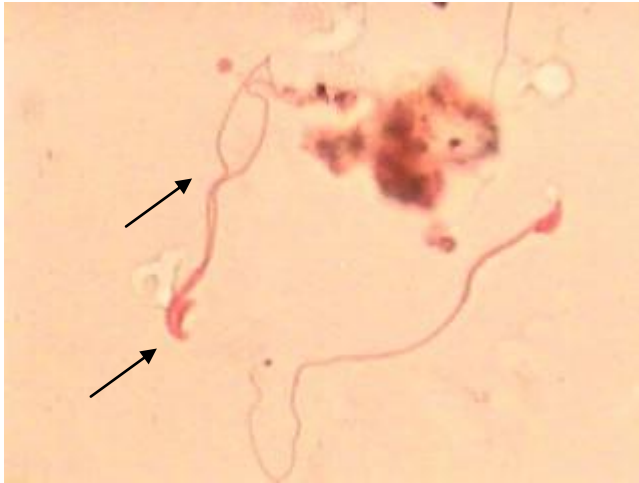
Figure 4.14: Sperm cell with double tail and banana shaped head. Sperms in this category have more than the normal one tail (Eosin-Y stain, Magnification x100).
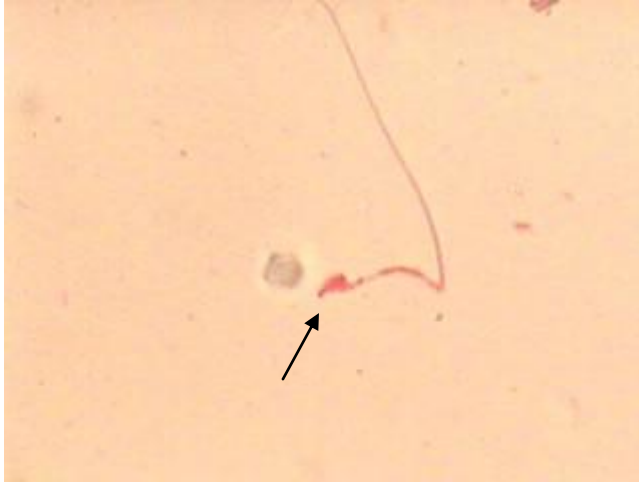
Figure 4.15: Sperm cell with hook at wrong angle. Sperm with hook at wrong angle has the hook but the location is quite different from that of a normal sperm (Eosin-Y stain, Magnification x100).
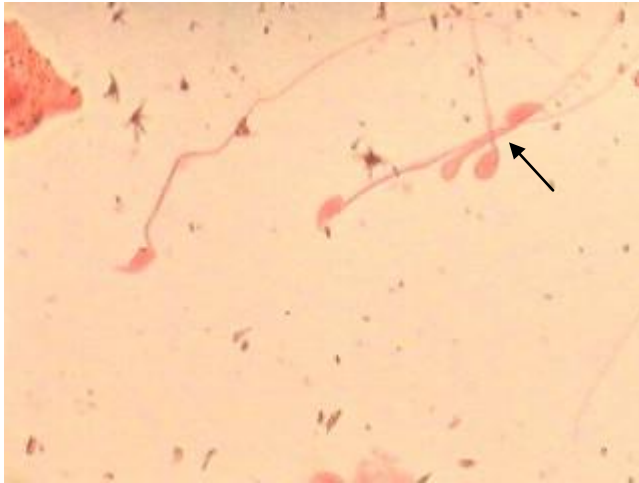
Figure 4.16: Folded sperm cell. Sperms that are folded are of different types ranging from those in which the head and the tail completely intertwine to those whereby the head and tail partially intertwine (Eosin-Y stain, Magnification x100).
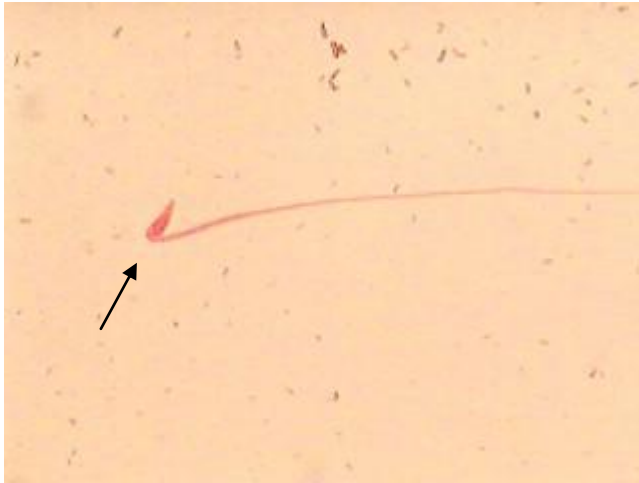
Figure 4.17: Sperm cell with banana shaped head. The shape of the head is like that of banana; hence the name banana shaped sperm (Eosin-Y stain, Magnification x100).

Table 4.19: The mean sperm count of mice exposed to raw and simulated leachates and well water from e-waste dump sites at Alaba International and Computer village markets, Lagos, Nigeria.

Conc (%)	Mean (/ml)			
	ASL	ARL	CSL	CRL
Distilled Water	17.201×10^6	17.632×10^6	17.411×10^6	17.961×10^6
1	16.921×10^6	17.101×10^6	17.142×10^6	17.168×10^6
5	$14.615 \times 10^{6*}$	$15.111 \times 10^{6*}$	$15.318 \times 10^{6*}$	15.416×10^6
10	$13.041 \times 10^{6*}$	$13.981 \times 10^{6*}$	$14.724 \times 10^{6*}$	$14.978 \times 10^{6*}$
25	$11.832 \times 10^{6*}$	$12.316 \times 10^{6*}$	$13.109 \times 10^{6*}$	$13.308 \times 10^{6*}$
50	$9.768 \times 10^{6*}$	$10.859 \times 10^{6*}$	$11.601 \times 10^{6*}$	$11.872 \times 10^{6*}$
Cyclophosphamide (20mg/kgbw)	$8.631 \times 10^{6*}$	$8.982 \times 10^{6*}$	$8.749 \times 10^{6*}$	$8.692 \times 10^{6*}$
Week(s)	AWW		CWW	
Distilled Water	17.101×10^6		17.101×10^6	
1	17.111×10^6		17.320×10^6	
2	16.187×10^6		16.791×10^6	
3	$15.661 \times 10^{6*}$		16.442×10^6	
4	$14.701 \times 10^{6*}$		$15.533 \times 10^{6*}$	
5	$14.221 \times 10^{6*}$		$15.014 \times 10^{6*}$	
Cyclophosphamide (20mg/kgbw)	$8.882 \times 10^{6*}$		$8.882 \times 10^{6*}$	

*Significant at $p < 0.05$.

ASL – Alaba simulated leachate; ARL – Alaba raw leachate; AWW- Alaba well water; CSL – Computer village simulated leachate; CRL – Computer village raw leachate; CWW- Computer village well water.

Well water

Table 4.19 shows the result of the mean sperm count in mice exposed to well water from both Alaba International and Computer Village markets. The negative and positive controls showed 17.101×10^6 mL^{-1} and 8.882×10^6 mean sperm count respectively. The mean sperm count significantly ($p<0.05$) decreased in an exposure-duration dependent manner at 3, 4 and 5 weeks of AWW and 4 and 5 weeks of CWW (Table 4.19).

4.8 Comet assay

Table 4.20 shows the result of the DNA damage induced in human peripheral blood lymphocytes exposed to leachate samples obtained from CSL and ASL. Cell viability was >80% in all the treatment groups utilised. Based on tail length (TL), percentage tail DNA (%TDNA) and olive tail moment (OTM), results indicate that the tested leachates induced significant ($p<0.05$) concentration-dependent increases in DNA damage in human lymphocytes at the higher concentrations of each of the leachates, indicating an increase in DNA damage (Table 4.20). Although samples from both markets showed a positive genotoxicity response, the %TDNA, OTM, and TL showed that the genotoxicity in ASL is greater than in CSL. There were significant positive correlations (0.82 and 0.98 for CSL and ASL respectively) between the total %TDNA, OTM, and TL of the leachates. The concentration with the highest genotoxicity was 7%. Figures 4.18 and 4.19 show the DNA damage induced in human peripheral blood lymphocytes exposed to leachate samples.

Spearman correlation analysis of the %TDNA, OTM, and TL of the samples and the PAHs, PCBs, heavy metals, and PBDEs showed that there was a positive correlation ($p<0.01$) with both PAHs ($r=1.00$ for %TDNA and TL, and 0.8 for OTM) and heavy metals ($r=0.06$ for %TDNA and TL and 0.8 for OTM). There was a positive, but not statistically significant trend with PCBs ($r=0.4$ for %TDNA and TL, and 0.14 for OTM), and a negative correlation ($r=-0.2$ for %TDNA and TL, and -0.4 for OTM) with PBDEs. The Pearson correlation analysis showed a strong positive significant ($r<0.01$) correlation between comet assay, sperm morphology and MN but strong negative correlation with the sperm count (Table 4.21).

Table 4.20: DNA damage in human peripheral blood lymphocytes treated with leachates obtained from e-waste dump sites in Lagos, Nigeria[a]

Test samples	Conc (%)	Comet assay parameters		
		% Tail DNA	Olive Tail Moment	Tail Length (μm)
Positive control(H₂O₂)	100μM	20.19±1.26*	6.70±0.51*	40.96±2.60*
CSL	7	10.73±0.94*	4.51±0.54*	30.50±2.08*
	5	12.63±0.65*	3.28±0.24*	29.50±2.08*
	2	11.12±0.99*	3.62±0.41*	21.50±1.62*
	1	9.72±1.29*	2.72±0.37*	18.08±1.62*
	0.5	2.81±0.83	0.90±0.33	8.32±1.75
ASL	7	19.58±1.38*	6.93±0.53*	40.34±2.30*
	5	15.53±1.15*	5.82±0.53*	31.74±2.08*
	2	14.01±1.12*	4.92±0.54*	31.42±2.29*
	1	11.76±1.08*	4.43±0.47*	29.92±1.99*
	0.5	5.58±0.81*	1.55±0.27	11.84±1.43*
Negative control (RPMI)		1.29±0.38	0.33±0.09	4.82±0.58

[a]Values are mean (±S.E.) of % Tail DNA, Olive tail moment and tail length.

