HEAVY METAL CONCENTRATIONS AND SYSTEMIC TOXICITY IN *Rattus norvegicus* EXPOSED *IN SITU* TO GROUNDWATER AT OLUSOSUN LANDFILL, LAGOS, NIGERIA

BY

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ABSTRACT

Landfilling remains the major method of municipal solid waste disposal in Nigeria. However, the inadequacy of personal protective equipment and proximity of residential quarters to waste dumpsites predispose workers and residents to landfill toxic heavy metals, which could pose public health risk. There is a plethora of *ex situ* studies on systemic toxicity of landfill soil, groundwater and leachates in prokaryotic and eukaryotic systems. However, paucity of information exist on body-burden of heavy metals and systemic effects on exposed populations *in situ*. This study was carried out to assess heavy metal accumulation in tissues, and systemic toxicity in rats (*Rattus norvegicus*) exposed *in situ* to groundwater at Olusosun landfill in Lagos, Nigeria.

Olusosun landfill and a residential environment (17.3 km southwest of the landfill) were chosen as the study and control sites, respectively. Male rats (weight = 69.2±4.1g; 8-9 weeks old; n=30 per location) were exposed daily to groundwater via drinking at three locations on the landfill, and the control for 4, 8, 12, 16, 20 and 24 weeks. Post-exposure, blood and organs were collected (5 rats per location per periods); lead, cadmium and chromium were analysed in blood, liver and kidney using standard procedures. The DNA damage was assessed using bone marrow micronucleus assay. Gastric physiopathological indices (Parietal Cell Count, PCC; Mucus Cell Count, MCC; and Gastric Mucous Secretion, GMS), serum hepatic and renal function parameters (Aspartate Aminotransferase, AST; Alanine Aminotransferase, ALT; urea; and creatinine) were assessed using standard procedures. Tissues (liver, kidney, lungs, spleen and stomach) histology and hepatic oxidative stress biomarkers (Catalase, CAT; Superoxide Dismutase, SOD; Reduced Glutathione, GSH; and Malondialdehyde, MDA) were also evaluated using standard procedures. Data were analysed using descriptive statistics, ANOVA and Pearson's correlation (r) at $\alpha_{0.05}$.

The concentrations (mg/L) of selected metals were higher in blood (Pb:0.04-0.61, Cd:0.03-0.49 and Cr:0.05-0.27), liver (Pb:0.22-0.56, Cd:0.03-0.50 and Cr:0.10-0.31) and kidney (Pb:0.38-0.69, Cd:0.32-0.45 and Cr:0.10-0.23) of exposed rats compared with control (blood-Pb:0.03-0.41, Cd:0.02-0.15 and Cr:0.02-0.08; liver-Pb:0.05-0.22, Cd:0.01-0.16 and Cr:0.05-0.13; kidney-Pb:0.10-0.31, Cd:0.07-0.14 and Cr:0.04-0.18) at all exposure periods. There was significant time-dependent (r=0.5) increased in

micronuclei induced in bone marrow cells of exposed rats (1.9-6.0 folds) suggesting that the accumulated heavy metals were genotoxic in the organ-system of the exposed rats. Compared with control, PCC significantly increased (1.1-1.5 folds), while MCC and GMS decreased (1.1-2.2 and 1.0-1.3 folds, respectively). Also, there was time-dependent significant decrease in PCC (r=-0.7), MCC (r=-0.3) and GMS (r=-0.4). There were significant increases in activities of AST (2.0-4.9 folds), ALT (1.1-3.1 folds), levels of urea (1.1-1.7 folds) and creatinine (1.3-2.2 folds). Tissue-lesions observed in exposed rats included hepatic necrosis and steatosis; tubular fibrosis and glomerular degeneration; alveolar hyperplasia; fusion of white pulps and haemosiderin deposition; and gastric mucosa desquamation. The CAT activities, levels of GSH and MDA increased significantly (1.2-3.5, 1.3-1.5 and 1.1-2.8 folds, respectively); while SOD decreased (1.3-2.4 folds) suggesting free radical generation in exposed rats.

Exposed *Rattus norvegicus* at Olusosun landfill had heavy metal accumulation in organs which induced genetic, histologic and gastric-physiological damage.

Keywords: *In situ* landfill-exposure, Heavy metal accumulation, Genotoxicity, Gastric physiopathology.

Word count: 493

CERTIFICATION

I certified that this work was carried out by Mr. Adeyinka Michael GBADEBO in the Ecology and Environmental Biology Unit of the Department of Zoology, University of Ibadan, Ibadan, Nigeria under my supervision.

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DEDICATION

I dedicate this project to my parents: my mum Mrs. Remi Friday, for her love, prayers and priceless support; my step-dad, Mr. M. T. Friday, for his extraordinary understanding and tireless support; and my dad, Mr. Adedayo Gbadebo, for his constant prayers; and to everyone God has used to positively touch my life.

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CHAPTER ONE

INTRODUCTION

Waste generation and management drive much of the global economies and it is in fact connected to the rate of urban and economic development. The global municipal solid waste (MSW) generation has been estimated to about 1.3 billion tonnes at the rate of 1.2 kg/capita/day (OECD/IEA, 2016). In Nigeria, waste generation is estimated to be about 25 million tonnes/year with a daily kg/capita rate of 0.44 – 0.66. The problem is not the quantity of waste generated, but the environmental and health impacts associated with the inadequate waste management systems (Ogwueleka, 2009).

There are four common methods of waste management practice in most developing countries including Nigeria. These are landfilling, incineration, anaerobic digestion and composting. The last three methods only reduces the volume of waste, the residues from these methods are ultimately disposed of in open dump sites/landfills. It is best to describe solid waste landfills in Nigeria as open dump sites, since there are no sanitary landfills in Nigeria. This is because landfills in Nigeria are not engineered, unlined and are usually located in abandoned excavated mining sites, wetlands, rivers (for purpose of land reclamation) and open lands (most often accidentally created) close to residential quarters. However, there are reports that these unlined waste disposal sites are releasing noxious contaminants (organic and inorganic chemicals) into the surrounding environment (air, soil) and groundwater in form of unpleasant odour, particulate matters, landfill gases, and complex mixtures in form of leachates (Ikem *et al.*, 2002; Bakare *et al.*, 2013a). The emissions from landfills could pose adverse environmental and public health risk.

Leachates which are complex mixtures of deleterious chemical and pathogenic microorganisms are produced when decomposing matters and leachable inorganic chemicals are mixed by infiltration of precipitation and/or groundwater underflow which then percolate into the surrounding soil, underground water aquifers and run-off into surface water, polluting them (Mor *et al.*, 2006). These complex mixtures have been characterized severally, and observed to contain high levels of heavy metals, persistent organic pollutants and pathogenic microorganism above permissible limits (Oshode *et al.* 2008; Bakare *et al.*, 2013a).

The leachates from MSW landfills have further been demonstrated to lead to mutagenic, genotoxic and systemic harmful effects in microorganisms, plants and animals of various ecological habitats (Bakare *et al.*, 2013b; Alimba and Bakare, 2016). Reports exist and evidences abound on leachate contamination of underground waters within 2 km radius of MSW landfills in Nigeria (Oyeku and Eludoyin, 2010; Akinbile and Yusoff, 2011; Oni and Hassan, 2013; 2014; Bakare *et al.* 2013a), thereby posing chemical accumulation and health threat to plants, animals and human populations who depends on these water sources for their sustenance.

Aside the potential dangers posed by its leachates, MSW landfills are major contributors to obnoxious gases in form of landfill gases (LGs). LGs consist of 50 – 60 % methane, 30 – 40 % CO_2, and minute quantities of many other chemical substances and heavy metals. LGs could specifically pose environmental and health hazards such as spontaneous fire/explosion and asphyxiation hazards and issues relating to nuisance obnoxious odours, smokes and fogs (ATSDR, 2001). Also, among the chemical compounds present in LGs, metal ions and volatile organic compounds (VOCs) (apart from NO_x and SO_x) are known to pose serious environmental and health risk because they have short- and long-term toxic effects, cancer causing properties and are persistent in the environment (Karthikeyan *et al.*, 2011).

Determination of the composition of ambient air emission from solid waste landfills has shown high concentration of metal ions in air particulate matters (PMs), elevated levels of hazardous VOCs and pathogenic bioaerosols (Karthikeyan *et al.*, 2011; Kumar *et al.*, 2015, Akpeimeh *et al*., 2019). Continuous and long term exposures to

atmospheric air containing these gases are harmful to the environment and public health.

1.1 Justification of study

In Nigeria, occupational and residential exposure to pollutants from landfill sites is on the increase due to poor waste management practice, close proximity of residential areas with landfill sites, and increasing informal dumpsites due to increased generation of solid waste. Living and/or working around waste landfills have been linked to diverse ill health effects such as respiratory tract inflammations, asthma, gastrointestinal diseases, birth defects, cancers and infectious diseases (Rachiotis *et al.*, 2012; Odewabi *et al.*, 2013; Alabi and Bakare, 2015; Gopalakrishnan *et al.*, 2016). However, epidemiological data to this effect are still scarce especially in developing countries due poor community health record, and challenges/limitations associated with carrying out research involving human participants.

Toxic substances enter the body through various routes which include the oral route via the gastrointestinal tract (ingestion), the airway route via the lungs (inhalation) and from skin/dermal contact with the toxic agents. Systemic tissues and organs in the mammalian body are sensitive to chemical induced toxicity due to their involvement in various metabolic activities like detoxification, regeneration, absorption, secretion, gaseous exchange, excretion and storage of chemicals. This makes the organs to be directly exposed to chemicals hence damage to visceral organs may be useful in explaining possible mechanisms of chemicals induced toxicity (Alimba, 2013). So far, there is a dearth of information in Nigeria on human body burden (of heavy metals and organic polutants) and systemic toxicity that could potentially result from living and/or working around solid waste dumpsites (Odewabi *et al.*, 2012; 2013). Also, there is scarcity of information on the potential genetic and systemic toxicity from animals exposed *in situ* to landfill sites.

The Olusosun landfill is an unlined dumpsite, with no engineered gas and leachate collection systems. Attempt to upgrade the site was done by construction of drainage around the heaps of waste which drains leachate and water run-off to a leachate

collection pond. However, this pond is without treatment facilities, and still subject to underground infiltration and evaporation. Also, there are the presence of hand dug wells and borehole on the landfill site and within 2 km radius of the site, which the workers and residents in this environs use for their day to day domestic and commercial activities. Bordering the Olusosun landfill are commercial and industrial hubs, a primary school, religious centres (churches and mosques), LAG-BUS terminus, motor parks and garages, filling station, mechanic workshop, residential buildings, etc. Occasionally, thick smokes and fogs are seen emanating from the site, either from deliberate burning of waste or by spontaneous fire outbreak. The above mentioned scenarios and activities, contribute significantly to potential sources of risks and daily human exposure and environmental contamination by large doses of toxic chemicals present in this massive dumpsite including heavy metals. These exposures can either be through inhalation of obnoxious odours and air emissions, ingestion of contaminated foods, fruits and vegetables, and leachate contaminated underground water or from direct contact to soil and waste matters from the site. Indirectly, vermin (houseflies, roaches, birds and rodents), roaming ruminant and domestic animals seen on the site could also transmit contaminants and pathogenic microorganisms that can constitute a potential exposure to health hazards and disease conditions from the Olusosun landfill.

Therefore, this study was designed to mimic *in situ* ambient exposure of waste workers and populations living around municipal waste landfill to groundwater (borehole water) and air emissions at the dumpsite in a rat model. In summary, the paucity of epidemiological information on the toxicity and genotoxicity, body burden of contaminants (especially metals) and public health impacts on workers and people living around solid waste dumpsites in Nigeria and also the need to strengthen environmental policies governing municipal solid waste management (MSWM) are the justification for this work.

1.2 Aim of the study

This study aims at assessing heavy metal concentrations, genetic and systemic toxicity in Wistar albino rats (*R. norvegicus*) exposed *in situ* to groundwater (borehole) and air emissions at Olusosun landfill (OL) in Lagos, Nigeria.

1.3 Objectives of the study:

1. Analysis of the heavy metal [chromium, cadmium, copper, lead, zinc and iron] levels in the underground water at Olusosun landfill and control site.

2. Determination of selected heavy metal (chromium, cadmium, copper, lead and zinc) concentrations in blood, liver, kidney and lungs of rats chronically exposed to ambient landfill air and borehole water source.

3. Evaluation of the cytogenotoxic effects on rats chronically exposed to ambient landfill air and borehole water source using bone marrow micronucleus assay.

4. Determination of the systemic toxic effects on rats chronically exposed to ambient landfill air and borehole water from body weight and organ weight indices, haematology, serum biochemistry, and histology of the liver, kidney, lungs and spleen.

5. Investigation of the gastric physiopathologic effects on rats chronically exposed to ambient landfill air and borehole water source from parietal cell count, mucous cell count, mucus secretion and gastric histopathology.

6. Assessment of oxidative damage and lipid peroxidative effects in liver of rats chronically exposed to ambient landfill air and borehole water source.

1.4 Hypotheses of the study

The following research hypotheses were developed to guide the study:

H0: 1 There is no significant relationship between the tissue and organ concentrations for selected heavy metals in rats exposed to ambient landfill air and borehole water source compared to the corresponding control groups.

H0: 2 There is no significant relationship between genetic and systemic damage (haematological, histopathological, biochemical and gastric pathophysiological) in rats exposed to ambient landfill air and borehole water source compared to the corresponding control groups.

CHAPTER TWO

LITERATURE REVIEW

Municipal solid wastes are waste collected from municipalities. They include waste from households, institutions and waste generated from small businesses (OECD/IEA, 2016). Engineers define municipal solid waste as substances that are disposed off from residential area and commercial centres, which have ceased to have value to their owners. Anthropologists believes that wastes are actual and real evidence of people's culture, what people had used and thrown away tell better story of their life style. Ecologists believe that there is nothing called waste in real sence and in nature while industrial ecologists see waste as "a right thing in a wrong place". (Vergara and Tchobanoglous, 2012). Irrespective of the definition of MSW, waste generation is inevitable, its presence is undisputable and the production cum composition is the result of anthropogenic activities and human life style; and the way waste is manage have a direct impact on environment and human health.

2.1 Global rate of generation of solid waste

Waste is the end product of human lifestyle and consumption. Waste generation and management drive much of the global economies and it is greatly associated with urbanization and economic growth. With progress in urbanization in countries; economic wealth and standard of living increase, and this in turn leads to corresponding increase in the quantity goods consumed and services available. Increase in consumption of good and services will invariably lead to corresponding increase in the quantity of waste and by-products generated.

It is estimated that about 1.3 billion tonnes of municipal solid waste are generated globally on a yearly basis at the rate of 1.2 kg/capita/day. This per capita rates actually varies from country to country, from city to city and even within cities. Also, it has been calculated that global urban MSW generation will increase from 1300 million

tonnes in 2013 to 2720 million tonnes in 2050 (Table 2.1, Hoornweg and Bhada-Tata, 2012; OECD/IEA, 2016).

The waste generation rate in rural community is usually lower. This is because the rural communities are poorer, consume more of farm produce, purchase less packaged items from the commercial stores and practice more reuse and recycling of items. Also, rural MSW generation was calculated to be 500 million tonnes in 2013, increasing to around 900 million tonnes in 2050 (OECD/IEA, 2016).

Sub-Saharan Africa generate an estimated 62 million tonnes of solid waste annually. The quantity of waste generated per person per day is usually very low in sub-Saharan Africa and this usually range from 0.09 – 3.00 kg/capita/day, with a mean of about 0.65 kg/capita/day. The Island countries are usually the countries with highest per capita rates and this is because of the high volume of waste generated by the tourism industries in these countries and also because there is more efficient recording of wastes generated. East Asia and the Pacific Region generate an estimated 270 million tonnes of waste per year. In this region, China alone contributes the biggest share and this makes up about 70% of the total waste generated in the region. The quantity of waste generated per person per day ranges from 0.44 – 4.30 kg/capita/day for the East Asia and the Pacific Region, with a mean of about 0.95 kg/capita/day (Hoornweg and Bhada-Tata, 2012).

The annual waste generation in Eastern and Central Asia is approximately 93 million tonnes, with a per capita rate ranging from 0.29 – 2.1 kg/capita/day and an estimated average of 1.1 kg per person per day. The Latin America and the Caribbean has been said to have the most comprehensive and consistent waste generation data (as given by the Pan-American Health Organisation's Regional Evaluation of SWM, (PAHO, 2005). The annual waste generation in this region is about 160 million tonnes, with quantity of waste generated per person per day ranging from 0.1 – 14 kg/capita/day and an average value of 1.1 kg/capita/day.

Also, about 63 million tonnes of waste is generated annually in the Middle East and North Africa. This is accounted for by an approximate range of 0.16 – 5.7 kg/capita/day, with a mean of 1.1 kg per person per day. Furthermore, with the OECD nations, a calculated 572 million tonnes of solid waste is generated annually, having a range of 1.1 – 3.7 kg/capita/day and a mean of 2.2 kg per person per day.

Table 2.1: An estimated summary of urban population, GDP and MSW generation for major countries or world regions.

Region	2013				2050			
	Urban population (millions)	Urban GDP (USD/cap.)	Urban waste generation (kg/cap./yr)	Urban waste generation (Mt/yr)	Urban population (millions)	Urban GDP (USD/cap)	Urban waste generation (kg/cap./day)	Urban waste generation (Mt/yr)
China	744	18 625	355	264	1 058	62 996	510	540
India	401	9 444	270	108	814	40 195	454	370
United States	260	56 399	497	129	350	91 396	558	196
European Union	378	39 395	453	171	424	67 552	521	221
Africa	423	9 243	234	99	1276	15 613	303	386
Middle East	152	28 810	363	55	270	55 166	423	114
Latin America	363	17 265	341	124	505	34 592	425	215
Other OECD	395	32 154	418	165	486	55 591	490	238
Other non-OECD	616	17 004	325	200	1 027	39 237	430	441
World	**3 732**	**22 760**	**353**	**1 316**	**6 210**	**45 025**	**438**	**2 720**

Source: OECD/IEA, 2016

Also, annually about 70 million tonnes of waste is generated in South Asia, with the quantity of waste generated per person per day ranging from 0.12 – 5.1 kg/capita/day at an average of 0.45 kg person per day (Hoornweg and Bhada-Tata, 2012). OECD countries generate almost half of the world's waste, while Africa and South Asia regions produce the least waste.

2.2 Solid waste generation in Nigeria

Solid waste generation in some urban cities in Nigeria is presented in Table 2.2. Waste generation rate has been estimated to be about twenty-five million tonnes annually in Nigeria, with a daily rate of 0.44 – 0.66 kg/capita/day and the waste density in the country range from 280 – 370 kgm^{-3} (Ogwueleka, 2009). The problem is not the quantity of waste generated, but the environmental impacts which such waste is causing to the quality of the environment due to inadequate waste management systems. The operations of the waste management authorities in Nigerian cities, where they exist, are inefficient and ineffective. Only about 30-50% of waste is collected in most cities, the remainder is often burned or dumped haphazardly on open plots of land particularly along main roads and on the streets where it causes blockage to drainages, contribute to flooding, distort city aesthetics and lead to public health hazards (Jaiyeoba, 2002).

The rapid changes and growth in industrial technology, the rapid increase in the country's population, the ever increasing rural-urban migration, the dramatic change in consumption patterns in favour of imported packaged can foods and other luxurious goods are substantially yielding high quantities of both degradable and non-degradable materials like metals, bottles, plastic materials, glasses, electrical and electronic equipment and other solids which transverse Nigerian cities today; hence complicating the problem of maintaining urban environment at a healthy level for human beings (Maton *et al.*, 2016).

Oladipo *et al.* (2011) in a report on policy direction for MSW management in Nigeria gave a summarized review on waste volume in some Nigerian cities. Lagos with population of 10.3 million in 1990 generated 3.7 million tonnes of solid waste; Kano with population of 1.4 million in 1994 generated 450 tonnes per day; Port Harcourt and Warri in 2002 generated 164,029 tonnes and 66,721 tonnes per year of solid wastes

Table 2.2: Selected urban cities in Nigeria, volume of waste generated and the waste management agencies

City	Population	Agency	Tonnage /Month	Density (kg/m^3)	Kg/Capita /Day
Lagos	8,029,200	Lagos State waste management authority	255,556	294	0.63
Kano	3,348,700	Kano State environmental protection agency	156,676	290	0.56
Ibadan	307,840	Oyo State environmental protection commission	135,391	330	0.51
Kaduna	1,458,900	Kaduna State environmental protection agency	114,443	320	0.58
Port Harcourt	1,053,900	Rivers State environmental agency	117,825	340	0.60
Makurdi	249,000	Urban development board	24,242	300	0.48
Onitsha	509,500	Anambra environmental protection agency	84,137	310	0.53
Nsukka	100,700	Enugu State environmental protection agency	12,000	370	0.44
Abuja	159,900	FCT environmental protection agency	14,785	280	0.66

Source: Ogwueleka (2009)

respectively; Maiduguri in 2002 generated 8.5 million tonnes of solid wastes; Makurdi generated an average of 0.54 kg per capita per day of solid wastes; Abuja generated between 0.55 kilogram and 0.58 kilogram per person per day of solid wastes; Kaduna, Onitsha, Aba, New Bussa and Uyo generated as much as 4,313,124 tonnes, 386,593 tonnes, 236,703 tonnes, 9,518 tonnes, and 20,923 tonnes of solid wastes per year respectively; Oyo generated about 55,200 kg per day of solid wastes; Uyo collected 4,079,000 kg of municipal wastes for disposal monthly; the projected per capita solid wastes generation in Ilorin by the year 2020 has been predicted to be about 0.43 kg per person per day (Oladipo *et al.*, 2011; Maton *et al.*, 2016).

2.3 Composition of solid waste

Knowledge of the composition of solid waste usually determines the method of collection, storage, and transportation and this invariably determines the most effective method for it treatment and disposal to be used in a municipality. There are many ways of classifying waste. There are some common groupings however. Based on origin, waste can be grouped as domestic waste, clinical waste, industrial waste, agricultural waste and nuclear waste. Based on form, waste can be grouped as liguid, gas and solid, and based on properties, waste can be grouped as toxic, inert and carcinogenic wastes. The majority of these wastes can as well fit into a number of groups (Kemp, 1998).

Municipal solid waste can be broadly divided into organic and inorganic components. However, waste can be further categorized into organic, plastic, papers, metals, glass and ‘others’ (Hoornweg and Bhada-Tata, 2012). Table 2.3 describes the different types of waste and their sources while Figure 2.1 shows the approximate values for percentage composition of the different types of waste in a global MSW stream. The most predominant type of MSW is the organic waste; this is followed by paper waste, metallic waste, other types of waste, plastic and glass wastes.

2.4 Municipal solid waste management methods

Municipal solid waste management (MSWM) is the various methods and processes of collecting, transferring, treating, recycling, recovering resources and disposing solid waste generated in urban centres. The objectives of solid waste management are to enhance and advance the quality of urban centres, create jobs and generate income, and to protect environmental health

Table 2.3: Types of waste and their sources

Types	Sources
Organic	Food scraps, yard (leaves, grass, brush) waste, wood, process residues
Paper	Paper scraps, cardboard, newspapers, magazines, bags, boxes, wrapping paper, telephone books, shredded paper, paper beverage cups. Strictly speaking paper is organic but unless it is contaminated by food residue, paper is not classified as organic.
Plastic	Bottles, packaging, containers, bags, lids, cups
Glass	Bottles, broken glassware, light bulbs, colored glass
Metals	Cans, foil, tins, non-hazardous aerosol cans, appliances (white goods), railings, bicycles
Others	Textiles, leather, rubber, multi-laminates, e-waste, appliances, ash, other inert materials

Source: Hoornweg and Bhada-Tata (2012)

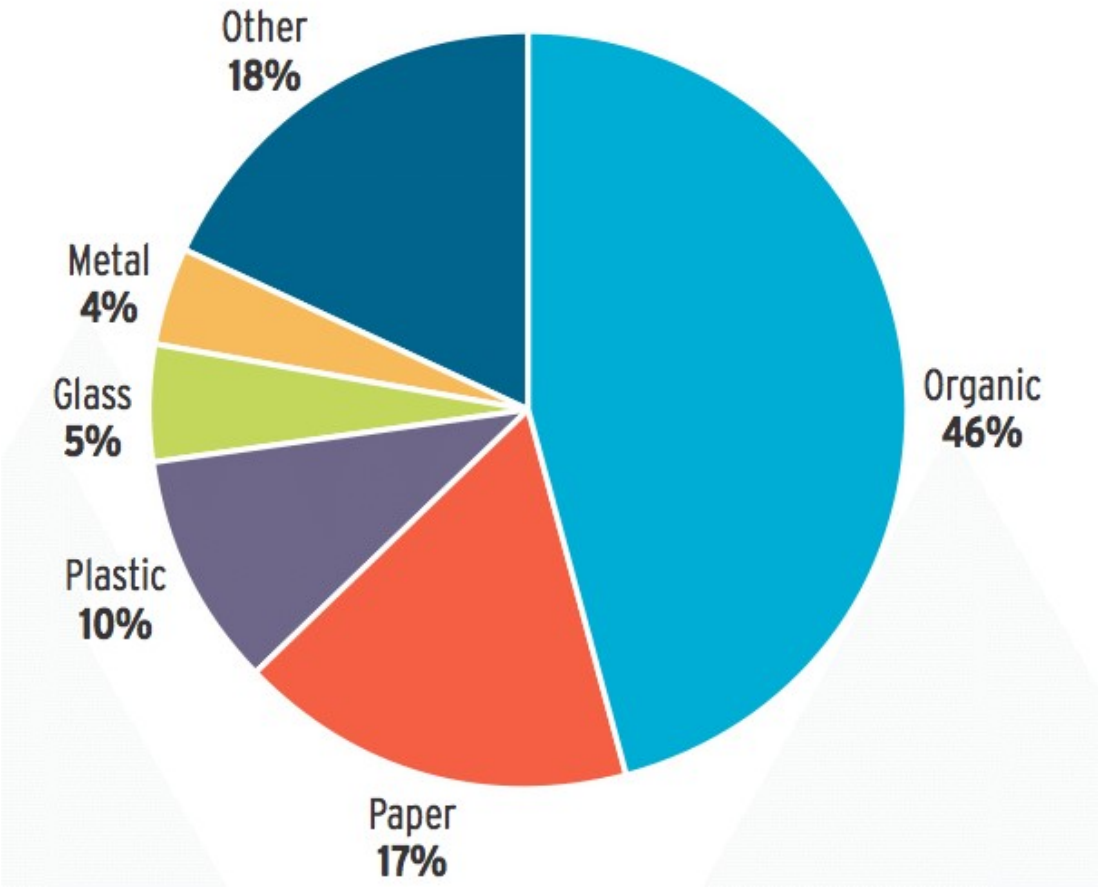

Figure 2.1: Percentage global solid waste composition

Source: Hoornweg and Bhada-Tata (2012)

and support economic growth. MSWM is a major duty of state and local government environmental agencies. The agencies are charged with the duties of handling, employing and disposing of solid waste produced within the state (Ogwueleka, 2009; Vergara and Tchobanoglous, 2012).

Municipal solid waste management is the most crucial service provided by a city's administrators for its populace. In low-income nations and many middle-income nations, waste is the largest single budget item for cities and one of the largest employers. If a city cannot manage its waste properly, it is most likely that such city will fail in managing more complex community services such as education, health, or transportation. Consequently, when waste is poorly managed, it produces impact on the economy, health, local and global environment (Hoornweg and Bhada-Tata, 2012).

Municipal solid waste management practices commonly used in different nations includes (a) landfilling of solid waste, (b) incineration of waste, (c) composting of organic solid waste, (d) open burning and (e) recycling or recovery from waste.

(a) Landfilling of solid waste: Landfilling apparently happened to be the main disposal method for MSW in both developing and developed countries. It is estimated to account for the management of about ninety-five percent of the total MSW collection globally. Co-disposal of MSW is usually what is in operation in most landfills. In developed countries, landfilling is done in an engineered and systematic manner. On the contrary, in developing nations, open dumping of waste in available lands in an unscientific manner is the common practice (Karak *et al.*, 2012). Usually, these dump sites in most developing countries are accidentally created, without appropriate lining/cover systems and no leachate collection systems. More still, they are sited in public places, close to residential quarters and in wetlands (Alimba, 2013).

(b) Incineration of waste: Disposing solid waste into landfill is now being discouraged due to depleting availability of land resources and the need to recycle some re-usable waste resources. However, separating and recycling of waste holds limited economic benefit, thus, resource recovery in the form of electric power and heat generation are rapidly gaining favour in the last twenty decade. Incineration of MSW is an attractive alternative for waste disposal and landfilling for the following reasons: (i) It reduces

waste volume (by 85-90%) and mass (about 70%) to a fraction of its original size and in the process create the chance of recovering energy; (ii) reduction of waste is straightaway and does not involve the long reaction time for biological decomposition; (iii) it removes the burden of transportation cost since an incineration plant can be constructed closed to the waste sources; (iv) it provides economic benefit from sales of energy generated and this can be used to offset the cost of operation; and (v) the operation can be carried out to meet environmental legislative limit and avoid air pollution. Regardless of these benefits, using incineration in waste management in developing countries holds some limitations as a result of high water content and low calorific value of the waste; making it not self-sustaining (Karak *et al.*, 2012).

(c) Composting of organic waste: Rather than incinerating or landfilling, organic part of MSW can be composted. For composting or anaerobic digestion to be done, source separation of organic and inorganic waste must be carried out. Composting usually leads to the production of a stabilized organic product (which can be used in agricultural soil enrichment) and also the generation of biogas.

(d) Open burning of waste: This is still a common practice in low-income countries, and usually, open burning is done to reduce waste volume or odours emanating from dumped or uncollected waste. This waste management method is highly discouraged because it is the main source of air pollution with hazardous gases such as furans and dioxins.

(e) Recycling of waste: recycling is the reuse or re-selling of materials recovered from waste. Items commonly recycled include paper, metal, and glass. For waste recycling to be effective waste separation from source must carried out. Developed countries usually employ curbside recycling options for the collection and sorting of wastes for the purpose of recycling. Whereas, in developing countries, low- to no-income group know as scavengers are involved in collecting recyclable materials that are dispersed everywhere in the urban area or at various waste dumpsites (Karak *et al.*, 2012).

In Nigeria today, besides the different types of solid waste management practice, waste management is characterized by inefficient collection methods, inadequate collection system and inappropriate waste disposal (Ogwueleka, 2009).

2.5 Groundwater and hydrogeology of Lagos metropolis, Nigeria

Groundwater is the water underneath the ground and below land surface. It found in pores between sedimentary particles and in the fissures of more solid rocks. Most groundwater lies at shallower depths, however, and plays a slow but steady part in the hydrologic cycle. Groundwater may have direct interaction with water from seepings due to run off and from the soil surface by an impervious stratum. The physical and chemical features of groundwater depend on the geological nature of the surrounding environment, which in turn determine the composition of the water. Water that circulates in a sandy organic substratum is acidic and has few minerals, while water that circulates in limestone contains bicarbonate thus making it alkaline (Lasisi, 2011).

In Nigeria, groundwater is a very vital water resource that is available to both the urban and rural dwellers. However, in cities, pipe borne water becomes an alternative. Specifically, in the rural areas, hand-dug wells are the main potable water source even if the streams dry up during the dry season. Sadly, due to human life style of poor hygiene practices in developing countries, these resources are constantly under threat of pollution (Ikem *et al.*, 2002).

Nigeria as a country is situated in West Africa and has different ecological regions. In the south the climate is equatorial, it is tropical in the centre and the climate is arid in the north. The vegetations of the country is influenced by the climatic variations, and this range from mangrove swamps in the south, tropical rainforest in the centre, savannah in the north and sahel savannah in the north-east. The annual rainfall varies from 4000 mm in the south and 250 mm in the north with a national average of 1180 mm (Alagbe, 2002). Geological reports gave rock types as Precambrian basement in the southwest, south-east and north-central. The rocks include gneisses, schists, migmatites, pegmatite, charnockitic and quartz-schist (Adediji and Ajibade, 2005). Lagos state (Latitudes $6^{o}23'$N and $6^{o}41'$N and Longitude $2^{o}42'$E and $3^{o}42'$E) with a landmass of 8345 kilometer square, is located in southwest Nigeria and is physically small in size. However, it is the most densely populated state in the country, with an estimated population of around ten million inhabitants (National Population Commission [NPC] 2006).

The hydrogeological nature of Lagos environment had been reported by Longe *et al.* (1987). Under the ground in Lagos area, the soil is compose mainly of laterite type of soil (rich in iron and aluminium), with reddish brown colour having sand and clay types of soil. The laterite section could vary in depth, usually with an average thickness of about 4 metres. Following this will then be an alternating sequence of sand and clay soil (Figure 2.2). After this is the water-bearing zone containing loose, medium to coarse sand having a depth of about 10.4 metres known as the first aquifer horizon. Groundwater movement in this aquifer zone is mainly of the lateral/sideways type which makes it prone to groundwater contamination. The hydraulic conductivity of the water-bearing zone has been estimated to be in the order of 0.001 cm/s. This aquifer horizon is directly under covered by a semi-permeable zone of reddish gray, silt with clay seal of varying depth with an average of about 4 metre thickness. The calculated Hazen estimates from grain size analysis showed that the hydaulitic conductivity for the semi-permeable layer is about 0.0000001 cm/s. This layer separates the first aquifer zone from the underlying aquifer zone. The second aquifer horizon is compose mainly of medium sized sand with traces of silt sand and this zone has estimated hydraulic conductivities in the order of 0.0004 to 0.001 cm/s. These aquifer horizons (the first and the second) have abundant water supply and is the main source of domestic and industrial water supply. The chemistry indicates that the groundwater is suitable for most domestic, industrial and agricultural purposes except for the occurrence of excessive iron. The iron content of the water from all the wells sampled in Lagos metropolis is of the order of 25 - 600 % higher than the recommended safe values (0.1 mgl^{-1}) (Longe *et al.*, 1987).

2.6 Impacts of landfill on groundwater and soil

Landfill/dumpsite (historically referred to as midden) is the oldest and most common method of waste management and is any site use for waste disposal by burying the waste in excavated land or depression (like closed mining sites). It is the only true disposal method of waste management and the most economical, especially in developing countries. A sanitary landfill is defined as a method of disposing of refuse on land without creating nuisances or hazards to public health or safety, by utilizing the principles of engineering to confine the refuse to the smallest practical volume, and

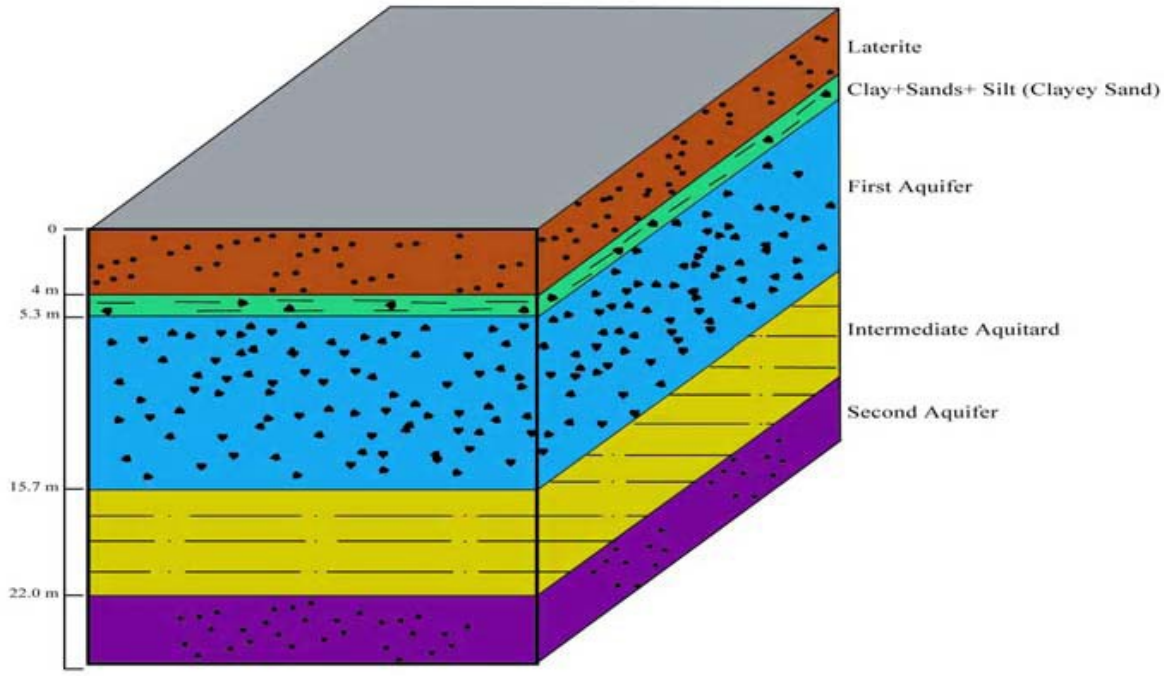

Figure 2.2: An illustrative diagram of subsurface geology of Lagos state.

Source: Longe *et al.* (1987)

to cover it with a layer of earth at the conclusion of each day's operation or at such more frequent intervals as may be necessary (ASCE, 1959).

Modern/sanitary landfill is usually constructed to reduce/eliminate environmental pollution. Here, the waste matters are laid down in layers. When the layer is about 3m in depth, it is cover with a thin layer of soil and compacted by a bulldozer. To reduce pollution of surface and groundwater, lining and contouring the site is done. Also, before siting a modern landfill, area that is not predisposed to flooding with very low soil water table and appropriate soil type is selected and upland drainages are diverted (as illustrated in figure 2.3a and figure 2.3b). Usually, due to anaerobic decomposition of organic waste on landfill, gases (e.g. methane) are produced. Proper venting must be done to avoid explosion hazard (Huang, 2009).

There have been reports that unlined sanitary landfills are emitting hazardous chemicals to surrounding groundwater, soil and to the air, via laechate and landfill gas respectively (Ikem *et al.*, 2002). The environmental safety of waste landfills as a management method is also raising concerns especially in the US and Europe. These concerns include long-term heavy metal build-up in the affected soil and adjacent environment, pollution of groundwater and increase incidence of diseases resulting from pathogens (Christensen *et al.*, 2001).

Wastes disposed on landfills usually experience either groundwater underflow or percolation from rainfall, and as water flow through the waste, it gathers and accumulate various types of organic and inorganic substances which settle at the base of the disposal site. The accumulated and pollutant-loaded liquid is termed 'leachate' (Mor *et al.*, 2006) and this can infiltrate and contaminate the soil. Leachates from MSW landfills are concentrated complex effluent containing inorganic (including heavy metals), organic and xenobiotic compounds (Christensen *et al.*, 2001). A number of persistent organic pollutants (POPs), polychlorinated dibenzofurans (PCDFs), dioxins and polyaromatic hydrocarbons (PAHs) have been characterized previously in leachates samples from obtained from landfills (Sanchez-Chardi *et al.*, 2007).

Ikem *et al.* (2002) evaluated the groundwater quality near two (2) waste sites in Ibadan and Lagos, Nigeria. In their study, 51 groundwater were sampled seasonality for one

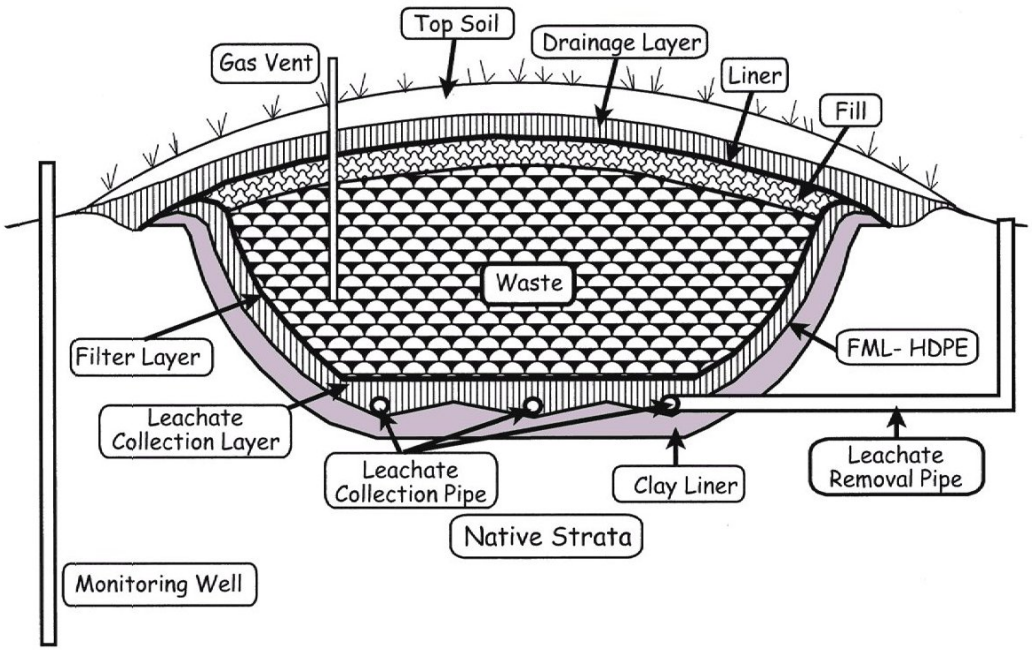

Figure 2.3a: Cross sectional diagram of a sanitary landfill.