*$p < 0.05$= level of significance of DNA damage in leachate-treated peripheral lymphocytes compared with the untreated control.

CSL-Computer Village market simulated leachate; ASL-Alaba International market simulated leachate.
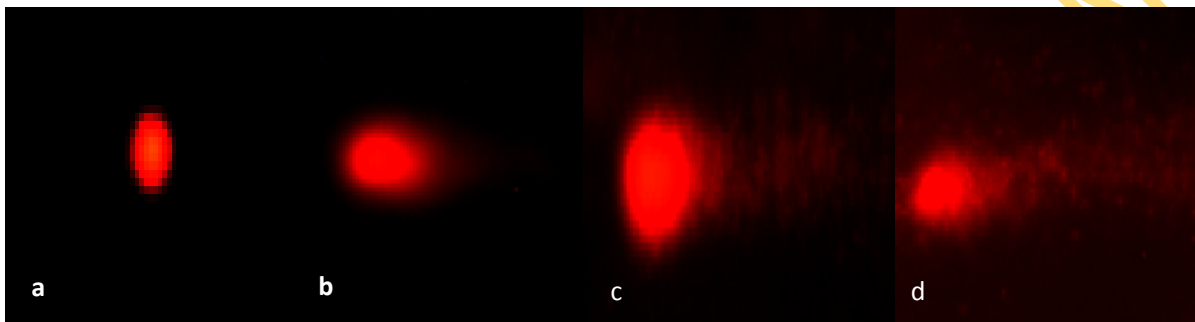
Figure 4.18: DNA damage in human peripheral blood lymphocyte (HPBL) treated with different concentrations of Alaba e-waste simulated leachate. (a) nucleus from an untreated HPBL (negative control), (b-d) DNA damage in nuclei from e-waste-treated HPBL (b=1%; c=5%; d=7%) (Propidium iodide stain, Magnification x100).

Table 4.21: Pearson correlation analysis of sperm morphology, micronucleus, comet assays and sperm count.

	ASL_{sh}	ASL_{mn}	ASL_{ca}	ASL_{sc}
ASL_{sh}	1			
ASL_{mn}	0.954**	1		
ASL_{ca}	0.972**	0.980**	1	
ASL_{sc}	-0.962**	-0.986**	-0.998**	1

	ARL_{sh}	ARL_{mn}	ARL_{sc}
ARL_{sh}	1		
ARL_{mn}	0.963**	1	
ARL_{sc}	-0.975**	-0.994**	1

	CSL_{sh}	CSL_{mn}	CSL_{ca}	CSL_{sc}
CSL_{sh}	1			
CSL_{mn}	0.970**	1		
CSL_{ca}	0.924**	0.969**	1	
CSL_{sc}	-0.964**	-0.985**	-0.977**	1

	CRL_{sh}	CRL_{mn}	CRL_{sc}
CRL_{sh}	1		
CRL_{mn}	0.959**	1	
CRL_{sc}	-0.963**	-0.985**	1

**significant at 0.01 (2-tailed); ASL – Alaba simulated leachate; ARL – Alaba raw leachate; CSL – Computer village simulated leachate; CRL – Computer village raw leachate; sh-sperm morphology; mn-micronucleus; ca-comet assay; sc-sperm count.

4.9 MTT assay

The IC_{50} value was used in this study as a parameter for cytotoxicity. Figure 4.19 shows the result of NIH/3T3 cells treated with different concentrations (5-100 %) of the Alaba e-waste simulated leachate. There was a concentration dependent decrease in cell viability from 5 to 50 % concentration. However, no significant decrease in cell viability was observed at 60 and 70 % concentrations of the sample. There was complete destruction of cells from concentrations above 70% (data not shown). The IC_{50} value for the e-waste simulated leachate was at 30% concentration.

4.10 Biochemical tests

4.10.1 Determination of catalase activity

Table 4.22 shows the effect of the various exposures on the liver CAT activity. All the test groups, except 1% concentration, had higher CAT activity but significantly ($p < 0.001$) only at 10-50 % concentrations of the leachate and 4 and 5 weeks well water exposure compared with the control group. However, CAT activity of groups treated with leachate and well water are concentration and exposure-duration dependent with 10, 25 and 50 % concentrations yielding 10.49%, 24.92% and 75.74% increase while 4 and 5 weeks well water exposure yielded 16.72% and 25.90% increase, relative to the control group.

4.10.2 Determination of SOD activities

Table 4.22 shows the effect of the various exposures on the liver super oxide dismutase (SOD) activity. Groups treated with 1% concentration and 1 week exposure elicited the highest activity while the 50% concentration and 5 week treated groups had the least SOD activity in the leachate and well water treatment respectively. The treated groups also had 49.89%, 59.57%, 68.17%, 23.66% and 52.69% significant reduction ($p < 0.001$) in liver SOD activities in mice exposed to 10, 25 and 50 % concentrations of the leachate sample and 4 and 5 weeks well water exposure respectively, compared with the control group.
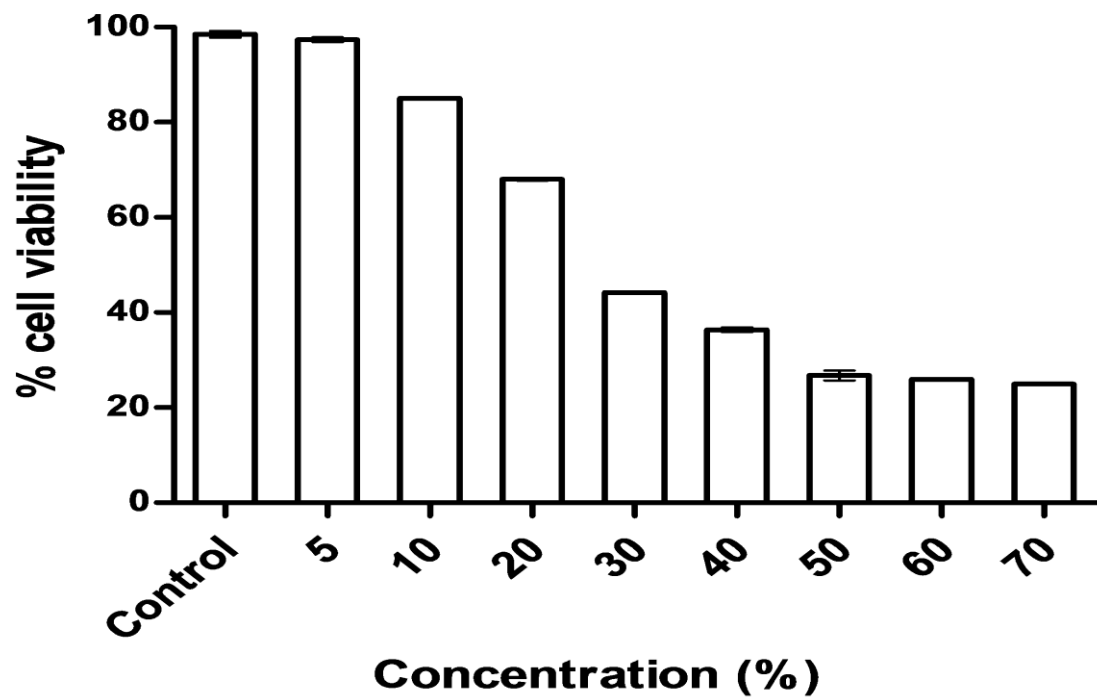
Figure 4.19: Percentage viability of NIH 3T3 cells exposed to Alaba e-waste simulated leachate. Cell viability was evaluated by MTT method. Each data point represents values from three independent experiments (n = 3).

Table 4.22: The effects of simulated e-waste leachate and well water on the liver lipid peroxidation (MDA), catalase, superoxide dismutase (SOD), reduced glutathione (GSH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.

Conc	MDA (μmol/ml)	Catalase (μm/mg)	SOD (U/ml/Min)	GSH (μm/g tissue)	ALT (U/ml)	AST (U/ml)
Alaba simulated e-waste leachate						
Normal	5.0±0.18	76.25±0.96	4.65±0.09	8.60±0.08	19.75±0.96	41.75±0.5
1%	5.4±0.14	76.25±0.96	4.58±0.05	8.65±0.09	21.00±0.82	42.50±0.58
5%	5.85±0.06	78.50±0.58	3.9±0.08	9.55±0.10	26.25±0.50*	45.25±0.96
10%	8.0±0.16*	84.25±1.71*	2.33±0.09*	10.85±0.19*	29.75±0.96*	49.25±0.96*
25%	11.98±0.15*	97.25±1.71*	1.88±0.10*	12.50±0.08*	40.75±1.26*	54.75±0.96*
50%	18.8±0.18*	134.0±1.41*	1.48±0.05*	14.15±0.13*	47.0±0.82*	61.0±0.82*
Well water						
1 week	5.08±0.13	78.0±0.82	4.45±0.06	8.71±0.06	20.00±0.82	41.25±0.5
2 weeks	5.38±0.13	81.75±0.96	4.08±0.05	8.95±0.06	25.00±1.41	43.50±0.58
3 weeks	5.78±0.09	83.02±0.96	3.55±0.13*	10.05±0.06*	28.75±0.50*	48.25±0.50
4 weeks	7.68±0.17	89.0±0.82*	3.58±0.15*	11.48±0.05*	32.00±1.41*	51.25±1.50*
5 weeks	8.35±0.10*	96.0±0.82*	2.20±0.08*	12.38±0.09*	36.75±0.50*	56.25±0.50*

*significant at 0.001

4.10.3 Determination of GSH activities

Table 4.22 shows the effect of various treatments on the liver reduced glutathione (GSH) level in the experimental mice. The GSH level was concentration and exposure-duration dependent with the highest GSH level measured from the groups treated with 50% concentration of the leachate and 5 week well water exposure. The treated groups had 26.16%, 45.35%, 64.54%, 16.86%, 33.49% and 43.95% GSH level significantly higher ($p<0.001$) for 10, 25 and 50 % concentrations of the leachate and 3, 4 and 5 week well water exposure respectively, than that of the control group.

4.10.4 Determination of AST activities

Table 4.22 shows the effect of various treatments on the serum aspartate aminotransferase (AST) level in the experimental mice. The AST level was concentration and exposure-duration dependent with the highest AST level measured from the groups treated with 50% concentration of the leachate and 5 week well water exposure. The treated groups had 17.96%, 31.14%, 46.11%, 22.76% and 34.73% AST level significantly higher ($p<0.001$) for 10, 25 and 50 % concentrations of the leachate and 4 and 5 week well water exposure respectively, than that of the control group.

4.10.5 Determination of ALT activities

Table 4.22 shows the effect of various treatments on the serum alanine aminotransferase (ALT) level in the experimental mice. The ALT level was concentration and exposure-duration dependent with the highest ALT level measured from the groups treated with 50% concentration of the leachate and 5 week well water exposure. The treated groups had 32.91%, 50.63%, 106.33%, 137.98%, 45.57%, 62.03% and 86.08% ALT level significantly higher ($p<0.001$) for 5, 10, 25 and 50 % concentrations of the leachate and 3, 4 and 5 week well water exposure respectively, than that of the control group.

4.11 Determination of mitochondrial membrane potential

In this study, a decrease in mitochondrial membrane potential in response to exposure to the leachate was observed, as evidenced by the decrease in the intensity of the red and green fluorescence ratio (Figure 4.20). The decrease in MMP was concentration dependent and significant ($p<0.05$) at 20 and 40 % concentrations compared to the negative control.
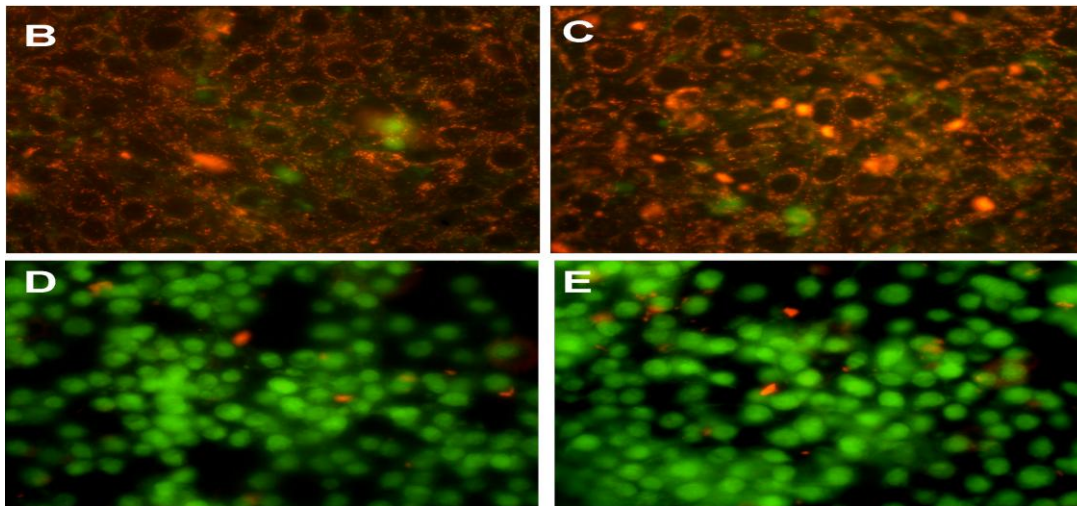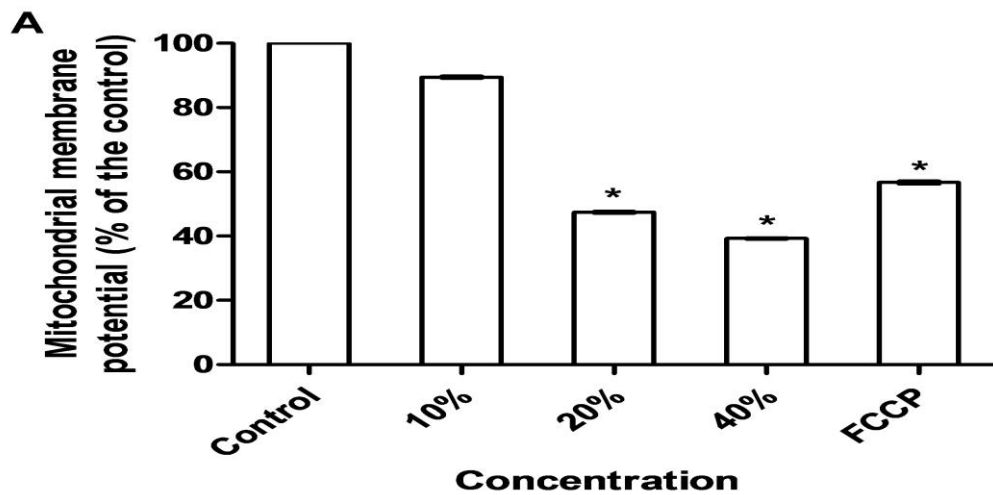
Figure 4.20: Effect of different concentrations of Alaba e-waste simulated leachate on the mitochondrial membrane potential of NIH 3T3 cells. NIH 3T3 cells were incubated for 24 h with 1 μM FCCP as positive control. The mitochondrial membrane potential was investigated using the JC-1 as a fluorescent probe. A) Quantitative analysis in spectrofluorimeter. The results are expressed as the mean ± SD (n = 3). The decrease of red/green ratio indicates a decrease in mitochondrial membrane potential, and the results are expressed as percentage of the control cells (100%), *$p<0.05$. (B-E) Representative images of at least three qualitative analyses in fluorescence microscope. B=control; C=10%; D=20% and E=40%.

4.12 *In vitro* determination of reactive oxygen species generation using DCFH-DA probe

The result of ROS generation induced by NIH/3T3 cell line exposure to Alaba e-waste simulated leachate is shown in Figure 4.21. The results indicate an induction of oxidative stress in the exposed cell lineage at tested concentrations of the leachate, which is significant at $p < 0.05$. The positive control (H_2O_2) induced a significant increase in oxidative stress in the exposed cell lineage.

4.13 Cell cycle analysis

Exposure of NIH/3T3 to different concentrations of the leachate showed different cell cycle distribution pattern (Figure 4.22a). Treatment with the leachate sample decreased the population of NIH/3T3 cells in G2/M and G1/G0 cell phases, which was significant ($p < 0.05$) at the tested concentrations. There was also a decrease in cell population in the S phase at the concentrations but significant ($p < 0.05$) only at the 20 and 40 % concentrations. This was accompanied by a markedly increase in Sub/G1 population at all tested concentrations of the leachate. As evident in Figure 4.22a, e-waste leachate clearly induced subdiploid (sub/G1) events, which are a hallmark of cell death by apoptosis. The percentage of apoptotic cells increased from 4.2% in control group to 72.22% in 40% concentration of leachate treatment ($p < 0.05$). The control cells showed a typical distribution of cell cycle phases. These indicate that the e-waste leachate induced cell death of NIH/3T3 lineage due to DNA fragmentation, which is suggestive of apoptosis.