Source: https://superstarfloraluk.com/3110043-Landfill-Layers-Diagram.html (accessed 10/04/2018)

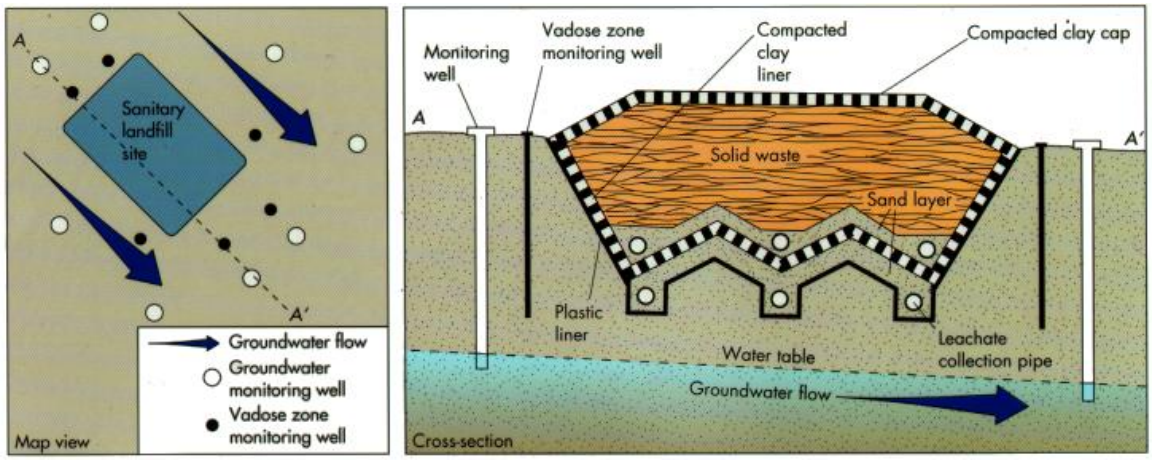

Figure 2.3b: Map and cross-sectional view of a sanitary landfill.

Source: http://nurulhanifahastuti.blogspot.com.ng/2013/01/sanitary-landfill.html (accessed 10/04/2018)

year in Lagos (21 wells) and for two years in Ibadan (30 wells). They observed that there was significant alteration in groundwater quality parameters exceeding WHO standard limits for portable drinking water. Parameters that were altered in the groundwater include pH, conductivity, TDS, chlorides, nitrates, NH_3, chemical oxygen demand, Pb, Cr, Cd, Al, Fe, Ni and total coliforms. Some wells downgradient of the waste dumpsites both in Lagos and Ibadan were observed to have direct significant impact from leachate migration making them unfit for immediate human consumption unless properly treated.

Longe and Enekwechi (2007) revealed the impact of MSW leachate from the Olusosun landfill in Lagos, Nigeria on groundwater quality. They observed higher concentrations of anions: sulphate, nitrate and chloride; and heavy metals (like copper, iron, cadmium and chromium) in groundwater down the slope of the landfill without fading in concentrations. Although, high Fe concentration is characteristic of groundwater in Nigeria in general and also, particularly in Lagos state (WHO/UNICEF, 2006), high iron concentration (range: 0.41 – 4.19 mg/L; mean: 0.94; WHO tolerance level: 0.30 mg/L) in the groundwater at Olusosun environment makes iron an important groundwater quality issue.

Oyeku and Eludoyin (2010) in another study on the effect of dumpsite on groundwater at Ikosi Ketu, Oregun Industrial Estates, Ojota residential areas and Olusosun landfill area, 20 hand dug wells and boreholes were randomly selected and sampled and leachate samples were also obtained from the dumpsite. They observed that the groundwaters were generally alkaline and contained copper, iron, lead and cobalt concentrations that were higher than WHO permissible limits. They resolved that once a major landfill is within 2 km radius, the groundwater around there will be subject to heavy metal contaminations from the landfill.

Akinbile (2012) assessed the impact of Ondo State Waste Management Authority dumpsite in Akure on the chemical, physical and bacteriological characteristics of borehole water and soil within 10 – 100 m distance. Higher concentrations ranges of iron, nitrate, nitrite and calcium were observed in the water. Out of the heavy metals, Zn ranged from 3.3 and 5.4 mg/l and Pb ranged between 1.1 to 1.2 mg/l. Porosity, pH, soil water holding capacity, organic C and N and organic matter ranged between 44 to

48, 6.9 to 7.5, 38 to 54, 1.42 to 2.48, 0.12 to 0.21 and 2.44 to 4.27 % respectively. The *E. coli* and thermotolerant coliform bacteria contents were higher and greater than those in all water samples analysed being an indication of faecal contamination from the landfill. The variance from the WHO standard was also more than 50% in 2/3 of water samples, which further confirmed bacteriological pollution. Akinbile (2012) noted that this observed impairment in water and soil quality parameters make them unsafe for consumption and agricultural purpose respectively.

Afolayan *et al.* (2012) studied the hydrological implication of solid waste disposal on groundwater in Lagos, Nigeria. Fifteen (15) well waters and 3 leachate samples were collected within 750 m distance around the Solous I (closed), II and III (operational) landfills. They observed that the concentration of Cl^-, Pb, Cd and Fe were greater in operational sites and above WHO limits for drinking water quality as compared to closed sited. Excess of chloride ions (Cl^-) is an index of groundwater contamination and proper monitoring of groundwater quality is necessary around closed and operational landfill sites to avoid the imminent public health implications.

Oni and Hassan (2013) characterized the groundwater around the area bordering Aba-Eku municipal dumpsite in Ibadan, Southwest Nigeria after it was upgraded in order to protect surface and ground waters. Two (2) wells – up-gradient and down-gradient of the dumpsite (600 m away) were sampled for twenty-month. Though fewer physicochemical parameters were elevated in the up-gradient well compared to the down-gradient well, the concentrations of chloride, sulphate, Pb, Cd and Fe exceeded regulatory limits in both wells. Oni and Hassan (2014) also noted that the principal contributors to metal levels (especially, lead and cadmium) in leachate and soil (and apparently in the groundwater) are the metal and metal containing wastes (plastic, glass, fine fractions and miscellaneous waste). Thus, they recommended that there is urgent need to remediate the groundwater around the dumpsite given the potential health implications of the pollutants (Oni and Hassan, 2013).

Adedosu *et al.* (2013) investigated the extent of environmental pollution from Olusosun landfill, Ojota, Lagos. Soil, leachate and underground water (UGW) samples were analysed. The concentrations of copper, iron, lead, zinc and cadmium in soil were far higher than the background concentrations suggesting influence from

anthropogenic landfilling activities. The average concentrations of metals in both leachate and UGW samples followed the trend iron > lead > copper > zinc > cadmium with levels of lead, cadmium and iron in all UGW samples greater than allowable limits for drinking water set by Nigerian Industrial Standards (NIS) and WHO. Correlation analysis revealed a common origin for the metals observed in the soil, leachate and UGW. Pollution and geoaccumulation indices revealed gross pollution of soil, leachate and UGW. They implored that an urgent attention is needed to mitigate the potential danger on human health.

Oyiboka (2014) studied the effects of Solous landfills (located at Igando, Alimosho LGA, in Lagos State) on groundwater quality. This dumpsite is the second largest by land mass and waste volume received in Lagos State. The results showed that coliform tests indicate the potential presence of pathogenic microorganisms in the wells, and of the eighteen (18) hand dug wells sampled, five (5) had DO, total alkalinity, nitrate, Fe, Pb and Cu to be above WHO limits for drinking water quality. This implies that some of the well waters are contaminated by leachate and pathogens from the landfill and may be unsafe for human consumption. In addition, there was evidence of acidification and nitrification of the groundwater, and these have been associated with the presence of the waste disposal site within the vicinity of their outlets, and many dumpsites have also been observed to contaminate drinking water with pathogenic bacteria leading to cases of poisoning, cancer, cardiovascular diseases and abnormal embryonic development (Sia Su, 2008).

2.7 Air emissions from landfills

Solid waste landfill usually produce landfill gas (LG) and particulate emissions during mechanical operations. LGs are produced from three processes on the landfill, namely: decomposition caused by bacteria on organic waste; volatilization of certain industrial aqueous and solid waste; and chemical reactions of some waste.

It is estimated that a typical landfill gas contain forty-five to sixty percent methane and forty to sixty percent carbon(IV)oxide by volume. LG also have minute quantities of N_2, O_2, NH_3, sulfides, H_2, CO, and non-methane organic compounds (e.g. vinyl chloride, trichloroethylenen and benzene). Table 2.4 gives the percentage volume of landfill gases and their characteristics. The rate and volume of landfill gases produced

Table 2.4: Typical landfill gas components

Component	Percent by Volume	Characteristics
Methane	45 – 60	Methane is a naturally occurring gas. It is colorless and odourless. Landfills are the single largest source of U.S. man-made methane emissions.
Carbon dioxide	40 - 60	Carbon dioxide is naturally found at small concentrations in the atmosphere (0.03%). It is colorless, odorless, and slightly acidic.
Nitrogen	2 – 5	Nitrogen comprises approximately 79% of the atmosphere. It is odorless, tasteless, and colorless.
Oxygen	0.1 - 1	Oxygen comprises approximately 21% of the atmosphere. It is odorless, tasteless, and colorless.
Ammonia	0.1 – 1	Ammonia is a colorless gas with a pungent odor.
NMOCs (Non-methane organic compounds)	0.01 – 0.6	NMOCs are organic compounds (i.e., compounds that contain carbon). (Methane is an organic compound but is not considered an NMOC.) NMOCs may occur naturally or be formed by synthetic chemical processes. NMOCs most commonly found in landfills include acrylonitrile, benzene, 1,1-dichloroethane, 1,2- cis dichloroethylene, dichloromethane, carbonyl sulfide, ethylbenzene, hexane, methyl ethyl ketone, tetrachloroethylene, toluene, trichloroethylene, vinyl chloride, and xylenes.
Sulfides	0 – 1	Sulfides (e.g., hydrogen sulfide, dimethyl sulfide, mercaptans) are naturally occurring gases that give the landfill gas mixture its rotten-egg smell. Sulfides can cause unpleasant odors even at very low concentrations.
Hydrogen	0 – 0.2	Hydrogen is an odorless, colorless gas.
Carbon monooxides	0 – 0.2	Carbon monoxide is an odorless, colorless gas.

Source: ATSDR (2001)

from specific sections of a landfill depends on the waste composition, age of the refuse, presence of oxygen, moisture content and temperature (ATSDR, 2001).

Karthikeyan *et al.* (2011) noted that apart from CH_4 and CO_2 gases, landfill air emissions contain minute quantities of other chemical substances including heavy metals. Among these other chemical constituents of LG, metals in their ionic forms and VOCs are of great public health concerns due to their acute toxicities, cancer causing effects and non-biodegradability.

Atmospheric particulate matters (PMs) are some form of liquid or solid complex substances hanging freely in the air and usually constitute a major source of air pollution. The PM, specifically the fine particulate matter ($PM_{2.5}$), with diameter of about 2.5µm, are very harmful to public health due to their intricate and complex composition, high adsorption properties, and their increasing levels in the atmosphere in relations to rapid industrial growth and landfilling activities (Zhang *et al.*, 2011). Their adsorption properties makes them easily absorb and transport even more dangerous substances such as heavy metals, PAHs, PCBs, pathogens (viruses and bacteria), other hazardous compounds and potential cancer inducing agents (Li *et al.*, 2015). On prolonged exposure, PM could aggravate many public health issues such as asthma and other lung diseases (Rice *et al.*, 2015).

Volatile organic compounds (VOCs) are odourous gases oozing out from landfill sites due to organic waste decomposition. Also, VOCs are chemical compounds which easily convert to gaseous state and have low solubility in water. They are by-products of industrial processes and end up in landfills with industrial wastes and can contribute to problem of indoor and outdoor pollution. These pollutants have the potentials of causing irritation to nasal nerve cells; create discomfort feeling and other psychosomatic effects. Odourous VOCs could also contribute to triggering asthma attack (Gebicki *et al.*, 2017).

2.8 Toxicity and hazards of air emissions from landfills

Landfill gases (LGs) can be toxic or could pose serious environmental and health hazards, specifically there is the possibility of spontaneous ignition, explosion and risk from asphyxiation. Also, there could be health and psychological issues relating to obnoxious odours coming out from the landfill sites. Methane (CH_4) and other landfill

gases such as ammonia (NH_3), hydrogen sulfide (H_2S) and NMOCs are flammable. However, for LGs to cause explosion, they must be in concentrations within their lower and upper explosive limits (LEL and UEL). Methane is the LG with the greatest risk of explosion hazard. It is explosive between its LEL of 5% by volume and its UEL of 15% by volume. However, because CH_4 concentrations within landfills are typically 50% (much higher than its UEL), frequent explosion are unlikely, but occasionally spontaneous explosion do occur (ATSDR, 2001).

Any of the LGs has the potential of creating an asphyxiation hazard if they occur at concentrations that could impose oxygen deficiency in the environment. For instance, when LGs (e.g. CO_2) escape from the landfill and are collected in a confined space such as a basement or an underground utility corridor, such confined spaces could become oxygen-deficient (ATSDR, 2001).

Recently, on Wednesday, 14 March, 2018, there was a spontaneous ignition on the Olusosun landfill, Ojota, Lagos. The fire covered the entire area of the dumpsite, releasing thick smokes for days which adversely affected lives, properties and socio-economic activities in the State (Figure 2.4). The Lagos State government had to order the close down of the landfill and advise residents within and around the site to relocate immediately to forestall any further environmental and public health disaster. The Director of the State Fire Service and The Cleaner Lagos Initiative (CLI) noted that, though the cause of the fire was not yet clear, however, there can be chemical reactions and build-up of pocket of unstable landfill gases due to the indiscriminate dumping, and this was further compounded by dry weather condition (Folarin, 2018).

The levels of PMs and VOCs emanating from solid waste landfill sites has aroused concerns because of their toxic effects on workers and residents within the vicinity of the sites. PMs could incorporate toxic heavy metals, enter the human body during breathing and cause severe respiratory health damage and can even accumulate in different body tissues and organs (Li *et al.*, 2015). Fine particulate matter ($PM_{2.5}$) containing mutagenic agents could increase death risk, compromise the immune and nervous systems and result in other severe health effects (Li *et al.*, 2015).

Figure 2.4: Recent fire explosion and environmental hazard at Olusosun landfill, Ojota, Lagos State, and its environs.

Source: Folarin (2018)

Karthikeyan *et al.* (2011) characterised the PMs and VOCs in ambient environment of two municipal solid waste dumpsites in India. They observed that black carbon, dust, sulphate, ammonia and nitrate are the most common species of PM_{10} and $PM_{2.5}$.

Important metal ions like B, Cd, Fe, Al, Cu, Ca, Mg, K, Pb, Sr, Ni and Zn were also characterized in the PMs. The ambient air sample also carries noxious compounds e.g. octane, decane, nonane, diethyl phthalate, cyclobutane, methanamine, acetone diperoxide and carbon disulfide. The respirable PM in samples of air from the dumpsites varied widely between 211 microgram per meter cube and 900 microgram per meter cube, and exceeded the upper limits of 150 microgram per meter cube standards prescribed by Central Pollution Control Board (CPCB, 2010), as well as USEPA.

Koshy *et al.* (2009) investigated the bioreactivity of respirable airborne PMs from a municipal landfill. They observed that both particulate matter fractions, $PM_{10\text{-}2.5}$ and $PM_{2.5\text{-}0.1}$ collected at the municipal landfill contained higher overall levels of metals than the corresponding urban PM. The landfill $PM_{2.5\text{-}0.1}$ fraction contained significantly ($p<0.01$) greater concentrations of lead and zinc than other samples. Furthermore, exposure of the supercoiled plasmid DNA to damaging oxidative PM caused a conformational change in the tertiary structure of the plasmid to the relaxed and/or linear form, and $PM_{2.5\text{-}0.1}$ were observed to be more bioreactive compared to larger $PM_{10\text{-}2.5}$ or PM_{10}. Landfill $PM_{2.5\text{-}0.1}$ showed a more greater oxidative capacity than the urban $PM_{2.5\text{-}0.1}$, with the former inducing fifty percent damage (TD_{50}) at 25 μgml^{-1}, while the urban $PM_{2.5\text{-}0.1}$ elicited the same level of damage at 185 μgml^{-1}. However, exposure of human tracheobronchial epithelial cells *in vitro* to landfill PM showed no significant adverse effects. Similarly, de Kok *et al.* (2005) showed that $PM_{2.5}$ containing low concentrations of heavy metals (Pb, Cd, Cr and Ni) produced genetic toxicity both *in vivo* and *in vitro*.

James and Stack (1997) observed that VOC levels at a landfill site in Ireland which received both municipal and industrial non-hazardous waste, were 5 – 13 times higher than the expected values for a typical rural location and among the elevated hazardous components identified were toluene (< 308 $\mu g/m^3$), tetrachloroethene (< 100 $\mu g/m^3$) and 1,1,1 –trichloroethane (< 27.7 $\mu g/m^3$). However, benzene constituted a potential

health hazard with seventy-five percent of the sample containing levels in excess of guideline limits of 5 μg/m^3 (WHO, 1987) and with a maximum recorded level of 166 μg/m^3. Urase *et al.* (2008) also observed that VOCs emitted from a solid waste disposal site contain elevated levels of the so-called BTEX compounds (benzene, toluene, xylene, ethylbenzene) and the concentration of chlorinated compounds were very low. However, benzene concentration ranged from below the detection limit to 20 mg/m^3. This upper limit of detected benzene was in the range of 10^4 times the environmental quality standard concentration (0.003 mg/m^3) in Japan.

The Datianshan landfill is the largest landfill in Guangzhou, South China, receiving both commercial and municipal solid waste. Zou *et al.* (2003) showed that most VOC levels at the landfill are 2 – 10 times higher than those from urban sites. They observed that the ambient VOC levels in different seasons in Datianshan landfill contain high levels of benzene (1.2 – 167 μg/m^3), toluene (1.7 – 202 μg/m^3) and chlorinated VOCs. Sixty (60) VOCs were identified in the summer sample as against 38 VOC species in the winter sample; and 16 of these compounds detected were USEPA priority pollutants. Benzene for instance, is a known carcinogen and exposure to 1 μg/m^3 of benzene produced a lifetime risk of 4 x 10^{-6} for leukaemia and usually, 5 μg/m^3 is considered as a practical limit of exposure (ENDS, 1996). Thus, the level of benzene observed in this study may pose a significant health hazard to site operators.

In the same study, Zou *et al.* (2003) reported 13 and 9 chlorinated compounds in the summer and winter samples respectively. The average concentrations of some USEPA priority chlorinated pollutant species detected were; chloroform (13 μg/m^3), carbon tetrachloride (3.7 μg/m^3), trichloroethane (9.3 μg/m^3), tetrachloroethene (16.8 μg/m^3) and chlorobenzene (2.2 μg/m^3). Though, compared to aromatic compounds, the concentrations of chlorinated species were lower in the landfill ambient air (0.1 – 31 μg/m^3 in winter and 0.2 – 35 μg/m^3 in summer), however, their effects on the environment should not be overlooked due to their toxicity. Chloroethene is a known human carcinogen and has been asserted to cause liver cancer. Thus, long-term exposure to landfill gases could pose detrimental risk to human health.

2.9 Exposure pathways from landfills to man

Exposure is the link between a health hazard and a health outcome. The route by which people are exposed to contaminants from landfills is as important as the amount/concentration to which they are exposed. A complete exposure pathway starts at the source of contamination, environmental media through which the contaminant moves through and the route of exposure to affected population (WHO, 2000).

Sanitary landfill is absent in Nigeria and most wastes produced within the country are dumped of in open dump sites and in unlined landfills. Co-disposal of solid waste is the common practice in most landfill, where the dumpsite receives a combination of all types of waste ranging from domestic refuse, market garbage, and agricultural waste to hazardous clinical, industrial and electronic wastes. Also, most major landfills are sites close to rivers and streams or located in area with higher water table such as swamps and wetlands; and to fill abandoned mining/excavated sites (Alimba, 2013). Therefore, these landfills are prone to leaking, and hazardous wastes disposed in them can leach heavy metals and other toxins into soil, air and water table from where they can enter food chains/webs. Also, these heavy metals such as mercury, cadmium and lead are liberated into the air and ashes when incinerated or burnt. Molecules of these toxic metals may settle on the surface of aquatic plants with no observable effect on the plants. However, when smaller aquatic animals including fishes feeds on these plants, a high level of these metals will be accumulated in their body. Larger aquatic animals and fishes feeding on the smaller fishes will end up bio-accumulating even greater and life-threatening quantity of the toxicants. From these bigger fishes, the bio-accumulated toxins may eventually get into the food chain and this become a major route of exposure to the general public (Don-Pedro, 2009).

In summary, the pathways of exposure among populations living around solid waste landfills are air transport and inhalation of contaminants, ingestion of water and food (contaminated with landfill chemicals, particulate matters and microbes), noise from heavy duty machines, dermal contact and transmissions of pathogens through vermin (vectors of diseases) (Alimba, 2013) as illustrated in figure 2.5. In other words, populations who dwell within the area around solid waste landfills could be exposed directly to air pollutants released from the sites (LG containing methane, H_2S, CO_2 and other contaminants including VOCs, PM and bioaresols) or to polluted soil, surface

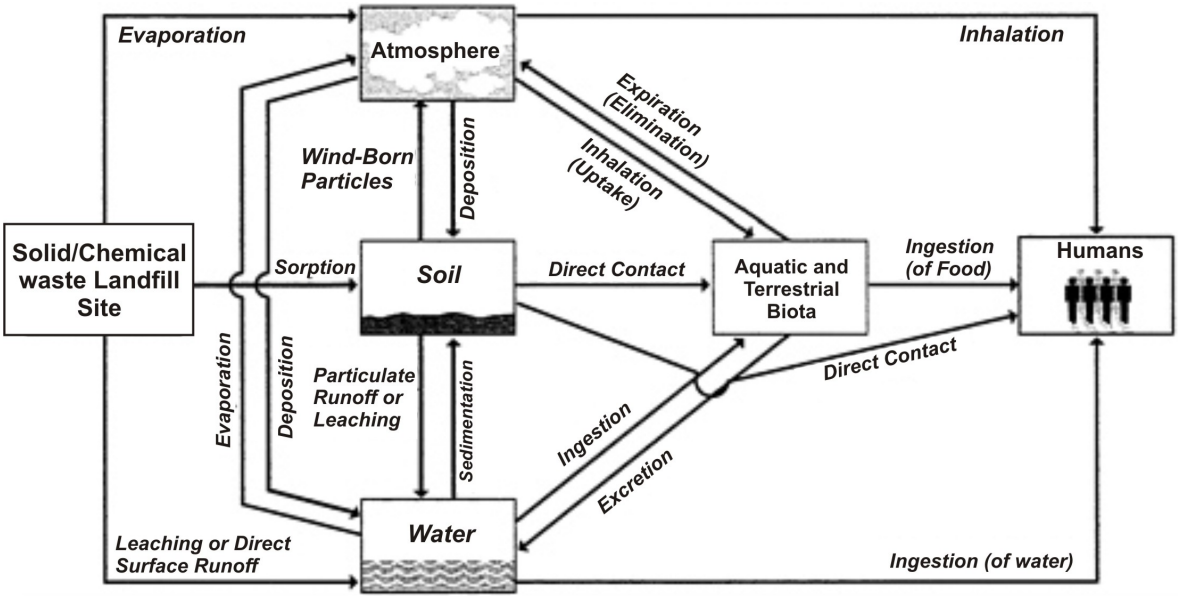

Figure 2.5: Schematic illustrations of potential exposure pathway from a landfill site to man.

Source: Grisham (1986)

and underground water (Mataloni *et al.*, 2016). Either directly or indirectly, human and animal exposure to landfill sites and pollutants has been observed to cause adverse environmental and public health effects. These effects could range from altering the natural aesthetic of the environment, psychologic to health related consequences and mortality (Porta *et al.*, 2009).

2.10 Genotoxicity and mutagenicity of solid waste landfill leachate in animals

Municipal landfills in Nigeria has been known to release pollutants and contaminants such as obnoxious odour, soot and smoke, particulate matters, heavy metals, leachate, VOCs, XOCs and bioaerosol into surrounding environment. Constituents of some of these pollutants have been shown to cause DNA damage (Bakare *et al.*, 2012a; Alabi *et al.*, 2012; Alimba *et al*., 2017). *In vivo* and *in vitro* bioassays in different living systems have been used to study the genotoxicity and mutagenicity of leachates and potential mechanistic mode of actions in inducing DNA damage.

In making prediction as regards human health and toxicological risk, testing in animals is usually more acceptable when compared to the use of microorganism and plant systems. The reason is that animals have similar physiological and enzymatic processes in metabolizing various environmental agents. Animal testing also allows for controlling the various environmental conditions during the experiment, to assess oxidative damages and the mechanisms of damage (Stegeman and Lech, 1991). There is a wide range of animal models and biomarkers in them that have been used in studying the genetic toxic and mutation causing effects of landfill chemicals and leachates.

Bakare *et al.* (2005), Alabi and Bakare (2011) and (2014) untilized the abnormal sperm morphology test and alterations in sperm count (biomarkers of genotoxicity) in mice to ascertain the DNA damaging effects of raw and simulated leachates and underground water collected from different solid waste and electronic waste dumpsites in South-west Nigeria. Abnormal sperm morphology had been validated to show correlation with fertility problems in males, preimplantation issues in embryos and congenital abnormalities at birth.

Increase frequencies of micronuclei (MN) in different organs/tissues and in peripheral blood in different animal models have also been used to show the genotoxicity of

leachate and contaminated underground water from MSW landfills. Deguchi *et al.* (2007) showed that untreated and treated domestic and industrial MSW leachate from Japan elevated the occurrence of MN in cells of gill in goldfish (*Carassius auratus*). Similarly, Alimba *et al.* (2011) and Bakare *et al*. (2013a) reported that MSW leachate obtained from Abule Egba in Lagos, Nigeria and electronic waste leachates obtained from Alaba International electronic market, Lagos induced increased frequencies of micronuclei in peripheral RBCs of African catfish (*C. gariepinus*). Similar increase in MN was observed in peripheral blood cells and PCE of mice (*Mus musculus*) and Japanese quail (*Coturnix japonica*) exposed to leachates obtained from dumpsites in two municipalities (Aba-Eku and Olusosun-Ojota) in South-west Nigeria (Alimba and Bakare, 2016).

Also, in *in vitro* evaluation, Amahdar *et al.* (2009) revealed that waste leachates obtained from Morocco (Settat town) caused MN formation and altered the rate of cell division of human peripheral blood lymphocytes. Similarly, Alimba *et al.* (2017) reported that simulated leachates from two developing countries induced DNA damage and cytotoxicity in human cell lines such as lymphoma (Jurkat), osteosarcoma (HOS) and hepatocarcinoma (HepG2) cells. The cytotoxicity showed evidence of apoptosis and other cytological alterations. Also, the DNA damage was evident with concentration dependent increase in Olive tail moment. Toxic metals and organic constituents identified in the leachates were believed to cause the genotoxic effects in cells obtained from humans.

Real-time quantitative polymerase chain reactions was also used to show increased expression of Cd-methallothionein-coding mRNA in earthworm (*Eisenia fetida*) exposed to soil contaminated with landfill leachate as an index of mutagenicity in the earthworm (Manier *et al.*, 2012). Other techniques in animal cell lines (e.g. human peripheral blood lymphocytes, *Daphnia magna* somatic cells, etc) such as comet assay have been used to show the DNA damaging effects of MSW leachates (Bakare *et al.*, 2007; Widziewicz *et al.*, 2012; Alabi *et al.*, 2012). The various genetic abnormalities observed in these studies suggests that MSW leachates and polluted groundwater contain metals and other xenobiotics which have the potential to cause DNA damage in both germ cells and somatic cells in different animal models and humans.

2.11 Systemic toxicity of solid waste landfill exposure

All living organisms are made of cells. Cells performing similar functions are organized together to form tissues, and different tissues come together to form organs (e.g. skin, liver, kidney, heart, lungs, etc.). The highest level of organization in living multicellular organisms is the organ-system; this comprise of organelles, cells, tissues, and organs working together in a coordinated manner to carry out a particular life supporting function (e.g. circulatory, digestive, nervous, excretory, etc. systems) (Taylor *et al.*, 1998). The components of the systems are involved in material transport, metabolism, modulation, assimilation, detoxification, immunity, storage, and elimination of xenobiotics from the body of organisms. This makes these organs to be at risk of pathological alterations and oxidative damage due to the actions of the xenobiotics.

Xenobiotic induced changes in organ-system of organism and this can be seen on the total functioning and coordination of the body. These changes can be studied from the alterations in organ-weight indices, haematological and biochemical parameters and histopathological observations of these organs (Michael *et al.*, 2007; Tayeb *et al.*, 2010). Thus, systemic toxicity can be used to understand and predict the possible mechanisms of xenobiotic induced organ and tissue damage.

Solid waste landfills have been known to release large amount of xenobiotics into the adjacent environments in the form of particulate matters (PMs), volatile organic compounds (VOCs), bioaerosols and leachates (Mor, *et al.*, 2006). The systemic effects of these contaminants have been widely studied both in animal and exposed human populations.

Silkworth *et al.* (1984) in a systemic study of mice sub-chronically (90 days) exposed by direct contact to soil samples of Love Canal Chemical dumpsite in Niagara Falls, New York, observed that the air sample above the soil contain 87 VOCs and toxic organochlorides were detected in liver tissues of exposed mice. Also, morphological alteration such as hepatocyte hypertrophy was seen in the liver of the mice. In another follow up study, raw leachate and soil extracts from Love Canal dumpsite were administered orally to rats to investigate the effects on maternal health and fetal development. They observed fourteen percent decreased maternal weight gain,

maternal mortality, an elevation in the occurrence of resorptions per dam, an elevation in the number of non-surviving implants per dam, a decrease in the number of viable fetuses per dam, a decrease in fetal birth weight, and signs of delayed fetal development (Silkworth *et al.*, 1986). They concluded that contaminants in the dumpsite could results in hepatic, maternal and fetal toxicity.

Bakare *et al.* (2003) reported that mice exposed to simulated and raw MSW leachates from three dumpsites in Southwest Nigeria (Orita-Aperin, Oworonsoki and Abadina) experienced loss of hair, weight loss, sluggishness and loss of appetite. Similar observation was made by Alimba *et al.* (2012) on rats exposed to leachates from two landfills in Southwest Nigeria.

Li *et al.* (2010) also observed that mice exposed to leachate from landfill experienced reduction in body weight and elevation in relative kidney and liver weight gain. They noted that loss of appetite (anorexia) is a common symptom of liver injury, together with reduced body weight gain. Sneezing and difficulty in breathing suggest a respiratory abnormality due to toxicosis of the chemicals present in the dirty water. Ungroomed hair, Hair loss, abscess, diarrhea, reduced activities and muscular disorder are common signs of immune and central nervous systems perturbation (Benlahcen *et al.*, 2009). Chemical toxicity from the leachates was believed to trigger these clinical signs and symptoms.

Oshode *et al.* (2008) carry out an ecotoxicological assessment of leachate from Aba-Eku municipal landfill, Southwest, Nigeria using mud catfish (*Clarias gariepinus*). They observed that PCV (%), RBC number, Hb concentration, lymphocyte and WBC count were elevated with increasing leachate levels. Histopathological lesions observed in liver of exposed fish include congestion of the portal vessels and central veins, hepatic necrosis and glycogen deposition infiltration of the hepatocytes. The kidney showed degeneration of renal tubules and tubular calcification and gills had stunted and sloughed secondary lamellae. They explained that the alterations in the haematological parameters indicate a compensatory erythropoiesis, resulting in the synthesis and multiplication of more red blood cells to make amend for the older ones that are quickly being damaged as a result of decline in blood oxygen carrying capacity. An unusually elevated leucocyte indicate inflammation, hypersplenism,

stress, trauma, and immune response of the fish to toxins in the leachate. Heavy metals and other toxicants in the leachate induced the observed haematological and histological changes.

Sanchez-Chardi *et al.* (2007) and Sanchez-Chardi and Nadal (2007) reported that wild rodents (wood mice - *Apodemus sylvaticus* and greater white-toothed shrew - *Crocidura russula*) exposed to Garraf landfill (NE, Spain) bioaccumulated metals in their tissues (liver and kidney), registered low residual index (RI), high relative renal weight, increased ALT activity and micronucleus (MN) frequencies in blood. Structural alterations such as inflammation , cell cycle arrest (necrosis and apoptosis), preneoplastic nodules, microsteatosis and vacuolation (in hepatic tissues); and tubular dilatation and necrosis, cylinders and inflammation (in renal tissues) were seen in the exposed rodents (Sanchez-Chardi *et al.*, 2009). Accumulated heavy metals from the polluted site was believed to induced the observed morphological, physiological and genetic alterations, and since the exposure route was through the diet, liver and kidney tissues are the first targets (Pereira *et al.*, 2006; Wlostowski *et al.*, 2008). The damage to the liver may be related to it metabolic functions in toxic substance bioaccumulation, excretion and transformation (Sanchez-Chardi *et al.*, 2009); and the induced damage to organs by accumulated metals might have been through the production of ROS (Wlostowski *et al.*, 2008).

Alimba *et al.* (2012) reported the hepatic and renal toxic effects of leachates in Wistar rats (*Rattus norvegicus*). There was significant reduction in body weight and elevation in absolute and relative liver and kidney weight gain; increase in AST, ALT, urea and creatinine but decrease in albumin and total protein. Increase relative organ weights could be due to bio-accumulation of toxic heavy metallic elements found in the leachates in these organs. This result indicates that toxic heavy metallic elements and other deleterious constituents found in the leachates could have bioaccumulate in these organs resulting in increased organ weights, hepatocellular injury and renal function impairment. In support of this observation, Alimba *et al.* (2012) also reported structural changes such as mild to severe multifocal degeneration of the hepatocytes, multiple periportal foci, cellular infiltration, interstitial haemorrhage, cortical congestion, degenerative epithelia of renal tubules and necrosis. They explained that the morphological alterations in the liver and kidney tissues must have been due to

hypoxic conditions in the tissues and elevated free radical species induced by metals or joint action of other constituent of the leachate (Stohs and Bagchi, 1995).

There are reports that activated municipal sewage sludge are rich source of nitrogenous matter and could be good and cheap supplements of animal protein. However, Bag *et al.* (1999) fed rats with varying concentrations (5 – 20 %) of domestic sewage sludge in their diet for 10 weeks. They observed that liver and muscle activities of lactate dehydrogenase, serum and liver aspartate aminotransferase, and serum and muscle alkaline phosphatase activities were significantly higher in rat treated with the sewage sludge. Heavy metals such as Zn, Ni, lead, copper, chromium and cadmium (1.820, 0.273, 0.017, 0.053, 0.006 and 0.005 mg/g respectively of dry sludge) were identified in the sludge. They noted that, increased level of liver and muscle LDH in rat treated with sludge-supplemented diets indicated that metal contaminants in the sludge caused the switchover in respiratory process at the tissue level to anaerobiosis due to severe impairment of the aerobic respiratory process. They concluded that, though sludge supplemented diet is in rich organic nitrogen, caution must be taken to avoid metal biomagnification and toxicity. Increased LDH activities in serum were also reported in fish *Channa punctatus* exposed to increasing concentrations of tannery wastewater (Parveen *et al.*, 2017). The result revealed that the waste water caused cellular hypoxia creating an anaerobic condition and inducing cellular damage.

Alimba and Bakare (2012) reported the haematotoxicity of leachates from two dumpsites in Nigeria in rats. They observed significant reduction in RBCs, platelets, WBC, lymphocytes, haemoglobin, HCT (%), MCHC, monocytes, eosinophil and basophil; but significant increase in MCV, MCH and neutrophils. Morphological lesions on erythrocytes include echinocytes, acanthocytes, schizocytes, target cells and tear drops cells. This finding showed that the leachate from solid waste landfill can cause direct impairment of bone marrow and blood cells and induce both haematotoxic and non-specific immunotoxic effects in rats and potentially exposed human populations. Metals were believed to provoke the observed toxic effects. The finding of Hounkpatin *et al.* (2013) on the haematological evaluation of Wistar rats exposed to Cd, Hg and their combination also support the haematotoxic and immunotoxic effects of metal contaminants from solid waste landfills.

Apart from DNA damage in somatic tissues and organs (blood, liver, kidney and spleen), municipal sludge leachate collected from the vicinity of a dumpsite in Lucknow, India caused oxidative stress in the hepatic tissues of mice (Bakare *et al.*, 2012a). Similarly, Bakare *et al.* (2013a) reported that mice exposed to e-waste contaminated well waters and leachates from Lagos, Nigeria induced significant elevation in catalase, decline in glutathione and malondialdehyde with corresponding decline in superoxide dismutase levels. These authors asserted that heavy metallic elements (As, Cd, Cu, Pb, Cr, etc.) and other toxic constituents present in the landfill leachates and water samples contributed to the observed oxidative stress; and that such tissues and organs may be potential targets for systemic toxicity in exposed animal and human populations.

Alimba *et al.* (2015) investigated the potential brain damage induced by two landfill leachates (Olusosun and Aba-Eku landfill) on rats. The leachate induced a decrease in body weight gain and superoxide dismutase level but elevation in absolute and relative brain weight gain, malondialdehyde level and catalase level in both brain and serum of treated rats. Alterations in brain and serum biochemistry was consistent with neurotoxic lesions observed in the brain. The neurotoxic constituents of the leachate such as heavy metals (lead, arsenic, cadmium, manganese and nickel), sulphates, NH_3, Cl^- and phosphate were above standard permissible limits and were believed to induce the neurotoxicity via oxidative damage.

Meyer (1983) studied the health effects of a massive toxic waste dump at Hardeman County, Tennessee, US on area residents exposed to contaminated drinking water. They observed multiple symptoms, evidence of hepatomegaly and significantly ($p<0.01$) elevated liver function parameters such as ALP and AST and lowered ($p<0.001$) albumin and total bilirubin. This result showed strong indications of a subclinical transitory liver insult associated with consumption of water from wells contaminated by leachate from the chemical dump sites.

In Ogun State, Nigeria, Odewabi *et al.* (2012) studied the response of inflammatory biomarker and oxidative stress markers in waste management workers (WMWs). They observed significant increase in C-reactive protein, ceruloplasmin, malondialdehyde (MDA) and uric acid, with reduction in catalase, glutathione and superoxide dismutase

as compared to the reference group. For markers of hepatic injury, there was significant decrease in albumin and elevated AST activity, but no significant change in the levels of ALT, ALP, total cholesterol, and triglycerides; also, there was no alteration in biomarkers of kidney functions (creatinine and urea) when compared to the control. Haematological alterations include significant decrement in Hb, PCV, and MCV and significant elevation in WBC count. These results implies that exposure to MSW could increase the risk of systemic inflammation and oxidative stress in WMWs.

Furthermore, systemic inflammation (elevated erythrocyte sedimentation rate (ESR) and CRP) was associated with immune response; increase in IgA, IgG and adenosine deaminase in WMWs. This observation was concomitant with haematological changes such as significant elevation in eosinophils, monocytes, total WBC and lymphocytes (Odewabi *et al.*, 2013). The implication of this is that, there are abundant microorganisms in the solid waste environment and this makes waste workers to be predisposed to infections that usually require activation of the immune system. Odewabi *et al.* (2013) noted that inadequate protective gears and poor compliance with safety measures among WMWs could be implicated for such level of systemic inflammation and immune response among waste workers.