In order to evaluate the relationship between cell death and ROS increment, antioxidant was added to the cells before exposure to the leachate. Figure 4.22b-h shows the histograms of the number of the cell channel (vertical axis) against the DNA content (horizontal axis), of exposed NIH/3T3 and control cells as observed under the flow cytometry. Interestingly, the presence of N-acetylcysteine protected cells against the oxidative stress caused by e-waste leachate after its administration prior to the exposure to e-waste leachate. This was observed by the significant increase in cells at G2/M, S and G1/G0 phases and a concomitant decrease in cells at sub/G1 phase.
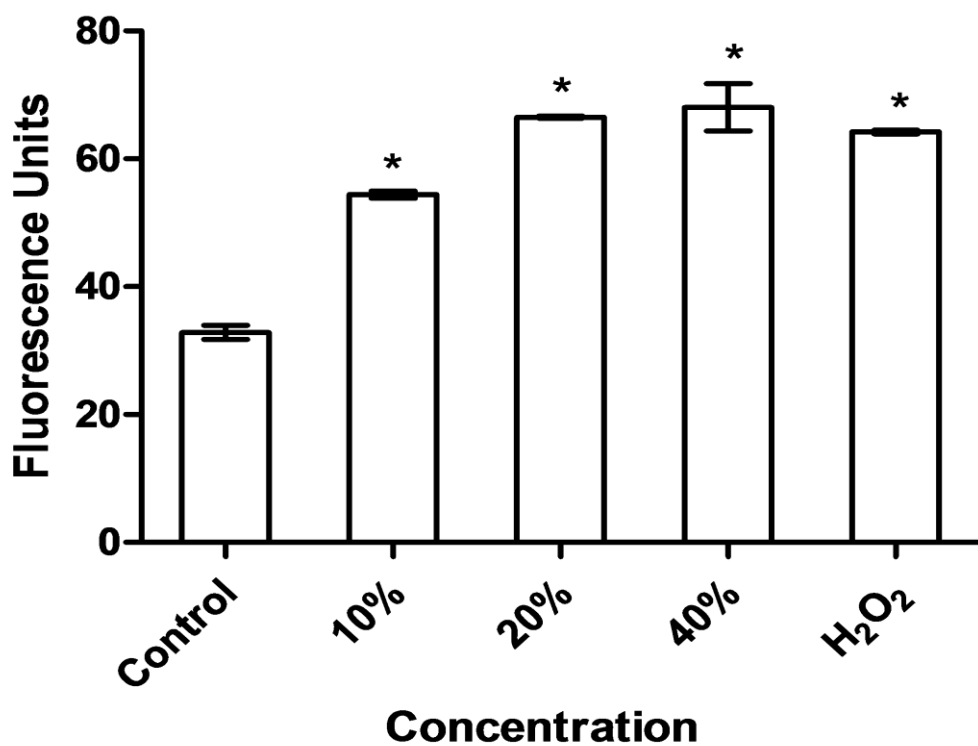
Figure 4.21: Effect of Alaba e-waste simulated leachate on free radical generation in NIH 3T3 cell lineage. The results were expressed as the percentage of fluorescent cells in comparison to control samples (zero % of fluorescence), the results were normalised by the protein concentration. 2000 μM of H_2O_2 was used as positive control. *$p < 0.05$.
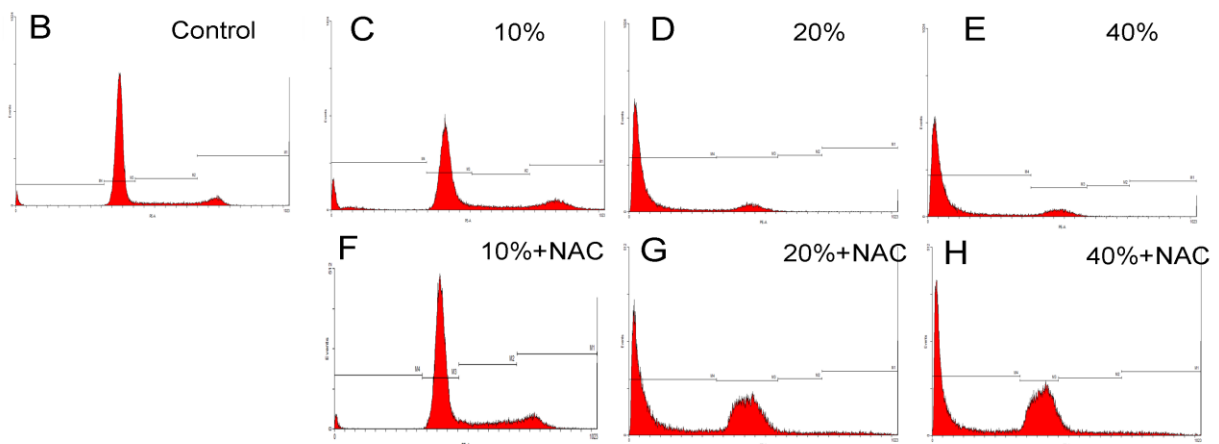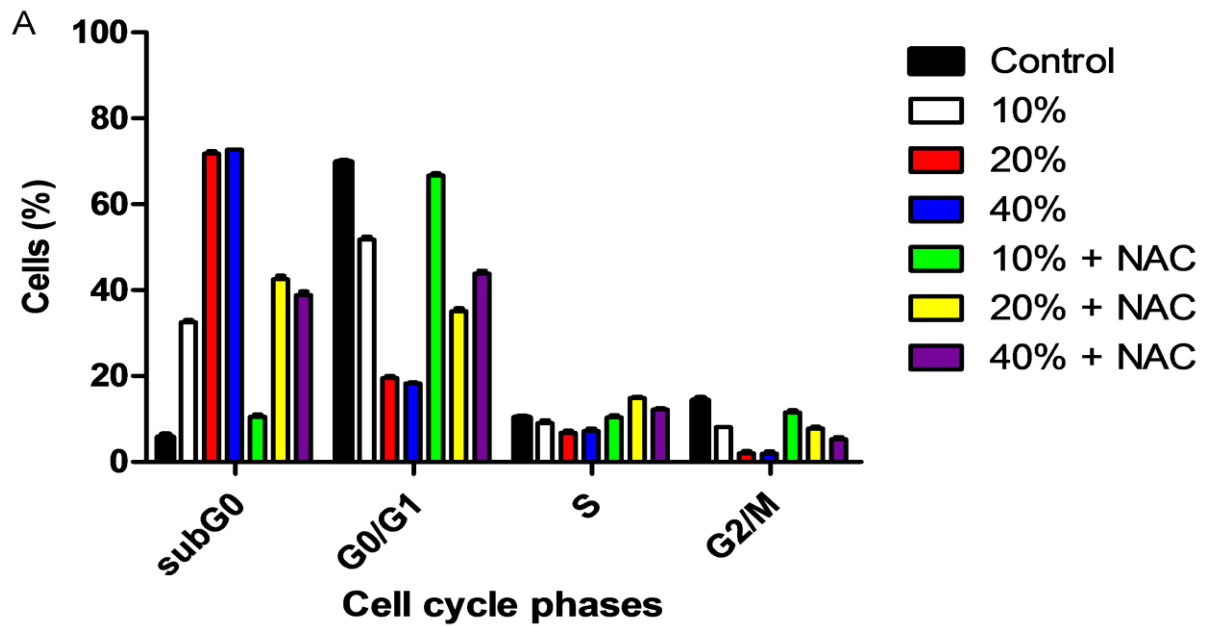
Figure 4.22: Effects of Alaba e-waste simulated leachate on cell cycle distribution in NIH 3T3 cell lineage. (A) G0/G1, G2/M and S indicate the cell phase, and sub/G1 DNA content refers to the proportion of apoptotic cells. Each phase was calculated by using the WinMDI 2.9 program. (B-H) Histograms showing the number of the cell channel (vertical axis) against DNA content (horizontal axis). NAC=N-Acetylcysteine.

However this protection does not produce a complete reversal of the toxic effects of the e-waste leachate (Figures 4.22f-h).

4.14 Acridine orange/ethidium bromide (AO/EB) double staining assay for differentiation between necrotic and apoptotic cells

Morphological features of apoptosis such as chromatin condensation, nuclear fragmentation, alterations in the size and the shape of cells, as revealed by fluorescence microscopic analysis, were observed predominantly after e-waste simulated leachate treatment at 20 and 40 % concentrations. The maximum increase in the number of apoptotic cells was observed in 40% concentration of the Alaba e-waste simulated leachate, which was significantly higher compared to the control. As shown in Figure 4.23, the different concentrations of the e-waste simulated leachate treatment also induced necrosis.
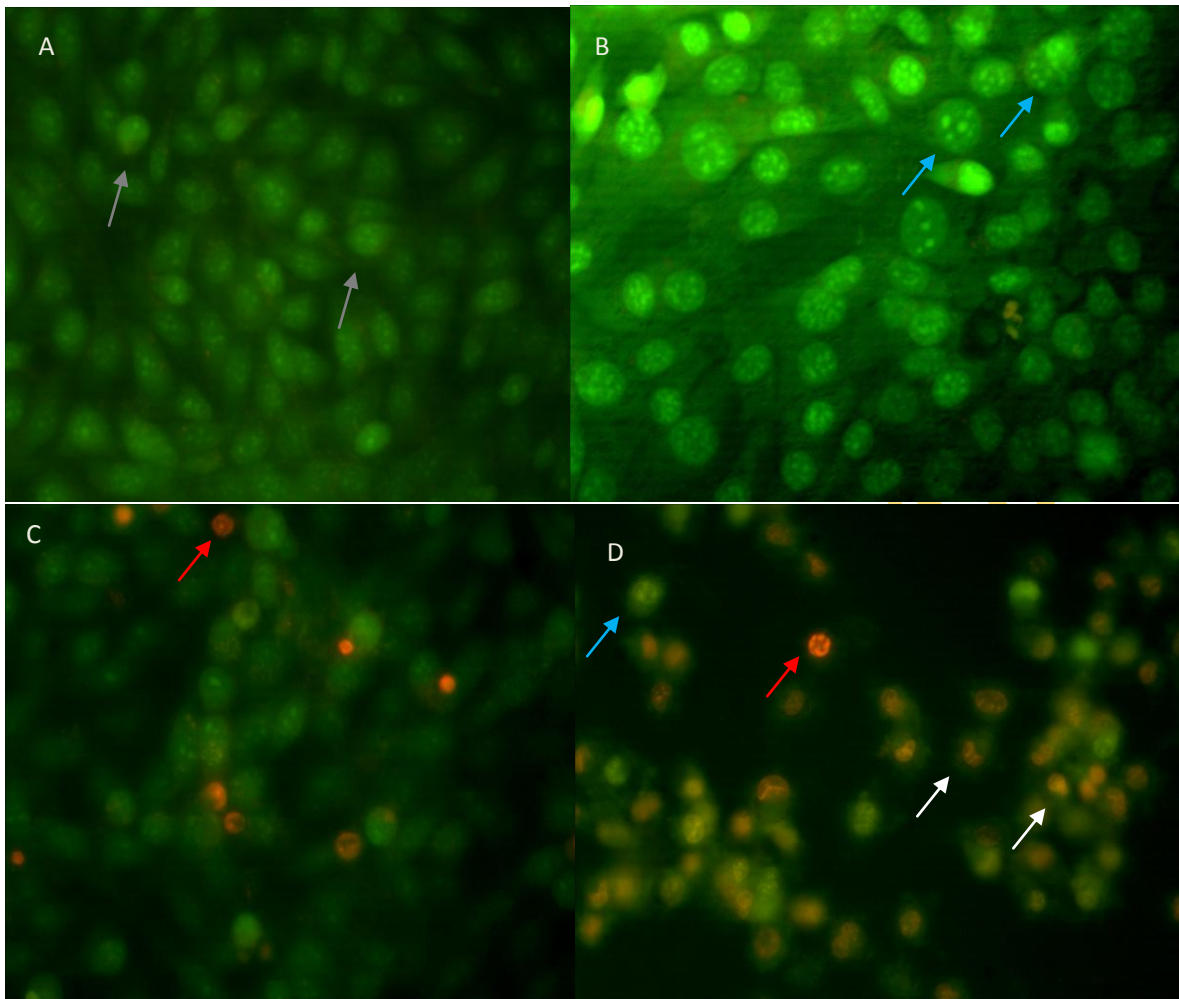
Figure 4.23: NIH 3T3 exposed to different concentrations of Alaba e-waste simulated leachate and double stained with Acridine orange and ethidium bromide. Ash-color arrow live cells; Blue arrow indicate apoptotic bodies; White arrow indicate apoptotic cell; Red arrow indicate necrotic cell. Representative images of at least three qualitative analyses in fluorescence microscope (Magnification at 400X). Concentrations of leachate: A(0%), B(10%), C(20%), D(40%).

CHAPTER FIVE

Discussion

The management and recycling of e-wastes has become a major global environmental problem. The improper processing of these e-wastes could release considerable quantities of toxic heavy metals and organic compounds into the workplace environment, surrounding soils and watercourses. Real-life exposures to toxic substances most often involve complex mixtures of hazardous chemicals than single toxicant. Therefore, e-waste leachates consisting of mixture of many chemicals including heavy metals and organics are a potential risk to human health. In the present study, the evaluation of environmental contamination, DNA damage and mechanism of e-waste-induced cytogenotoxicity was carried out.

The results showed that the physico-chemical characteristics of e-waste leachates were significantly higher than acceptable limits by regulatory authorities (USEPA, 2009; NESREA, 2007). The high BOD observed in the e-waste leachates and well water indicates that organics measured as BOD can cause taste and odor problems and oxygen depletion in the groundwater, thereby posing threat to aquatic organisms. In addition, the presence of high concentrations of PAHs, PBDEs and PCBs in some of these samples can serve as co-substrate for microorganisms, which may facilitate the conversion of these hazardous chemicals into more hazardous chemicals. The elevation in the physico-chemical characteristics of the tested samples above the normal values can cause severe degradation of groundwater quality and preclude its use for domestic water supply purposes (Lee and Jones-Lee, 1993). The TDS is a valuable indicator of the total dissolved salt content of water. The high concentrations of TDS observed in the groundwater suggest a downward transfer of leachate into groundwater as reported earlier by Mor *et al*. (2006), Al-khaldi (2006) and Longe and Enekwechi (2007). High concentrations of TDS decrease the palatability of water and may also cause gastrointestinal irritation in humans and laxative effects particularly upon transits (WHO, 1997).

The high concentration of heavy metals in the leachates, *A. hybridus* and well water is an indication of environmental contamination by e-waste-indiscriminate disposal in these areas. This is of public health concern as the values for the heavy metals in the samples exceeded the NESREA allowable limit. The high heavy metal contamination obtained in this study is in agreement with previous

reports from e-waste recycling sites in China and its surroundings (Leung *et al*., 2006). Heavy metal contamination in the plant samples was a reflection of their surrounding soils, as the pattern was similar. Plants from Alaba International market were the most contaminated, similar to the level of contamination in the leachates from the soil. The significant high levels of heavy metals in the plants reported in this study is of great concern as the plants utilised (*A. hybridus*) are edible, which is consumable by free range animals and can be toxic to them and subsequently the humans who consume these animals as a result of bioaccumulation or cause a damage to the exposed populace who feed directly on these contaminated vegetables. Moreover, the high concentration of Cd, Cr, Cu, Pb and As in the tested samples is of great health risk. This elevated level of heavy metals is as a result of constant open e-waste burning from the electronic markets where the samples were derived.

The accumulation of non-functional electronics, primitive repair processes and their subsequent open burning, have contributed to the elevated concentrations of heavy metals in these areas. Pb can be released from old solder, CRT monitor glass, lead-acid batteries, and some formulations of PVC. A typical cathode ray tube in a TV or computer monitor contains between 4 and 8 lb Pb, monitor glass contains 20% Pb by weight, and a typical Pb battery contains about 18 lb Pb (Royte, 2005; Russell, 2006; Huo *et al*., 2007). High level of Cu in the leachates were probably obtained from burning of copper wire, printed circuit board tracks, and lead components; Zn from steels; Cd from light-sensitive resistors, corrosion-resistant alloys, nickel–cadmium batteries; and Cr from steel plates. These electronic materials were seen at the dumpsites and their leaching may have been responsible for the high concentration of these heavy metals in this analysis.

The results also demonstrated a higher concentration of heavy metals in the leachates from the dumpsite at Alaba international market compared to those from Computer village market dumpsite. This is probably because the former has more electronic materials, shops, and wastes; and has been a site for electronic activities longer than the latter. These reports of high heavy metal contamination by e-wastes are in accordance with previous reports on heavy metal contamination from some other e-waste polluted sites in Philipines, China and Switzerland (Roman and Puckett, 2002; Hicks *et al*., 2005; Leung *et al*., 2006; Wang and Guo, 2006; Guo *et al*., 2009).

The well waters were also contaminated with heavy metals. Drinking water can be obtained from a number of sources; the one used often depending on the relative availability of surface water (such as rivers, lakes, and reservoirs) and ground water aquifers. In Nigeria ground water from wells is an important source of drinking water. However, in some cases ground water may contain chemical constituents hazardous to the health. This study showed that e-waste activities are gradually contaminating the ground water as evident in the concentrations of heavy metal levels ranging from 0.001 mg/L to 0.41 mg/L in the well water studied. Pb and Cd were present in higher concentrations than the allowable standards. E-waste contaminants can enter aquatic systems via leaching from dumpsites where processed or unprocessed e-wastes may have been deposited. Similarly, the disposal of acid following hydrometallurgical processes into waters or onto soils, as well as the dissolution or settling of airborne contaminants, can also result in the contamination of aquatic systems. Chemical compounds of toxic potential entering a public water supply may impose an immediate risk on human health. Interactions of heavy metals with the organic environmental pollutants can be more toxic than the health effects of heavy metals alone and this offers an important premise of study to ecotoxicologists (Atif *et al*., 2005).

This study showed a high contamination level of soils and plants by organics in the studied areas. Since AMS and CMS are e-waste dumpsite soils, the high PAH level is attributed to the constant open e-waste burning in these areas. The higher concentration of PAHs in AMS compared to CMS however might be because Alaba International market contains more electronic shops and hence more e-waste is generated there than in the Computer village market. Also, the former has been in operation longer than the latter; hence has been involved in open e-waste burning longer than the latter.

Similar to the report of Bakare *et al*. (2009b), the values obtained in this study showed that both plant and soil samples analysed were more contaminated than the reports of previous studies in China and other related countries (Zhang *et al*., 2006; Shen *et al*., 2009a; Maskaoui and Hu, 2009). Since Nigeria has no available soil and plant standards for PAHs, criteria constituted in other countries were used to evaluate the extent of contamination. In the Dutch list (VROM, 1994), soil should not exceed the optimum value of 1 mg/kg when the concentrations of ten VROM PAHs [naphthalene, anthracene, phenanthrene, fluoranthene, benzo(a)anthracene, chrysene, benzo(a)pyrene,

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benzo(g,h,i)perylene, benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene] are summed together. The PAH concentration of the samples collected from Alaba International and Computer Village markets far exceeded this limit, indicating that samples from both markets were contaminated. The concentration of carcinogenic PAHs exceeded the limit of 300 µg/kg for sensitive land use (SEPA, 2002), only in e-waste dumpsite of Alaba International market. Since the target set by the Dutch government for unpolluted soil is 20–50 µg/kg (Van Brummelen *et al*., 1996), therefore, since all the soils sampled far exceeded 50 µg/kg, they are considered to be highly polluted by PAHs.

In the plant samples, the highest concentration was recorded from plants collected at Alaba International market. Three-ring compounds dominated the concentration profile in all the samples. The result herein is similar in pattern (petrogenic source) to the soil samples from where the plants were derived, an indication of a strong correlation between plant and soil PAHs in both markets. The level of PAHs in these plants was higher than previously reported in similar sites in China (Shen *et al*., 2009a), an indication of extensive contamination of the soil samples and subsequent absorption of this organic pollutant by the root of the edible plants growing on these contaminated soils.

High concentrations of PBDEs were detected in the samples from both markets. The major PBDE congeners in the soil samples were BDE-99 and BDE-209. BDE-209 was the main contaminant in AMS while BDE-99 was the main contaminant in CMS. The contamination by these congeners is in accordance with previous reports of PBDE soil contamination in China (Julander *et al*., 2005; Cai and Jiang, 2006). The concentration of Σ*penta*-BDE is the most predominant in all the samples. This congener distribution pattern was similar to that previously reported by Wang *et al.* (2005). The high level of the PBDE congeners in these soil samples was probably derived from the commercial penta-BDE product used in fire retardants applied to electronics.

Similar to the soil, the major PBDE congener in the plant samples was BDE-209. The percentage BDE-209 was higher in the plant samples than in the soil samples. It is therefore suggested that consumption of contaminated plants is probably one of the major sources of the high human burden of BDE-209 previously reported in similar areas in China, Europe and Brazil (Rind, 2002). Such study of human burden of PBDE has not been conducted in Nigeria in spite of the high level of e-waste contamination. This observation is in accordance with previous reports, where BDE-209 had

been found to be the predominant congener in tree bark and vegetables in China (Zhu and Hites, 2006; Yang *et al*., 2008). The general patterns of percentage of the sum of tri- to hexa-BDE distribution in plant samples were similar to those in soil samples, an indication of a correlation between the plants and the soil from their surroundings. This type of correlation is in accordance with the report of Liu *et al*. (2008).

The PCB concentrations of the soils exceeded the Dutch action value and the Australian and New Zealand ecological investigation level of 1000 µg/kg, and the Canadian soil guideline for residential areas (1300 µg/kg) (VROM, 1994). All the samples consisted of di- to deca-PCBs and contained high concentrations of di- to tetra-chlorinated substituted homologues. It therefore appears that the PCBs may have originated from contamination by Aroclor 1242, 1254, and 1260 because of the larger presence of lower chlorinated biphenyl congeners. In the past, Aroclor was widely used as the dielectric medium in transformers and capacitors. Previous studies have also reported high concentrations of lower chlorinated PCB congeners in contaminated soils (Zhao *et al*., 2006; Shen *et al*., 2009a). In general, very similar profiles of PCB congeners, pattern of indicator PCBs, and WHO-toxic PCBs were observed at both locations with tetra-PCBs being the predominant contaminant. This is an indication that the PCBs in these sites are from the same type of contaminants. There are higher PCB contamination levels in the study areas than previously reported for contaminated soils in China (Zhao *et al*., 2006; Shen *et al*., 2009a). The ΣPCBs in plants in this study showed a higher PCB contamination than those observed by Zhao *et al*. (2006). The plant samples from the two markets have similar PCB congener pattern with the soil samples from where they were derived. The ΣPCBs in plants in this study showed a higher PCB contamination than those observed by Zhao *et al*. (2006).