2.12 Gastric anatomy and physiology in rats

The stomach wall consists of a mucous layer, mucosa, sub-mucosa, muscularis and serosa. The mucous layer protects the mucosal surface from harmful components in the lumen (HCl and pepsin) capable of damaging the epithelial barrier and the bulk of the gastric mucosa is occupied by the gastric glands. The anatomy of gastric mucous membrane of rat is illustrated in figure 2.6. The following cells are component of the gastric gland (Figure 2.7):

1. *The Surface Epithelial (Mucous) Cells*: These cells constitute the lining epithelium covering the inner surface of the stomach being in direct contact with the lumen and secret a protective coat of alkaline mucus. These cells are irregular shapes tend to a pyramidal with an ovoid nucleus having a basal locations surrounded by clear

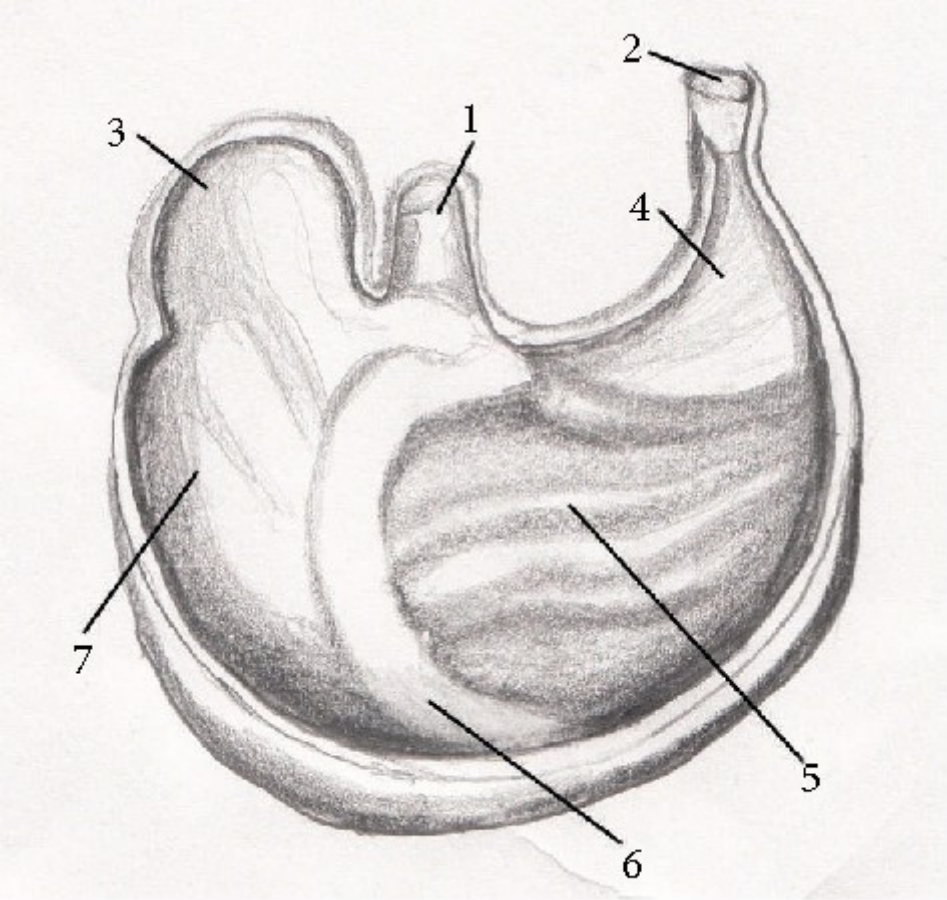

Figure 2.6: Anatomy of gastric mucous membrane of rat.

1: pars cardiaca, 2: pars pylorica, 3: fundus ventriculi, 4: glandulae pyloricae, 5: glandulae gastricae propriae, 6: glandulae cardiacae, 7: pars nonglandularis.

Source: Vdoviaková *et al.* (2016)

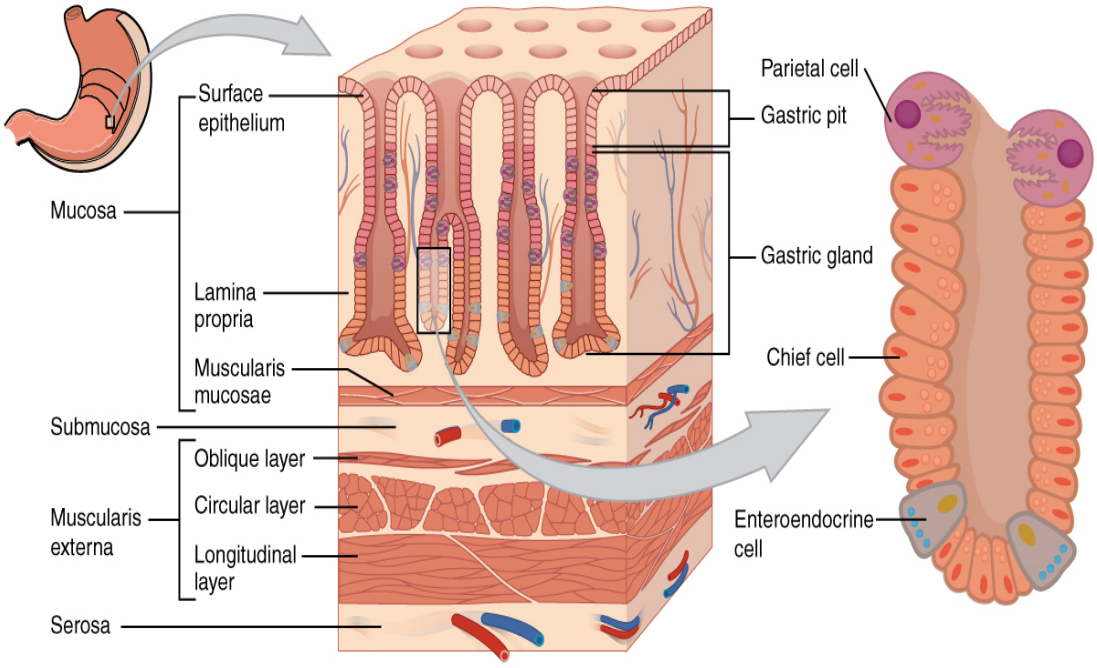

Figure 2.7: Histology of the Stomach of rat.

Source: https://opentextbc.ca/anatomyandphysiology/chapter/23-4-the-stomach/ (Accessed 16/04/2018)

cytoplasmic mass. The apical portions of these cells are occupied by dense discrete granules.

2. *Chief (Zymogenic) Cells*: These cells are mainly located at the bases of the gastric glands and are responsible for the secretion of propepsinogen. These cells have a conical or pyramidal outline; with a basophilic cytoplasm and basally situated spherical nuclei.

3. *Parietal (Oxyntic) Cells*: These cells known as acid-forming cells and are the principle secretors of hydrochloric acid in the stomach. They are scattered among the other cell types. The parietal cells are large in size with a spherical or pyramidal outline and with acidophilic cytoplasm and centrally spherical nucleus.

4. *Argentaffin (Enteroendocrine) Cells*: These cells are found at the bases of the gastric glands, they are small sizes and conical or pyramidal shapes. The cytoplasm contains secretory granules at the basal regions with spherical nuclei in the basal regions. They secrete various hormones into the interstitial fluid of the lamina propria. These include gastrin, which is released mainly by enteroendocrine G cells (Khattab, 2007) as shown in figure 2.7.

The rat stomach performs a variety of functions, including serving as a reservoir for food, exposing ingested food to acid secreted by the parietal cells and pepsin secreted by the chief cells. It also provides a barrier that prevents microorganisms from entering the intestine. The diverse physiological functions of the stomach depend on an intact gastric mucosal integrity (Duan *et al.*, 2006).

The major gastric secretions are hydrochloric acid (HCl), pepsin, intrinsic factor, mucus and bicarbonate ion. Gastric juice is corrosively acidic and its protein-digesting enzymes can digest the stomach itself. Therefore, the stomach is protected by the mucosal barrier and anything that breaches the gel-like mucosal barrier producers can induce inflammation of the underlying layers of the stomach wall (Shereen-Lehman, 2014).

2.12.1 Effects of xenobiotics on the stomach

One route of exposure to environmental chemicals is ingestion through the oral cavity and any substance that passes through this cavity get to the reservoir and digestive organ called the stomach.

Adewoye and Salami (2013) studied the anti-ulcerogenic mechanism in gastric ulcer induced rats. They reported those ulcerated rats that were pre-treated with magnesium had significantly decreased parietal cells number when compared with ulcerated untreated group. Also, mucous cell number of ulcerated rats that were pre-treated with magnesium significantly increased. They concluded that the ability of magnesium to reduce or inhibit the number of the acid producing parietal cells and to stimulate the production of mucus producing cells accounts for it anti-ulcerogenic properties. Similarly, Oluwole *et al.* (2008) observed the gastro-protective effects of methanolic extracts of two plants (*Entandrophragma angolense* and *Tetracera potatoria*) in Albino rats. At low doses the extracts stimulated increase in mucus cells count with concomitant increase in gastric mucus secretion. Same observation was made for high dose of *T. potatoria* but long duration of treatment with high dose of *E. angolense* decreased mucus cell count and decreased mucus secretion. They explained that at long duration and high dose, the extract may have initiate desensitization of gastric mucus cells involved in the gastric mucus secretion.

Rats were exposed to high cadmium (Cd) concentration in their drinking water for 30 days (Asar *et al.*, 2000). They observed significantly increased mean blood and mucosa Cd levels, while there was significantly decreased mucus thickness and mucin content. Conversely, parietal cell count and basal acid output was reduced. Also, zymogenic cells were observed to have focal enlarged endoplasmic reticulum, dilated Golgi cisternae, degenerated mitochondria, broken tubulovesicles, lysosomal structures as well as dense nuclei. They explained that the observed reductions in mucus thickness and parietal cell counts were due to loss of zymogenic unit's cell population.

The impact of pesticide endosulfan (an organochlorine contaminant of the aquatic ecosystem) on histology of stomach of *Channa punctatus* (Bloch) was studied by Haloi *et al.* (2013). They observed histological changes such as haemorrhage, swelling of cell (oedema), breakage of outer membrane, sloughing of mucosa layer and

vacuolization, amongst others. Haloi *et al.* (2013) noted that pathogenicity in the stomach and intestine may be because organochloride contaminants in the presence of HCl produced in the stomach usually generate organochloride acid. This organochloride acid are known to exhibit strong corrosive properties which could destroy mucous secreting cells of the stomach lining to induce inflammation and pathologic conditions.

Bais and Lokhande (2012) reported the histological alterations in the freshwater fish *Ophiocephalus striatus* (*Channa*) treated with cadmium chloride ($CdCl_2$). They observed that the stomach showed hyperchromatic epithelial cells, desquamation of stomach mucosa and destruction of glandular epithelial duct. Desquamation was described to show sloughing of cells on the stomach epithelium which is as a result of necrosis or some degenerative alterations or post- mortem autolysis. Similarly, it have been noted that lesions due to toxicity that are usually frequent in the intestine of various fishes exposed to $CdCl_2$ include degenerative changes in the tips of villi, hyperemia, loss of structural integrity of mucosal folds, necrosis, cellular debris, degenerative mucosal epithelium (vacuolation, hypertrophy, hyper-chromasia), desquamation of mucosal epithelium, excessive mucus in gut of lumen, inflammatory infiltration of submucosal and necrosis of submucosa in intestine (Newman and MacLean, 1974; Gutierrez *et al.*, 1978).

Olaleye *et al.* (2007) observed that rat orally exposed to low and high doses of lead (Pb) for 15 weeks significantly increased the gastric mucosal damage evidenced with significantly increased gastric lipid peroxidation. They concluded that Pb in the stomach interfer with oxidative metabolism to induce the formation of gastric ulcer. Also, Dai *et al.* (2009) and Abdallah *et al.* (2010) asserted that Pb in the stomach causes increase in the generation of free radicals or reactive oxygen species which usually will be mopped up by free radical scavengers. However, if not mopped up, this will exposed the stomach lining to inflammation and subsequent mucosal damage. These harmful effects of Pb as well as its inhibition of enzyme activities might be the main inducer of intestinal histopathological damage in exposed animals. Also, histopathological damages such as surface epithelium or mucosal erosions, swollen cells, and irregular nuclear shape of the cells were induced in the stomach of rat orally

administered with ethylene glycol (a known contaminant in household and food products) as observed by Khattab (2007).

Furthermore, many researches have suggested the role of heavy metal exposure in increasing the incidence and mortality of gastric cancer (Yuan *et al.* 2016). The International Agency for Research on Cancer (IARC) has classified some metals (chromium, lead, arsenic, cadmium, and mercury) as certain or probable carcinogens (Jarup, 2003; Welling *et al.*, 2015). The highest death rate associated with gastric cancer was reported in the cities of Divandareh, Bijar and Saghez where there is high environmental contamination due to mineral deposition of Pb, As and Sb in the area (Eskandari *et al.*, 2015). Also, high concentration of As in drinking water in villages located in Simav Plain in Turkey was associated with the majority of liver, bladder, and stomach cancer cases (Gunduz *et al.*, 2015) and mortality statistics collected from this region from 1995 to 2005 showed that the rate of gastrointestinal cancers was higher than the Turkish average (Gunduz *et al.*, 2010). Wang *et al.* (2011) reported that long-term exposure to Cd and Pb from waste water and downstream river in a region surrounding a multi-metal sulphide mine in Southeast of Guangdong Province, China, enhanced the mortality risk of several cancers, including lung, esophageal, and gastric cancer.

2.13 Toxic effects of heavy metal exposure in man

Heavy metals are those metallic elements that are toxic to a biological system. These metals are very difficult to be broken down into harmless forms by microorganism and may even become bioamplified in living system. The myriads of heavy metals are grouped into non-essential and essential metals. The essential groups are essential because they are important as prosthetic groups in enzymes and key metabolic processes in organisms. Examples are copper, cobalt, manganese, vanadium, iron, , zinc and molybdenum. However, the excessive intake of essential elements can also cause damage. The non-essential elements, such as cadmium, lead, mercury, arsenic are not required in the physiological bodily processes of organisms, so they are known to be toxic at comparatively low concentrations in living organisms (Raymond and Felix, 2011). Metals occur naturally in the environment; however, their concentration in environmental compartments (air, soil and water) has been significantly increased

by anthropogenic activities such as industrial processes and landfilling (Pereira *et al.*, 2006; Sanchez-Chardi *et al.*, 2007).

Lead (Pb) is common toxicant from municipal waste landfills and environmental intake of Pb in children has been reported to be neurotoxic. Pb can cross the blood-brain barrier and disrupt the normal functioning and development of the brain in children (Needleman, 2004). Though there is no safety margin for lead exposure in children, Guo *et al.* (2014) reported that average blood lead level (BLL) of 7.06 µg/dl was observed in children from Guiyu, China, an e-waste dominated town, with a subgroup of 24. 80% of children exceeding the recommended 10 µg/dl BLL elevated limit in children. In 2010, there was lead poisoning epidemic in Zamfara State, Northern Nigeria, due to artisanal gold ore processing in residential compounds in villages. At least 17, 000 people were affected and more than 400 children (≤ 5 years) died in seven villages. The mining activities contaminated the soil, streams and other environmental compartments with mercury and lead (Tirima *et al.*, 2016). Elevated levels of lead exposure have been associated with decreased cognitive function, increased bone resorption, delayed physical development, and intellectual impairment (Canfield *et al.*, 2003).

Cadmium (Cd) is a highly toxic metal with no observable function in the animal body system (Mirani *et al.*, 2012). It is a component of batteries, dyes, paints, plastics, smelted and electroplated material (Kaplan *et al.*, 2011) which are usually co-disposed in most dumpsite in Nigeria and other developing countries. Leachate and groundwater from municipal landfills and surrounding environment have been characterized with high concentrations of Cd (Alabi and Bakare, 2014; Alimba *et al.*, 2011) and this toxic element has been reported to affect the lungs, liver, kidney and testes following acute intoxication (Mirani *et al.*, 2012). Also to be noted is that cadmium has greater tendencies to bioconcentrate in tissues of living organisms when compared to zinc and lead. A case study for cadmium (Cd) poisoning epidemic was the '*itai-itai*' disease incidence in Japan. The '*itai-itai*' disease is particularly known for osteomalacia and osteoporosis, having a high incidence to result in bone fractures together with serious pain in the bone and renal tubular dysfunction (Aoshima, 2016).

Copper (Cu) is an essential trace element; however, too much consumption of Cu can induce a toxicological reaction. When Cu is present in the body, it is mainly accumulated in the liver and when the Cu level exceeds the detoxification ability of the liver, excess copper will be released into the blood. Chronic Cu poisoning can cause abnormal liver function, hepatomegaly, lung fibrosis and nervous system disorders (Cadet, 1988). A study by Li *et al.* (2015) observed selective hepatic lodging of copper and they asserted that long-term exposure to Cu in $PM_{2.5}$ would lead to liver damage.

Chromium (Cr) is also an essential element and chronic exposure to its compounds can induce hepatocellular carcinoma and lung cancer (Arita and Costa, 2009).

Arsenic (As) is a toxic element and the main route of exposure to it is through drinking water that is contaminated by it. Arsenic has been associated with several diseases, including diabetes, hypertension and tumors of the skin, bladder, liver and lungs. Cobalt (Co) also usually contaminate water and Co ions concentration greater than or equal to 0.0002 mg/L can lead to erythropoietic damage and increase cases of goiter in mammals and in humans (Oyeku and Eludoyin, 2010).

The usage of nickel (Ni) as a metal is greatly increasing in modern day technologies. Health effect resulting from exposure to Ni and its compound include but not limited to lung fibrosis, cardiovascular system deterioration, kidney injuries, skin allergies and so on (Denkhaus and Salnikow, 2002). Also, iron (Fe) as a metal is widely known to impart plumbing and other appliances from their corrosion properties, and this can cause alteration in taste and odour of water (Oni and Hassan, 2013).

Manganese (Mn) is also an essential trace and important element needed in various metabolic pathways, and as a necessary agent in oxidative phosphorylation, it is important as a key factor in the series of reactions involved in oxidative processes. Richardson *et al.* (2012) noted that manganese in systemic circulation is usually absorbed by cells that are rich in mitochondria which are largely in abundance in organs/tissues such liver, brain, and hair. However, elevated concentrations of Mn in the body system can cause damage to many organs, such as liver dysfunction, cardiovascular problems, reproductive issues, immune system compromise and central nervous system disorders (Lu *et al.*, 2015).

Mercury (Hg) is an element that is present everywhere at once in the environment. Natural and anthropogenic events has contributed to it release and mobilization in the environment. Mercury is widely and well known for it toxic effects in living organisms. High environmental contamination with either elemental Hg, organic or inorganic mercury, can result in serious ecological and public health hazards. Known clinical and subclinical consequences of exposure to elemental mercury includes but not limited to tremor, over-sensitivity, respiratory and renal failures, cardiac arrest and cognitive dysfunctions. Organic mercury (e.g. methylmecury) is even more extremely toxic due to it lipophilic nature (Wong *et al.*, 2006). A good case study of the hazard from exposure to organic mercury is the Minamata disease incidence. In May 1956, the first official cases of Minamata disease were reported in Japan. It is an incidence of severe mercury (Hg) poisoning in humans (with over 2217 confirmed cases) resulting from the consumption of shellfish and fish that has accumulated methylmercury (MeHg) which were discharged in waste water from a chemical plant (Chisso Co. Ltd.). The disease affected the brain and resulted in complications such as sensory disturbances, dysarthria, ataxia, auditory disturbances, constriction of the visual field and tremor (Hachiya, 2006).

2.13.1 Mechanism of metal-induced toxicity

Many factors have been implicated in the mechanism of metal-induced toxicity, however, well known factors include the production of reactive oxygen species (ROS) which is usually observed with redox-active metals (e.g. Fe, Cr, Cu, etc.). Also, another factor is when the cell's sulfhydryl supplies are used up and this is a vital indirect mechanism with redox-inactive metals (e.g. Pb, Cd, As, Hg) in inducing oxidative stess. In living system, both redox-active and redox-inactive metals usually lead to the enhanced generation of reactive oxygen species (ROS). Examples of these ROS are superoxide radical (O_2^-), hydroxyl radical (HO^-) and hydrogen peroxide (H_2O_2) (Stohs and Bagchi, 1995). However, the body of animals produces physiological defense antioxidant systems which help to mop up free radicals. Exposure to environmental metals can cause increase stimulation of ROS and this can over-power the cells' physiological antioxidant defense system leading to a phenomenon known as oxidative stress. Oxidative stress conditions can result in various lesion and dysfunctions on cells/tissues which are due to damages caused by

ROS on DNA, lipids and proteins (Ercal *et al.*, 2001). The possible mechanisms involved in metal-induced toxicity are summarized in figure 2.8.

2.14 Public health impacts of solid waste landfills

Evidences are growing in developed and developing countries on the public health consequences in residents who are living (or who once lived) in close proximity to municipal and chemical landfills. Some of these landfills have either been closed or still in operations, yet their effects on adjacent populations persist.

Pukkala and Ponka (2001) in Finland found association of excess cases of cancer of the pancreas and skin in males living in houses built on former dumpsites. Jarup *et al.* (2002) found no excess risk of developing bladder, brain, hepatobiliary cancers and leukaemia in people who are living within 2 km of 9,565 landfills in Great Britain from 1982 to 1992. Michelozzi *et al.* (1998) in Italy observed association between mortality caused by laryngeal cancer especially as the distance from the waste disposal sites and incineration plant decrease, though, no association was found with cancer of the liver, lungs and lymphatic vessels. There is insufficient proof of an increased risk of cancer for populations and communities in close proximity to landfills and results from studies have been inconsistent (Porta *et al.*, 2009).

Dolk *et al.* (1998) and Vrijheid (2000) conducted independent research on the hazardous waste landfill sites in Europe. They observed that there was no excessive risk associated with living in close proximity (radius of 3 km) to the landfills, although significant associations exist between proximity and both non-chromosomal and chromosomal congenital problems respectively. Elliott *et al.* (2001) and (2009) found increased incidence of cardiovascular anomalies, neural tube problems, abdominal wall defects, epispadias, hypospadias and low birth weights in populations living within 2 km radius of 9,565 landfills in Great Britain (starting from 1982 to 1997) compared with reference population. In a combined scientific review of twenty-nine papers on the association between residential proximity to landfills and the danger of an abnormal birth results, Saunders (2007) noted that while most studies showing positive relationship were well carried out, over half showed no associations with any adverse birth outcome and most of the latter are also well conducted. The review concluded

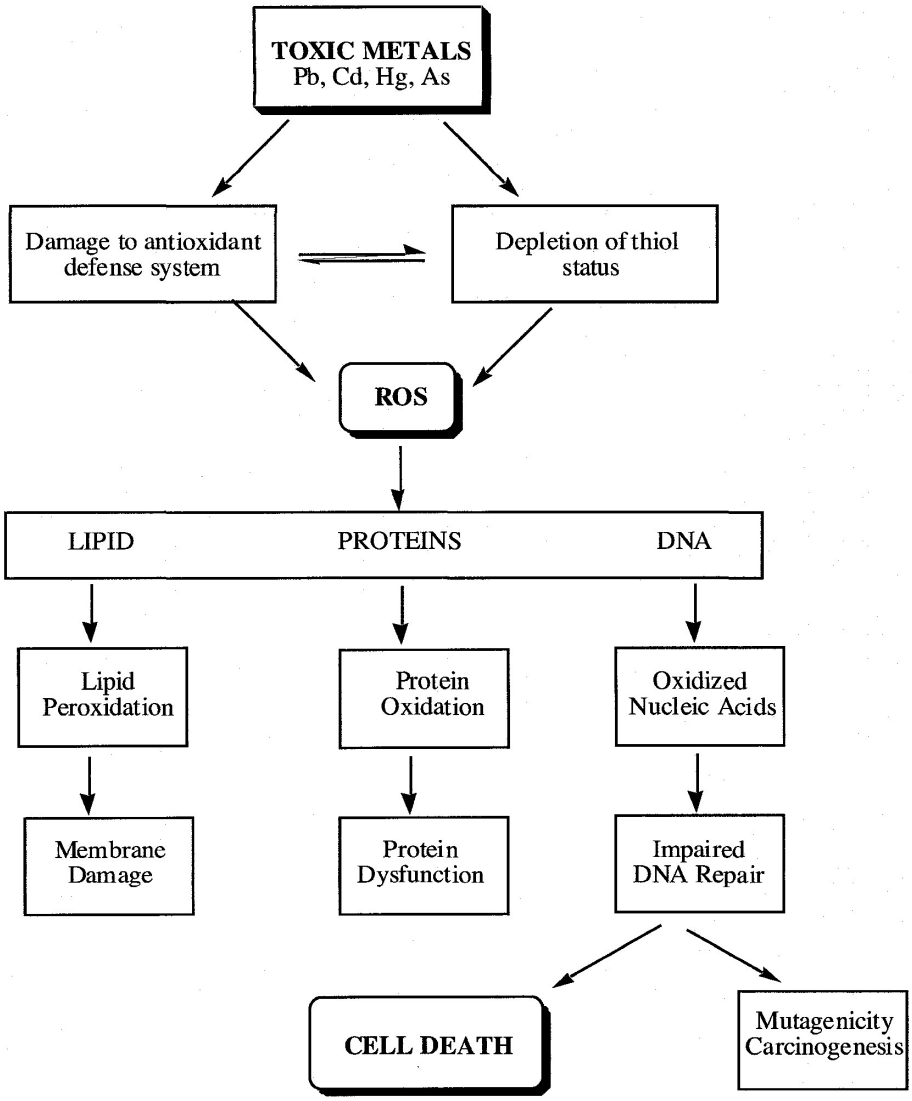

Figure 2.8: Possible mechanisms for metal-induced oxidative stress.

Source: Ercal *et al.* (2001)

that the proof of an association of residence near a waste landfill with anomalies at birth is not strong enough.

Furthermore, Pukkala and Ponka (2001) also reported that there is a significant prevalence of asthma in a population living in an area that was formerly a dumpsite for domestic and industrial wastes. In another study, ecological investigation on cohort around an industrial waste in the UK revealed an elevation in rate of hospitalization due to respiratory diseases (Fielder *et al.* 2001). Also, in a retrospective study carried out in the United State on a cohort, the findings suggested an elevation in the number of patients visiting the hospitals due to asthma and other respiratory anomalies (Ma *et al.* 2007). Gelberg (1997) reported that landfill workers had significantly higher prevalence of work-related respiratory, dermatological, neurologic and hearing problems in a cross-sectional study of employee at the New York City Department of Sanitation as compared to controls. More recently in Lagos, Nigeria Alabi and Bakare (2015) reported significantly higher occurrence of aches, migraine, nausea, spontaneous abortion and cancer among workers and residents within 100 m of two informal electronic waste dumpsites as compared to control cohort.

Generally, the assessment of health impacts associated with proximity to waste landfills is clouded with a lot of uncertainties and further compounded with the difficulty of excluding confounding factors (Vrijheid, 2000; Mattiello *et al.*, 2013).

2.15 Review of methodology

There have been significant advances in toxicological studies in the recent past, and the use of systemic toxicity, cytotoxicity, genotoxicity, mutagenicity, carcinogenicity, immunotoxicity and histopathological end-points/biomarkers have been adopted in evaluating toxicity of environmental chemicals and pollutants. The ultimate goal of these toxicological evaluations is to use biological data to indicate symptoms of early diseases and to possibly predict the risk of development of long-term health outcomes (Alimba, 2013).

2.15.1 *In vivo* micronucleus test in rat

The *in vivo* and *in vitro* micronucleus assay has been accepted and used to a great extent in genetic toxicology. The *in vivo* micronucleus test is of greater importance in

assessing genotoxicity risk in whole animal/human because it put into consideration other internal physiological factors such as metabolism of the agent, pharmacokinetics and possibilities of DNA repair after exposure to genotoxic agent. Micronuclei, also called Howell-Jolly bodies are usually small, smooth and round remnants of nuclear material left within the cytoplasm of erythrocytes after a DNA/chromosome damaging event. The technical term for young/immature erythrocyte is polychromatic erythrocyte (PCE) and this usually last for approximately 24 hours before transforming into matured erythrocyte known as normochromatic erythrocyte (NCE). The PCE are known to yet have RNA, they are basophilic and with Giemsa stain, they appears light blue to blue-gray. The NCE are usually smaller than PCE, they no longer contain RNA, are acidophilic and with Giemsa stain, they appears light organge to orange-pink (Krishna and Hayashi, 2000).

A micronucleus can be about $^{1}/_{20}$ to $^{1}/_{5}$ of the diameter of an erythrocyte. Even after treatment with high doses of chromosome-breaking agents, most of the micronucleated cells will contain just one micronucleus (MN). However, there are cases of cells having two or more micronuclei as well as some with almond-shaped micronuclei (Schmid, 1975). Naturally, the number of PCEs is almost unlimited and the spontaneous rate of micronucleated cells is low and consistent (average of three per thousand), but in the presence of very low dosages of standard chromosome-breaking mutagen, a positive result can be recorded (Matter and Grauwiler, 1974).

In rodent, especially mouse and rat, *in vivo* micronucleus assay has to a great extent been used to confirm genotoxicity from bone marrow erythropoietic cells and peripheral blood erythrocyte. However, MN evaluation is less sensitive with peripheral blood erythrocyte in adult rats. This is because the spleen in rat selectively removes micronucleated RBC. Notwithstanding, regulatory agencies around the world has recommened that estimating micronuclei induction should be among the first set of *in vivo* genotoxicity test in assessing the toxicity of a substance, and the test is effective in detecting both clastogenicity and aneugenicity (Krishna and Hayashi, 2000).

Micronuclei in PCEs are formed primarily from acentric pieces or centric chromosomes that are unable to migrate and follow the mitotic spindle during cell division in erythroblast (Figure 2.9a and 2.9b). An elevation in the occurrence of

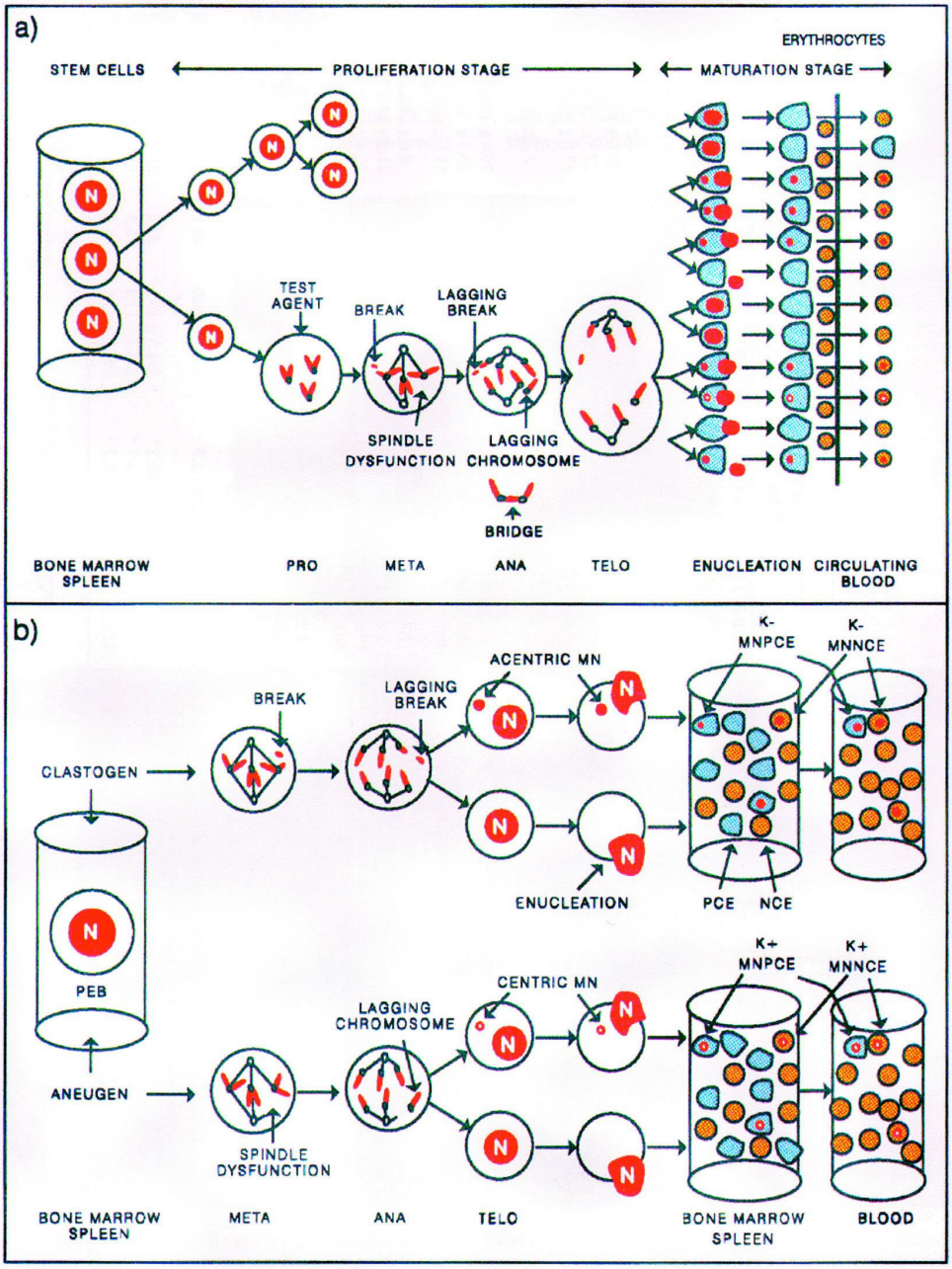

Figure 2.9: (a) The process of erythropoiesis *in vivo*; (b) the mechanism of micronucleus formation in the polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs).

K+ = Kinetochore-positive erythrocytes

K- = Kinetochore-negative erythrocytes

N, nucleus; PEB, proerythroblast; MN, micronucleus.

Source: Krishna and Hayashi (2000)

micronucleated polychromatic erythrocytes (MNPCE) implies that there must have been chromosome damage either by an aneugenic or clastogenic agents or spindle dyfunction. Also, the PCE to NCE ratio between test agent-treated animals and vehicle-control animals provides a cytotoxicity index. Micronuclei induction has been reported as a predictive index for evaluating the carcinogenic potential of environmental and occupational chemical exposure (Krishna and Hayashi, 2000).

2.15.2 Systemic toxicity tests in rat

2.15.2.1 Haematology

Haematological parameters have been validated in determining the diseased state of domestic animals and humans. Alterations in haematological indices can provide information about the physiological changes and pathological changes caused by environmental toxic substance exposure. The blood is the main transport tissue in the circulatory system of animals and the blood cells are exposed to any agent absorbed or injected into the body of the animal. Haematological data from *in vivo* experimental studies is a very good way of determining injuries and infections in tissues and organs, and to predict human health risk (Alimba and Bakare, 2012).

2.15.2.2 Serum markers of liver and kidney damage and cytotoxicity

The main route of exposure to environmental toxic substances is via inhalation, ingestion and dermal contact and all substances that enter the body via these routes will eventually get to the liver and other organ of the body. The liver helps primarily to metabolize and/or detoxify any substance that gets to it, and so the liver is subject to the deleterious effects from the chemicals. In cases of liver tissue damage and hepatocellular necrosis some biochemical enzymes (for example, alanine aminotransferase [ALT], aspartate aminotransferase [AST] and alkaline phosphatase [ALP]) are ususlly released into the blood circulatory system and this enzymes can be measured as an index of liver injury and cell membrane damage (Friedman *et al.*, 1996).

The main excretory organ of the animal system is the kidney and the kidney is known to have faster metabolic rate than other organs (Li *et al.*, 2015). Serum urea and creatinine levels are biochemical indicators of renal injury. Their elevation is

associated with impairment of renal function (Gowda *et al.*, 2010). Serum albumin and total protein are supplementary biomarkers of hepatic production functions and nephrotoxicity (Gowda *et al.*, 2010). It has been observed that biochemical analysis correlates well with histological lesions to confirm the induction of organ-specific effects of xenobiotics.

Similarly, lactate dehydrogenase (LDH) is a cytoplasmic enzyme which is reliable as a marker of metabolic abnormalities (Karthikeyan and Bavani, 2009) and tissue levels of respiratory anaerobiosis. Also, in cases of damaged cells or necrosis, cytoplasmic enzyme such as LDH leak out into the extracellular milieu through the impaired plasma membrane and can be measured as an index of cytotoxicity (Chan *et al.*, 2013).

2.15.2.3 Gastric physiopathology

For the stomach to carry out effectively its many physiological functions the integrity of the gastric mucosal epithelium must not be compromised and an imbalance between the protective mechanism (mucin, bicarbonate and prostaglandins) and aggressive factors (high gastric acid - HCl, pepsin, *Helicobacter pylori*, etc.) dictates alterations (lesion) in mucosal cells and peptic ulcer development. The gastric mucus is a crucial factor in gastric mucosal protective and defensive function and a decline in gastric mucus secretion makes the mucosa more susceptible to injury caused by different factors. Elevation in gastric acid secretion, pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation, diminished gastric blood flow and gastric motility have been said to be the possible inducer of pathogenic conditions of the stomach such as gastric inflammation and ulcer (Duan *et al.*, 2006; Abd El-Rady *et al*., 2021).

2.15.2.4 Antioxidant enzymes and lipid peroxidation

Antioxidants are molecules in the body that work to prevent the oxidation of other molecules. Oxidation reactions can lead to the generation of free radicals which could overwhelm the cells' intrinsic antioxidant defenses, leading to cell damage or death; a condition known as oxidative stress. In the body of both plants and animals, there are complex systems of antioxidants and these include non-enzymatic forms such as glutathione and vitamins (C, A, and E). Other enzymatic forms are catalase (CAT), superoxide dismutase (SOD) and different types of peroxidases. When these systems

of antioxidants fail, the resultant effect is oxidative stress; and oxidative stress plays majoy role in the etiology of many human and animal diseases (Ercal *et al.*, 2001; Varjovi *et al.*, 2015).

Antioxidant enzymes work as a complex to remove all types of ROS. First step is to convert ROS into less active molecules and block them to be transformed into harmful forms such as hydroxyl radicals. SOD, glutathione peroxidase (GPx) and CAT are known as primary antioxidant enzymes and each one performs reduction of particular ROS. Superoxide radicals are reduced by SOD so that H_2O_2 and O_2 are formed. GPx is the next player in which has a role to reduce H_2O_2 or organic hydro-peroxides to water and alcohol respectively, using reduced glutathione (GSH) as the electron donor. CAT also plays a role in protecting cells against the toxic effects of H_2O_2, through acting catalytically to convert the harmful H_2O_2 to water (H_2O) and oxygen (O_2). CAT is recruited, when high concentration of H_2O_2 is found (Varjovi *et al.*, 2015). GSH contain sulfhydryl group and act as a first line of defense against oxidative stress by acting as non-enzymatic antioxidant. GSH can also be involved in the enzymatic detoxification reaction of ROS as a co-factor or as a co-enzyme (Lushchak, 2012).

Lipid peroxidation (auto oxidation) is a chain reaction providing a continuous supply of free radicals that initiates further peroxidation in the lipid rich membrane of lipoproteins by ROS like $OH^{\cdot}$ (Hydroxyl radical), $NO^{\cdot}$ (nitric oxide radical), O^{2-} (superoxide radical), $ROO^{\cdot}$ (Peroxyl radical) etc resulting in generation of lipid peroxidation products like Malondialdehyde (MDA) (Nagamani *et al.*, 2015). MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations and has been widely used for many years as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA). Lipid peroxidation has been implicated in various diseases and pathological conditions such as carcinogenesis, cardiovascular diseases, neurodegeneration, and aging. Increased levels of the lipid peroxidation product, Malondialdehyde (MDA) play a very important role in the pathogenesis of these diseases (Ayala *et al.*, 2014).

2.15.2.5 Histopathology

Histopathological examinations of specific tissues are useful as a biomarker that give important qualitative and quantitative information about acute or chronic effects of toxic chemicals, which are not easily predicted by other biomarkers (Gurcan *et al*., 2009). Histopathological data correlates well with biochemical analysis and change in organ weight, and are vital to gain insight into how structural alterations could lead to disorders of organ functions and subsequently resulting in disease conditions (Amacher *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study sites

The study site was Olusosun landfill in Lagos State and a control site at a residential neighbourhood in Olodi Apapa, Lagos, Nigeria. The Olusosun landfill is located at Ojota, Oregun area of Kosofe Local Government Area, Lagos State (Latitude 6°35′N and Longitude 3°22′E) (Figure 3.1). The landfill was a swampy area prior to the landfilling activities. It has been an active dumpsite since 1992 and receives an estimated 10,000 tons of solid waste each day. It is about 42 hectares of land with 18m deep excavation, which makes it the largest landfill in the nation (Aderibigbe, 2010). It has no engineered leachate collection system (except for a leachate pond) hence leachates produced during decomposition of biodegradable matter in the wastes are released into the environment and may find their way into the ground and surface water. The leachate pond on the site is not engineered for leachate treatment, thus only serving as a temporary reservoir where leachate and water run-off on the site are gathered through drainages and gutters dug around the dumpsite. Eventually, the leachate either slowly evaporates or seep and percolate into the ground.