The MN assay showed that the tested leachates and well water contained constituents capable of inducing clastogenic and aneugenic effects in the exposed mice. Chromosomes, the carriers of genetic material, have been widely recognised as cytogenetic biological markers which manifest as micronuclei in damaged dividing cells (Fenech, 2002). Induction of MN might be as a result of the leachates and contaminated well water absorption into the cells and induction of a change in the pH within and outside the cells, which might affect the activities of enzymes and change the structure of DNA (Meng *et al*., 2002). A MN is formed during the metaphase/anaphase transition of mitosis

(Schmid, 1975). MN may arise from a whole lagging chromosome or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei (Hayashi *et al*., 2007). Since acentric fragments do not have a centromere, they are not pulled toward the daughter nuclei at the time of nuclear division. These acentric fragments are left in the cytoplasm which appears as MN (Hosseinimehr *et al*., 2003). Administration of a given substance during cell proliferation may produce chromosome damage and also act on the macromolecules related to the function of chromatid disjunction (e.g., tubulin) inducing spindle dysfunction, depending on the mechanism of action (Krishna and Hayashi, 2000). MN assay is a sensitive, rapid, and extensively used tool for the detection of mutagenic and genotoxic effects of chemicals in the environment (Tucker *et al*., 1996) and has been used extensively in situ (Al-Sabti and Metcalfe, 1995).

An increase in NCE was observed compared to PCE in the tested samples. Krishna and Hayashi (2000) concluded that the PCE-to-NCE ratio between test agent-treated animals and vehicle-control animals provide a cytotoxicity index. This is an indication that the leachates and well water constituents increased the rate of aging of the exposed erythrocytes from PCE to NCE, thereby decreasing their normal life span and increasing the risk of genotoxicity.

In the sperm morphology assay, the criteria for a positive response were satisfied: there was an increase in abnormal sperm morphology to at least double the negative control level. There was also evidence of a concentration-dependent rise in the number of aberrant sperm cells. Sperm morphology test provide a direct measure of the quality of sperm production in chemically treated animals. Studies evaluating the genetic consequences of chemically induced sperm changes have mainly focused on understanding the genetic basis of chemically shaped abnormalities in mice. A number of lines of evidence suggest that an induced change in sperm morphology is reflected by genetic damage in the male germ cell (Topham, 1980). The induction of the sperm head abnormality can be either due to impaired spermatogenesis or damage in the genetic material of spermatogonia and spermatocytes. Tasdemir *et al*. (1997) reported that abnormality in the sperm head morphology reflects abnormality in spermatogenesis. Wyrobek *et al*. (1983) also noted that when male germ cells are exposed *in vivo* to a test sample, a positive result demonstrates the sample ability to damage spermatogenesis. Among

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the various etiologies attributed to the cause of male infertility, sperm DNA damage is highly significant. The integrity of sperm DNA is of vital importance for the successful fertilisation, embryogenesis and embryo development. Damage to sperm DNA may result in the abnormalities of sperm morphology by affecting the differentiation of spermatogenetic stem cells. Sperm DNA damage may be caused due to an abnormal package and segregation of chromatin material, oxidative stress or abnormal cell apoptosis (Aitken and De luliis, 2010).

Thus, the sperm abnormalities observed herein are indications that the leachates constituents exerted an effect on sperm from treated spermatogonia cells. Data showed that the leachates constituents were capable of interacting with the genetic processes involved in spermatogenesis in mice. This was corroborated by a significant decrease in the mean sperm count of exposed mice when compared with the negative control. This indicates that the leachates were not only capable of altering spermatogenesis, but also of reducing or destroying the viability of sperm cells. This therefore suggests that the samples contained constituents which are not only able to produce damaged sperm cells which might be unable to fertilize ovum or produce mutated zygote but are also capable of reducing viable sperm cells which is a major factor leading to infertility.

The result of the sperm abnormalities and induction of MN observed in the well waters showed the level of the e-waste contamination and pollution of drinking water and the possible genotoxic effects it might have on the public health. Although the increase in sperm abnormalities and induction of MN observed were only significant after 4 and 5 weeks exposure in the tested animals, the bio-accumulative effects in humans and animals constantly exposed to these contaminated wells over a long period of time cannot be over-emphasized. This is of greater concern as children and minors are found as scavengers in these markets and child labor is on the increase. The exposure to these mutagens, possible teratogens and carcinogens present in these e-wastes at this early stage might have a deleterious effect on these children in later years.

The damage to DNA by the constituents of the leachate samples was further confirmed by the result of the single-cell gel electrophoresis assay on human lymphocytes. The single cell alkaline gel electrophoresis method was adopted to determine the effects of e-waste contamination on DNA. The alkaline comet assay is sensitive for a wide variety of DNA lesions. Among them are single- and

double-strand breaks, oxidative DNA base damage, and alkali-labile sites including incomplete repair sites, in any eukaryotic cell (Tice, 1995). These results confirm the fact that comet assay is a very sensitive and rapid technique for measuring DNA damage in individual cells which implies that the observed induction of MN and sperm abnormalities in this study is as a result of damages to the DNA of the affected cells. To the best of our knowledge, this report constitutes the first study of e-waste leachate induced DNA damage in human lymphocytes, using the comet assay. This report is consistent with the report of Liu *et al*. (2009), where significant DNA damaged was observed in human populations exposed to the processing of e-waste and also to that of Bakare *et al*. (2012), wherein industrial and municipal solid waste leachates induced DNA damage in human peripheral blood lymphocytes.

The Pearson correlation analysis further confirmed this conclusion by showing that the results of the assays are positively correlated in the tested samples. Sperm morphology, MN and comet assays demonstrated a strong positive correlation. The positive correlation between sperm morphology, MN and comet assays is an indication that the observed sperm abnormalities and MN in this study arose from clastogenic events leading to damaged chromosomes. The correlation between these assays also shows that increase in DNA damage as shown by the comet assay leads to rise in the induction of MN and abnormal sperm morphology. Negative correlation between the assays and the sperm count is an indication that the overall viability of the sperm cell is directly dependent on the quality of the genetic make-up as well as the morphology.

This study further showed that the simulated leachates induced more genetic damage than raw leachates. This is probably due to the presence of higher concentrations of heavy metals in the simulated samples compared with raw leachates. There is also the possibility of other constituents in the simulated leachates (though not analysed) that might be responsible for the observed higher mutagenicity and genotoxicity in this study. Generally, e-waste was shown to contain PAHs, PCBs, and PBDEs (Leung *et al*., 2006; Bi *et al*., 2007; Zheng *et al*., 2008), which are capable of interacting with DNA; and some of these have been classified by USEPA as priority carcinogens (USEPA, 1996b).

PAHs have been shown to be genotoxic to *Escherichia coli* (Mersch-Sundermann *et al*., 1992),

Drosophila (Frölich and Würgler, 1990), and humans (Gamboa *et al*., 2008), and are potential carcinogenic agents (Ruíz and Rizo, 2007; Tuntawiroon *et al*., 2007). The USEPA has designated 16 PAHs as priority pollutants (USEPA, 1999). This classification considers the hazardous level to health and the degree of carcinogenic and mutagenic potential. The presence of these priority pollutants in high concentrations in the samples studied, suggests that PAHs are capable of inducing the observed DNA damage. It is well documented that PAHs form adducts with several proteins and nucleic acids (Perera, 1996; Jeffrey *et al*., 2006). For example, benzo(a)pyrene and its metabolites induce genetic damage in germinal and somatic cells, expressing itself as chromosomal aberrations, mutations, adduct formation in DNA, sister chromatid exchange, and micronuclei formation (Hininger *et al*., 2004; Lemiere *et al*., 2005; Lewtas, 2007; Platt *et al*., 2008). It is for this reason that it has been employed as comparison agent to demonstrate genotoxicity in diverse biomarker tests of early damage. Thus, this compound serves as a reference indicator to predict genotoxicity/carcinogenicity (Ruchirawat *et al*., 2005; Neri *et al*., 2006).

PCBs have also been shown to be toxic in mammalian embryonic and neurological development and organogenesis (Fishbein, 1974; Beckman and Brent, 1984; Rogan and Gladen, 1992). PCBs have also been found to induce cellular proliferation (Soto *et al*., 1992), to be carcinogenic (Silberhorn *et al*., 1990), clastogenic (Sargent *et al*., 1989), and caused birth defects (Safe, 1994) in both animals and humans. The mechanism of action of PCB genotoxicity includes production of intermediates capable of forming nucleic acid adducts and acting as alkylating agents. For instance, PCB metabolites such as arene oxide intermediates are known alkylating agents (Hargraves and Allen, 1979) and are capable of forming nucleic acid adducts (Wyndham and Safe, 1978). It is also possible that genotoxicity was by recombinogenesis, as bioactivated PCBs have been shown to be genotoxic through this mechanism (Butterworth *et al*., 1995).

There are contradictory reports on the genotoxicity of PBDEs. While PBDE mixtures did not show mutagenic activity in *Salmonella* or *Saccharomyces* cerevisiae (IPCS, 1994) assays, or in a mouse lymphoma assay (NTP, 1986). Barsien *et al*. (2006) reported induction of micronuclei and other nuclear abnormalities in mussels exposed to BDE-47. Cytotoxicity and genotoxicity of BDE-47 were also observed in human neuroblastoma cells exposed *in vitro* (He *et al*., 2008). The observed

genotoxic damage in our study might have been because there was higher concentration of less brominated congeners in the samples. Several studies have reported that effect on cell viability and cell apoptosis caused by less brominated congeners was greater than that of more brominated congeners (He *et al*., 2008; Jin *et al*., 2010). Less brominated congeners are more bioactive than the more brominated PBDE and are more potent at producing reactive oxygen species, which in turn causes greater genotoxicity (Jin *et al*., 2010).