People live (in shanty town) on and work in the dumpsite as scavengers, cart pusher and civil servants of Lagos State Waste Management Authority (LAWMA). The scavengers/waste pickers and most other individuals on the site were seen daily with little or no safety or protective gears; they wore dirty and tattered clothes, most with no safety boot, nose mask, goggles or helment. Also, there are the presence of hand dug wells and borehole on the landfill site and within 2 km radius of the site, which the workers and residents in this environs use for their day to day domestic and commercial activities. Furthermore, bothering the Olusosun landfill are commercial and industrial hubs, a primary school, religious centres (churches and mosques), LAG-BUS terminus, motor parks and garages, filling station, mechanic workshop,

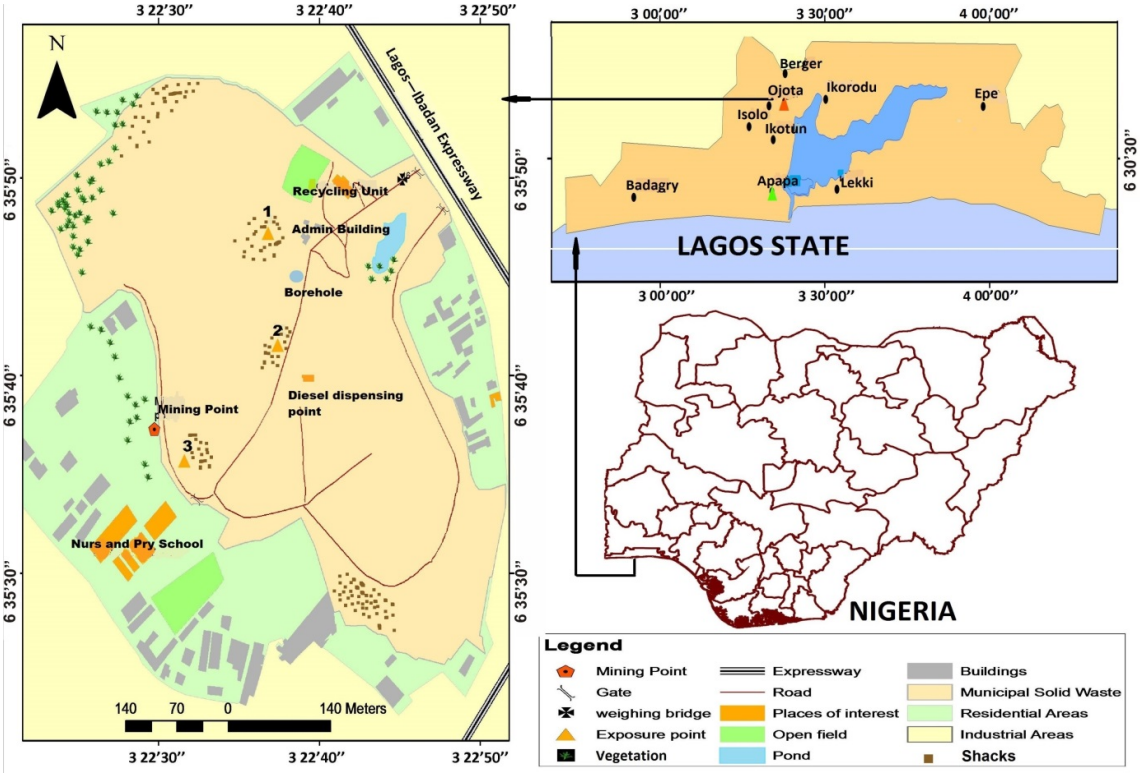

Figure 3.1: Olusosun landfill site showing exposure points (marked as 1, 2 and 3) and map of Lagos State showing relative position of the landfill at Ojota (orange triangle) and the control point at Apapa (green triangle) (straight distance between them is approximately 17.25km).

residential buildings, etc. Occasionally, thick smokes and fogs are seen emanating from the site, either from deliberate burning of waste or by spontaneous natural occurrence.

Lagos State (Latitudes 6°23′N and 6°41′N and Longitudes 2°42′E and 3°42′E) is a densely populated state in Nigeria and small in physical size with a total landmass of approximately 8345 km^2 (Figure 3.1). The population of the state is estimated at 10 million people (Nigeria Population Commission, 2006).

3.1.1 Exposure points at Olusosun landfill and control sites

On Olusosun landfill, three different exposure points (Pt1, Pt2 and Pt3) were selected and the criteria for choosing these points are (i) must be on the dumpsite, (ii) a secured position, (iii) close to locations where people carry out their day to day activities and rest/live on the dumpsite and (iv) the points were distanced apart to have a fair coverage of exposure to ambient air emissions emanating from the landfill. Some activities around these points were as described below.

Point 1 (Pt1) exposure unit (Lat. 06°35′ 47.22″N, Long. 3°22′ 36.81″E) was on the dumpsite at the extreme back of the LAWMA administrative block. Activities like active dumping and layering of refuse by waste disposal trucks and tractor machines, active waste picking by scavengers, sewing of collection bags, waste sorting (into metal cans, bottles, plastics, rubbers, polythene/nylon, electronics, etc), food cooking and on-site food selling, wooden shops (selling of consumable and drinks) and shacks (where people rest and live) (Figure 3.2).

Point 2 (Pt2) exposure unit (Lat. 06°35′ 41.57″N, Long. 3°22′ 37.42″E) was on the dumpsite, however, activities like diesel and engine oil dispensing for LAWMA trucks takes place some few meters away from this point. Adjacent to this point is a tarred road that gives access to the heavy trucks and machines that comes there to refill their gas and change their oil. Light servicing of these trucks also take place here. Little settlements (shacks and shops) and sorting of wastes also take place in this point (Figure 3.3).

Figure 3.2: Point 1 at Olusosun landfill site.

Showing rat exposure units (red arrow), heap of waste dumps (Black arrow), food selling point (green arrow), bag collectors (blue arrow) and settlements/shacks (white arrows).

Figure 3.3: Point 2 at Olusosun landfill site.

Showing rat exposure units (red arrow), heap of waste dumps cover with red sand (Black arrow), LAWMA diesel container office (Yellow arrow), abandoned diesel storage tank, (green arrow) and sorted waste and tyres (blue arrow).

Point 3 (Pt3) exposure unit (Lat. 06°35′ 34.73″N, Long. 3°22′ 30.18″E) was on the dumpsite and the closest structure to this point was wooden shops/shacks (commercial activities) and the mechanic village. This point is also close to the active red sand mining site (sand used in periodic layering of the landfill were mined here). The main activities here are heavy duty waste disposal truck servicing and repairing, diesel selling/dispensing, sand mining, waste sorting, local settlements/shacks and local food cooking and selling (Figure 3.4).

The control point was located at a residential neighbourhood in Olodi Apapa, Lagos, Nigeria (Lat. 06°26′ 42.86″N, Long. 3°20′ 19.36″E), an area with no solid waste dumping within 4 km radius of the exposure set up (Figure 3.5). The straight distance between the control point and the Olusosun landfill in Ojota was determined from Global Positioning System (GPS) coordinates (etrex 8, GARMIN©, 2000-2004) to be approximately 17, 250m (17.25km).

3.2 Water sample collection and heavy metal analysis

Water samples were obtained from underground borehole taps at the landfill and control sites into separate 10 L pre-cleaned plastic containers and these were collected fresh every week. Aliquots of 200 mL from the water samples collected weekly were stored in clean 500 mL water bottles from August 2015 to January 2016. These were mixed together to form a monthly composite sample and the composite samples were used for heavy metal determination. The concentrations of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn) and iron (Fe) were analysed in the monthly composite samples following the methods of United States Environmental Protection Agency (USEPA 1996) and APHA (1998). Briefly, 100 mL of each of the monthly composite underground water samples (Olusosun and control) were digested by heating with concentrated HNO_3, and the volume was reduced to 3–5 mL. This volume was made up to 10 mL with 0.1 N HNO_3. Concentrations of the metals for each month were estimated in three replicates using Perkin Elmer Atomic Absorption Spectrophotometer (AAS-200).

3.4 Biological materials

Male Wistar albino rats (*Rattus norvegicus*) (6 - 7 weeks old), which had been inbred for several generations, were obtained from the animal house of the College of

Figure 3.4: Point 3 at Olusosun landfill site.

Showing rat exposure units (red arrow), heap of waste dumps (green arrow) and mechanic village (yellow arrow).

Figure 3.5: Control exposure units.

A residential neighbourhood in Olodi Apapa, Lagos State (red arrow).

Medicine, University of Ibadan, Nigeria. They were transported to the control point at a residential neighbourhood in Olodi Apapa, Lagos, Nigeria until they were about 8 – 9 weeks old with a mean ± SD weight of 69.22 ± 4.13g. The rats were given the borehole underground water collected from the control site as their drinking water and were fed with standard pellet rodent chow purchased from Ladokun feed Nigeria® *ad libitum*. All the rats were free from ailment and were maintained in pathogen free plastic cages for a minimum of 14 days to allow them acclimatize to their new environments (the control point) before taking some to the landfill for exposure to the landfill air emissions and underground water.

3.5 Study design

3.5.1 Systemic toxicity

One hundred and twenty male Wistar Albino rats (*Rattus norvegicus*) (8 – 9 weeks old, 69.22 ± 2.71g) were divided randomly into 4 groups of 30 rats each. Three groups were exposed to emissions at three different points (Pt1, Pt2 and Pt3) on Olusosun landfill and the 4th group was placed at the control site in Olodi Apapa, Lagos. The exposure units were plastic cages (55 x 36 x 20 cm) perforated with wire gauze on two sides (Figures 3.2, 3.3 and 3.4) to allow for in and out flow of emissions (landfill gases, volatile organic compounds, particulate matters and dust, microorganisms) with air through the cages. Rats were cared for on the dumpsite; bedding (wood shavings) were changed every 3 days, they were fed with rodent chow (Ladokun pelleted feed®) and given borehole water collected from the dumpsite as their daily drinking water. After 4, 8, 12, 16, 20 and 24 weeks post exposures, 5 rats from each points and the control were randomly selected and transported to the laboratory (Biochemistry Laboratory at Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria) in cages and carried with an air conditioner (AC) regulated bus to avoid stress.

3.5.2 Clinical observations and body weight measurement

The rats were observed twice weekly for signs of clinical toxicity in the physical appearance, behaviours, mortality and morbidity. The weight of the body of each rat in the exposed groups and control were determined for initial, weekly and terminal weights using an electronic compact scale (KERRO BL30001).

3.5.3 Blood collection and organ weight measurement

Once rats were brought to the laboratory (in the morning, between 8 – 10 am), rats were weighed prior to blood collection to determine the terminal body weight. Blood were collected by cardiac puncture using light anesthesia (1 mg Xylazine and 7 mg Ketamine/70g bwt; intramuscularly) and aliquoted into EDTA bottles and plain bottles. Blood samples in EDTA bottles (for haematological analysis and heavy metal quantification) were immediately mixed by rolling along the body of the container to prevent coagulation and proper mixing with the EDTA anticoagulant. Blood collected into plain bottles were allowed to stand and clot for thirty minutes and then centrifuged at three thousand revolution per minute for ten minutes to part away the serum and the sera were then preserved at −70^{o}C until used for biochemical analysis. Cervical dislocation was used to sacrifice the rats; liver, kidneys, lungs, spleen and stomach were surgically removed using a pair of scissors and forceps, washed with ice-cold normal saline, dried with Whatman filter paper and weighed to determine the absolute weight of organs. The relative organ weight (absolute organ weight/terminal body weight X 100 g) was calculated for all the viscera. Part of the liver, kidney (left), lungs and spleen were immediately fixed in 10% formalin for histological examination. Another part of the liver, kidney (right), and lungs were refrigerated (−4^{o}C) for heavy metal quantification. The remaining part of the liver samples was immediately frozen at −70^{o}C until used for antioxidant enzyme analysis.

3.5.4 Heavy metal determination in blood and viscera

Rat whole blood in EDTA bottle, liver, kidney (right) and lungs were analysed for heavy metal accumulations. Heavy metals such as lead (Pd), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) were quantified in the samples following acid digestion and atomic absorption spectrometry.

Briefly, 0.5 mL of blood was transferred into 100 mL digestion flask and 5 mL nitric acid (HNO_3) and 1.5 mL of concentrated (70%) perchloric acid ($HClO_4$) was added. Also, 0.2 g of liver, lungs, and right kidney were transferred into their respective digestion flasks and 2 mL nitric acid was added (USEPA, 1996). These preparations were then moved into an automated digestion block (QBlock Digestion System, Questron Technologies Corp.) which is operated from a wireless screen. The digestion

system provides heating up to 230 ^{o}C and take about 45 minutes or less to complete the digestion process. The digested sample obtained was cooled and made-up with 25 mL ultra-pure (deionized) water. A blank was also prepared by digesting 0.5 mL of deionized water (instead of samples) with 5 mL nitric acid (HNO_3) and 1.5 mL of concentrated (70%) perchloric acid ($HClO_4$). The digested sample blank was cooled and made-up with 25 mL ultra-pure (deionized) water. The resulting sample and blank volume was filtered with Whatman filter paper into storage container and stored at room temperature until needed for quantification. This digestion process was carried out at Dr. D. K. Olukoya Central Research Laboratory, University of Lagos (UNILAG), Akoka-Yaba, Lagos, Nigeria. The quantification of the selected heavy metals (Pb, Cd, Cr, Cu and Zn) was done at the Multi-disciplinary Central Research Laboratory (MCRL), University of Ibadan, Ibadan, Nigeria using Perkin Elmer Atomic Absorption Spectrophotometer (AAS-200).

3.5.5 Bone marrow micronucleus assay

At 4, 8, 12, 16, 20 and 24 weeks post exposure to ambient air emissions and groundwater from the landfill site and control, 3 rats/point/exposure periods were randomly selected and sacrificed for micronucleus analysis in bone marrow cells in accordance to Schmid (1975) and as modified by Alimba and Bakare (2016). Rats were sacrificed by dislocating the cervical bone of the neck and the two femurs of the hind-limbs were harvested and immediately dropped into petri dishes filled with normal saline. The bones were then carefully freed of adherent tissues using a pair of scissors, forceps and clean pieces of white silk material. The proximal and distal epiphyses were carefully cut off with scissors until there are openings to the marrow canal on both ends. Using 2 mL syringe and needle, 1 mL of fetal bovine serum (FBS) was used to flush out the bone marrow cells from the proximal end of the bone marrow canal through the opened end at the distal part of the bones into 2.5 mL eppendoff tube. The marrow cells in the eppendoff tube were then homogenized using a micropipette (1000 µL). It was then centrifuge at one thousand revolutions per mins for ten mins after which the supernatant was poured away. Another 1 mL FBS was added to the cells and homogenised with a micropipette, after which it is centrifuge at one thousand revolutions per mins for ten mins. The supernatant was poured away and the cells were again homogenised to re-suspend cells with 0.05 mL (50 µL) FBS.

Five slides were prepared per rat by evenly spreading a drop of the cell suspension over a clean and pre-label glass slides. Slides were placed flat on a wooden rack and in a dust free environment to air-dry for 24 hours. Next, slides were fixed in 70% methanol for 2 mins and allowed to air dry. Fixed slides were stained in 100 % May-Grunwald's solution for 3 - 4 mins in coplin jars, and immediately transferred into another coplin jar containing 1:1 May-Grunwald/distilled water and allowed to stain for another 3 – 4 mins. Slides were then rinsed thoroughly in distilled water and allowed to air dry completely. Slides were double stained in 5 % Giemsa for 5 mins, rinsed in distilled water and allowed to air dry completely overnight while standing on a wooden rack. Slides were then mounted by first clearing in xylene and mounted with DPX and coverslips. 1000 cells of polychromatic erythrocytes (PCE) were scored for micronucleated PCE (MNPCE) as an index of genotoxicity. Also, from each slide, on the same microscopic fields 1000 cells were counted to ascertain PCE to NCE ratio, which is an index of cytotoxicity (Krishna and Hayashi, 2000).

3.5.6 Haematological analysis

Aliquot of blood sample collected into EDTA bottles from the rats was used to determine the recommended haematological indices including: Red Blood Cell count (RBC), Haemoglobin content (Hb), Haematocrit/PCV (Ht), Mean Corpuscular Haemoglobin (MCH), total white blood cell count (WBC) and differentials [lymphocytes, neutrophils and Mixed Cell Count (MXD), eosinophils, basophils and monocytes] at the Clinical and Diagnostic Laboratory, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria following standard protocols (Cheesbrough, 2005).

3.5.7 Serum biochemical analysis

Clinical chemistry biomarkers of hepatic injury, renal injury and cell membrane damage were determined from serum samples of rats exposed *in situ* to ambient air and underground water from Olusosun landfill environment and the corresponding control site. Liver function was assessed by measuring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) in serum. Renal function was assessed by measuring serum urea and creatinine concentrations. The levels of cytotoxicity in the animal system were

assessed with lactate dehydrogenase (LDH). These were analyzed using diagnostic kits (Randox Laboratory Ltd, UK).

Aspartate aminotransferase and ALT activities were analysed following the protocol of Reitman and Frankel (1957). Aspartate aminotransferase levels is based on the principle that oxaloacetate formed from the aspartate aminotransferase catalysed reaction between alpha ketoglutarate (α - oxoglutarate) and aspartate is coupled with chromogen (2, 4 – dinitrophenyl hydrazine) in alkaline medium to form coloured hydrazone. The intensity of the coloured hydrazone measured with spectrophotometer is proportional to the aspartate aminotransferase activities.

Following the kit procedure, a reagent blank was prepared by pipetting 0.5 mL of reagent 1 (R1) [a solution of phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100mmol/L) and α-oxoglutarate (2 mmol/L)] and 0.1 mL of distilled water into a test tube. The sample was prepared by pipetting 0.5 mL of R1 and 0.1 mL of sample serum into another test tube. These preparations were mixed properly, and the test tubes covered with aluminium foil and incubated in a water bath for exactly 30 minutes at 37 ^{o}C. After incubation, 0.5 mL of reagent 2 (R2) [2, 4 – dinitrophenyl hydrazine (2 mmol/l) - chromogen solution] was added into the preparations. They were mixed properly and allowed to stand for exactly 20 minutes at room temperature after which 5.0 mL of 0.4 mol/L sodium hydroxide solution was added, properly mixed and the absorbance was read against the reagent blank (from a glass cuvette) after 5 minutes using a spectrophotometer (Spectrum Lab S23A, GLOBE MEDICAL ENGLAND) at 546 nm wavelength. AST activity (U/I) was determined from the standard curve provided in the kit manual (Appendix III).

Alanine aminotransferase activity is determined from the principle that pyruvate was formed from alanine aminotransferase catalysed reaction between alpha ketoglutarate (α – oxoglutarate) and L-alanine. The pyruvate formed couples with chromogen (2, 4 – dinitrophenyl hydrazine) in alkaline medium to form coloured hydrazone. The intensity of the coloured hydrazone measured with spectrophotometer is proportional to the alanine aminotransferase activities.

Following the kit procedure, a reagent blank was prepared by pipetting 0.5 mL of reagent 1 (R1) [a solution of phosphate buffer (100 mmol/L, pH 7.4), L-alanine

(100mmol/L) and α-oxoglutarate (2 mmol/L)] and 0.1 mL of distilled water into a test tube. The sample was prepared by pipetting 0.5 mL of R1 and 0.1 mL of sample serum into another test tube. These preparations were mixed properly, and the test tubes covered with aluminium foil and incubated in a water bath for exactly 30 minutes at 37 ^{o}C. After incubation, 0.5 mL of reagent 2 (R2) [2, 4 – dinitrophenyl hydrazine (2 mmol/L) - chromogen solution] was added into the preparations. They were mixed properly and allowed to stand for exactly 20 minutes at room temperature after which 5.0 mL of 0.4 mol/L sodium hydroxide solution was added, properly mixed and the absorbance was read against the reagent blank (from a glass cuvette) after 5 minutes using a spectrophotometer (Spectrum Lab S23A, GLOBE MEDICAL ENGLAND) at 546 nm wavelength. Alanine aminotransferase activity (U/I) was determined from the standard curve provided in the kit manual (Appendix III).

Alkaline phosphatase (ALP) activity is based on the principle that ALP catalyses the hydrolysis of p – nitrophenylphophate at pH 9.8, liberating p – nitrophenol and phosphate. The rate of p – nitrophenol formation, measured photometrically is proportional to the catalytic concentration of ALP in the sample (Roy, 1970). Using the kit protocol, the reagent buffer (R1a) solution [containing diethanolamine buffer (1 mol/L, pH 9.8) and $MgCl_2$ (0.5 mmol/L)] and a substrate solution (R1b) [p – nitrophenylphosphate (10 mmol/L)] was reconstituted by mixing 3 mL of substrate solution (R1b) with 20 mL of buffer (R1a). 0.01 mL of the serum sample was pipette into a cuvette and 0.5 mL of the reconstituted substrate mixture was pipette into the test cuvette at room temperature. This preparation was mixed properly and the initial absorbance was read immediately at 405 nm against air. With a timer, absorbance was read again at 1, 2 and 3 minutes. The ALP activity was calculated using the formulae:

$$\mathbf{U/I = 2760 \times \Delta A\ 405\ nm/min}$$

Where:

$$\mathbf{\Delta A = [(A_1 - A_o) + (A_2 - A_1) + (A_3 - A_2)] / 3 \qquad OR \qquad \Delta A = A_3 - A_2}$$

Serum total protein (TP) (Biuret test) was determined based on the principle that cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex. The intensity of this coloured complex is measure photometrically and is proportion to the amount of protein present in the sample

(Treitz, 1970). Using the kit protocol, the kit contains the reagent one (R1 – Biuret reagent) [a solution of sodium hydroxide (100 mmol/L), Na-K-tartrate (16 mmol/L), potassium iodide (15 mmol/L) and cupric sulphate (6 mmol/L)] and blank reagent (R2) [solution containing sodium hydroxide (100mmol/L) and Na-K-tartrate (16 mmol/L)]. Also, the kit comes with a CAL. Standard (usually a protein like FBS). The Biuret reagent (R1) was diluted with double distilled water with a 1 in 4 dilution (100 mL of R1 in 400 mL of ddH_2O). Also, R2 was diluted with double distilled water (1:4 dilution, R2/ddH_2O). A blank sample was prepared by diluting 1.0 mL of Biuret reagent (R1) with 0.02 mL of distilled water. The serum sample was prepared by pipetting 0.02 mL of serum and 1.0 mL of R1 into a test tube. Also, The CAL. Standard was prepared by pipetting 0.02 mL of FBS and 1.0 mL of R1 into a test tube. All preparations were mixed properly and incubate for 30 minutes at room temperature. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) were measured with a spectrophotometer against the blank sample at 546 nm. Total protein (TP) concentration (in g/L or g/dL) was calculated using the formulae:

$$\mathbf{TP = (A_{sample}/A_{standard}) \text{ X Standard concentration}}$$

The standard concentration is given in manual (RANDOX MANUAL TP 245) as 57.86 g/L (or 5.79 g/dL).

Serum albumin (ALB) level was assayed based on the principle that albumin quantitatively binds to the indicator bromocresol green (BCG) (3, 3', 5, 5' – tetrabromo-m cresol sulphonephthalein) to produce a coloured complex which can be measured spectrophotometrically at 578 nm. The intensity of the coloured complex formed is directly proportional to the albumin concentration present in the sample (Doumas *et al.*, 1971). Following the kit procedure, the kit contain BCG concentrate (R1) [a solution containing succinate buffer (75 mmol/L; pH 4.2), Bromocresol green (17 mmol/L) and Brij 35 preservative] and a CAL standard (a serum). Reconstitute R1 by diluting 13.5 mL of R1 with 87 mL of distilled water. Prepare a reagent blank by pipetting 0.01 mL of distilled water and 3.0 mL of the reconstituted R1 into a test tube. Prepare a standard by pipetting 0.01 mL of the CAL standard and 3.0 mL of R1 reagent into a test tube. The serum sample is prepared by pipetting 0.01 mL of the serum and 3.0 mL of the R1 reagent. All these preparations were mixed and incubated

for 5 minutes at room temperature. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) were measured against the reagent blank at 578 nm using a spectrophotometer (Spectrum Lab S23A, GLOBE MEDICAL ENGLAND) and the albumin concentration is calculated thus;

Albumin conc. = ($A_{sample}/A_{standard}$) X Standard concentration

The standard concentration is given as 45.9 g/L (or 4.59 g/dL) (RANDOX MANUAL/RX MONZA AB 362)

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyses the interconversion of pyruvate and lactate with the concomitant interconversion of NADH and NAD^+. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. In this kit, LDH oxidises NADH to NAD^+, which is specifically detected by colorimetric (365 nm) assay (Rec. GSCC [DGKC], 1970; Weisshaar *et al.*, 1975). The reagent includes buffer/substrate [containing phosphate buffer (50 mmol/L; pH 7.5) and pyruvate (0.6 mmol/L)] and NADH (0.18 mmol/L). One vial of the NADH was reconstituted with 3 ml of Buffer/substrate. The sample was prepared by pipetting 0.04 mL of the serum sample into a cuvette and adding 1.0 mL of the reconstituted reagent at room temperature. This was mixed properly, and the absorbance read 1, 2 and 3 minutes with a spectrophotometer. Air was used as blank at 365 nm. The LDH activity was calculated with the formulae:

LDH activity (U/I) = 7647 x ΔA Where, $\Delta A = A_2 - A_3$

Serum creatinine (CREA): Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease and it is measured primarily to assess kidney function. The principle involved in creatinine quantification is that creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration (Bartels *et al.*, 1972). The reagents include picric acid (35 mmol/L), sodium hydroxide (0.32 mol/L) and CAL standard. Following kit protocol, equal volume of the picric acid and sodium hydroxide was mixed to get a working reagent. 1.0 mL of the working reagent and 0.1 mL of the CAL standard solution was mixed in a cuvette and the

absorbance was read immediately against air at 492 nm after 30 seconds (A_1) and 2 minutes later (A_2). Also, 1.0 mL of the working reagent and 0.1 mL of the serum sample was mixed and absorbance read immediately after 30 seconds and 2 minutes against air. Concentration of creatinine is calculated thus:

$A_2 - A_1 = \Delta A_{sample}$ or $\Delta A_{standard}$

Conc. of CREA (mg/dl) = (ΔA_{sample} / $\Delta A_{standard}$) x Standard concentration (mg/dl)

Where the Standard concentration = 1.95 mg/dL

OR

Conc. of CREA (µmol/L) = (ΔA_{sample} / $\Delta A_{standard}$) x Standard concentration (µmol/L)

Where the Standard concentration = 173 µmol/L

Serum urea concentration was determined according to Weatherburn (1967). This is based on the principle that ammonia (produced from the urease catalysed hydrolysis of urea to ammonia and CO_2) is converted to indophenols blue in the presence of sodium nitroferricyanidephenol (sodium nitroprusside) and hypochlorite reagents (in a reaction referred to as Berthelot's reaction). The absorbance was read spectrophotometrically at 546 nm. The RANDOX kit contain reagent 1 (R1) [a solution containing EDTA (116 mmol/L), sodium nitroprusside (6 mmol/L) and urease (1 g/L)], reagent 2 (R2) is diluted phenol (120 mmol/L), reagent 3 (R3) [diluted sodium hypochlorite (27 mmol/L) and sodium hydroxide (0.14N)] and CAL standard.

Following the kit procedure, prepare R1 solution by gently mixing 1.0 mL of urease (in a vial) with 37.0 mL sodium nitroprusside. Dilute reagent 2 (R2 – 110 mL of phenol) with 660 mL of distl-H_2O. Dilute reagent 3 with 750 mL of distl-H_2O. A reagent blank was prepared by pipetting 10 µL of distilled water and 100 µL of R1 into a test tube. The standard was prepared by pipetting 10 µL of the CAL standard and 100 µL of R1 into a test tube. The sample was prepared by pipetting 10 µL of serum and 100 µL of R1 into test tube. All these preparations were mixed and covered with aluminium foil and incubated in a water bath at 37 ^oC for 10 minutes. After incubation, 2.5 mL of reagent 2 and 3 were simultaneously pipette into all preparations. These

were mixed and immediately covered with aluminium foil and incubated again at 37 ^{o}C for 15 minutes. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were read against the reagent blank at 546 nm.

The concentration of urea is calculated thus;

Conc. of urea (mg/dL) = (A_{sample} / $A_{standard}$) x Standard concentration (mg/dL)

Where the Standard concentration = 76.87 mg/dL

OR

Conc. of urea (mmol/L) = (A_{sample} / $A_{standard}$) x Standard concentration (mmol/L)

Where the Standard concentration = 12.79 mmol/L

3.5.8 Oxidative damage analysis in liver

Antioxidant enzyme biochemistry and lipid peroxidation was carried out from liver homogenates to determine the level of oxidative damage done to the liver being one of the main organs for detoxification of xenobiotics entering the body. Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation (LP) (measured from malondialdehyde (MDA) level) levels were determined following standard laboratory protocols. 0.5 g of the liver was weighed and homogenized with 4.5 ml ice-cold 0.1M phosphate buffer (pH 7.4) and centrifuge at 4000 rpm for 10 minutes. The resultant supernatants were stored at – 70 ^{o}C prior to subsequent biochemical analysis.

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as mmoles of H_2O_2 consumed/min/mg protein as described by Sinha (1972). The reaction mixture (1.5 mL) pipette into a cuvette contained 1.0 mL of 0.01M phosphate buffer (pH 7.4), 0.1mL of liver homogenate and 0.4 mL of 2M H_2O_2. The mixture was quickly turned up-side-down to mix properly and then inserted into a spectrophotometer. The initial absorbance was read at 620 nm and subsequent change in absorbance at 30 seconds, 1 minute and 2 minutes. An extinction coefficient of 40.0 $M^{-1}cm^{-1}$ was used.

Calculation

Catalase activity = ΔA_{620}/min x reaction volume x dilution factor

$$\frac{\text{40.0 x sample volume x mg protein/mL}}{}$$

= mmoles H_2O_2/min/mg protein (or CAT/min/mg protein)

Superoxide dismutase: The activity of superoxide dismutase (SOD) was quantified following the protocol of Misra and Fridovich (1972).

0.1 mL of liver homogenate was dissolved in 0.9 mL of distl-H_2O so the volume became 1 in 10 dilution. A portion of 0.2 mL of the new solution was added to 2.5 mL of 0.05M carbonate buffer (pH 10.2). Immediately the reaction was started by the addition of 0.3 mL of freshly prepared 0.3mM adrenaline to the mixture.

This was quickly mixed by inversion. The reference cuvette contained 2.5 mL carbonate buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. The SOD enzymatic activity was calculated as nmol of epinephrine protected from oxidation mg^{-1} protein min^{-1} using molar extinction coefficient of 4.02 $x10^3$ $M^{-1}cm^{-1}$.

Calculation

Increase in absorbance per minute (ΔA_{480}/min) = $\frac{A_3 - A_0}{t}$

Where A_0 = absorbance at 0 second

A_3 = absorbance at 150 seconds (2.5 min)

t = time of final absorbance (2.5 min)

SOD activity = $\frac{\Delta A_{480}\text{/min x reaction volume x dilution factor}}{\text{4.02 x } 10^3 \text{ x sample volume x mg protein/mL}}$

= nmoles of epinephrine/min/mg protein (or SOD/min/mg protein)

Reduced glutathione (GSH): The protocol of Ellman (1959) was employed in determining the concentration of reduced glutathione.

The sample was prepared by pipetting 1.0 mL of liver homogenate (supernatant) with 1.0 mL of 10% trichloroacetic acid (TCA) into a test tube and centrifuging at 3000 rpm for 10 minutes. The reaction mixture contain 0.5 mL of the eventual sample

supernatant treated with 0.25 mL of Ellman's reagent [19.8 mg of 5, 5' – dithiobisnitrobenzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate] and 1.5 mL of phosphate buffer (0.2M, pH 8.0). A blank was prepared with 1.0 mL of distilled water replacing the liver homogenate. All readings were taken within 5 minutes at 412 nm, as colour developed is not stable. An extinction coefficient of 13, 600 litre mol^{-1} cm^{-1} was used in calculated the concentration of GSH in the sample (Ellman, 1959).

Calculation

$$\text{GSH concentration} = \frac{A_{412} \text{ x reaction volume x } 10^6}{13,\ 600 \text{ x sample volume}}$$

= nmoles GSH formed ml^{-1} (nmol/mL)

Lipid peroxidation: Lipid peroxidation as evidence from the production of thiobarbituric acid reactive substances (TBARS) was quantified using the procedure of Niehaus and Samuelsson (1968).

The sample was prepared by mixing 0.4 mL of liver homogenate with 1.6 mL of Tris-HCl buffer (pH 7.5). Then, an aliquot of 0.1 mL of the eventual mixture was treated with 2 mL of (1:1:1) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA). The mixture was boiled in a water bath (at 100^{o}C) for 15 minutes and allowed to cool. It was then centrifuged at room temperature for 10 minutes at 3500 rpm. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The reference blank contain the reagents and 0.1ml distilled water (without the sample). The MDA concentration was estimated following the procedure of Adam-Vizi and Sergi (1982). Lipid peroxidation (MDA) in nmol/mL $gram^{-1}$ of tissue was computed with a molar extinction coefficient of 1.56 x $10^5 M^{-1} cm^{-1}$.

$$\text{MDA (nmol/mL)} = \frac{\text{Absorbance x Volume of mixture x } 10^6}{E_{532nm} \text{ x Volume of sample}}$$

3.5.9 Gastric physiopathological analysis: Mucous and parietal cell counts and gastric mucus secretion analysis

Following sacrifice of rats by brisk cervical dislocation, two stomachs from each group (Pt1, Pt2 and Pt3) and control were surgically removed, opened along the lesser curvature, rinsed in normal saline to remove food materials, blotted dry on Whatsman paper and weighed. They were then immediately fixed in 10% formalin and used for

histological analysis for mucous and parietal cell counts and general histology. Other three stomachs from each group and control were removed and the glandular portion of the stomach was excised and opened along the lesser curvature. These were prepared following standard protocol as described by Corney *et al.* (1974) to determine the gastric mucus secretion and absorbance was read at 605nm.

The absorbance of each solution was then used to calculate the various concentrations of dye and the weight of dye (expressed in mg) deduced, using a standard curve. The weight of dye was then expressed over the weight of the stomach, to give the weight of mucus secreted. Thus,

$$\text{Gastric mucus secretion (mg/g tissue)} = \frac{\text{Weight of dye (mg)}}{\text{Weight of stomach (g)}}$$

3.5.10 Histological analysis

Small pieces of livers, kidney, lungs and spleen that were fixed in 10% formalin were dehydrated through ascending grades of alcohol (50%, 70%, 90% and 100%). They were cleared in xylene, impregnated in and embedded in molten paraffin wax using Embedding system (Leica EG 1160). Sections were cut at 4 μm on a rotator microtome. The sections were floated on water using water bath at 45^{o}C and then picked on frosted end slide. The slides were fixed on hot plate for about 30 minutes. Nuclei were stained with the Erlich's haematoxylin for 5 minutess, rinsed in running tap water and differentiated with 0.5% acid alcohol for 1-2 seconds. This was stained with eosin for 2 minutes, dehydrated, cleared and mounted in DPX. This was later observed and evaluated for morphological alteration using research microscope (Olympus, Tokyo) at x100 and photomicrographs were taken in bright field at x400. This examination was done by a trained pathologist.

3.6 Government approval and ethical guideline

The field experiment was approved by the Lagos Waste Management Authority (LAWMA) (Appendix I) and the ethical approval for this study was obtained from the University of Ibadan Animal Care and Use in Research Ethics Committee (UI-ACUREC/App/2015/037) (Appendix II).

3.7 Statistical analysis

Data were presented as mean ± standard error (SEM) and one way analysis of variance (ANOVA) was used to determine the significant (at $p<0.05$) differences among the landfill exposed groups and control group; and Multiple Comparison Procedure between the mean of the treated groups and control group was done using Dunnett Post hoc Test at $p<0.05$. Student t-test was used for the pooled data. Pearson's correlation (r = correlation coefficient [corr-coeff]) was done to determine the strength of linear relationship between and within metal concentrations (in tissue and organs) and systemic/genetic alterations. GraphPad Prism 5.0, SPSS 16.0 and Microsoft excel were the statistical tools used.

CHAPTER FOUR

RESULTS

4.1 Heavy metal analysis in underground water

The monthly concentrations of selected heavy metals analysed in Olusosun landfill underground water (OGW) and the Olodi-Apapa underground water (AGW) (control) are presented in Table 4.1.

The observed concentrations (mg/L) of Pb in AGW ranged from 0.07±0.03 (August, 2015) to 0.19±0.02 (October, 2015), while the concentrations (mg/L) in OGW ranged from 0.29±0.04 (August, 2015) to 0.52±0.06 (December, 2015). The monthly concentrations of Pb analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW [except January, 2016 ($p>0.05$)], and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

The observed concentrations (mg/L) of Cd in AGW ranged from 0.01±0.001 (November, 2015) to 0.03±0.006 (September, 2015), while the concentrations (mg/L) in OGW ranged from 0.11±0.02 (November, 2015) to 0.31±0.03 (January, 2016). The monthly concentrations of Cd analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW, and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

The observed concentrations (mg/L) of Cr in AGW ranged from 0.03±0.002 (September and November, 2015) to 0.05±0.004 (January, 2016), while the concentrations (mg/L) in OGW ranged from 0.06±0.004 (September, 2015) to 0.30±0.03 (January, 2016). The monthly concentrations of Cr analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW [except August, 2015 ($p>0.05$)], and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

Table 4.1: Monthly heavy metal concentrations (mg/L) in Olusosun borehole water and control

Year	Month	AGW	OGW	AGW	OGW	AGW	OGW	AGW	OGW	AGW	OGW	AGW	OGW
		Pb		Cd		Cr		Cu		Zn		Fe	
2015	August	0.07±0.03	0.29±0.04*	0.02±0.004	0.26±0.02*	0.04±0.007	0.13±0.08	0.05±0.001	2.97±0.11*	0.21±0.01	3.04±0.12*	1.06±0.43	30.93±1.31*
	September	0.09±0.03	0.43±0.05*	0.03±0.006	0.18±0.02*	0.03±0.002	0.06±0.004*	0.04±0.002	1.82±0.03*	0.21±0.03	2.61±0.06*	1.61±0.31	25.59±1.61*
	October	0.19±0.02	0.33±0.01*	0.02±0.004	0.12±0.04*	0.04±0.003	0.07±0.008*	0.02±0.002	1.82±0.25*	0.16±0.01	2.96±0.17*	0.95±0.23	28.85±1.05*
	November	0.16±0.07	0.46±0.04*	0.01±0.001	0.11±0.02*	0.03±0.002	0.27±0.02*	0.02±0.002	1.70±0.07*	0.19±0.01	3.94±0.08*	1.27±0.23	8.79±1.11*
	December	0.12±0.01	0.52±0.06*	0.02±0.005	0.30±0.02*	0.04±0.003	0.20±0.03*	0.02±0.006	2.76±0.18*	0.24±0.05	4.22±0.08*	0.91±0.17	7.45±0.49*
2016	January	0.18±0.05	0.39±0.04	0.03±0.005	0.31±0.03*	0.05±0.004	0.30±0.03*	0.04±0.005	3.25±0.13*	0.27±0.02	4.53±0.02*	1.12±0.13	62.52±7.39*
Mean Total		0.13±0.05	0.40±0.09*	0.02±0.005	0.21±0.09*	0.04±0.008	0.17±0.10*	0.03±0.01	2.29±0.68*	0.21±0.04	3.55±0.78*	1.15±0.26	27.35±19.98*
[a]NESREA		0.01		0.003		0.05		1.00		3.00		1.00	
[b]USEPA		0.015		0.005		0.10		1.30		5.00		0.30	

Units of the metal concentrations are in mg/L. Values are given as mean ± SD. Significant difference between the two groups were analyzed by unpaired t test with Welch's correction ($p<0.05$) and denoted with asterisk (*).

AGW = Olodi-Apapa groundwater; OGW = Olusosun groundwater. [a]NESREA (2011); [b]USEPA (2017)

The observed concentrations (mg/L) of Cu in AGW ranged from 0.02±0.002 (October, 2015) to 0.05±0.001 (August, 2015), while the concentrations (mg/L) in OGW ranged from 1.70±0.07 (November, 2015) to 3.25±0.13 (January, 2016). The monthly concentrations of Cu analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW, and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

The observed concentrations (mg/L) of Zn in AGW ranged from 0.16±0.01 (October, 2015) to 0.27±0.02 (January, 2016), while the concentrations (mg/L) in OGW ranged from 2.61±0.06 (September, 2015) to 4.53±0.02 (January, 2016). The monthly concentrations of Zn analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW, and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

The observed concentrations (mg/L) of Fe in AGW ranged from 0.91±0.17 (December, 2015) to 1.61±0.31 (September, 2015), while the concentrations (mg/L) in OGW ranged from 7.45±0.49 (December, 2015) to 62.52±7.39 (January, 2016). The monthly concentrations of Fe analysed in OGW were significantly ($p<0.05$) higher than those observed in AGW, and these were also heigher than permissible limits for drinking water by standard organizations (NESREA, 2011; USEPA, 2017).

4.2 Cytogenotoxicity of underground water and air emissions in rats that were exposed on Olusosun landfill

The frequencies of micronucleated polychromatic erythrocyte (MNPCE) and micronucleated normochromatic erythrocyte (MNNCE) induced in the bone marrow of rats exposed *in situ* to borehole water and ambient air at three different points (Pt1, Pt2 and Pt3) on Olusosun landfill for 4, 8, 12, 16, 20 and 24 weeks are presented in Table 4.2. Using ANOVA and Dunnett post hoc test, there was significant ($p<0.05$) increase in the MNPCE induced in the bone marrow of rats exposed to the underground water and air emissions at the three points and for all periods of exposure on the landfill compared to their corresponding controls. The exception is with Pt3 (4 and 8 weeks exposure periods; though higher frequencies of MNPCE were induced in the exposed rats, these were not significant [$p>0.05$] compared to their corresponding control). The

frequency of MNPCE was duration dependent with higher MN being induced as the duration of exposure increased from 8 weeks to 24 weeks at most points. However, rats exposed for 4 weeks had exceptionally higher MNPCE compared to those of 8 and 12 weeks exposure.

There was increase in the frequencies of MNNCE in the bone marrow of exposed rats for all periods of exposures compared to their corresponding control and these increase were significant ($p<0.05$) at Pt1 (8 weeks), Pt1 and Pt3 (12 weeks), Pt3 (16 weeks), Pt1, Pt2 and Pt3 (20 weeks), and Pt1 and Pt2 (24 weeks) (Table 4.2).

The PCE/NCE ratio decreased in all exposed rats compared to the corresponding controls (Figure 4.1). These decreases were significant ($p<0.05$) at Pt3 (4 weeks), Pt1, Pt2 and Pt3 (8 weeks), Pt1 and Pt2 (12 weeks), Pt2 (20 weeks), and Pt1 (24 weeks). Figure 4.2 shows the MNPCE, MNNCE and binucleated micronucleated PCE (BNMNPCE) observed in the exposed animals.

Pearson's correlation was used to evaluate the linear relationship between duration/period of exposure (4, 8, 12, 16, 20 and 24 weeks) and the pooled data (combination of Pt 1, 2 and 3) on cytogenotoxic effects for *in situ* exposure to Olusosun landfill contaminants. The correlation between exposure periods and the induced MNPCE ($p = 0.001$; corr-coeff = 0.450) and MNNCE ($p = 0.000$; corr-coeff = 0.472) were positive and significant; correlation between exposure periods and PCE/NCE ratio ($p = 0.000$; corr-coeff = -0.676) was also negative but significant.

Also, the correlation between MNPCE and MNNCE was a strong positive relationship ($p = 0.000$; corr-coeff = 0.665). Correlation between MNPCE and PCE/NCE ratio was insignificant weak negative correlation ($p = 0.268$; corr-coeff = -0.190); and MNNCE showed insignificant weak negative correlation with PCE/NCE ratio ($p = 0.134$; corr-coeff = 0.255).

Table 4.2: The mean of MNPCE and MNNCE induced in bone marrow cells of landfill exposed rats

Duration of Exposure (Weeks)	Sampling point	Mean±SE	
		MNPCE	MNNCE
4	Cn	2.33±0.88	4.33±0.33
	Pt1	16.33±2.60*	15.00±5.77
	Pt2	16.00±3.46*	11.33±1.45
	Pt3	9.33±3.18	15.00±2.89
8	Cn	4.33±0.33	3.33±0.33
	Pt1	9.00±0.58**	7.33±0.88**
	Pt2	9.00±1.16**	4.33±0.33
	Pt3	6.00±0.58	4.33±0.33
12	Cn	1.67±0.33	1.67±0.33
	Pt1	8.33±0.33***	5.67±1.20*
	Pt2	6.00±0.58***	3.33±1.20
	Pt3	6.33±0.33***	6.33±0.33*
16	Cn	6.33±0.88	5.33±0.33
	Pt1	13.33±1.45**	8.00±1.16
	Pt2	15.00±1.16**	8.33±0.88
	Pt3	13.00±1.16*	13.33±0.88***
20	Cn	6.33±0.33	4.33±0.33
	Pt1	13.33±0.88**	9.33±0.33**
	Pt2	14.33±0.88**	18.33±1.45***
	Pt3	22.33±1.45***	17.33±0.67***
24	Cn	6.33±0.67	10.33±0.67
	Pt1	18.33±3.18**	25.67±2.73**
	Pt2	17.00±1.16**	22.33±3.18*
	Pt3	15.00±1.16*	13.00±2.08

MNPCE = Micronucleated polychromatic erythrocyte; MNNCE = Micronucleated normochromatic erythrocyte; Cn = control; Pt1, Pt2 and Pt3 (point 1, 2 and 3).

Data with (*) are different significantly when compare with corresponding control (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$).

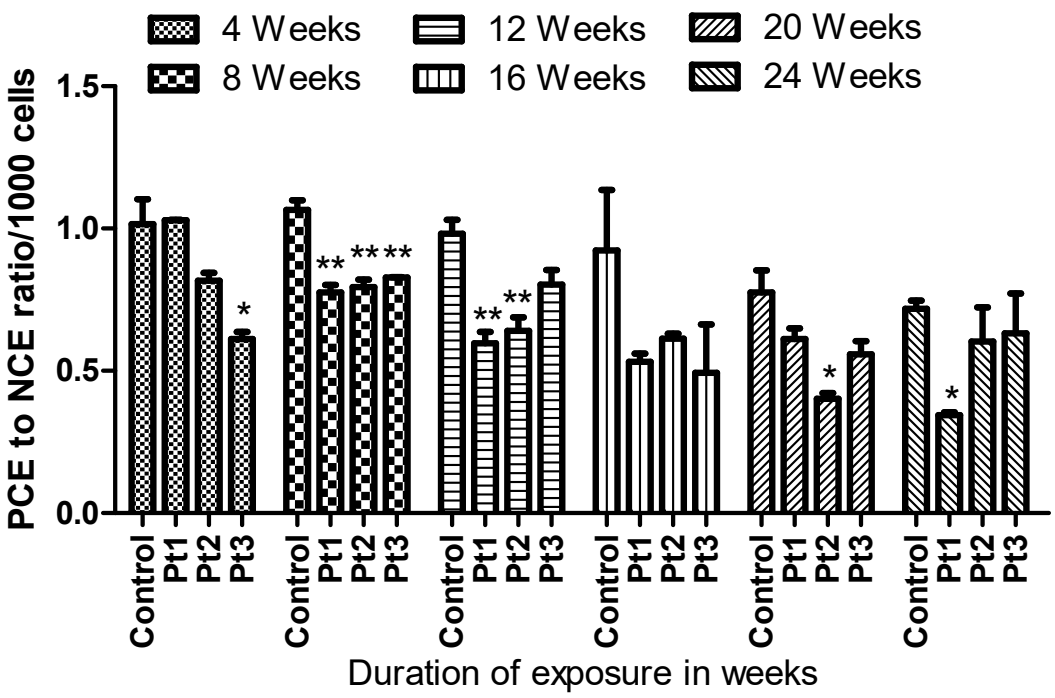

Figure 4.1: PCE to NCE ratio in bone marrow cells of rats exposed to borehole water and ambient air at OL (Pt1, Pt2 and Pt3) and control.

End points are mean (± standard error). Data with (*) are significantly different (* = $p < 0.05$; ** = $p < 0.01$).

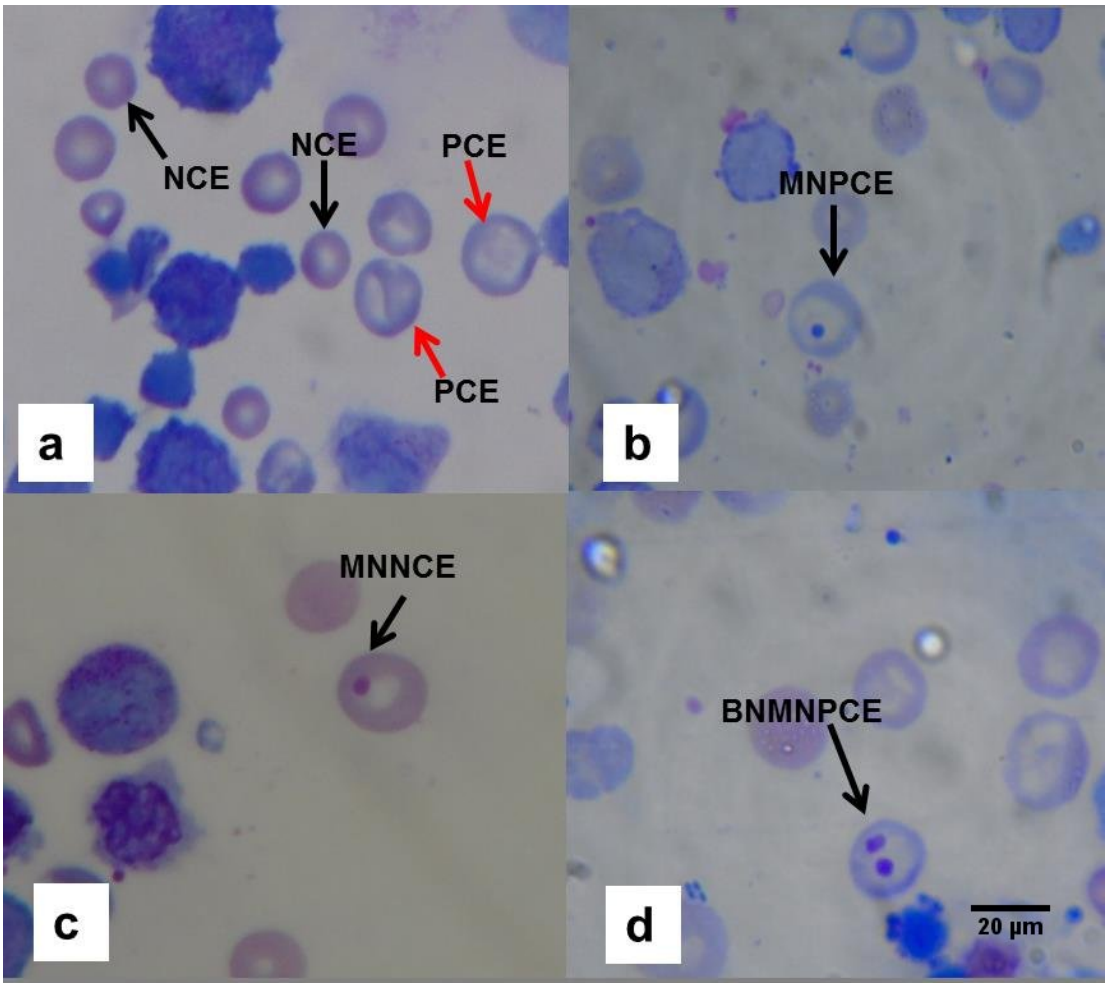

Figure 4.2: Micronucleus induced in bone marrow cells of landfill exposed rats.

(a) Normochromatic erythrocytes (NCE - black arrows) and Polychromatic erythrocytes (PCE – red arrows), (b) Micronucleated Polychromatic erythrocyte (MNPCE), (c) Micronucleated Normochromatic erythrocyte (MNNCE) and (d) binucleated micronucleated polychromatic erythrocyte (BNMNPCE). X100 oil immersion.

4.3 Systemic toxicity of underground water and air emissions in rats that were exposed on Olusosun landfill

4.3.1 Clinical signs of toxicity in landfill exposed rats

Rats exposed to borehole water and ambient air at OL showed obvious clinical signs of toxicity especially during the first four (4) weeks of introduction of rats to the dumpsite. Clinical signs such as anorexia, diarrhoea, highly pigmented faecal pellets (brown-black colour for rats in Olusosun landfill while light brown colour for rats in the control), reduced activities, abscesses, ungroomed fur and fur turning yellow were observed in the rats exposed to Olusosun landfill environment as compared to the control (Figure 4.3).

Another notable sign of toxicity that was observed after the rats exposed to the landfill air emissions and groundwater at the three different points were sacrificed and dissected were the consistent appearance of hepatic cyst on the liver samples of rats exposed for 8 weeks (fewer cases), 12, 16, 20 and 24 weeks durations (Figure 4.4). Whereas, liver cyst was not present in the liver samples of rats from the control site. Also observed were bloated stomach and intestine in some rats exposed to the dumpsite (Figure 4.5).

4.3.2 Terminal weight and percentage (%) change in body weight gain in landfill exposed rats

Rats exposed to the borehole water and ambient air at OL had significant ($p<0.05$) higher terminal body weight compared to their corresponding control sets at the end of each exposure periods (Table 4.3). The exceptions were 4 weeks exposure (Pts 1, 2 and 3), 8 weeks (Pt1), 20 weeks (Pt2) and 24 weeks (Pt3): which were all higher [except 4 weeks (Pt3); which was lower] but not significantly ($p>0.05$) different compared to their corresponding control sets. Also, the percentage (%) changes in body weight gain in rats exposed to the landfill were duration dependent and higher compared to their corresponding control at the end of each exposure periods (Table 4.3). The exceptions were rats exposed for 4 weeks (Pts 1 and 3), which had lower percentage (%) changes in body weight gain compared to their corresponding control set.

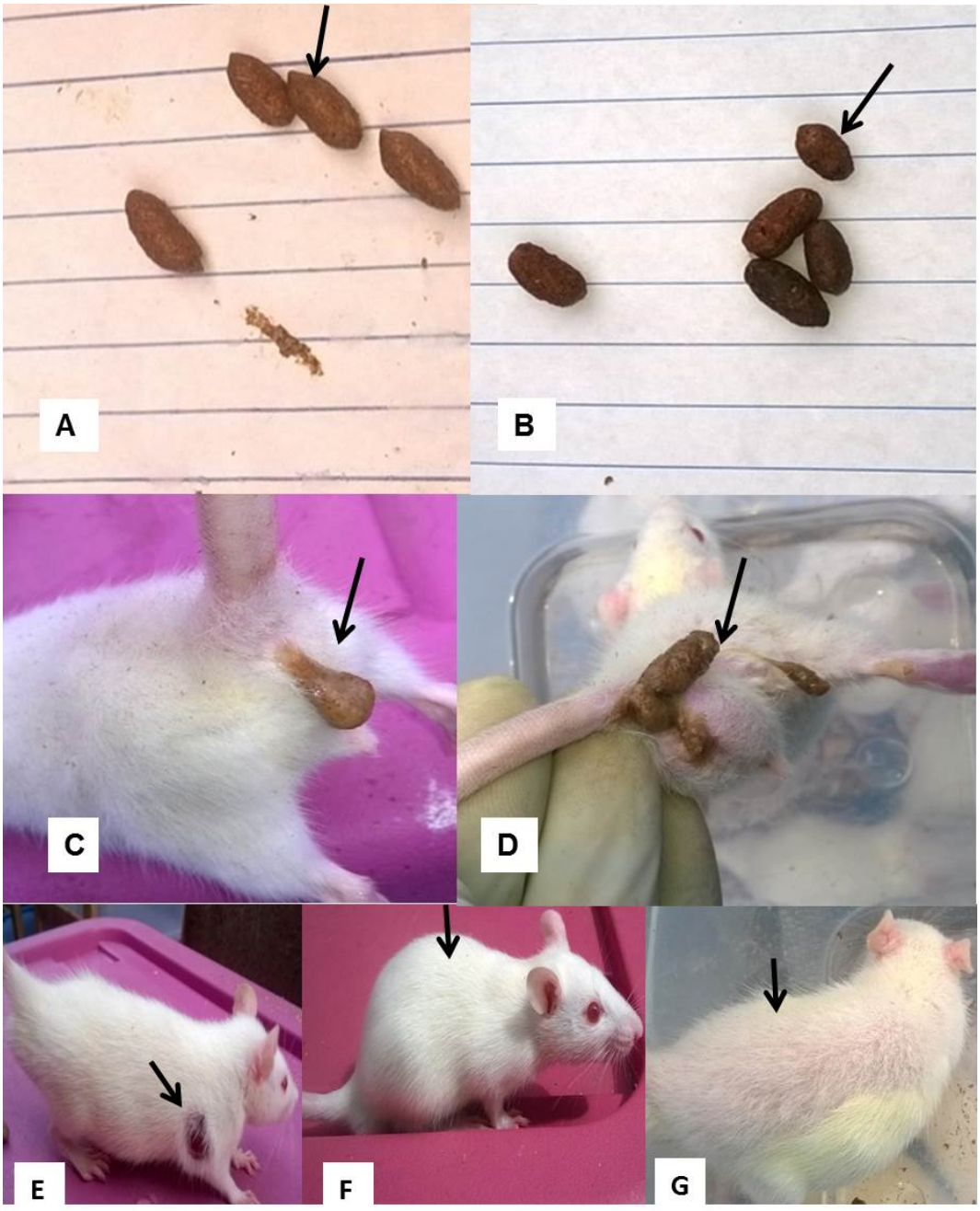

Figure 4.3: Some clinical signs of toxicity observed in landfill exposed rats.

(A) Light brown feaces observed in control rats, (B) Brown-black pigmented feaces observed in rats during the first 3 weeks of exposure to dumpsite, (C) & (D) Watery and mucus feace (Diarrhoea), (E) Abscesses, (F) Well groomed rat from control site, and (G) Poorly groomed rat with yellow colouration on fur from dumpsite.

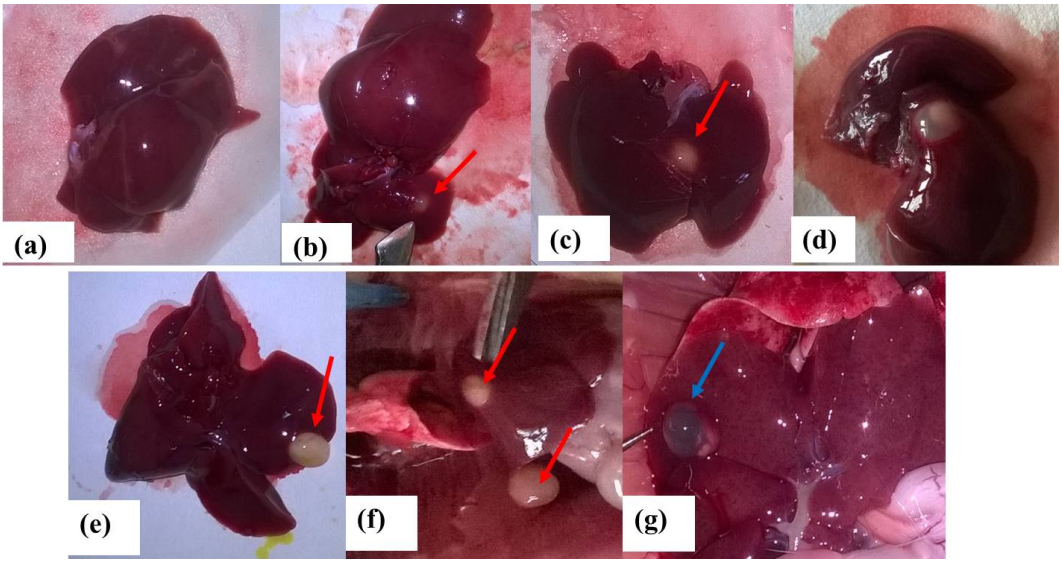

Figure 4.4: Progressive out growth (hepatic cyst/tumor) on the liver samples (red arrows) of rats exposed to Olusosun landfill.

(a) Liver from control rat with no out growth, (b) and (c) Out growth on liver as observed in the 8 and 12 weeks exposure, (d) and (e) Out growth on liver as observed in the 12, 16 and 20 weeks exposure duration, (f) and (g) Out growth on liver as observed in the 20 and 24 weeks; some out growths have turned black (necrotic) (blue arrow).

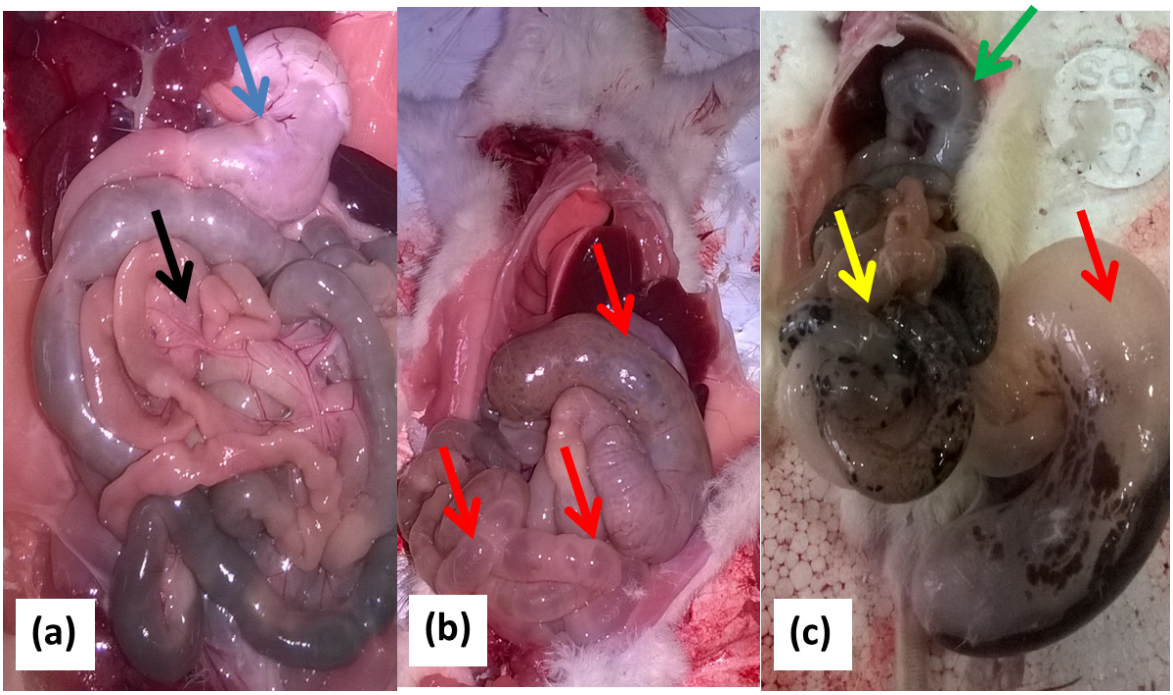

Figure 4.5: Bloated stomach and intestine in landfill exposed rats.

(a) Normal anatomy of the stomach (blue arrow) and intestine (black arrow), (b) Moderate bloating (air filled space) of the small intestine and colon (red arrows) observed in a rat exposed for 12 weeks (Pt1) (c) Severe bloating of the stomach (green arrow), small intestine (yellow arrow) and colon (red arrow) observed in a rat exposed for 20 weeks (Pt3).

Table 4.3: Initial and terminal body weight of landfill exposed rats

Exposure points	No of rat exposed	Initial body weight (g)	No of rat sacrificed	Terminal body weight (g)	% Change in body weight
	0 week exposure			4 weeks exposure	
Cn	30	66.88±2.99	5	74.96±5.33	12.08
Pt1	30	75.19±2.19	5	78.02±3.72	3.76
Pt2	30	68.46±3.83	5	84.68±7.17	23.69
Pt3	30	62.32±2.90	5	67.10±4.62	7.67
	0 week exposure			8 weeks exposure	
Cn	30	66.88±2.99	5	91.24±5.87	36.42
Pt1	30	75.19±2.19	5	116.50±10.50	54.94
Pt2	30	68.46±3.83	5	143.80±8.47**	110.05
Pt3	30	62.32±2.90	5	134.30±10.15**	115.50
	0 week exposure			12 weeks exposure	
Cn	30	66.88±2.99	5	96.65±6.33	44.51
Pt1	30	75.19±2.19	5	153.00±9.03***	103.48
Pt2	30	68.46±3.83	5	126.40±7.79*	84.63
Pt3	30	62.32±2.90	5	137.40±7.17**	120.47
	0 week exposure			16 weeks exposure	
Cn	30	66.88±2.99	5	105.30±7.26	57.45
Pt1	30	75.19±2.19	5	181.50±6.97***	141.39
Pt2	30	68.46±3.83	5	139.90±14.89*	104.35
Pt3	30	62.32±2.90	5	155.10±4.91**	148.88
	0 week exposure			20 weeks exposure	
Cn	30	66.88±2.99	5	134.20±7.68	100.66
Pt1	30	75.19±2.19	5	197.20±8.64***	162.27
Pt2	30	68.46±3.83	5	163.60±9.87	138.97
Pt3	30	62.32±2.90	5	169.10±4.54*	171.34
	0 week exposure			24 weeks exposure	
Cn	30	66.88±2.99	5	164.70±9.33	146.26
Pt1	30	75.19±2.19	5	219.00±6.59***	191.26
Pt2	30	68.46±3.83	5	196.30±6.48*	186.74
Pt3	30	62.32±2.90	5	181.10±6.34	190.60

End points are mean ± SE. Data with (*) are different significantly (*P<0.05; **P<0.01; ***P<0.001) when likened to the corresponding control.

4.3.3 Absolute and relative organ weights gain in landfill exposed rats

The results for the liver and kidney absolute and relative weights in rats exposed to the landfill water and ambient air are presented in Table 4.4. The absolute liver weights (ALW) increased in all exposed groups and these were significant ($p<0.05$) with rats exposed for 4 weeks (Pts 1 and 3), 8 weeks (Pts 2 and 3), 12 weeks (Pts 1, 2 and 3), 16 weeks (Pts 1 and 2), 20 weeks (Pts 1, 2 and 3) and 24 weeks (Pt 1), as compared to their corresponding controls. Also, the relative liver weights (RLW) increased in all exposed groups and these were significant ($p<0.05$) with rats exposed for 4 weeks (Pts 1 and 3), 8 weeks (Pts 1, 2 and 3), 12 weeks (Pts 2 and 3), 16 and 20 weeks (Pts 1, 2 and 3) and 24 weeks (Pt 1), as compared to their corresponding controls. For the pooled data on exposure at Olusosun, Pearson's correlation analysis revealed that there was significant strong positive correlation between exposure periods (in weeks) and absolute liver weight (g) ($p=0.000$; $r=0.570$). Also, there was significant strong negative correlation between exposure periods (in weeks) and relative liver weight (g) ($p=0.000$; $r=-0.647$).

The absolute kidney weights (AKW) increased in all groups exposed to Olusosun landfill air emissions and underground water and these were significant ($p<0.05$) with rats exposed for 8 weeks (Pts 1 and 2), and 16, 20 and 24 weeks (Pts 1, 2 and 3), as compared to their corresponding controls. Also, the relative kidney weights (RKW) were significantly ($p<0.05$) increased with the groups exposed for 4 weeks (Pt 1), 16 weeks (Pt 1 and 2) and 20 and 24 weeks (Pts 1, 2 and 3), as compared to their corresponding controls (Table 4.4). For the pooled data on exposure at Olusosun, Pearson's correlation analysis revealed that there was significant strong positive correlation between exposure periods (in weeks) and absolute kidney weight (g) ($p=0.000$; $r=0.424$). Also, the correlation between the exposure periods (in weeks) and relative kidney weight (g) was a strong significant negative correlation ($p=0.000$; corr-coeff= -0.453).

The results for lungs and spleen absolute and relative weights in rats exposed to the landfill are presented in Table 4.5. The absolute lung weights (ALgW) increased in rats exposed for 4, 8, 12, and 20 weeks (at Pts 1, 2 and 3) and 16 weeks (Pts 1 and 2); though insignificantly ($p>0.05$) different from the corresponding controls groups. Also, the relative lung weight (RLgW) increased in rats exposed to Olusosun landfill air

Table 4.4: Liver and kidney absolute and relative weights in rats exposed to the landfill

Exposure point	ALW	RLW	AKW	RKW
		4 weeks exposure		
Cn	2.21±0.24	2.39±0.20	0.53±0.05	0.70±0.08
Pt1	3.79±0.19*	4.88±0.23*	1.11±0.32	1.87±0.23*
Pt2	3.40±0.47	3.99±0.38	0.79±0.07	0.96±0.11
Pt3	3.46±0.37*	5.26±0.67*	1.02±0.37	1.47±0.46
		8 weeks exposure		
Cn	3.39±0.28	3.21±0.26	0.78±0.05	0.86±0.04
Pt1	4.00±0.68	4.07±0.17*	1.06±0.07*	0.92±0.04
Pt2	5.67±0.39*	4.08±0.07*	1.13±0.08*	0.79±0.05
Pt3	6.03±0.38*	4.68±0.23*	1.00±0.05	0.76±0.04
		12 weeks exposure		
Cn	3.34±0.20	3.25±0.22	0.75±0.04	0.78±0.02
Pt1	5.48±0.57*	3.74±0.12	1.11±0.19	0.71±0.09
Pt2	5.31±0.16*	4.25±0.25*	0.88±0.08	0.69±0.04
Pt3	5.89±0.82*	4.23±0.37*	1.05±0.18	0.75±0.09
		16 weeks exposure		
Cn	3.63±0.29	3.13±0.18	0.75±0.04	0.68±0.04
Pt1	5.74±0.76*	3.78±0.06*	1.41±0.04*	0.83±0.03*
Pt2	5.40±0.64*	3.84±0.05*	1.17±0.11*	0.84±0.04*
Pt3	5.30±0.33	3.77±0.10*	1.09±0.08*	0.70±0.04
		20 weeks exposure		
Cn	4.17±0.26	2.99±0.09	0.82±0.08	0.60±0.06
Pt1	6.75±0.29*	3.56±0.04*	1.53±0.07*	0.80±0.02*
Pt2	5.42±0.29*	3.80±0.12*	1.15±0.12*	0.81±0.05*
Pt3	5.50±0.34*	3.46±0.21*	1.18±0.03*	0.78±0.03*
		24 weeks exposure		
Cn	4.91±0.24	2.86±0.12	0.96±0.05	0.56±0.05
Pt1	7.11±0.41*	3.47±0.09*	1.55±0.04*	0.71±0.03*
Pt2	5.86±0.48	3.23±0.16	1.40±0.04*	0.72±0.04*
Pt3	5.94±0.38	3.27±0.15	1.27±0.04*	0.72±0.02*

Data are in mean ± SE. * = mean values differ significantly ($p<0.05$) from corresponding control (Cn) using Dunnett's multiple post hoc test.

ALW- Absolute liver weight (g); RLW- Relative liver weight (g); AKW - Absolute kidney weight (g); RKW- Relative kidney weight (g). Exposure points at Olusosun landfill (Pt1, Pt2 and Pt3); Cn- Control point.

Table 4.5: Lungs and spleen absolute and relative weights in rats exposed to the landfill

Exposure point	ALgW	RLgW	ASW	RSW
4 weeks exposure				
Cn	0.37±0.02	0.51±0.05	0.30±0.06	0.43±0.06
Pt1	0.65±0.13	0.82±0.13	0.47±0.03	0.61±0.03
Pt2	0.70±0.17	0.82±0.15	0.40±0.10	0.43±0.07
Pt3	0.48±0.02	0.73±0.06	0.64±0.34	0.85±0.44
8 weeks exposure				
Cn	0.79±0.09	0.86±0.07	0.47±0.01	0.47±0.01
Pt1	0.90±0.17	0.81±0.16	0.55±0.07	0.44±0.03
Pt2	1.02±0.07	0.71±0.02	0.66±0.03	0.47±0.04
Pt3	1.16±0.15	0.85±0.06	0.58±0.08	0.40±0.07
12 weeks exposure				
Cn	0.79±0.05	0.83±0.06	0.42±0.05	0.38±0.03
Pt1	0.99±0.19	0.63±0.08	0.62±0.13	0.37±0.06
Pt2	1.08±0.07	0.87±0.08	0.48±0.07	0.38±0.08
Pt3	1.30±0.33	0.93±0.21	0.69±0.10	0.46±0.05
16 weeks exposure				
Cn	0.93±0.15	0.98±0.23	0.44±0.07	0.43±0.04
Pt1	1.00±0.01	0.57±0.02	0.71±0.06	0.40±0.03
Pt2	0.95±0.03	0.71±0.07	0.52±0.04	0.36±0.04
Pt3	0.84±0.06	0.54±0.03	0.52±0.12	0.32±0.09
20 weeks exposure				
Cn	0.93±0.08	0.70±0.07	0.43±0.03	0.34±0.04
Pt1	1.18±0.10	0.60±0.05	0.72±0.10*	0.36±0.04
Pt2	1.03±0.13	0.62±0.05	0.64±0.12	0.40±0.08
Pt3	1.09±0.11	0.65±0.07	0.63±0.01	0.37±0.02
24 weeks exposure				
Cn	1.60±0.23	1.02±0.18	0.64±0.06	0.40±0.05
Pt1	1.30±0.15	0.59±0.05	0.70±0.01	0.33±0.02
Pt2	1.17±0.11	0.60±0.06	0.75±0.10	0.40±0.03
Pt3	1.58±0.37	0.87±0.19	0.83±0.14	0.46±0.04

Data are in mean ± SE. * = mean values differ significantly ($p<0.05$) from corresponding control (Cn) using Dunnett's multiple post hoc test.

ALgW- Absolute lungs weight (g); RLgW- Relative lungs weight (g); ASW - Absolute spleen weight (g); RSW- Relative spleen weight (g). Exposure points at Olusosun landfill (Pt1, Pt2 and Pt3); Cn- Control point.

emissions and underground water for 4 weeks (Pts 1, 2 and 3) and 12 weeks (Pts 2 and 3); though insignificantly ($p>0.05$) different from the corresponding controls groups. There were insignificant ($p>0.05$) decreases in the RLgW in rats exposed for 8 weeks (Pts 1, 2 and 3), 12 weeks (Pt1), and 16, 20 and 24 weeks (Pts 1, 2 and 3) as compared to the corresponding control groups. For the pooled data on exposure at Olusosun, Pearson's correlation analysis revealed that there was significant strong positive correlation between exposure periods (in weeks) and absolute lungs weight (g) ($p=0.000$; $r=0.484$). Also, there was significant strong negative correlation between exposure periods (in weeks) and relative lungs weight (g) ($p=0.011$; $r=-0.282$).

The absolute spleen weights (ASW) increased in all exposed groups and this was significant ($p<0.05$) only with rats exposed for 20 weeks (Pt1), as compared to their corresponding controls. Also, there were insignificant ($p>0.05$) increase in relative spleen weights (RSW) in rats exposed for 4 weeks (Pts 1 and 3), 12 weeks (Pt3), 20 weeks (Pts 1, 2 and 3) and 24 weeks (Pt3); and insignificant ($p>0.05$) decreases in RSW in rats exposed for 8 weeks (Pts 1 and 3), 12 weeks (Pt1), 16 weeks (Pts 1, 2 and 3) and 24 weeks (Pt1) as compared to their corresponding control groups (Table 4.6). For the pooled data on exposure at Olusosun, Pearson's correlation analysis revealed that there was significant strong positive correlation between exposure periods (in weeks) and absolute spleen weight (g) ($p=0.013$; $r=0.363$). Also,

the correlation between the exposure periods (in weeks) and relative lungs weight (g) was a strong significant negative correlation ($p=0.012$; $r=-0.369$).

4.3.4 Heavy metal concentrations in tissue and organs

4.3.4.1 Metal concentrations in rat's blood exposed in situ to the landfill

Figure 4.6 to 4.10 shows the concentration (mg/L) of Pb, Cd, Cr, Cu and Zn measures in the whole blood sample of rats exposed to Olusosun landfill for 4 to 24 weeks. Lead (Pb) concentrations in blood of exposed groups (Figure 4.6) were higher than the corresponding control groups throughout the exposure periods [except at 4 weeks (Pt1), 12 weeks (Pt2) and 24 weeks (Pt3) exposure periods and points; which were lower] and these were statistically significant ($p<0.05$) at 8 weeks (Pts 1, 2 and 3), 12 weeks (Pts 1 and 2), 16 and 20 weeks (Pts 1 and 2) and 24 weeks (Pt2). However, blood Pb concentration was significantly ($p<0.05$) lower at 24 weeks exposure (Pt3).

Also, the average blood Pb concentration (±SE in mg/L) measured during the study ranged from 0.027±0.003 (12 weeks exposure) to 0.343±0.01 (24 weeks) for the control groups; and from ND (4 weeks; Pt1) to 0.640±0.01 (8 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Pb concentrations in blood showed weak positive insignificant correlation with exposure periods ($p=0.722$; corr coeff = 0.063).

Cadmium (Cd) concentrations in blood of exposed groups (Figure 4.7) were higher than the corresponding control groups throughout the exposure periods [except 4 weeks (Pt2), 12 weeks (Pt3), 20 and 24 weeks exposures (Pt3); which were lower] and these were statistically significant ($p<0.05$) at 4 weeks (Pt3), 8 weeks (Pts 1, 2 and 3) and 20 weeks (Pts 1 and 2). Also, the average blood Cd concentration (±SE in mg/L) measured during the study ranged from 0.015±0.003 (4 weeks exposure) to 0.150±0.06 (20 weeks) for the control groups; and from 0.010±0.00 (4 weeks; Pt2) to 0.595±0.03 (20 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cd concentrations in blood showed weak positive insignificant correlation with exposure periods ($p=0.293$; $r=0.183$).

Chromium (Cr) concentrations in blood of exposed groups (Figure 4.8) were higher than the corresponding control groups throughout the exposure periods [except 16 weeks (Pts 1 and 2), and 20 and 24 weeks (Pt1); which were lower] and these were statistically significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3), 12 weeks (Pts 1 and 3) and 20 and 24 weeks exposures (Pt3). Also, the average blood Cr concentration (±SE in mg/L) measured during the study ranged from ND (8 weeks exposure) to 0.081±0.02 (24 weeks) for the control groups; and from ND (8 weeks; Pts 1 and 2) to 0.353±0.03 (12 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cr concentrations in blood showed weak negative insignificant correlation with exposure periods ($p=0.120$; $r=-0.285$).

Copper (Cu) concentrations in blood of exposed groups (Figure 4.9) were higher than the corresponding control groups throughout the exposure periods [except at 12 weeks (Pt2) and 24 weeks (Pt3); which were lower] and these were statistically significant ($p<0.05$) at 8 weeks (Pts 2 and 3), 16 weeks (Pt2), and 20 weeks (Pts 1 and 2). Also, the average blood Cu concentration (±SE in mg/L) measured during the study ranged

from ND (4 weeks exposure) to 0.157±0.02 (24 weeks) for the control groups; and from 0.010±0.00 (4 weeks; Pt2) to 0.335±0.03 (20 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cu concentrations in blood showed a significant strong positive correlation with exposure periods ($p=0.006$; $r=0.470$).

Similarly, zinc (Zn) concentrations in blood of exposed groups (Figure 4.10) were higher than the corresponding control groups throughout the exposure periods [except at 4 weeks (Pts 1 and 2), 12 weeks (Pt2) and 24 weeks (Pt3); which were lower] and these were statistically significant ($p<0.05$) at 8 weeks (Pts 1 and 3), 20 weeks (Pt1) and 24 weeks (Pt1). However, blood Zn concentration was significantly ($p<0.05$) lower at 24 weeks exposure (Pt3). Also, the average blood Zn concentration (±SE in mg/L) measured during the study ranged from 0.267±0.03 (8 weeks exposure) to 0.510±0.04 (20 weeks) for the control groups; and from 0.145±0.02 (24 weeks; Pt3) to 1.275±0.33 (20 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Zn concentrations in blood showed a significant strong positive correlation with exposure periods ($p=0.005$; $r=0.462$).

In addition, for the pooled data for rat exposed *in situ* to Olusosun landfill, with increasing exposure periods, Pb concentrations in blood showed significant strong positive correlation with blood Cd ($p = 0.000$; corr coeff = 0.791), blood Cu ($p = 0.001$; corr coeff =0.562) and blood Zn ($p = 0.001$; corr coeff = 0.540); and a significant strong negative correlation with blood Cr ($p = 0.000$; corr coeff = -0.797).

Also, Cd concentrations in blood showed significant strong positive correlation with blood Cu ($p = 0.005$; corr coeff = 0.489) and blood Zn ($p = 0.000$; corr coeff = 0.657); and a significant strong negative correlation with blood Cr ($p = 0.000$; corr coeff = -0.601).

Cr concentrations in blood showed significant strong negative correlation with blood Cu ($p = 0.008$; corr coeff = -0.489) and blood Zn ($p = 0.001$; corr coeff = -0.560).

Finally, Cu concentrations in blood showed significant strong positive correlation with blood Zn ($p = 0.000$; corr coeff = 0.725).

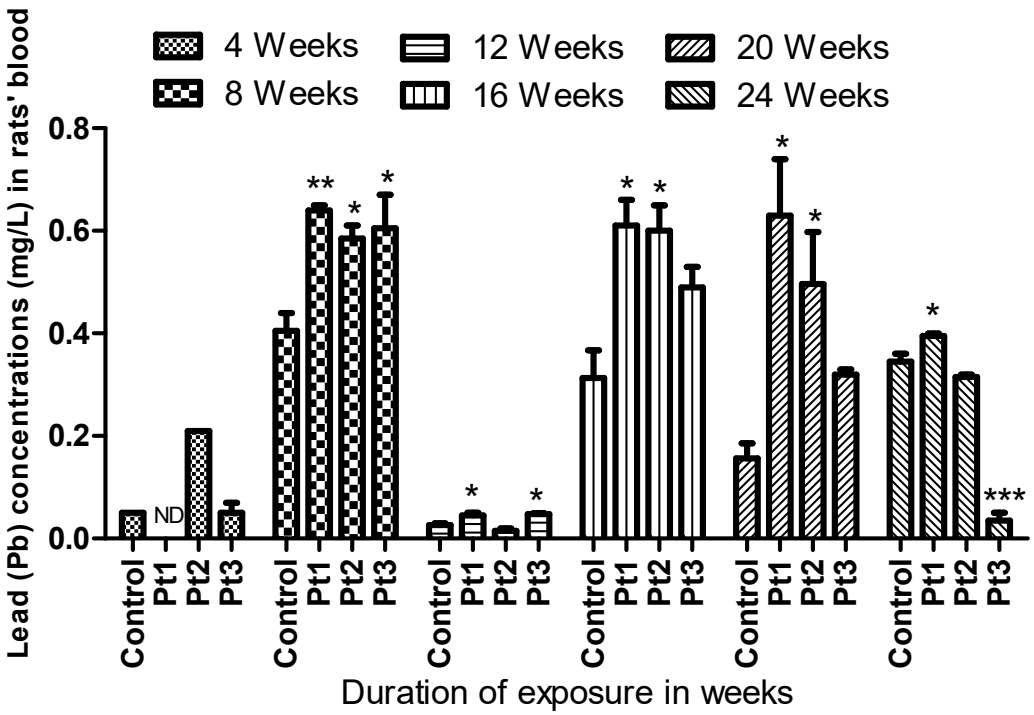

Figure 4.6: Lead (Pb) concentrations in the blood of rats exposed to borehole water and ambient air at OL for 4, 8, 12, 16, 20 and 24 weeks.

End points are mean (± SE). Data are significantly different (* = P<0.05; ** = P<0.01; *** = P<0.001) when likened to the corresponding control.