The observed genotoxicity could also be as a result of the presence of high concentrations of the heavy metals. It has been suggested that DNA damage induced by leachates might be due to the presence and interactions of heavy metals with DNA. Sa´nchez-Chardi *et al*. (2007) reported bioaccumulation from a municipal landfill of heavy metals such as Cd, Fe, Zn, Cu, Mn, Mo, and Cr in organs and tissues of the wood mouse. These heavy metals have the potential to induce mutations and cancer in living cells. Epidemiological studies and studies in experimental animals indicate that Pb is both genotoxic (Shaik *et al*., 2006) and carcinogenic (Fowler *et al*., 1994), and that Ni (Haugen *et al*., 1994) and Cd (Elinder and Jarup, 1996) are carcinogenic. Hexavalent Cr was reported to induce chromosomal aberrations, micronuclei, and single-strand DNA breaks in mammalian cells (Wise *et al*., 2002), and gene mutations in bacteria (DeFlora *et al*., 1990). Welders exposed to both Ni and Cr exhibit sister chromatid exchange (SCE) frequencies that represent an additive response for these two genotoxins (IARC, 1990). Cu produces free radicals, and when present in an unbound form, it produces reactive oxygen species that cause DNA, protein, and lipid damage (Galaris and Evangelou, 2002). The genotoxic activities of heavy metals were reported to be the result of formation of DNA-DNA and DNA-protein cross-links (DeFlora *et al*., 1990; Costa *et al*., 1994). DNA damage might also be due to the effects of other inorganic, organic, as well as unidentified xenobiotics present in the leachate.

Although the exact mechanism of leachate-induced genetic damage is not clear, some studies are suggesting that it could be via free-radical-damage mechanism (Radetski *et al*., 2004; Li *et al*., 2006; Koshy *et al*., 2007). In this study, we observed that e-waste leachate induced discernible oxidative stress in mouse liver as assessed by LPO measurement, a significant elevation in the activities of CAT and GSH, and a concomitant concentration-dependent decrease in SOD level. The e-waste

leachate and well water exposure also resulted in significant increase in the activities of serum ALT and AST. These enzymes are localised in periportal hepatocytes, reflecting their role in oxidative phosphorylation and gluconeogenesis and their serum activities presumably increase as a result of cellular membrane damage and leakage (Kaplan, 1993). This underlines their usage as biochemical markers for early acute hepatic damage (Adedara et al., 2010).

Elevated levels of AST and ALT in circulation were indicative of a hepatic injury after leachates and well water exposure. Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to cope with the ROS and other free radicals. However, when a condition of oxidative stress is established, the defence capacities against ROS become insufficient (Halliwell and Gutteridge, 2000). ROS attack cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995). Once formed, peroxyl radicals (ROO$^-$) can be rearranged via a cyclization reaction to endoperoxides (precursors of MDA) with the final product of the peroxidation process being MDA (Wang et al., 1996; Marnett, 1999). Determination of malondialdehyde (MDA) is considered to be an excellent index of lipid oxidation. The MDA is the end product of lipoperoxydation, considered as a late biomarker of oxidative stress and cellular damage (Kim et al., 2000; Dotan et al., 2004). Administration of e-waste leachate and contaminated well water resulted in dose-dependent increase in MDA level in treated animals. This result is consistent with previous studies on oxidative damage induced in heart, kidney and spleen of mice treated with landfill leachate (Li et al., 2006; Shokunbi and Odetola, 2008; Bakare et al., 2012).

The GSH plays a crucial role in protecting the cells from oxidative damage (Guoyao et al., 2004). In this study, hepatic GSH content was considerably increased after e-waste leachate and contaminated well water administration. This increase in GSH content under the present experimental model suggests its utilisation to challenge the prevailing oxidative stress under the influence of ROS generated from e-waste leachate and contaminated well water. Changes in levels of GSH were observed during increased oxidative stress (Bray and Taylor, 1993). Activities of SOD were markedly decreased by e-waste leachate and contaminated well water treatment but resulted in an increase in CAT activity. Superoxide radicals by themselves or after their transformation to hydrogen peroxide (H_2O_2) caused oxidation of the cysteine in the enzyme and decreased the SOD activity (Dimitrova et

al., 1994). The degradation of H_2O_2, a potent oxidant at high cellular concentration, is effected by CAT. The rise in the activity of CAT could be due to its induction to counter the effect against increased oxidative stress. This result is consistent with earlier report of Guangke *et al*. (2005).

From these findings, e-waste leachate and contaminated well water induced hepatic oxidative damage in a dose-dependent manner in mice that may be due to the leachate or its metabolites. It has been shown that glutathione response varies depending on the nature of the pollutant, response duration, and species (Lackner, 1998). Exposure to leachate has been shown to increase lipid peroxidation and activities of antioxidant enzymes such as glutathione peroxidase and catalase (CAT) in heart, kidney and spleens in mice (Guangke *et al*., 2005; Li *et al*., 2006). Tire leachates have been shown to have profound effects both on cytochrome P450 A1 content and on ethoxyl-resorufin-o-deethylase (EROD) activity, as well as upregulation of antioxidant defences in mice (Stephensen *et al*., 2003). Several xenobiotics enter the body through gastrointestinal tract and after absorption are transported by the hepatic portal vein to the liver; thus the liver is the first organ perfused by chemicals that are absorbed in the gut (Adedara *et al*., 2011). To date, research has largely concentrated on hepatotoxicity, since the liver plays a major role in the metabolism of xenobiotics and consequently the primary target of most toxic responses. The induction of oxidative stress herein suggests an increase in the production of free radicals within the liver cells following exposure to different concentrations of the e-waste leachate and contaminated well water. Thus, for leachate-induced genotoxicity, the hypothesis of leachate generated free radical-induced DNA damage is then reinforced.

The test sample contained some heavy metals and other inorganic constituents with known genotoxic and carcinogenic potentials. As described elsewhere, leachates contain a number of heavy metals and host of other organic compounds that are reported for their pro-oxidant potential (Lackner, 1998; Lewis *et al*., 2003; Sa´nchez-Chardi *et al*., 2007). Since the test leachate is a complex mixture, it may be difficult to pinpoint single most significant constituent responsible for oxidative damage. Several hydrocarbons and N-, Cl-, Cn- and P-containing compounds such as pesticides, phenols, phthalates, or polyvinyl aromatic hydrocarbons, all of which are usually present in leachates may reach toxic levels (Sa´nchez-Chardi *et al*., 2007). However, we believe that heavy metals may have contributed

significantly to induction of oxidative stress in this study. In this regard, it would be noteworthy to state that Cd, Cu, and Fe have been reported to induce the production of ROS (Radetski *et al*., 2004) in eukaryotic cells. Cu produces free radicals and when present in an unbound form, it produces ROS that cause DNA, protein, and lipid damages (Galaris and Evangelou, 2002). The concentration of Cd in the leachate samples is higher than the regulatory environment standards for cadmium (CPCB, 2010). 10 mg/L of Cd was sufficient to induce oxidative stress in the aquatic plant *Hydrilla verticillata* (Radetski *et al*., 2004). Cd, Fe, and Cu are known to interact with cellular redox processes or may even directly generate ROS, such as superoxide radical, singlet oxygen, hydrogen peroxide, or lipid free radicals (Galaris and Evangelou, 2002). Cd may act indirectly by depleting the antioxidant levels and thereby cause an increase in intracellular H_2O_2 (Misra *et al*., 1998). Increase in H_2O_2 may then catalyze Fe-/Cu-mediated redox reactions, which can produce subsequent ROS. Previous studies have reported that DNA breaks induced by Cd are mediated by the formation of ROS only in animal cells (Snyder, 1988; Tsuzuki *et al*., 1994).

Moreover, Cd also interferes with DNA repair enzymes (Hartwig, 1998). This observation on DNA damage in mouse somatic tissues corroborates previous studies on leachate genotoxicity in mice (Bakare *et al*., 2005; Sang and Li, 2005; Sa´nchez-Chardi *et al*., 2007), goldfish (Deguchi *et al*., 2007) and *Salmonella typhimurium* (Singh *et al*., 2007) and is one of the few reports on leachate-induced systemic genotoxicity in mouse somatic and germ tissues. The possible mechanism of induction of DNA damage evident by induced oxidative stress in the liver is in concert with the reports of Li *et al*. (2006) who showed oxidative damage induced by a municipal landfill leachate in the heart, kidney, and spleen of the mouse. It is also in support of induction of oxidative stress in freshwater fish by fly ash leachate (Ali *et al*., 2004). Other similar reports are from Ferrari *et al*. (1999) and Radetski *et al*. (2004) but with plant systems. This study is of relevance to humans as there are reports of adverse health effects in relation to exposure to e-wastes (Roze *et al*., 2009; Liu *et al*., 2009; Herbstman *et al*., 2010; Harley *et al*., 2010). Exposure to chemical substances in solid waste leachate can lead to genomic disruptions in the organs and tissues of human beings. Differential DNA damage and repair, as well as physiological roles in various organs and tissues could lead to an increase in the susceptibility toward disease and disorders.

The poisoning effects of heavy metals are due to their interference with the normal body biochemical processes. When ingested, in the acid medium of the stomach, the metals are converted into their stable oxidation states, which subsequently combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds, thus, mutilating their structures and hampering their functions. Although individual metals, when consumed above the bio-recommended limits, exhibit specific signs of toxicity such as gastrointestinal disorders, diarrhoea, stomatitis, tremor, haemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, asthma and pneumonia. The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Fowler *et al*., 1994).