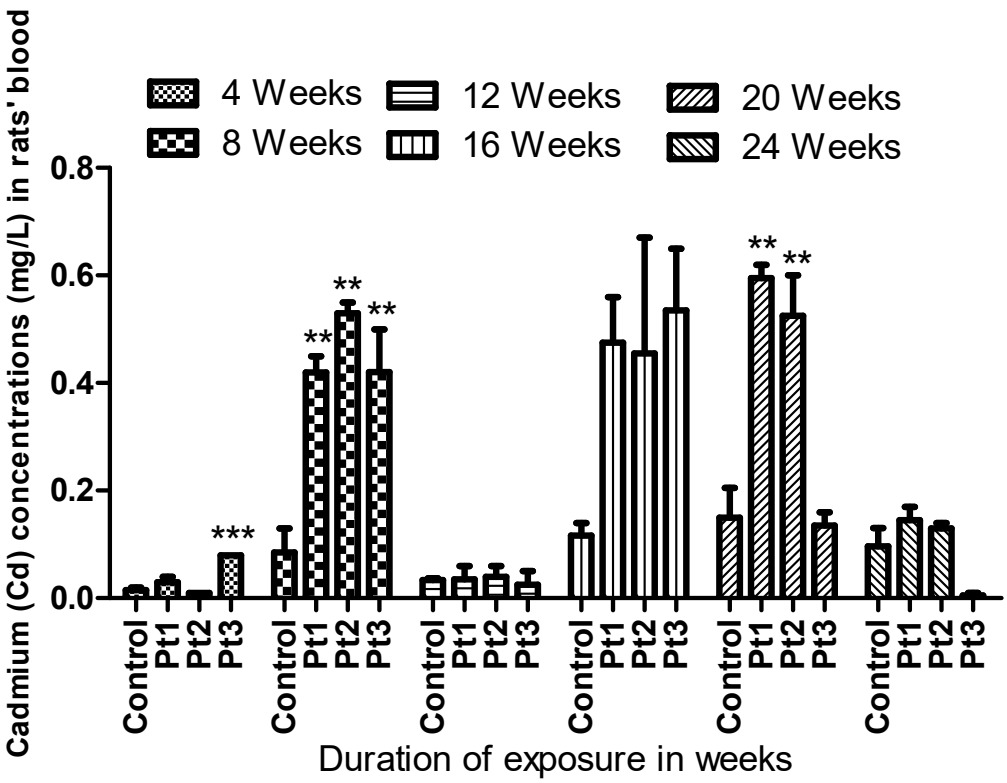

Figure 4.7: Cadmium (Cd) concentrations in the blood of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) compared to corresponding control.

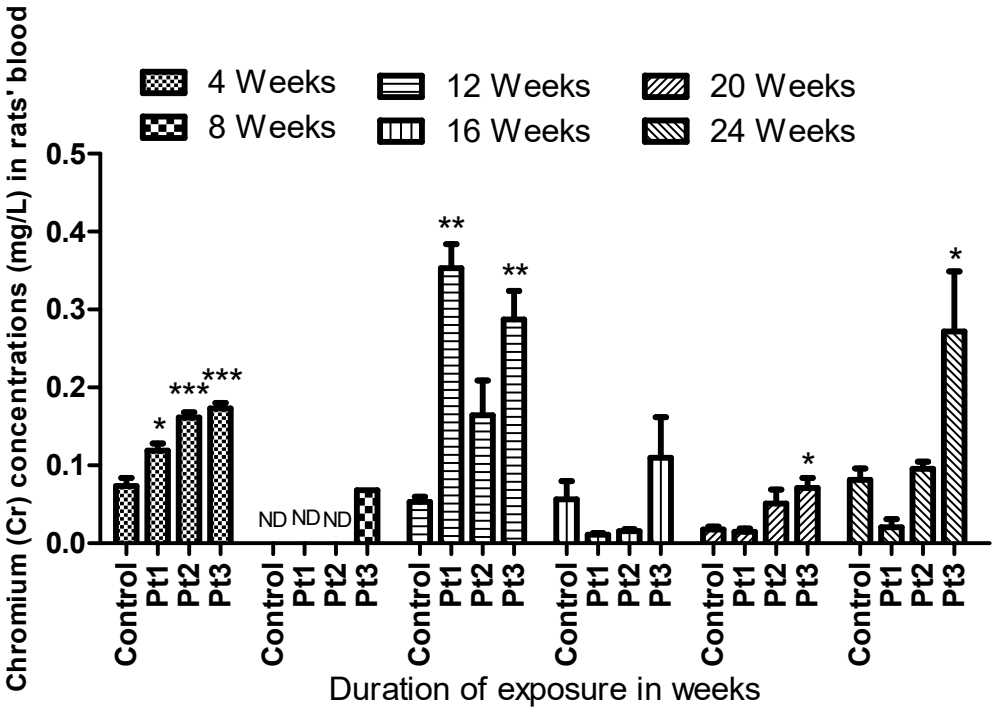

Figure 4.8: Chromium (Cr) concentrations in the blood of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± SE). Data are significantly different (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) when likened to the corresponding control.

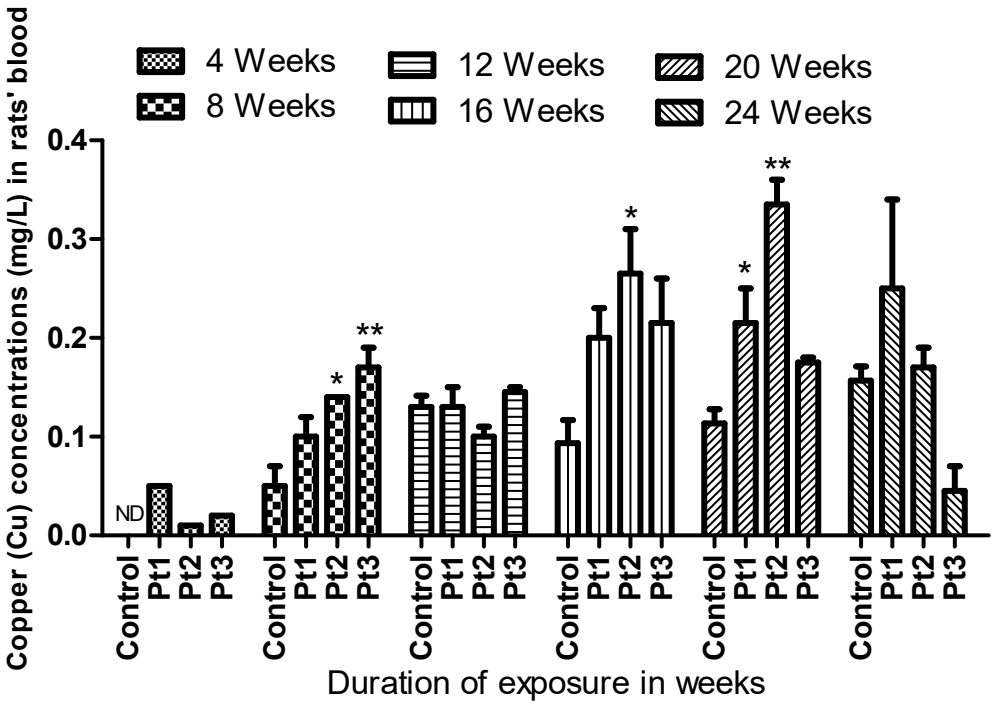

Figure 4.9: Copper (Cu) concentrations in the blood of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± stardard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to corresponding control.

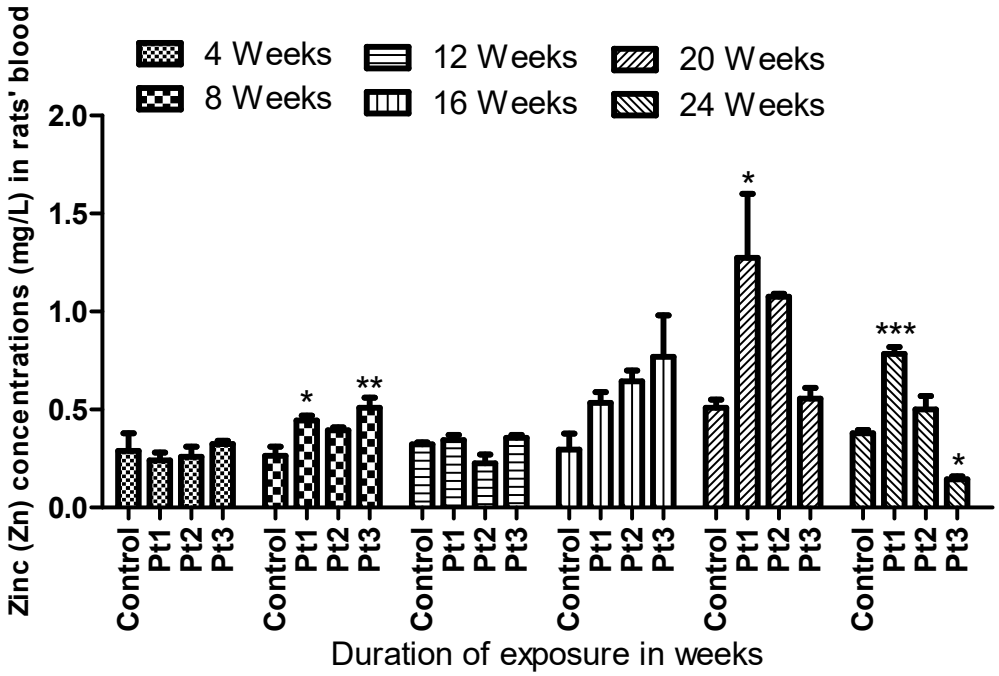

Figure 4.10: Zinc (Zn) concentrations in the blood of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

4.3.4.2 Metal concentrations in rat's liver exposed in situ to the landfill

Figure 4.11 to 4.15 shows the concentrations (mg/L) of Pb, Cd, Cr, Cu and Zn measures in the liver of rats exposed to Olusosun landfill for 4 to 24 weeks. Lead (Pb) concentrations in liver of exposed groups (Figure 4.11) were higher than the corresponding control groups throughout the exposure periods and these were statistically significant ($p<0.05$) at 4 weeks (Pt1), 8 weeks (Pts 2 and 3) and 12, 20 and 24 weeks (Pts 1, 2 and 3). Also, the average liver Pb concentration (±SE in mg/L) measured during the study ranged from 0.047±0.02 (12 weeks exposure) to 0.217±0.02 (24 weeks) for the control groups; and from 0.165±0.02 (12 weeks; Pt1) to 0.660±0.11 (8 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Pb concentrations in liver showed a weak positive insignificant correlation with exposure periods ($p=0.055$; $r=0.322$).

Cadmium (Cd) concentrations in liver of exposed groups (Figure 4.12) were higher than the corresponding control groups throughout the exposure periods and these were statistically significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3), 8 weeks (Pts 2 and 3), 16 weeks (Pts 1 and 2), 20 weeks (Pts 2 and 3), and 24 weeks (Pt3). Also, the average liver Cd concentration (±SE in mg/L) measured during the study ranged from 0.007±0.01 (12 weeks exposure) to 0.140±0.02 (20 weeks) for the control groups; and from 0.025±0.01 (12 weeks; Pt1) to 0.500±0.05 (20 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cd concentrations in liver showed a weak positive insignificant correlation with exposure periods ($p=0.722$; $r=0.061$).

Chromium (Cr) concentrations in liver of exposed groups (Figure 4.13) were higher than the corresponding control groups throughout the exposure periods and these were statistically significant ($p<0.05$) at 12 weeks (Pt2), 16 weeks (Pts 2 and 3), and 24 weeks (Pt3). Also, the average liver Cr concentration (±SE in mg/L) measured during the study ranged from not detected (ND) (4 and 8 weeks exposure) to 0.126±0.01 (20 weeks) for the control groups; and from 0.013±0.00 (4 weeks; Pt1) to 0.402±0.01 (12 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cr concentrations in liver showed a weak negative insignificant correlation with exposure periods ($p = 0.757$; corr coeff = -0.067).

Copper (Cu) concentrations in liver of exposed groups (Figure 4.14) were higher than the corresponding control groups throughout the exposure periods and these were statistically significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3), 8 weeks (Pt1), 12 weeks (Pts 1, 2 and 3), 16 and 20 weeks (Pt3), and 24 weeks (Pts 1 and 2). Also, the average liver Cu concentration (±SE in mg/L) measured during the study ranged from 0.055±0.01 (4 weeks exposure) to 0.260±0.05 (24 weeks) for the control groups; and from 0.160±0.02 (4 weeks; Pt3) to 0.837±0.27 (20 weeks; Pt3) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cu concentrations in liver showed a significant strong positive correlation with exposure periods ($p = 0.000$; corr coeff = 0.581).

Similarly, zinc (Zn) concentrations in liver of exposed groups (Figure 4.15) were higher than the corresponding control groups throughout the exposure periods and these were statistically significant ($p<0.05$) at 4 weeks (Pt13) and 12 weeks (Pts 1 and 3). Also, the average liver Zn concentration (±SE in mg/L) measured during the study ranged from 0.105±0.03 (8 weeks exposure) to 3.703±1.08 (24 weeks) for the control groups; and from 0.885±0.14 (4 weeks; Pt3) to 15.610±13.39 (20 weeks; Pt3) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Zn concentrations in liver showed a significant strong positive correlation with exposure periods ($p = 0.000$; corr coeff = 0.607).

In addition, for the pooled data for rat exposed *in situ* to Olusosun landfill, with increasing exposure periods, Pb concentrations in liver showed significant strong positive correlation with liver Cd ($p = 0.000$; corr coeff = 0.594) and liver Cu ($p = 0.027$; corr coeff = 0.368); insignificant weak positive correlation with liver Zn ($p = 0.214$; corr coeff = 0.222); and a significant strong negative correlation with liver Cr ($p = 0.035$; corr coeff = -0.432).

Also, Cd concentrations in liver showed insignificant weak negative correlation with liver Cr ($p = 0.058$; corr coeff = -0.392), liver Cu ($p = 0.870$; corr coeff = -0.028) and liver Zn ($p = 0.216$; corr coeff = -0.221).

Cr concentrations in liver showed insignificant weak negative correlation with liver Cu ($p = 0.389$; corr coeff = -0.184) and liver Zn ($p = 0.227$; corr coeff = -0.268).

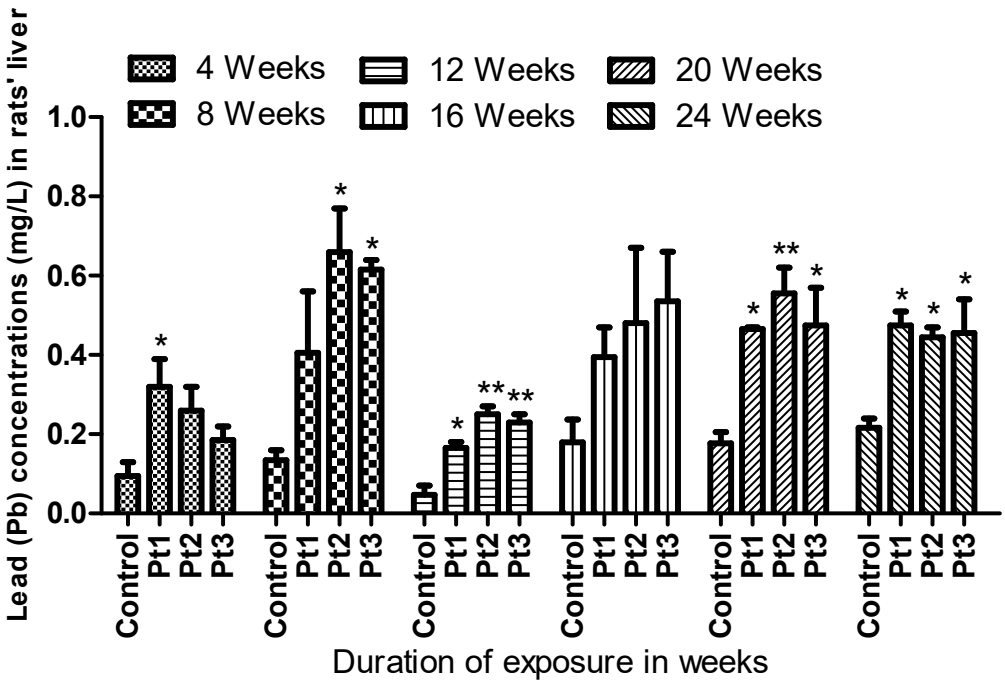

Figure 4.11: Lead (Pb) concentrations in the liver of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points represent mean (± SE). Values are significantly different (* = P<0.05; ** = P<0.01; *** = P<0.001) compared to corresponding control.

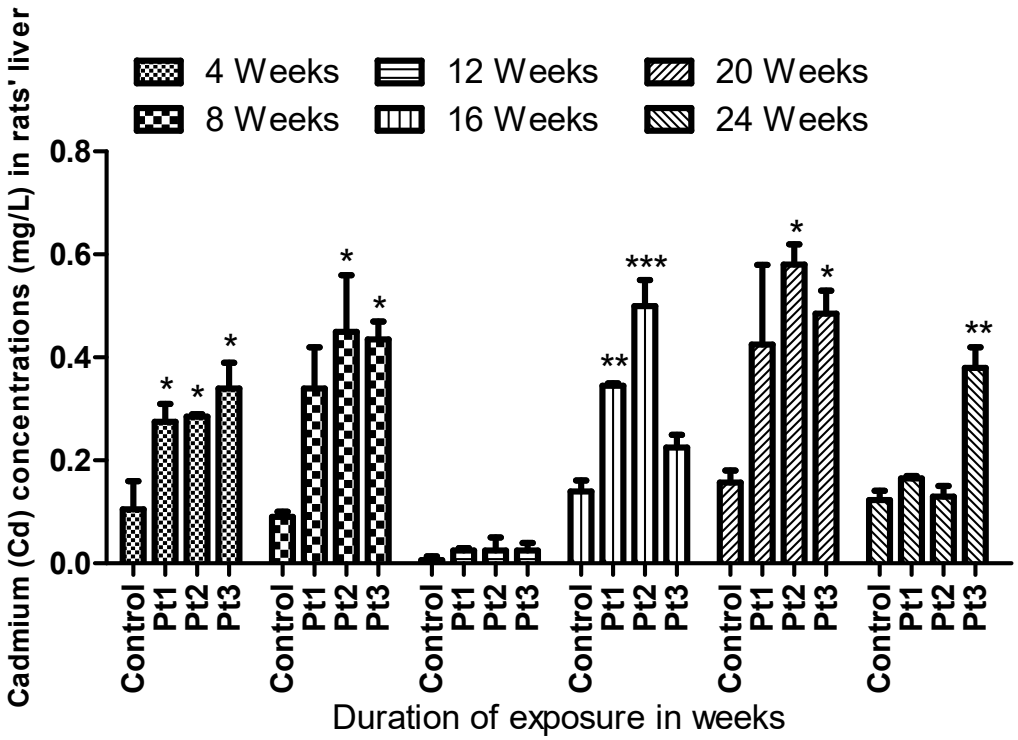

Figure 4.12: Cadmium (Cd) concentrations in the liver of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± SE). Data are significantly different (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) when likened to the corresponding control.

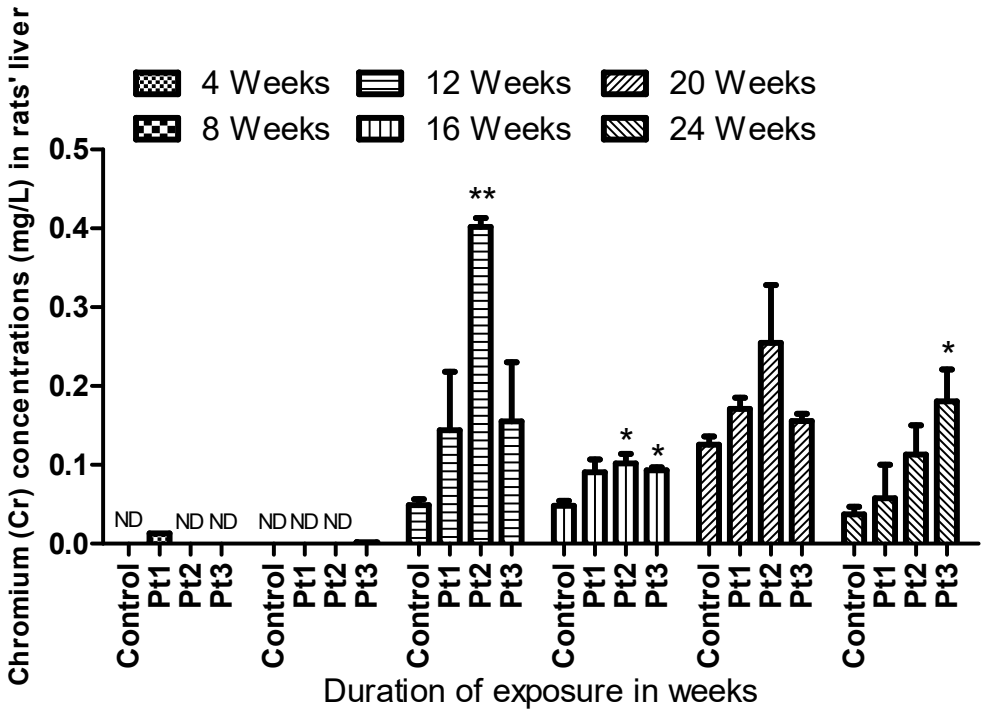

Figure 4.13: Chromium (Cr) concentrations in the liver of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

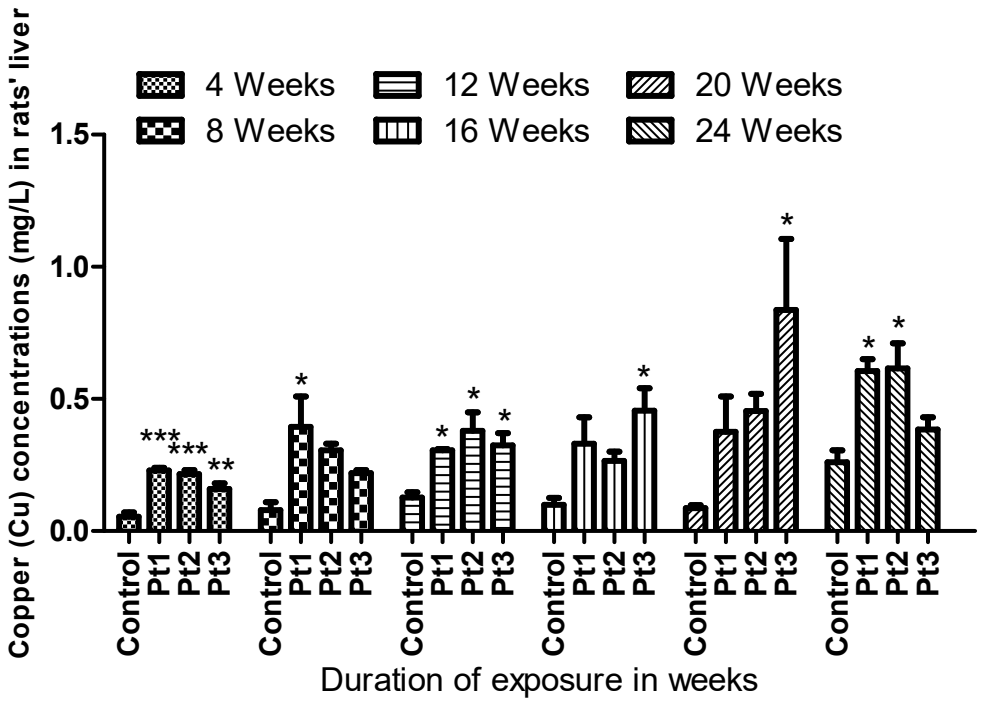

Figure 4.14: Copper (Cu) concentrations in the liver of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± SE). Data are significantly different (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) when likened to the corresponding control.

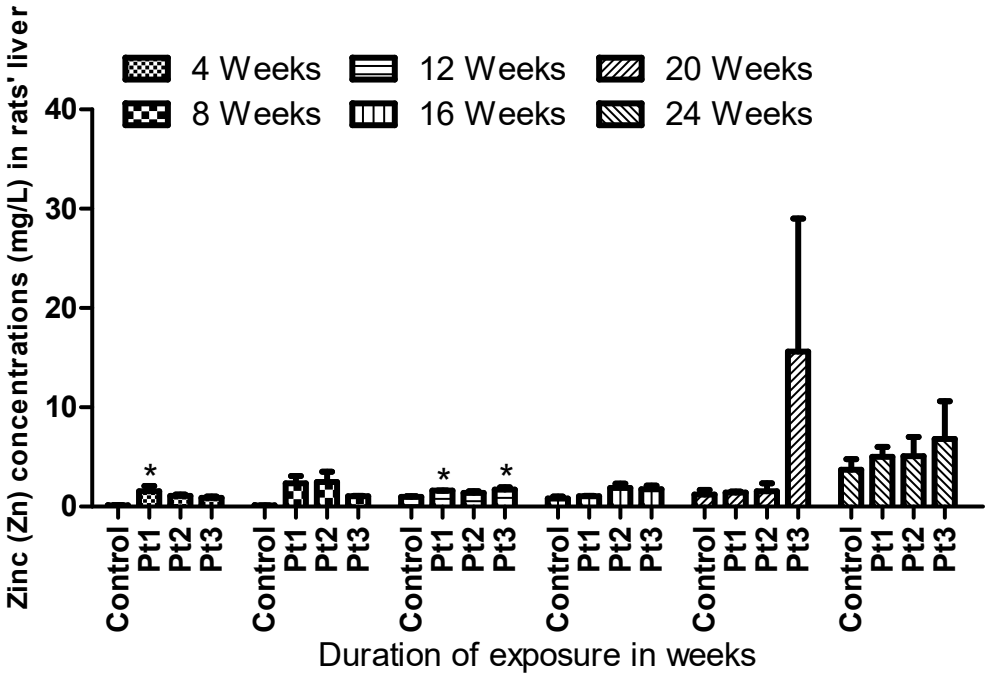

Figure 4.15: Zinc (Zn) concentrations in the liver of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

Finally, Cu concentrations in liver showed significant strong positive correlation with liver Zn ($p = 0.000$; corr coeff = 0.715).

4.3.4.3 Metal concentrations rat's kidney exposed in situ to the landfill

Figure 4.16 to 4.20 shows the concentrations (mg/L) of Pb, Cd, Cr, Cu and Zn measures in the kidney of rats exposed to Olusosun landfill for 4 to 24 weeks. Lead (Pb) concentrations in kidney of exposed groups (Figure 4.16) were greater when compared to the corresponding control groups throughout the exposure periods and these were significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3), 8 weeks (Pt1) and 12 and 16 weeks (Pts 1, 2 and 3). Also, the average kidney Pb concentration (±SE in mg/L) measured during the study ranged from 0.063±0.04 (12 weeks exposure) to 0.313±0.04 (24 weeks) for the control groups; and from 0.295±0.04 (12 weeks; Pt2) to 0.800±0.14 (8 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Pb concentrations in kidney showed an insignificant weak negative correlation with exposure periods ($p=0.151$; $r=-0.245$).

Cadmium (Cd) concentrations in kidney of exposed groups (Figure 4.17) were greater when compared to the corresponding control groups throughout the exposure periods and these were significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3), 12 weeks (Pt1), 20 weeks (Pt3), and 24 weeks (Pts 1 and 3). Also, the average kidney Cd concentration (±SE in mg/L) measured during the study ranged from 0.070±0.02 (12 weeks exposure) to 0.140±0.04 (16 weeks) for the control groups; and from 0.195±0.04 (20 weeks; Pt2) to 0.555±0.13 (8 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cd concentrations in kidney showed an insignificant weak negative correlation with exposure periods ($p=0.652$; $r=-0.078$).

Chromium (Cr) concentrations in kidney of exposed groups (Figure 4.18) were greater when compared to the corresponding control groups throughout the exposure periods [except 16 and 24 weeks (Pts 2 and 3); which were lower] and these were significant ($p<0.05$) at 8 weeks (Pts 2 and 3), and 16 weeks (Pt1). Also, the average kidney Cr concentration (±SE in mg/L) measured during the study ranged from ND (4 weeks exposure) to 0.177±0.03 (20 weeks) for the control groups; and from ND (4 weeks; Pts 1, 2 and 3) to 0.238±0.004 (20 weeks; Pt1) for the exposed groups. The pooled data for

rat exposed *in situ* to Olusosun landfill revealed that Cr concentrations in kidney were significantly strongly positively correlated with exposure periods (p=0.003; corr-coeff=0.497).

Copper (Cu) concentrations in kidney of exposed groups (Figure 4.19) were greater when compared to the corresponding control groups throughout the exposure periods except 24 weeks (Pt3; which was lower) and these were significant ($p<0.05$) at 4 weeks (Pt1), 8 weeks (Pt2), 12 weeks (Pts 1 and 2), 20 weeks (Pt1), and 24 weeks (Pt2). Also, the average kidney Cu concentration (±SE in mg/L) measured during the study ranged from 0.137±0.003 (12 weeks exposure) to 0.303±0.08 (24 weeks) for the control groups; and from 0.170±0.01 (12 weeks; Pt3) to 0.860±0.14 (20 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cu concentrations in kidney were significantly strongly positively correlated with exposure periods (p=0.002; corr-coeff=0.497).

Similarly, zinc (Zn) concentrations in kidney of exposed groups (Figure 4.20) were greater when compared to the corresponding control groups throughout the exposure periods and these were significant ($p<0.05$) at 4 weeks (Pts 1, 2 and 3) and 8 weeks (Pts 1 and 2), 16 weeks (Pt2), and 20 weeks (Pts 1, 2 and 3). Also, the average kidney Zn concentration (±SE in mg/L) measured during the study ranged from 0.190±0.02 (8 weeks exposure) to 0.910±0.16 (24 weeks) for the control groups; and from 0.765±0.17 (12 weeks; Pt2) to 3.050±0.75 (16 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Zn concentrations in kidney showed a non significant but weak-positive correlation with exposure periods (p=0.081; corr-coeff=0.294).

In addition, for the pooled data for rat exposed *in situ* to Olusosun landfill, with increasing exposure periods, Pb concentrations in kidney showed significant strong positive correlation with kidney Cd (p=0.018; corr-coeff=0.391); insignificant weak positive correlation with kidney Cu (p=0.802; corr-coeff=0.043) and kidney Zn (p=0.384; corr-coeff=0.150); and an insignificant weak negative correlation with kidney Cr (p=0.351; corr-coeff=-0.168). Also, Cd concentrations in kidney showed insignificant weak positive correlation with kidney Cu (p=0.990; corr-coeff=0.002) and kidney Zn (p=0.355; corr-

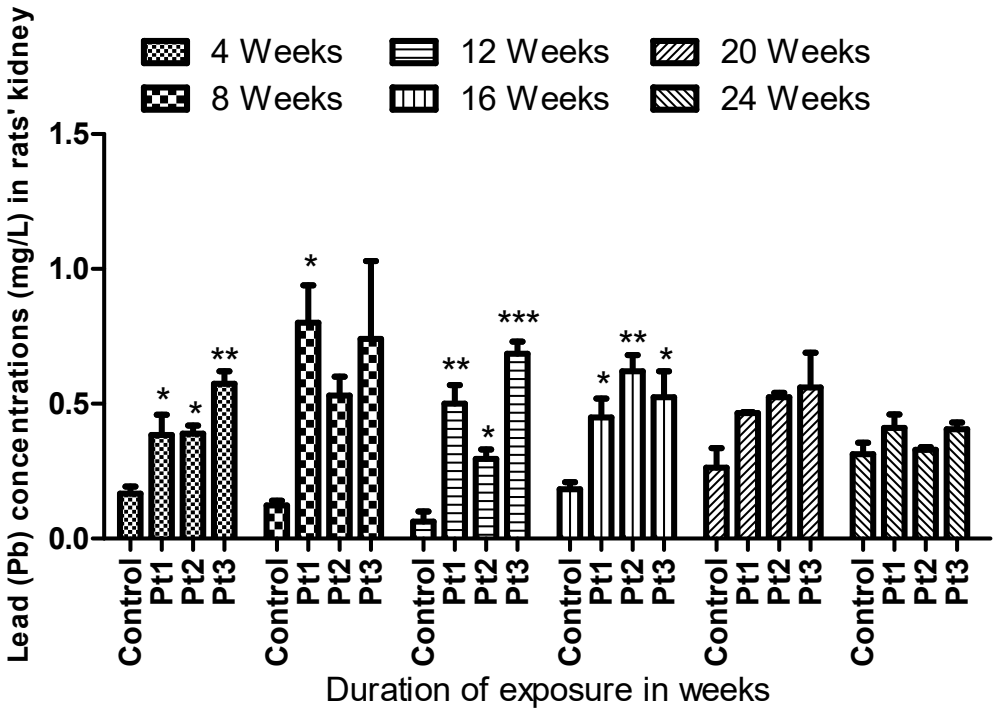

Figure 4.16: Lead (Pb) concentrations in the kidney of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± SE). Data are significantly different (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) when likened to the corresponding control.

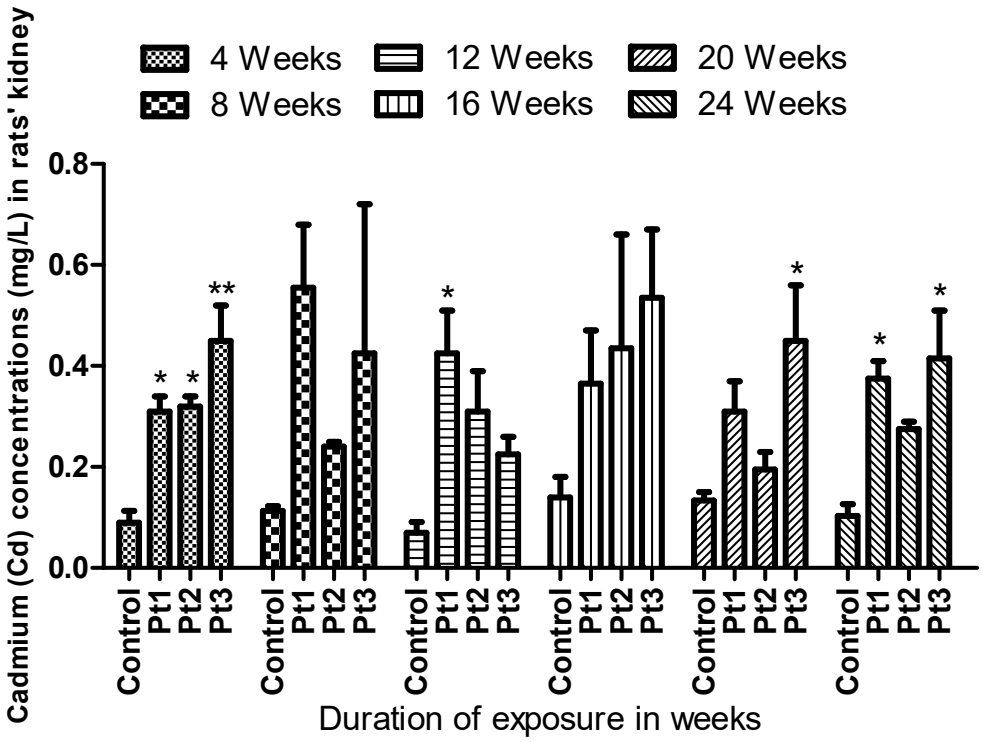

Figure 4.17: Cadmium (Cd) concentrations in the kidney of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

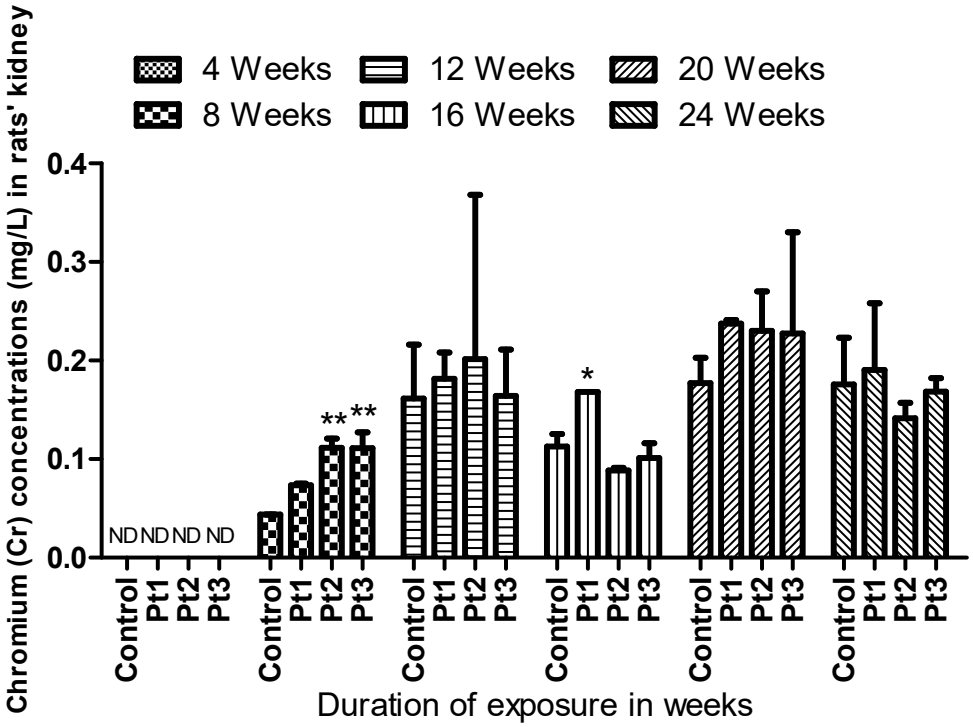

Figure 4.18: Chromium (Cr) concentrations in the kidney of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

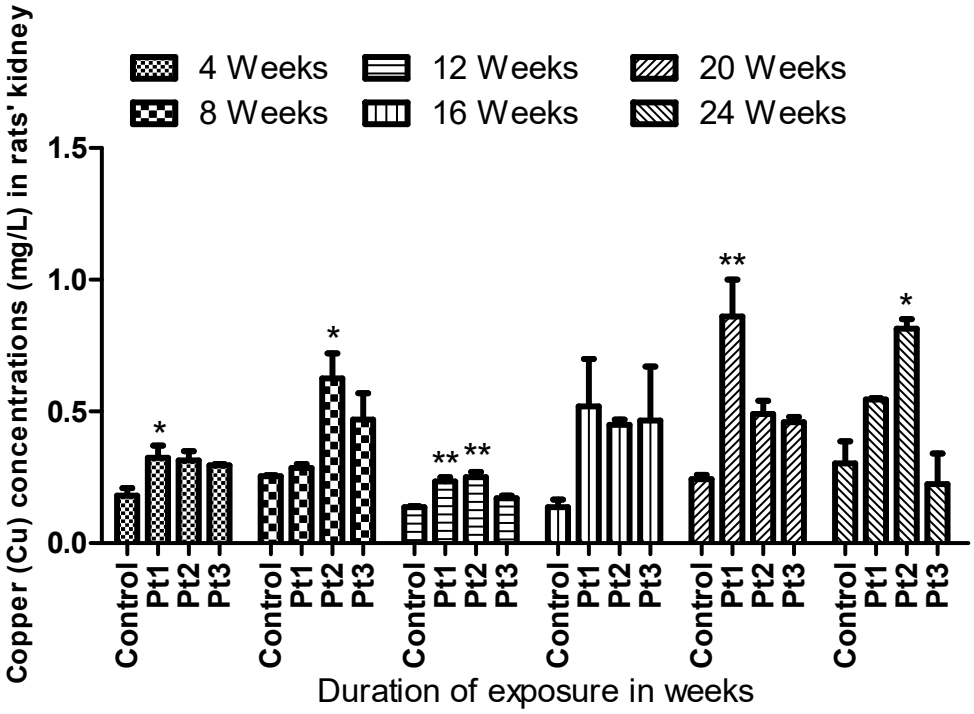

Figure 4.19: Copper (Cu) concentrations in the kidney of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

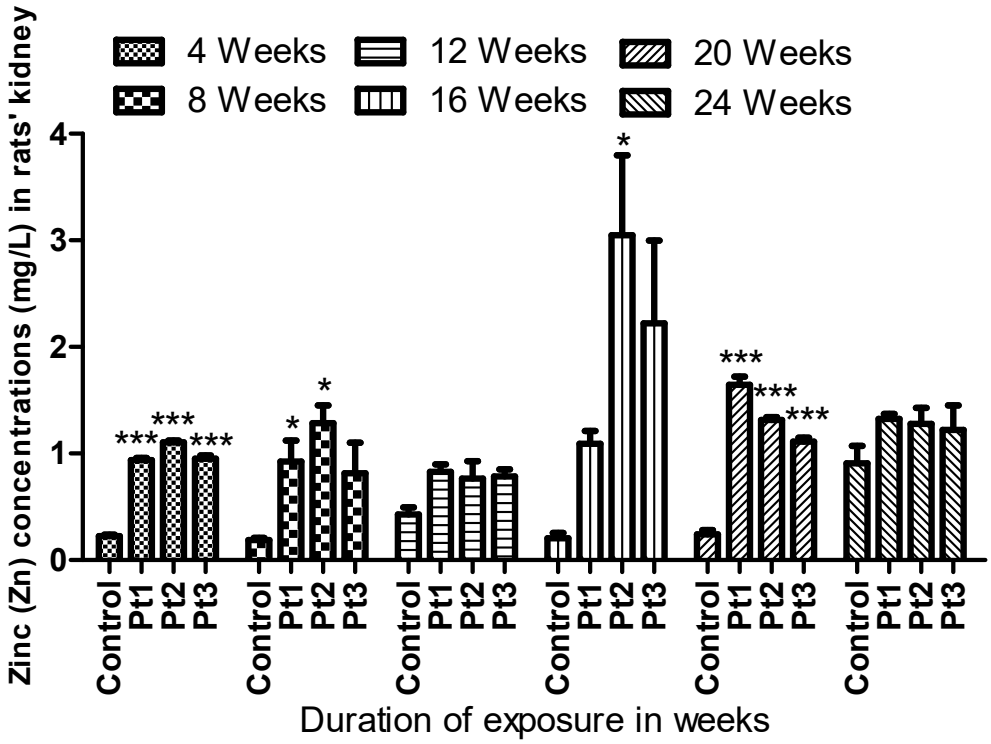

Figure 4.20: Zinc (Zn) concentrations in the kidney of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

coeff=0.159); and an insignificant weak negative correlation with kidney Cr (p=0.150; corr-coeff=-0.256).

Cr concentrations in kidney showed a non significant and weak-positive correlation with kidney Cu (p=0.895; corr-coeff=0.024) and an insignificant weak negative correlation with kidney Zn (p=0.358; corr-coeff=-0.165). Finally, Cu concentrations in kidney showed significant strong positive correlation with kidney Zn (p=0.028; corr-coeff=0.366).

4.3.4.4 Metal concentrations rat's lungs exposed in situ to the landfill

Figure 4.21 to 4.25 shows the levels (mg/L) of metals measured in the lungs of rats exposed to Olusosun landfill for 4 to 24 weeks. Lead (Pb) concentrations in lungs of exposed groups (Figure 4.21) were greater when compared to the corresponding control groups throughout the exposure periods except at 24 weeks exposure (Pt3; which was lower) and these were significant ($p<0.05$) at 8 weeks (Pt2), 16 weeks (Pt1) and 20 weeks (Pts 1, 2 and 3). Also, the average lungs Pb concentration (±SE in mg/L) measured during the study ranged from 0.030±0.006 (12 weeks exposure) to 0.340±0.02 (24 weeks) for the control groups; and from 0.050±0.05 (12 weeks; Pt2) to 0.780±0.13 (16 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Pb concentrations in lungs showed a non significant but weak positive correlation with exposure periods (p=0.584; corr-coeff=0.106).

Cadmium (Cd) concentrations in lungs of exposed groups (Figure 4.22) were greater when compared to the corresponding control groups throughout the exposure periods and these were significant ($p<0.05$) at 20 weeks (Pts 2 and 3) and 24 weeks (Pt3). Also, the average lungs Cd concentration (±SE in mg/L) measured during the study ranged from 0.087±0.03 (24 weeks exposure) to 0.250±0.05 (16 weeks) for the control groups; and from 0.105±0.005 (24 weeks; Pt2) to 0.535±0.03 (20 weeks; Pt3) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cd concentrations in lungs showed an insignificant weak negative correlation with exposure periods (p=0.400; r=-0.160).

Chromium (Cr) concentrations in lungs of exposed groups (Figure 4.23) were greater when compared to the corresponding control groups throughout the exposure periods

[except 8 weeks (Pt1), 12 weeks (Pt2), 20 weeks (Pt2), and 24 weeks (Pts 1 and 3); which were lower] and this was significant ($p<0.05$) at 8 weeks (Pt3) alone. Also, the average lungs Cr concentration (±SE in mg/L) measured during the study ranged from 0.028±0.01 (16 weeks exposure) to 0.238±0.04 (12 weeks) for the control groups; and from 0.025±0.004 (8 weeks; Pt1) to 0.336±0.08 (12 weeks; Pt1) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cr concentrations in lungs showed a non significant but weak positive correlation with exposure periods ($p=0.031$; corr-coeff=0.394).

Copper (Cu) concentrations in lungs of exposed groups (Figure 4.24) were greater when compared to the corresponding control groups throughout the exposure periods except 24 weeks (Pt3; which was lower) and these were significant ($p<0.05$) at 16 weeks (Pts 2 and 3), 20 weeks (Pt2), and 24 weeks (Pts 1 and 2). Also, the average lungs Cu concentration (±SE in mg/L) measured during the study ranged from 0.0637±0.03 (12 weeks exposure) to 0.170±0.03 (20 weeks) for the control groups; and from 0.080±0.01 (12 weeks; Pt1) to 0.475±0.06 (20 weeks; Pt2) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Cu concentrations in lungs showed a non significant but weak positive correlation with exposure periods ($p=0.111$; corr-coeff=0.297).

Similarly, zinc (Zn) concentrations in lungs of exposed groups (Figure 4.25) were greater when compared to the corresponding control groups throughout the exposure periods except at 8 weeks (Pt1) and 24 weeks (Pt3) (which were lower) and these were significant ($p<0.05$) at 8 weeks (Pt2) and 20 weeks (Pt2).

Also, the average lungs Zn concentration (±SE in mg/L) measured during the study ranged from 0.500±0.13 (12 weeks exposure) to 0.780±0.09 (24 weeks) for the control groups; and from 0.600±0.06 (12 weeks; Pt1) to 6.980±5.42 (16 weeks; Pt3) for the exposed groups. The pooled data for rat exposed *in situ* to Olusosun landfill revealed that Zn concentrations in lungs showed a non significant but weak positive correlation with exposure periods ($p=0.840$; corr-coeff=0.038).

In addition, for the pooled data for rat exposed *in situ* to Olusosun landfill, with increasing exposure periods, Pb concentrations in lungs showed an insignificant weak positive correlation with lung Cd ($p=0.487$; corr-coeff=0.134) and lung Zn (p-

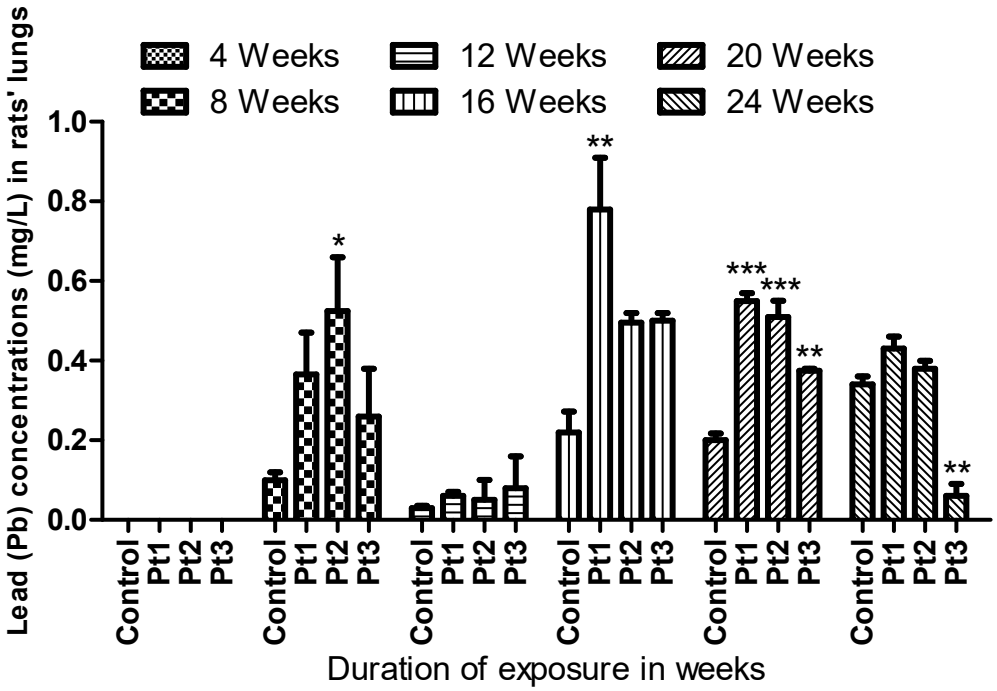

Figure 4.21: Lead (Pb) concentrations in the lungs of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

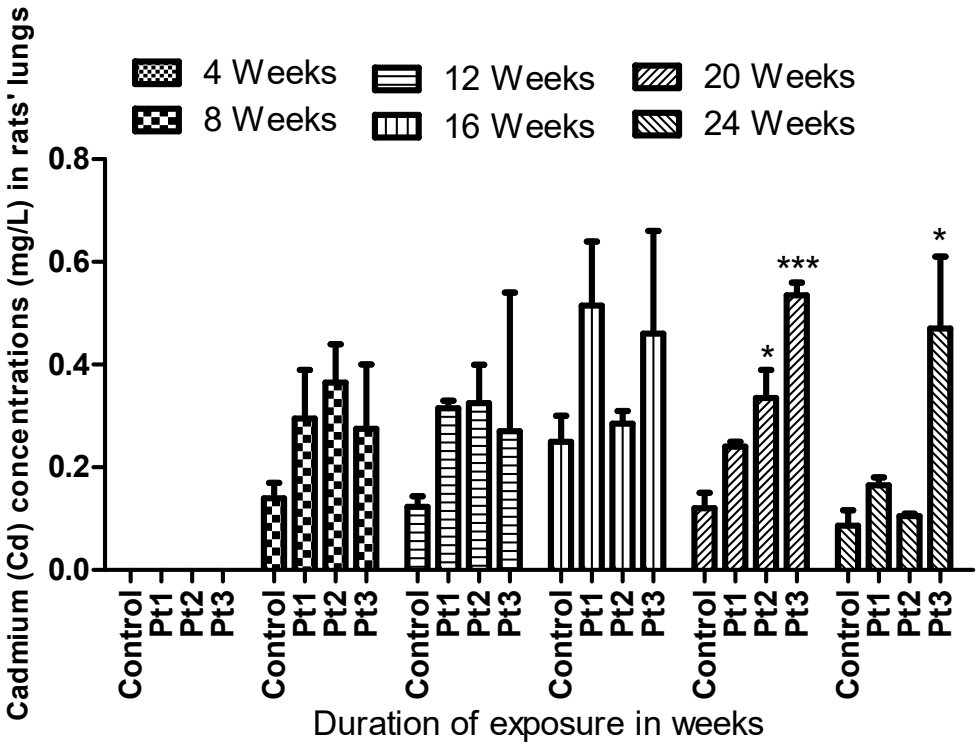

Figure 4.22: Cadmium (Cd) concentrations in the lungs of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

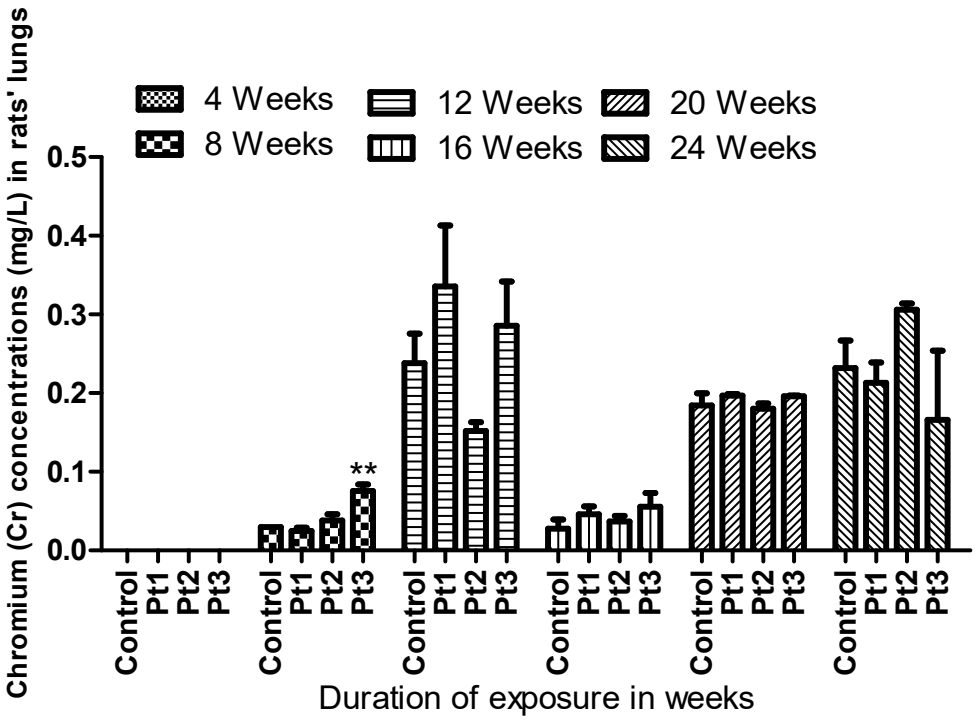

Figure 4.23: Chromium (Cr) concentrations in the lungs of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

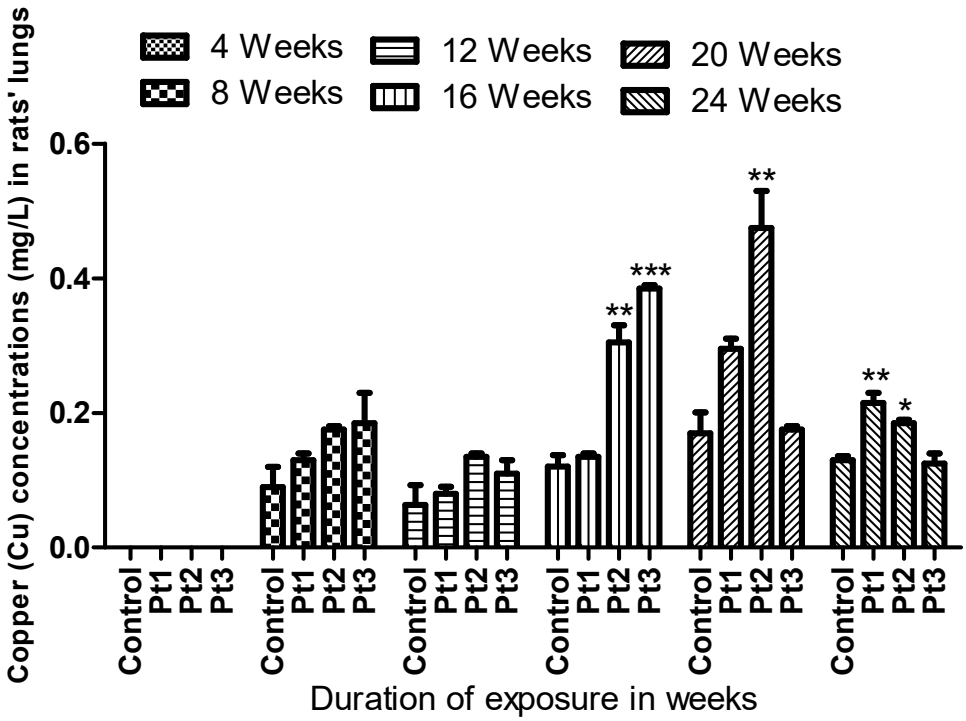

Figure 4.24: Copper (Cu) concentrations in the lungs of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

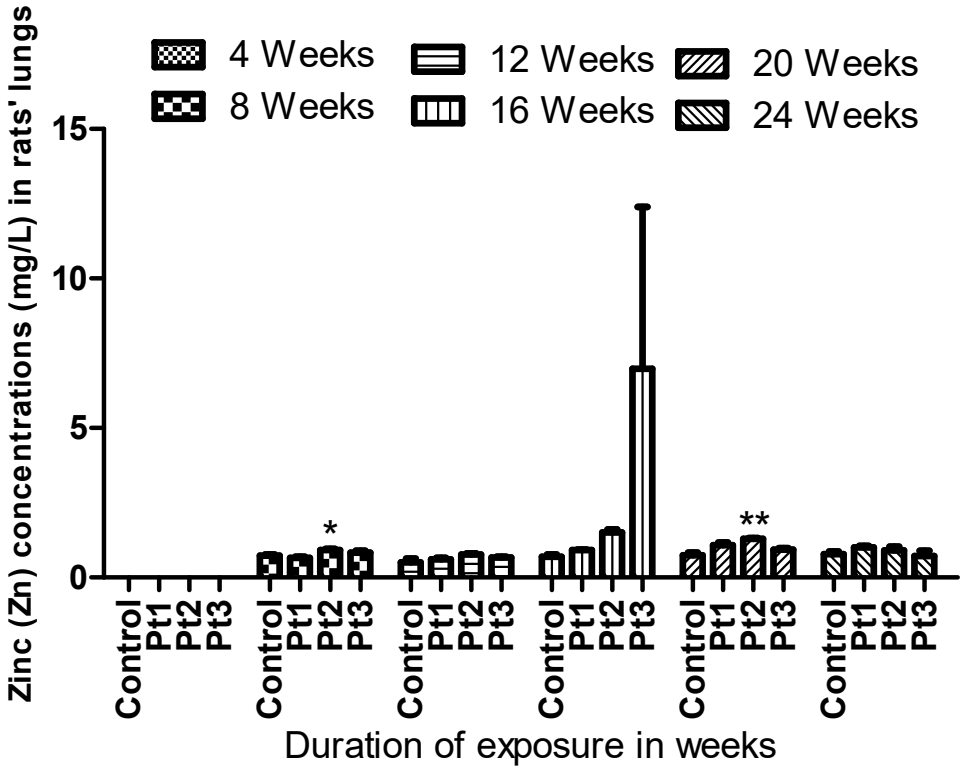

Figure 4.25: Zinc (Zn) concentrations in the lungs of rats exposed to borehole water and ambient air at OL for 4 - 24 weeks.

End points are mean (± standard error). Data are significantly different (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$) when likened to the corresponding control.

value=0.313; corr-coeff=0.194); significant strong positive correlation with lung Cu (p=0.011; corr-coeff=0.464); and a significant strong negative correlation with lung Cr (p=0.022; corr-coeff=-0.424). Also, Cd concentrations in lungs showed significant strong negative correlation with lung Cr (p=0.020; corr-coeff=-0.422); insignificant weak negative correlation with lung Cu (p=0.832; corr-coeff=-0.040); and a non significant but weak positive correlation with lung Zn (p=0.421; corr-coeff=0.152).

Cr concentrations in lungs showed an insignificant weak negative correlation with lung Cu (p=0.267; corr-coeff=-0.209) and lung Zn (p=0.345; corr-coeff=-0.178).

Finally, Cu concentrations in lungs showed significant and strong positive correlation with lung Zn (p=0.025; corr-coeff=0.409).

4.3.4.5 Correlation of metal concentrations in blood of rat with cytogenotoxic effects

Pearson's correlation analysis was used to measure the strength of the linear relationship for pooled data (combination of Pt 1, 2 and 3) between metal concentrations in blood of landfill exposed rats and corresponding cytogenotoxic effects.

Pb concentration (mg/L) in blood showed insignificant weak positive correlation with MNPCE (p=0.840; corr-coeff=0.036); and insignificant weak negative correlation with MNNCE (p=0.221; corr-coeff=-0.216) and PCE/NCE ratio (p=0.322; corr-coeff =-0.178).

Cd concentration (mg/L) in blood showed insignificant weak negative correlation with MNPCE (p=0.778; corr-coeff =-0.050), MNNCE (p=0.197; corr-coeff =-0.224) and PCE/NCE ratio (p=0.190; corr-coeff =-0.227).

Cr concentration (mg/L) in blood showed significant weak negative correlation with MNPCE (p=0.040; corr-coeff =-0.372), insignificant weak negative correlation with MNNCE (p=0.450; corr-coeff =-0.141) and significant weak positive correlation with PCE/NCE ratio (p=0.043; corr-coeff =0.366).

Cu concentration (mg/L) in blood showed insignificant weak positive correlation with MNPCE (p=0.314; corr-coeff =0.181), insignificant weak negative correlation with MNNCE (p=0.756; corr-coeff =-0.056) and significant strong negative correlation with PCE/NCE ratio (p=0.000; corr-coeff =-0.580).

Zn concentration (mg/L) in blood showed insignificant weak positive correlation with MNPCE (p=0.432; corr-coeff =0.135) and MNNCE (p=0.815; corr-coeff =0.040); and significant strong negative correlation with PCE/NCE ratio (p=0.003; corr-coeff =-0.479).

4.3.4.6 Correlation of absolute and relative organ weights with metal accumulation in organs

For the pooled data for rat exposed *in situ* to Olusosun landfill, there was positive correlations between absolute liver weight and concentrations of Pb (p=0.180; corr-coeff =0.229), Cu (p=0.077; corr-coeff=0.294) and Zn (p=0.043; corr-coeff =0.354) in the liver, however, this was only significant with concentration of Zn in the liver. There was insignificant negative correlations between absolute liver weight and concentrations of Cd (p=0.799; corr-coeff=-0.044) and Cr (p=0.931; corr-coeff=-0.019) in the liver. Also, relative liver weight showed negative correlations with Pb (p=0.356; corr-coeff=-0.159), Cd (p=0.806; corr-coeff=-0.042), Cr (p=0.281; corr-coeff=-0.230), Cu (p=0.075; corr-coeff=-0.296) and Zn (p=0.088; corr-coeff=-0.302), though not significant.

For the pooled data for rat exposed *in situ* to Olusosun landfill, there was positive correlations between absolute kidney weight and concentrations of Cu (p=0.013; corr-coeff=0.412) and Zn (p=0.183; corr-coeff=0.227) in the kidney, however, this was only significant with concentration of Cu in the kidney. There was negative correlation between absolute kidney weight and concentrations of Pb (p=0.041; corr-coeff=-0.343), Cd (p=0.566; corr-coeff=-0.099) and Cr (p=0.943; corr-coeff=-0.013) in the kidney; and this was significant only with Pb concentration. Also, relative kidney weight showed negative correlations with Pb (p=0.397; corr-coeff=-0.146), Cd (p=0.822; corr-coeff=-0.039), Cr (p=0.000; corr-coeff=-0.608), Cu (p=0.189; corr-coeff=-0.224) and Zn (p=0.386; corr-coeff=-0.149) concentrations in kidney, and this was only significant with Cr concentration.

For the pooled data for rat exposed *in situ* to Olusosun landfill, there was positive correlations between absolute lung weight and concentrations of Cr (p=0.241; corr-coeff=0.221) and Cu (p=0.492; corr-coeff=0.130) in the lungs, although not significant. There was negative correlation between absolute lung weight and

concentrations of Pb (p=0.660; corr-coeff=-0.085), Cd (p=0.066; corr-coeff=-0.340) and Zn (p=0.852; corr-coeff=-0.035) in the lungs; though not significant. Also, relative lung weight showed negative correlations with Pb (p=0.541; corr-coeff =-0.118), Cd (p=0.550; corr-coeff =-0.114), Cr (p=0.438; corr-coeff =-0.147), Cu (p=0.558; corr-coeff =-0.111) and Zn (p=0.515; corr-coeff =-0.124) concentrations in lung, but these were not significant.

4.3.7 Haematological alterations in rats exposed to landfill

Table 4.6 presents the effects of exposure of rats to air emissions and groundwater at three different points in Olusosun landfill on red blood cell (RBC) and RBC indices as compared to the corresponding negative control. In general, the mean red blood cell count (RBC), pack cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) observed throughout the study periods showed no significant difference ($p>0.05$) as compared to the values from the control rats. The only exception is in the 4 weeks exposure where there is consistent decrease in the RBC and RBC indices in the rats exposed to the landfill as compared to the control, however, the decrease were only significant ($p<0.05$) with rats from the Point 2 on the Olusosun dumpsite in their pack cell volume (PCV), haemoglobin concentrations (Hb) and mean corpuscular haemoglobin (MCH).

The white blood cells (WBCs), lymphocytes and neutrophils are presented in figures 4.26 to 4.28. There was a consistent increase in total leucocyte count (mm^{-3}) at all points (Pts 1, 2 and 3) after the 4, 8, 12 and 24 weeks exposure periods on Olusosun landfill (Figure 4.26). There was also increase in leucocyte count after the 20 weeks exposure at points 1 and 3. However, a significant ($p<0.05$) increase was only observed at 12 weeks (Pts 1, 2 and 3) and 24 weeks exposure (Pt2). Sixteen (16) weeks exposure (Pts 1, 2 and 3) and 20 weeks exposure (Pt2) showed an insignificant ($p>0.05$) decrease in leucocyte count; all as compared to their corresponding control groups.

Percentage lymphocyte (Figure 4.27) followed almost similar trend with leucocyte counts. There was a consistent increase in lymphocyte (%) at all points (Pts 1, 2 and 3) after the 4, 8, 12 and 24 weeks exposure periods. There was also increase in

Table 4.6: Alterations in haematological parameters in rats exposed to borehole water and ambient air at OL

Sample	RBC (x 10^6/L)	PCV (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
			4 Weeks exposure			
Cn	5.26 (0.06)	45 (0.58)	15.95 (0.22)	85.63 (0.21)	30.35 (0.01)	35.45 (0.03)
Pt1	4.88 (0.24)	37 (5.0)	12.79 (1.13)	75.50 (6.53)	26.18 (1.05)	34.78 (1.66)
Pt2	3.74 (0.83)	22 (10.0)*	7.31 (3.18)*	55.71 (14.47)	18.60 (4.41)*	33.56 (0.87)
Pt3	5.06 (0.00)	42.5 (1.50)	14.50 (0.92)	84.00 (2.97)	28.65 (1.81)	34.07 (0.95)
			8 Weeks exposure			
Cn	4.37 (0.43)	38.5(2.60)	12.79 (0.84)	88.95 (2.87)	29.55 (1.00)	33.22 (0.06)
Pt1	5.19 (0.03)	43.5 (0.50)	14.27 (0.17)	83.81 (0.48)	27.49 (0.16)	32.79 (0.00)
Pt2	4.51 (0.92)	38.5 (8.50)	13.20 (3.36)	85.14 (1.58)	28.98 (1.57)	34.02 (1.22)
Pt3	5.34 (0.03)	45.0 (0.00)	15.33 (0.43)	84.27 (0.48)	28.70 (0.64)	34.06 (0.95)
			12 Weeks exposure			
Cn	4.97 (0.03)	47.5 (0.29)	16.75 (0.08)	95.67 (0.08)	33.73 (0.009)	35.26 (0.04)
Pt1	5.45 (0.04)**	52.0 (1.00)	17.79 (0.58)	95.49 (1.22)	32.67 (0.86)	34.21 (0.46)
Pt2	5.45 (0.15)**	52.5 (2.50)	18.12 (0.57)	96.37 (2.56)	33.27 (0.34)	34.53 (0.57)
Pt3	5.45 (0.04)**	51.5 (0.50)	17.61 (0.56)	94.34 (1.61)	32.25 (0.79)	34.21 (1.42)
			16 Weeks exposure			
Cn	5.18 (0.05)	47.0 (1.53)	16.43 (0.60)	90.78 (3.53)	31.74 (1.44)	34.96 (1.01)
Pt1	5.12 (0.11)	44.5 (0.50)	14.66 (0.09)	87.02 (0.81)	28.67 (0.41)	32.95 (0.15)
Pt2	5.05 (0.03)	47.5 (0.50)	15.78 (0.22)	94.06 (0.43)	31.25 (0.26)	33.22 (0.11)
Pt3	5.14 (0.02)	49.0 (1.00)	16.23 (0.34)	95.33 (1.58)	31.57 (0.53)	33.11 (0.01)
			20 Weeks exposure			
Cn	5.17 (0.03)	51.0 (1.16)	17.48 (0.69)	98.65 (2.35)	33.81 (1.34)	34.22 (0.60)
Pt1	5.17 (0.04)	47.0 (2.00)	16.12 (0.22)	90.98 (3.26)	31.20 (0.21)	33.33 (0.00)
Pt2	5.22 (0.05)	48.5 (2.50)	17.11 (0.78)	92.97 (4.00)	32.80 (1.21)	35.29 (0.21)
Pt3	5.12 (0.11)	49.5 (4.50)	17.32 (1.57)	96.63 (6.81)	33.80 (2.37)	34.98 (0.02)
			24 Weeks exposure			
Cn	5.0 (0.06)	48.0 (1.53)	16.37(0.22)	96.21 (2.08)	32.75 (0.41)	34.16 (0.81)
Pt1	5.14 (0.02)	50.5 (0.50)	17.85 (0.05)**	98.24 (0.59)	34.72 (0.05)	35.30 (0.30)
Pt2	5.14 (0.03)	52.5 (0.50)	17.21 (0.16)*	102.2 (1.57)	33.49 (0.51)	32.78 (0.01)
Pt3	5.17 (0.19)	48.5 (0.50)	16.88 (0.15)	93.99 (2.4)	32.70 (0.89)	34.79 (0.06)

End points are mean (± SE) values. Values are significantly different from control at: *P<0.05, **P<0.01 and ***P<0.001. Cn: Control rat samples exposed to a residential area at Olodi-Apapa, Lagos. Pt1, Pt2 and Pt3 are rat samples exposed to Point 1, Point 2 and Point 3 on Olusosun landfill site.

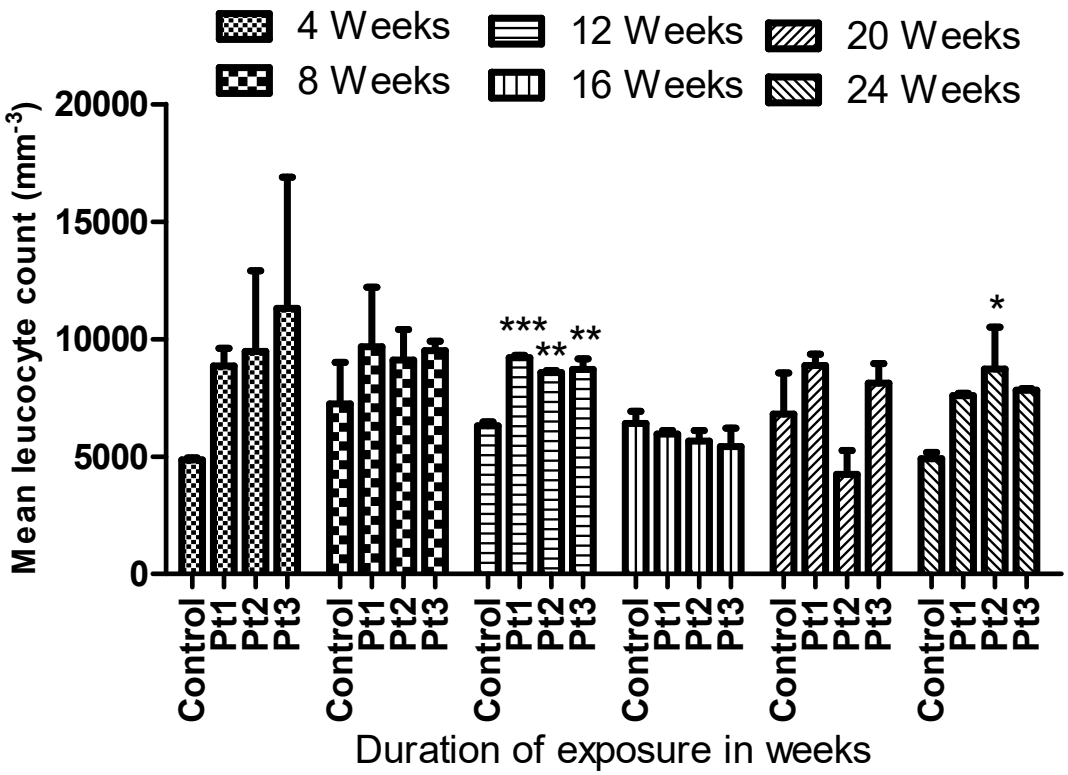

Figure 4.26: Effects of exposure to borehole water and ambient air at OL on rats' white blood cell (leucocyte) count as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05, **p<0.01 and ***p<0.001.

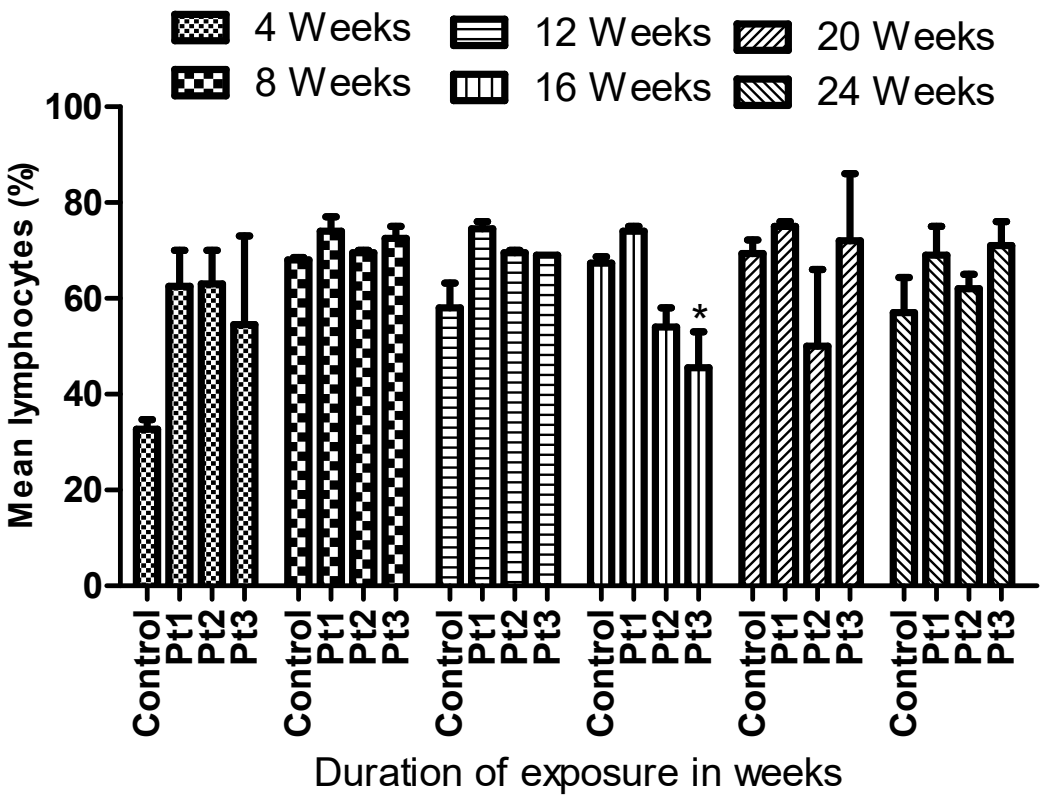

Figure 4.27: Effects of exposure to borehole water and ambient air at OL on rats' lymphocytes as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05, **p<0.01 and ***p<0.001.

lymphocytes after 16 weeks exposure (Pt1) and 20 weeks exposure at points 1 and 3. However, these increases in lymphocytes were not significant ($p>0.05$). Sixteen (16) weeks exposure (Pts 2 and 3) and 20 weeks exposure (Pt2) showed decreases in lymphocyte (%) and this was only significant ($p<0.05$) at 16 weeks exposure (Pt3); all as compared to their corresponding control groups.

Neutrophils (%) showed a complete inverse relationship with lymphocytes (%) (Figure 4.28). There was a consistent decrease in neutrophil at all points (Pts 1, 2 and 3) after the 4, 8, 12 and 24 weeks exposure periods. There was also decrease in neutrophils after 16 weeks exposure (Pt1) and 20 weeks exposure at points 1 and 3. Sixteen (16) weeks exposure (Pts 2 and 3) and 20 weeks exposures (Pt2) showed increases in neutrophils and this was only significant ($p<0.05$) at 16 weeks exposure (Pt3); all as compared to their corresponding control groups.

4.3.7.1 Correlation of exposure periods, metal concentration in blood and haematological alterations

The pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods indicated positive correlation with PCV ($p=0.000$; corr-coeff=0.651), RBC ($p=0.231$; corr-coeff=0.205), Hb ($p=0.000$; corr-coeff=0.627), MCV ($p=0.000$; corr-coeff=0.780), MCH ($p=0.000$; corr-coeff=0.731), MCHC ($p=0.553$; corr-coeff=0.102) and lymphocyte ($p=0.914$; corr-coeff=0.019); and this was significant ($p<0.001$) with PCV, Hb, MCV and MCH. Also, exposure periods indicated negative correlation with WBC ($p=0.006$; corr-coeff=-0.450) and Neutrophil ($p=0.926$; corr-coeff=-0.016); and this was significant ($p<0.05$) with WBC.

Generally, in this study, with increasing exposure periods, RBC showed positive correlations with PCV ($p=0.000$; corr-coeff=0.731), Hb ($p=0.000$; corr-coeff=0.757), MCV ($p=0.009$; corr-coeff=0.429), MCH ($p=0.017$; corr-coeff=0.397), MCHC ($p=0.563$; corr-coeff=0.100), WBC ($p=0.840$; corr-coeff=0.035) and lymphocyte ($p=0.849$; corr-coeff=0.033); and these were significant ($p<0.05$) with PCV, Hb, MCV and MCH. However, RBC showed insignificant negative correlation with neutrophil ($p=0.735$; corr-coeff=-0.059).

Also, with increasing exposure periods, WBC showed negative correlations with PCV ($p=0.048$; corr-coeff=-0.332), Hb ($p=0.334$; corr-coeff=-0.166), MCV ($p=0.010$; corr-

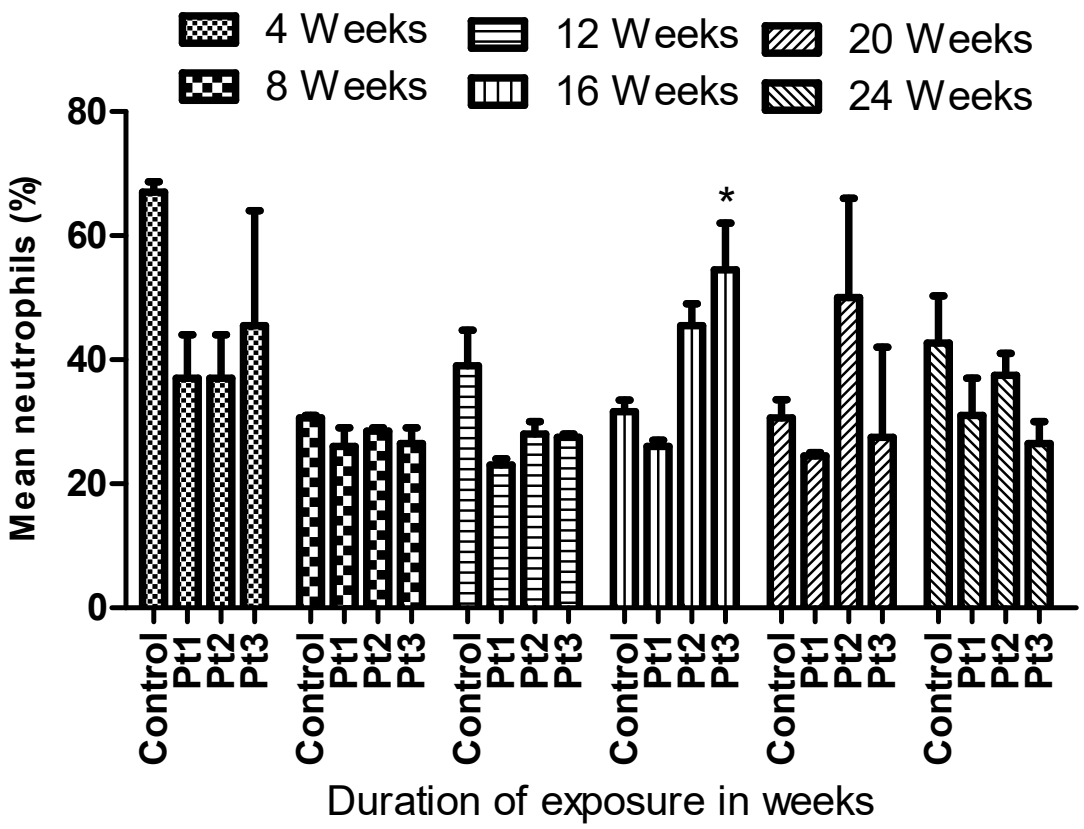

Figure 4.28: Effects of exposure to borehole water and ambient air at OL on rats' neutrophils as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05, **p<0.01 and ***p<0.001.

coeff=-0.426), MCH (p=0.035; corr-coeff=-0.353), MCHC (p=0.866; corr-coeff=-0.029) and neutrophil (p=0.717; corr-coeff=-0.063); these were significant (p<0.05) with PCV, MCV and MCH. However, WBC also showed insignificant positive correlation with lymphocyte (p=0.744; corr-coeff=0.056).

Lead (Pb) concentrations observed in blood of landfill exposed rats showed a negative correlation with PCV (p=0.296; corr-coeff=-0.188), RBC (p=0.178; corr-coeff=-0.240), Hb (p=0.168; corr-coeff=-0.246), MCV (p=0.590; corr-coeff=-0.097), MCH (p=0.737; corr-coeff=-0.061), MCHC (p=0.023; corr-coeff=-0.396), WBC (p=0.081; corr-coeff=-0.308) and lymphocyte (p=0.953; corr-coeff=-0.011); and this was only significant (p<0.05) with MCHC. However, Pb concentrations in blood showed an insignificant positive correlation with neutrophil (p=0.696; corr-coeff=0.071).