The result of MTT assay further confirms the cytotoxicity of the e-waste leachate. Cytotoxicity assays are useful to indicate the ability of a compound to cause cell death as a consequence of damage to one or more cellular functions (Weyermann *et al*., 2005; Fotakis and Timbrell, 2006). MTT is a cell viability method, based principally on mitochondrial activity. In this study, the assay revealed the cytotoxicity of e-waste simulated leachate with an IC_{50} of 30% in NIH/3T3 cell line. The e-waste simulated leachate was very toxic such that cells exposed to concentrations higher than 70% of the leachate resulted in 100% cell death. This high toxicity of the e-waste leachate is believed to be as a result of the presence of the high concentration of the toxic heavy metals and organic contaminants. The low IC_{50} in this study is of public health concern considering the current level of occupational and residential exposure of the Nigerian population to e-waste materials in and around the study area. Human exposure to e-wastes can exert dire consequences. Indeed, there are reports of human-induced adverse effects attributed to exposure to e-waste in China (Bi *et al*., 2007; Huo *et al*., 2007; Li *et al*., 2008a; Zheng *et al*., 2008) and Singapore (Tan *et al*., 2009).

Mitochondria have a crucial function in controlling the cell death process, besides playing a crucial role in maintaining the bio-energetic status of cells (Szabo *et al*., 2011). Mitochondrial membrane potential is a key indicator of cell health and mitochondrial permeability transition, which is an important step in the induction of cellular apoptosis. The result herein showed a decrease in mitochondrial potential in the exposed cell lineage. This suggests the involvement of the intrinsic pathway of apoptosis in the mechanism of cell death induction by the leachate. The impairment of

mitochondrial function by e-waste leachate can be of drastic consequences, upsetting the balance and cellular functions (Teodoro *et al*., 2011). Mitochondrial membrane potential collapse may result in the release of cytochrome c into the cytosol, where it would participate in the mechanism of apoptosis (Bossy-Wetzel and Green, 1999).

Although the susceptibility of proliferating cells to mutagens and carcinogens has been extensively investigated (Fairbairn *et al*., 1995; Anderson *et al*., 1998; Henderson *et al*., 1998), DNA damage by genotoxins is rarely studied as a function of cell cycle phase. In this study, the cytotoxicity observed by MTT assay was confirmed by flow cytometry and AO/EB assays. Cells exposed to simulated e-waste leachate decreased the population of NIH/3T3 cells in G0/G1 and G2/M cell phases, which was accompanied by a marked increase of a Sub/G1 population after 24 h. This showed the DNA damaging potential of the leachate with an apoptotic induction of 72.22% cells in the sub/G1 phase, compared with the control within 24 h of exposure. G0/G1, G2/M and S indicate the cell cycle phases, and Sub/G1 DNA content refers to the proportion of cells with DNA fragmentation suggesting late stage of apoptotic event (Ormerod, 2002; Yang *et al*., 2007; Silva *et al*., 2012).

Apoptotic cell death is often described as occurring as a consequence of oxidative insults. The result herein showed that simulated e-waste leachate induced oxidative stress at tested concentrations. Therefore, it seems reasonable to infer that the cytotoxic, mutagenic and genotoxic effects of simulated e-waste leachate may be the result of oxidative damage to cells because the simulated leachate was able to generate significant quantity of ROS in NIH/3T3 cell line. Moderate or high concentrations of ROS can become cytotoxic by blocking cell proliferation and inducing apoptotic or necrotic cell death (Dreher and Junod, 1996).

Study has shown that there is a complex system of enzymatic and non-enzymatic antioxidants that protect cells against harmful pro-oxidants (Reuter *et al*., 2010), and that only the increase in ROS production without a proportional increase in the production of antioxidants, can induce mitochondrial membrane permeability and damage to the respiratory chain which may trigger the apoptotic process (Valko *et al*., 2006). The hypothesis was tested by the introduction of an antioxidant, N-acetylcysteine, into the cell culture an hour prior to the e-waste exposure and the effect on induction of apoptotic cell by flow cytometry was studied. N-acetylcysteine significantly protected

the cells against the effects of e-waste simulated leachate as observed by the significant decrease in the cells at sub/G1 phase compared to the non-N-acetylcysteine exposed groups, thereby confirming the relationship between ROS and cell death in this case.

NIH/3T3 cells analysed in the presence of acridine orange and ethidium bromide staining (AO/EB staining) corroborated that apoptosis has been induced by simulated e-waste leachate. Staining of apoptotic cells with fluorescent dyes such as AO and EB is considered the correct method for evaluating the changed nuclear morphology (Leite *et al*., 1999; Gasiorowski *et al*., 2001; Savitskiy *et al*., 2003). As demonstrated in the results, AO/EB staining, based on nuclear morphology (perinuclear chromatin condensation, nuclear collapse and eventual fragmentation), showed a significant induction of apoptosis by simulated e-waste leachate at all concentrations. Acridine orange is taken up by both viable and nonviable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA) or red fluorescence if bound to single stranded nucleic acid (RNA). Ethidium bromide is taken up only by nonviable cells and emits red fluorescence by intercalation into DNA. The result showed that apoptotic cells were produced and a stronger apoptosis signal was induced with higher concentrations of the simulated e-waste leachate. To the best of our knowledge, these findings constitute the first report on the possible mechanism of toxicity of electronic wastes *in vitro*.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This study was carried out to assess environmental contamination, DNA and oxidative damage and mechanisms of cytogenotoxicity induced by e-wastes in Lagos, Nigeria. The results showed contamination of soils, plants and well water by e-wastes in the study areas. The major contaminants analysed were Pb, Cd, Cr, Cu, Zn, As, PAHs, PBDEs and PCBs. It was also observed that the e-waste leachates and well water were cytotoxic, mutagenic and genotoxic *in vivo* and *in vitro,* using a battery of assays. Reactive oxygen species (ROS) generation and apoptotic pathway were observed as possible mechanisms of e-waste induced cytogenotoxicity.

The high concentration of heavy metals and organics in the soils and plants provides evidence of environmental contamination by indiscriminate disposal and open burning of e-wastes in the study areas, and the subsequent uptake of these contaminants by plants. These provide a baseline data on the level of e-wastes contamination in Lagos, Nigeria, and the cytogenotoxicity potential of this type of waste. This study further suggests that e-waste may be a potential agent of genetic mutation in exposed plant, animal and human population. It also provides background information on the mechanism of induction of cytogenotoxicity by e-waste leachate. These are of environmental and public health relevance and may serve as basis for further studies on the impact of e-wastes on the Nigerian environment, especially the human body-burden of e-waste contaminants.

The use of outdated (and unsafe) ways to deal with e-wastes can lead to exposure to a variety of substances harmful to human health. The inappropriate methods employed in the waste management practices in Nigeria create the potential for a negative impact on the health of the residents as well as the entire ecosystem. In order to safeguard the health of Nigerians, the following recommendations are of importance:

1. Human body-burden of e-waste contaminants has not been studied in Nigeria. It is therefore suggested that this should be given priority considering the high level of the contaminants in soil, well water and edible plant samples as reported in this study.

2. The induction of MN and increased sperm abnormalities by the well waters calls for further

study on the workers and residents of these markets whose sole source of drinking and cooking is the contaminated well water. This will help to determine the level of damage in these exposed groups and help foster the immediate treatment of the affected. Adequate steps should be taken to protect the current and future bio-generations from the effects of e-waste pollution.

3. The NESREA should implement a well-coordinated management strategy to check the indiscriminate disposal of e-wastes into the environment. This will reduce if not eliminate the open dumping and burning of e-wastes.

4. The Government should carry out a remediation of the contaminated soil and water in the study sites and similar areas so as to prevent both workers and residents from further exposure to the e-waste contaminants.

5. The Federal Government need to separate residential area from electronic markets as this is at present, the case in the two study sites. This will help to prevent the exposure of residents to e-waste contaminants.

6. There is need for a public enlightenment on the hazards of e-waste exposure. This is necessary for both the workers and residents of these study sites and similar areas. The citizens also have to be educated not to dispose their e-wastes with municipal solid wastes or to store them in their attics or backyards.

7. The workers in the study sites work with little or no protective gear. There is need for constant education on the need for such individuals to wear protective gear while working with electronic material. Also, a legislation enforcing a minimum protective standard for workers in these markets should be put in place.

8. As a result of the influx of transboundary e-wastes and the low-end management practices in the developing countries, there is an urgent need for introduction of legislation dealing specifically with e-wastes in Nigeria. Established legislation such as EU Directives and draft legislation of National Development and Reform Commission (NDRC), China, should be

guidelines for such new legislations. Among other things, the following should be taken into cognizance:

a. E-waste collection, storage, recycling and/or disposal should be adequately funded.

b. Encouragement of e-waste recycling and disposal enterprises through legislative measures.

c. Development of best technology for e-waste management should be encouraged.

d. Have measures that will encourage the use and importation of Electrical and Electronic Equipment (EEE) manufactured with non-toxic, non-hazardous substances and recyclable materials in accordance with the EU RoHS Directive.

e. Implementation of Extended Producer Responsibility (EPR) mandating producers, importers/retailers the responsibility of collection, recycling and disposal of EoL EEE.

f. Introduction of standards and a certification system for second-hand appliances as well as recycling and disposal enterprises in ensuring safety and environmentally friendly processing of e-wastes.

9. The 3R initiative: the 3R initiative is one approach in dealing with the e-waste problem. It is summarize as follows:

a. Reduce: the amount of waste generated should be reduced and the use of toxic substances (such as Hg and Pb) should also be reduced or eliminated.

b. Reuse: electronics or their components which are still useable should be reused.

c. Recycle: the use of waste as a resource for the manufacture of other items.
The success of this initiative however depends on the organized collection and
transportation of e-waste as well as the public recognition of hazards involved in e-waste.

10. Citizen's responsibility: The success of EPR depends on the full cooperation of the citizenry. Each citizen should be responsible for the type of EEE they purchase, whether it contains toxic substances or it is toxic-free, and the subsequent release of such items after usage, to foster recycling by the appropriate bodies.

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