Cadmium (Cd) concentrations observed in blood of landfill exposed rats showed a negative correlation with PCV (p=0.930; corr-coeff=-0.015), RBC (p=0.550; corr-coeff=-0.104), Hb (p=0.691; corr-coeff=-0.070), MCHC (p=0.189; corr-coeff=-0.227), WBC (p=0.040; corr-coeff=-0.350) and lymphocyte (p=0.572; corr-coeff=-0.099); and this was only significant (p<0.05) with WBC count. However, Cd concentrations in blood showed insignificant positive correlations with MCV (p=0.747; corr-coeff=0.057), MCH (p=0.595; corr-coeff=0.093) and neutrophil (p=0.470; corr-coeff=0.126).

Chromium (Cr) concentrations observed in blood of landfill exposed rats showed insignificant negative correlations with MCV (p=0.960; corr-coeff=-0.009), MCH (p=0.974; corr-coeff=-0.006) and neutrophil (p=0.222; corr-coeff=-0.226). However, insignificant positive correlations were observed with PCV (p=0.573; corr-coeff=0.105), RBC (p=0.081; corr-coeff=0.318), Hb (p=0.435; corr-coeff=0.145), MCHC (p=0.405; corr-coeff=-0.155), WBC (p=0.098; corr-coeff=0.302) and lymphocyte (p=0.379; corr-coeff=0.164).

Copper (Cu) concentrations observed in blood of landfill exposed rats showed positive correlations with PCV (p=0.058; corr-coeff=0.333), RBC (p=0.708; corr-coeff=0.068), Hb (p=0.073; corr-coeff=0.317), MCV (p=0.014; corr-coeff=0.424), MCH (p=0.017; corr-coeff=0.412) and neutrophil (p=0.097; corr-coeff=0.294); and these were significant (p<0.05) with MCV and MCH. However, negative correlations were

observed with MCHC (p=0.605; corr-coeff=-0.093), WBC (p=0.000; corr-coeff=-0.575) and lymphocyte (p=0.136; corr-coeff=-0.265); and this was significant (p<0.001) with WBC.

Zinc (Zn) concentrations observed in blood of landfill exposed rats showed insignificant positive correlation with PCV (p=0.202; corr-coeff=0.218), RBC (p=0.744; corr-coeff=0.056), Hb (p=0.195; corr-coeff=0.221), MCV (p=0.110; corr-coeff=0.271), MCH (p=0.070; corr-coeff=0.306) and neutrophil (p=0.353; corr-coeff=0.159). However, negative correlation were observed with MCHC (p=0.715; corr-coeff=-0.063), WBC (p=0.033; corr-coeff=-0.356) and lymphocyte (p=0.489; corr-coeff=-0.119); and this was only significant (p<0.05) with WBC count.

4.3.5 Serum biochemical changes in rats that were exposed on the landfill

The results of serum clinical biochemical markers of hepatic and renal damage in rats that were exposed to borehole water and ambient air at OL are presented in Table 4.7. Aspartate aminotransferase (AST) activities increased in all exposed groups throughout the periods of exposure, and these were significant (p<0.05) at 4 weeks (Pt1), 8, 12, 16, 20 and 24 weeks exposures (Pts 1, 2 and 3), as compared to their corresponding control.

Alanine aminotransferase (ALT) activities increased in all exposed groups throughout the periods of exposure (except 4 weeks, Pts 2 and 3; where it decreased), and these were significant (p<0.05) at 12 weeks (Pts 2 and 3), 16 weeks (Pts 1, 2 and 3), 20 weeks (Pt2), and 24 weeks exposures (Pt3), as compared to their corresponding control.

Alkaline phosphatase (ALP) activities increased in all exposed groups throughout the periods of exposure (except 4 weeks, Pt1; where it decreased), and these were significant (p<0.05) at 8 weeks (Pts 1, 2 and 3), 12 weeks (Pts 1 and 2), 16 weeks (Pts 1, 2 and 3), and 24 weeks exposures (Pt3), as compared to their corresponding control.

Creatinine (Crea) increased in all exposed groups throughout the periods of exposure and these were significant (p<0.05) at 4 weeks (Pt3), 8 and 12 weeks (Pts 1, 2 and 3), 16 weeks (Pt1), 20 weeks (Pts 1, 2 and 3) and 24 weeks exposures (Pts 1 and 2), as compared to their corresponding control.

Similarly, urea increased in all exposed groups throughout the periods of exposure and these were significant ($p<0.05$) at 16 weeks (Pts 1, 2 and 3), 20 weeks (Pt3), and 24 weeks exposures (Pts 1, 2 and 3), as compared to their corresponding control.

Total protein (TP) decreased at most points and periods of exposure and these decrease were significant ($p<0.05$) at 16 weeks (Pt3) and 24 weeks exposures (Pt1), as compared to their corresponding control.

Albumin (ALB) decreased at most points and periods of exposure, however, these decrease were not significant ($p>0.05$), as compared to their corresponding control.

Furthermore, lactate dehydrogenase (LDH) activities increased in all exposed groups throughout the periods of exposure and these were statistically significant ($p<0.05$) at 4 and 8 weeks (Pts 2 and 3), 12 weeks (Pts 1, 2 and 3), 16 weeks (Pts 2 and 3), and 24 weeks exposures (Pts 1 and 3), as compared to their corresponding control.

4.3.5.1 Correlation of exposure periods, metal accumulation in liver and liver function parameters

For the pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods showed insignificant positive correlation with serum AST activities ($p=0.113$; corr-coeff=0.239).

Also, AST activities showed positive correlations with Pb ($p=0.128$; corr-coeff =0.258), Cr ($p=0.021$; corr-coeff =0.468) and Cu ($p=0.372$; corr-coeff =0.151) concentrations in the liver, and this was only significant ($p<0.05$) with Cr concentration in liver. However, AST activities showed insignificant ($p>0.05$) negative correlations with Cd ($p=0.642$; corr-coeff =-0.080) and Zn ($p=0.916$; corr-coeff=-0.019) concentrations in the liver.

The combined data of rat exposed *in situ* to Olusosun landfill, exposure periods showed insignificant negative correlation with serum ALT activities ($p=0.793$; corr-coeff =-0.040). Also, ALT activities showed significant positive correlations with Cr ($p=0.021$; corr-coeff =0.468) concentration in the liver and insignificant negative correlation with Pb ($p=0.628$; corr-coeff =-0.084), Cd ($p=0.974$; corr-coeff=-0.006), Cu ($p=0.578$; corr-coeff =-0.094) and Zn ($p=0.182$; corr-coeff =-0.238) concentrations in the liver.

Table 4.7: Alterations in serum biochemical markers of liver and kidney functions in landfill exposed rats

Sampling point	AST (U/I)	ALT (U/I)	ALP (U/I)	LDH (U/I)	TP (g/l)	ALB (g/dl)	Crea (mg/dl)	Urea (mg/dl)
				4 Weeks exposure				
Cn	31.85 (9.68)	33.75 (4.38)	107.60 (30.53)	117.30 (26.61)	76.56 (11.45)	4.06 (0.19)	1.39 (0.12)	31.55 (4.91)
Pt1	92.28 (1.79)*	36.27 (3.55)	79.12 (47.65)	232.00 (33.43)	79.31 (9.90)	4.16 (0.09)	1.66 (0.10)	37.53 (3.66)
Pt2	69.51 (22.12)	29.60 (5.93)	133.90 (73.14)	367.10 (122.40)*	80.80 (15.96)	4.43 (0.12)	1.85 (0.03)	31.73 (8.88)
Pt3	66.39 (25.76)	29.73 (1.39)	245.60 (22.08)	485.60 (19.12)**	63.10 (21.20)	4.08 (0.09)	1.98 (0.10)*	33.00 (8.34)
				8 Weeks exposure				
Cn	52.01 (6.52)	46.28 (1.74)	90.16 (4.87)	165.70 (6.74)	30.64 (7.09)	3.23 (0.04)	0.59 (0.08)	31.77 (0.63)
Pt1	138.90 (5.87)**	53.80 (5.93)	120.50 (2.43)*	203.90 (5.10)	28.41 (4.17)	3.31 (0.21)	1.20 (0.04)**	35.33 (0.32)
Pt2	144.50 (24.04)**	45.34 (1.46)	170.20 (11.31)***	262.50 (18.38)**	35.48 (7.69)	3.43 (0.41)	1.59 (0.13)***	34.24 (0.44)
Pt3	144.10 (5.06)**	54.70 (7.99)	144.40 (7.53)**	244.70 (13.24)**	33.45 (6.92)	3.55 (0.11)	1.17 (0.12)**	34.18 (3.92)
				12 Weeks exposure				
Cn	41.00 (4.04)	18.98 (0.80)	77.28 (8.87)	229.40 (20.23)	72.41 (0.21)	2.39 (0.10)	0.66 (0.08)	26.15 (6.62)
Pt1	144.20 (7.59)**	41.64 (10.36)	150.40 (4.14)*	523.80 (80.29)**	80.58 (6.03)	2.43 (0.20)	1.07 (0.08)*	32.42 (3.05)
Pt2	146.10 (16.23)**	82.18 (11.08)**	190.40 (22.08)**	638.50 (19.12)**	71.17 (2.31)	2.22 (0.05)	1.19 (0.04)**	49.08 (4.14)
Pt3	132.20 (22.25)**	53.91 (5.72)*	120.10 (4.14)	600.30 (11.47)**	70.64 (3.55)	2.17 (0.08)	1.26 (0.00)**	41.00 (0.39)
				16 Weeks exposure				
Cn	27.01 (9.59)	17.78 (1.97)	103.20 (4.98)	229.40 (7.65)	46.31 (2.40)	3.79 (0.14)	0.41 (0.05)	27.57 (0.70)
Pt1	118.70 (6.30)***	39.97 (3.81)***	136.20 (0.92)**	288.00 (6.74)	45.92 (3.71)	4.19 (0.12)	0.86 (0.12)**	49.97 (2.39)***
Pt2	122.10 (3.83)***	34.95 (2.53)**	124.20 (4.22)*	331.40 (29.40)**	28.95 (7.26)	3.66 (0.22)	0.59 (0.05)	42.56 (3.73)**
Pt3	170.40 (17.54)***	43.95 (3.53)***	122.80 (1.38)*	321.20 (30.59)*	22.24 (0.31)*	3.22 (0.05)	0.63 (0.05)	43.05 (0.36)*
				20 Weeks exposure				
Cn	42.02 (9.83)	23.30 (0.61)	93.84 (4.09)	252.40 (13.02)	24.28 (0.68)	3.69 (0.14)	0.65 (0.03)	34.23 (0.73)
Pt1	126.50 (4.73)**	43.40 (15.25)	102.10 (16.79)	267.60 (4.42)	30.60 (7.85)	3.20 (0.07)	0.94 (0.09)*	46.72 (2.75)
Pt2	131.10 (0.79)**	66.01 (3.48)*	124.20 (8.28)	279.10 (11.47)	34.38 (14.93)	3.18 (0.15)	1.10 (0.12)**	36.99 (1.18)
Pt3	125.80 (18.21)**	41.21 (6.13)	104.90 (4.78)	282.90 (57.73)	62.24 (5.53)*	3.21 (0.11)	1.39 (0.09)***	36.81 (3.58)**
				24 Weeks exposure				
Cn	60.15 (4.48)	19.55 (0.55)	75.62 (5.42)	186.10 (6.74)	77.91 (1.63)	3.50 (0.46)	0.81 (0.07)	37.98 (1.56)
Pt1	115.20 (9.22)**	30.24 (6.56)	103.00 (9.34)	303.30 (22.66)**	64.64 (2.31)**	3.30 (0.37)	1.26 (0.05)*	59.90 (3.81)***
Pt2	125.70 (10.47)**	34.42 (8.25)	99.36 (8.28)	240.90 (11.47)	71.19 (2.63)	3.44 (0.39)	1.48 (0.23)**	52.77 (1.85)*
Pt3	125.40 (4.45)**	47.24 (3.38)*	151.80 (27.60)**	604.10 (30.59)***	70.99 (3.55)	2.32 (0.10)	1.19 (0.12)	59.03 (4.24)**

End points are mean (± SE) values. Values are significantly different from corresponding control at: *p<0.05, **p<0.01 and ***p<0.001. Cn = control point

The pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods showed insignificant negative correlation with serum ALP activities (p=0.051; corr-coeff =-0.293). Also, ALP activities showed significant positive correlations with Cr (p=0.005; corr-coeff=0.551) concentration in the liver and insignificant negative correlation with Pb (p=0.281; corr-coeff =-0.185), Cd (p=0.591; corr-coeff =-0.093), Cu (p=0.134; corr-coeff=-0.251) and Zn (p=0.073; corr-coeff =-0.316) concentrations in the liver.
Similarly, exposure periods showed insignificant positive correlation with pooled data for serum LDH activities (p=0.867; corr-coeff=0.026). Also, LDH activities showed significant positive correlations with Cr (p=0.001; corr-coeff=0.652) concentration in the liver and negative correlation with Pb (p=0.002; corr-coeff=-0.507), Cd (p=0.001; corr-coeff=-0.540), Cu (p=0.897; corr-coeff=-0.022) and Zn (p=0.290; corr-coeff=-0.190) concentrations in the liver; which were significant (p<0.01) with Pb and Cd.

The pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods showed significant negative correlation with serum albumin (ALB) levels (p=0.017; corr-coeff=-0.355). Also, ALB levels showed negative correlations with Cr (p=0.001; corr-coeff=-0.641), Cu (p=0.297; corr-coeff=-0.176) and Zn (p=0.395; corr-coeff=-0.153) concentrations in the liver; and this was only significant with Cr. However, ALB levels showed positive correlation with Pb (p=0.354; corr-coeff=0.159) and Cd (p=0.007; corr-coeff=0.442) concentrations in the liver; and this was only significant with Cd.
Exposure periods showed insignificant negative correlation with the pooled data for serum total protein (TP) levels (p=0.711; corr-coeff=-0.057). Also, TP levels showed negative correlations with Pb (p=0.000; corr-coeff=-0.578), Cd (p=0.003; corr-coeff=-0.482) and Cu (p=0.860; corr-coeff=-0.030) concentration in the liver; and this were significant with Pb and Cd. However, TP levels showed insignificant positive correlation with Cr (p=0.129; corr-coeff=0.319) and Zn (p=0.447; corr-coeff=0.137) concentrations in the liver.

4.3.5.2 Correlation of exposure periods, metal accumulation in kidney and kidney function parameters

For the combined data of rat exposed *in situ* to Olusosun landfill, exposure periods showed significant negative correlation with serum creatinine (CREA) levels (p=0.004; corr-coeff=-0.430). Also, CREA levels showed negative correlations with

Pb (p=0.582; corr-coeff=-0.095), Cd (p=0.481; corr-coeff=-0.121), Cr (p=0.262; corr-coeff=-0.201), Cu (p=0.118; corr-coeff=-0.265) and Zn (p=0.003; corr-coeff=-0.484) concentrations in the kidney; and this was only significant (p<0.01) with Zn.

The pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods showed significant positive correlation with serum urea (UREA) levels (p=0.000; corr-coeff=0.677). Also, UREA levels showed positive correlations with Cr (p=0.309; corr-coeff=0.183), Cu (p=0.036; corr-coeff=0.350) and Zn (p=0.102; corr-coeff=0.277) concentrations in the kidney; and this was only significant with Cu. However, UREA levels showed insignificant negative correlation with Pb (p=0.078; corr-coeff=-0.298) and Cd (p=0.556; corr-coeff=-0.102) concentrations in the kidney.

LDH activities showed negative correlation with Pb (p=0.635; corr-coeff=-0.082), Cd (p=0.482; corr-coeff=-0.121), Cu (p=0.003; corr-coeff=-0.475) and Zn (p=0.106; corr-coeff=-0.274) concentrations in the kidney; and this was significant with Cu. However, LDH activities showed significant positive correlation with Cr concentrations in the kidney (p=0.003; corr-coeff=0.494).

4.3.6 Oxidative damage and lipid peroxidation in rats' liver that were exposed to landfill

The level of antioxidant enzymes and lipid peroxidation (MDA levels) measured in liver of rats exposed to borehole water and ambient air at OL and the control site are presented in Table 4.8. There was increase in CAT activities in the liver of rats exposed at the three points on the Olusosun landfill and at all exposure periods. The increases in CAT activities were significant (p<0.05) with rat exposed for 8 weeks (Pt 2), 12 weeks (Pts 2 and 3), 16 weeks (Pts 2 and 3), 20 and 24 weeks (Pts 1, 2 and 3) as compared to their corresponding controls. However, SOD activities decreased significantly (p<0.05) at most points and periods of exposure [this includes 4 weeks (Pts 1 and 2), 12 weeks (Pts 2 and 3), 16 weeks (Pts 1 and 2), and at 20 and 24 weeks (Pts 1, 2 and 3)] as compared to their corresponding control. GSH levels increased throughout the study periods (except at 16 weeks: Pts 1 and 2) and these were significant (p<0.05) at 12 weeks (Pts 2 and 3), 20 and 24 weeks exposures (Pts 1, 2 and 3) as compared to their corresponding controls. Also, MDA levels increased throughout the exposure periods and were significant (p<0.05) in rats exposed for 8

Table 4.8: Oxidative damage and lipid peroxidation in liver of landfill exposed rats

Duration of Exposure (Weeks)	Sampling point	Total Protein (g/L)	CAT (CAT/min/mg-protein)	SOD (SOD/min/mg-protein)	GSH (nmol/mL)	MDA (nmol/mL)
4	Cn	53.49±4.13	1.74±0.076	3.11±0.07	36.51±0.58	4.76±0.32
	Pt1	64.05±7.70	2.07±0.216	2.22±0.30*	42.79±5.59	6.06±0.28
	Pt2	62.77±3.82	2.00±0.093	2.25±0.22*	38.05±0.66	5.65±0.81
	Pt3	55.68±2.89	2.15±0.224	2.65±0.08	55.92 ±8.61	4.53±0.86
8	Cn	44.76±5.16	0.42±0.089	3.14±0.36	37.39±1.49	8.21±0.74
	Pt1	55.31±2.21	1.10±0.080	2.19±0.29	47.43±9.83	18.08±2.40**
	Pt2	41.85±4.56	1.34±0.220*	2.41±0.36	54.71±13.70	19.25±1.48**
	Pt3	40.76±4.77	0.81±0.326	2.46±0.56	40.81±2.30	10.86±1.56
12	Cn	46.45±0.42	2.21±0.65	4.92±0.30	33.20±3.22	3.68±1.26
	Pt1	44.96±0.00*	5.67±1.03	3.57±0.50	40.04±0.99	9.56±0.67*
	Pt2	44.55±0.14*	8.56±1.63*	2.47±0.20**	46.49±1.82*	11.71±0.14**
	Pt3	44.69±0.27*	6.87±0.10*	2.00±0.16**	45.99±2.65*	9.02±0.94*
16	Cn	46.72±1.06	1.41±0.380	3.26±0.12	42.09±2.98	7.16±0.41
	Pt1	53.86±2.84	2.32±0.087	2.47±0.26*	40.04±1.38	10.72±0.66**
	Pt2	42.94±5.10	3.19±0.425*	2.11±0.25**	40.70±2.65	11.85±0.47***
	Pt3	44.21±4.91	3.23±0.317*	2.73±0.22	45.00±11.58	11.38±0.88**
20	Cn	51.74±2.99	2.37±0.09	4.10±0.42	35.21±0.63	5.95±0.66
	Pt1	50.22±2.75	3.68±0.14*	2.32±0.27*	55.04±6.13**	11.89±0.32***
	Pt2	47.49±0.55	4.14±0.51*	2.25±0.46*	50.96±7.28*	11.78±1.01**
	Pt3	45.55±0.91	7.70±0.62***	0.70±0.16***	48.75±0.96*	11.08±1.09**
24	Cn	45.47±1.06	3.50±0.66	5.84±0.29	35.47±0.74	5.63±0.66
	Pt1	45.14±0.76	13.76±1.85***	4.51±0.27*	45.99±1.91***	9.69±0.96*
	Pt2	45.64±0.54	9.58±0.15*	4.12±0.40*	47.32±0.66***	9.36±0.47*
	Pt3	43.67±0.34	12.56±0.16**	2.30±0.58***	45.50±1.82**	9.69±1.08*

End points represents mean± SE. Values are significantly different (* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$) compared to control. Cn = Control, Pt1, Pt2 and Pt3 are Point 1, Point 2 and Point 3 respectively on Olusosun landfill, Ojota, Lagos State.

weeks (Pts 1 and 2) and 12, 16, 20 and 24 weeks (Pts 1, 2 and 3) as compared to their corresponding controls.

4.3.8 Gastric physiopathological alterations in rats exposed on the landfill

Figure 4.29, 4.31 and 4.33 present the results for gastric physiopathological indices for rats exposed at three points (Pt1, Pt2 and Pt3) to borehole water and ambient air at OL and corresponding controls for 4, 8, 12, 16, 20 and 24 weeks exposure periods and figure 4.30, 4.32 and 4.34; and table 4.9 present the mean data for the three points at Olusosun landfill as compared to the corresponding control (at Olodi-Apapa).

Alterations in parietal cell counts

Parietal cell counts (cells/HPF) of rats exposed to the landfill at the three points (Pts 1, 2 and 3) increase throughout the exposure periods (Figure 4.29), and these increases were significant ($p<0.05$) with rats exposed for 4 weeks (Pts 1 and 3), 16 weeks (Pt2) and 20 weeks (Pts 1 and 2) as compared to the corresponding controls. Also, the combined data (Figure 4.30 and Table 4.9) showed that the average parietal cell count (cells/HPF) were higher with rats exposed to Olusosun landfill at each exposure periods as compared to their corresponding control set at Olodi-Apapa. Student t-test showed that, these were significant ($p<0.05$) at 4, 8 and 24 weeks exposures.

Alterations in mucous cell counts

Mucous cell counts (cells/HPF) significantly ($p<0.05$) increased in rats' stomach exposed for 4 weeks (Pts 1, 2 and 3), but subsequently decreased in rats exposed for 8 and 12 weeks (Pts 1, 2 and 3), 16 and 20 weeks (Pts 1 and 2) and these decrease were significant ($p<0.05$) at 8 weeks exposure (Pts 1, 2 and 3), 12 weeks (Pts 1 and 2), and 20 weeks (Pt1). This was followed with increases in mucous cell counts in rats exposed for 24 weeks and these increases were significant ($p<0.05$) with Pts 2 and 3 rats; as compared to the corresponding controls (Figure 4.31).

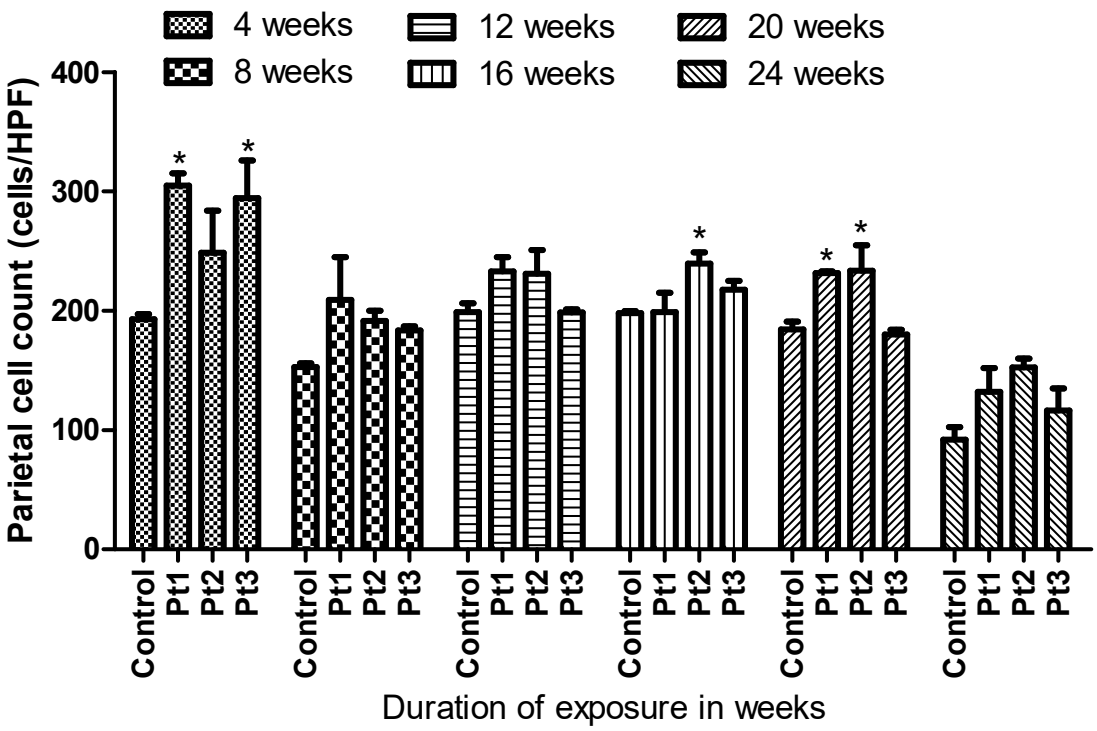

Figure 4.29: Effects of exposure to borehole water and ambient air at OL on rats' gastric parietal cell count as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05. Pt 1, 2 and 3 = Points 1, 2 and 3 at Olusosun landfill. HPF – high power field.

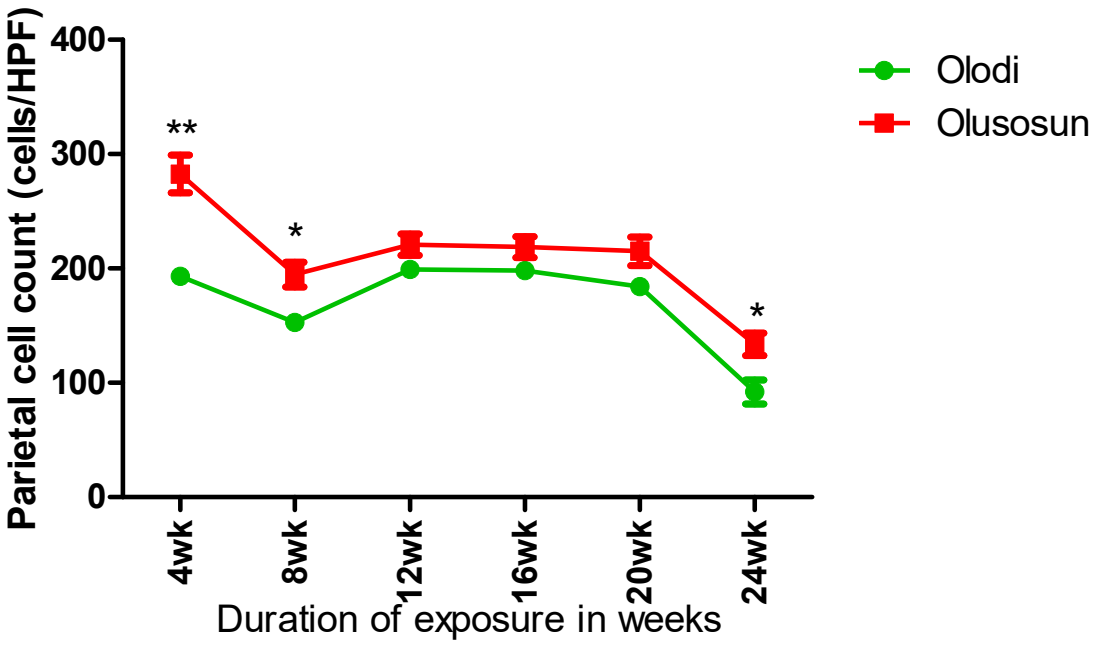

Figure 4.30: Pooled data for effects of exposure to borehole water and ambient air at OL on rats' gastric parietal cell count as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05 and **p<0.01. HPF – high power field.

Also, the pooled data (Figure 4.32 and Table 4.9) showed that the average mucous cell count (cells/HPF) were higher with rats exposed to Olusosun landfill for 4, 16 and 24 weeks exposure periods, and using Student t-test, these were significant ($p<0.05$) at 4 and 24 weeks exposures. Similarly, the average mucous cell count (cells/HPF) were lower with rats exposed to Olusosun landfill for 8, 12 and 20 weeks exposure periods, and using Student t-test, these were significant ($p<0.05$) at 8 and 12 weeks exposures; as compared to their corresponding control set at Olodi-Apapa.

Alterations in gastric mucus secretion

Gastric mucus secretion (mg/g tissue) was almost concomitant with mucous cell count. There was increase in mucus secretion at 4 weeks exposure (Pts 1, 2 and 3), which was significant ($p<0.05$) at Pt1. Subsequently, there were decreases in mucus secretions in rats exposed for 8 weeks (Pts 1, 2 and 3), 12 weeks (Pts 1 and 2), 16 and 20 weeks (Pt1) and these decrease were significant ($p<0.05$) at 8 weeks exposure (Pts 2 and 3), 12, 16 and 20 weeks (Pt1). This was followed with increase in gastric mucus secretion in rats exposed for 24 weeks (Pts 1, 2 and 3) and these increases were significant ($p<0.05$) at Pts 2 and 3; as compared to the corresponding controls (Figure 4.33). Also, the mean data (Figure 4.34 and Table 4.9) showed that the average gastric mucus secretion (mg/g tissue) were higher with rats exposed to Olusosun landfill for 4, 16 and 24 weeks exposure periods, and using Student t-test, these were significant ($p<0.05$) at 4 and 24 weeks exposures. Similarly, the average mucus secretion (mg/g tissue) were lower with rats exposed to Olusosun landfill for 8, 12 and 20 weeks exposure periods, and using Student t-test, these was significant ($p<0.05$) with rats exposed for 8 weeks; as compared to their corresponding control set at Olodi-Apapa.

4.3.8.1 Correlation of exposure periods, metal concentrations in blood and gastric indices

The pooled data of rat exposed *in situ* to Olusosun landfill, exposure periods showed negative correlations with parietal cell counts ($p=0.000$; corr-coeff=-0.656), mucus cell counts ($p=0.091$; corr-coeff=-0.286) and gastric mucus secretions ($p=0.006$; corr-coeff=-0.381); and these were statistically significant ($p<0.01$) with parietal cell counts and gastric mucus secretions. Also, parietal cell count showed a significant ($p<0.05$)

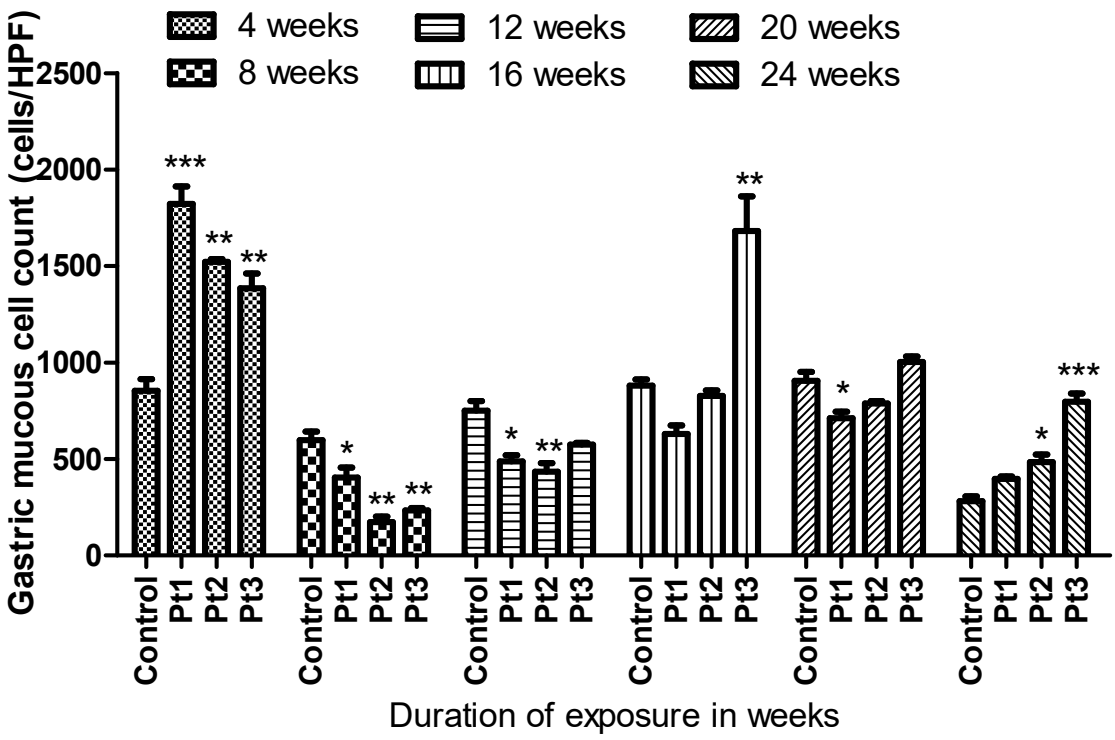

Figure 4.31: Effects of exposure to borehole water and ambient air at OL on rats' gastric mucous cell count as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05, **p<0.01 and ***p<0.001. Pt 1, 2 and 3 = Points 1, 2 and 3 at Olusosun landfill. HPF – high power field.

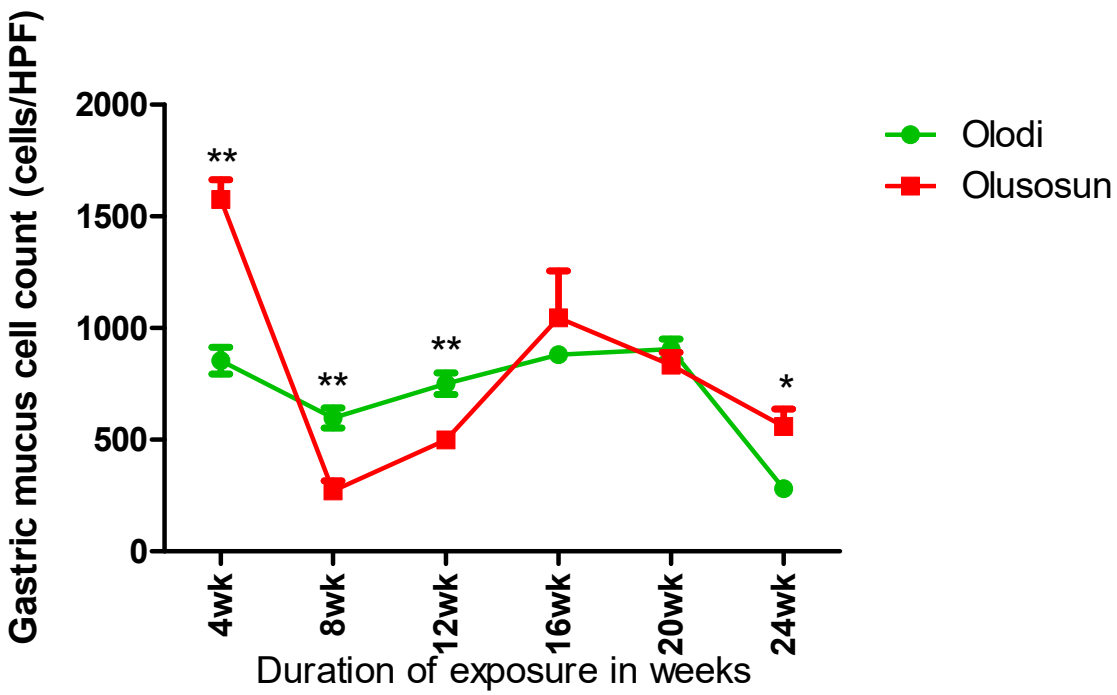

Figure 4.32: Pooled data for effects of exposure to borehole water and ambient air at OL on rats' gastric mucous cell count as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05 and **p<0.01. HPF – high power field.

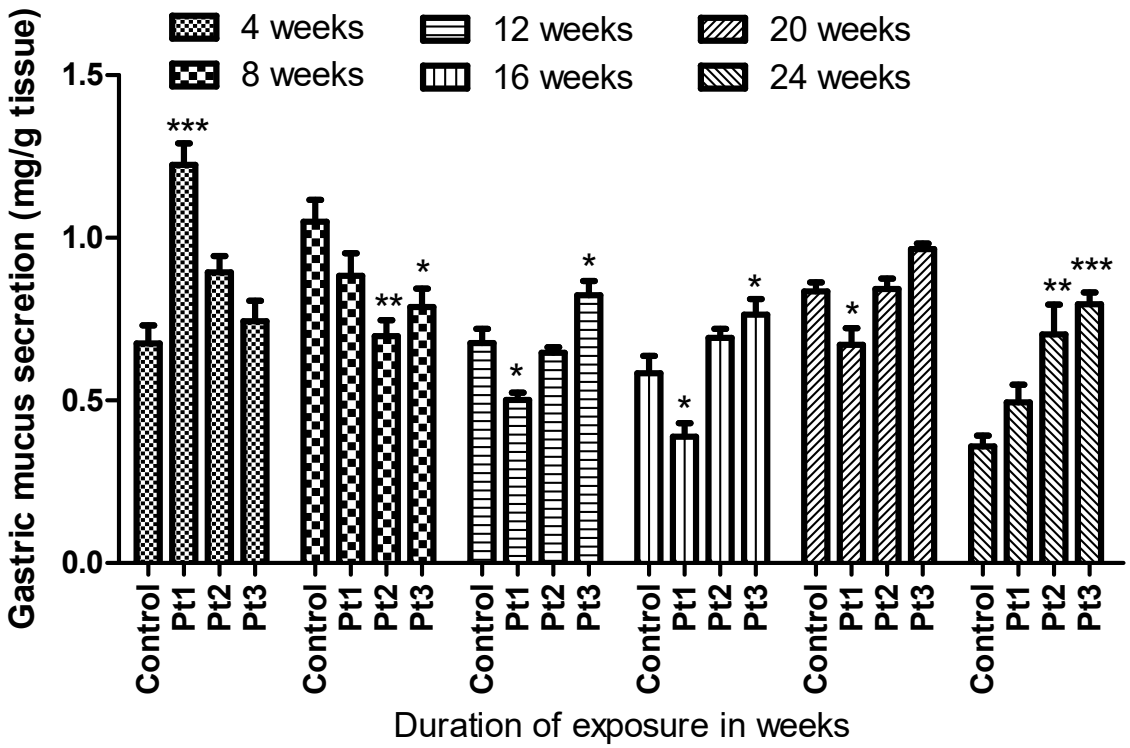

Figure 4.33: Effects of exposure to borehole water and ambient air at OL on rats' gastric mucus secretion as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05, **p<0.01 and ***p<0.001. Pt 1, 2 and 3 = Points 1, 2 and 3 at Olusosun landfill.

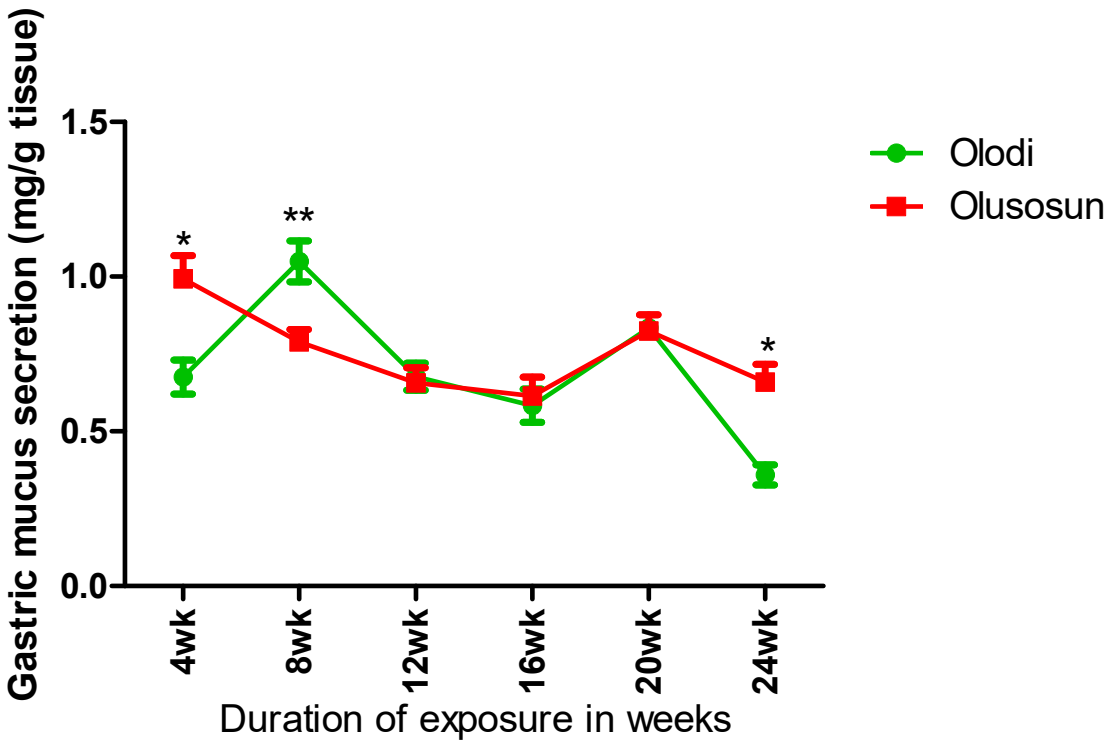

Figure 4.34: Pooled data for effects of exposure to borehole water and ambient air at OL on rats' gastric mucus secretion as compared to control.

End points are mean (± SE) values. Values are significantly different from control at: *p<0.05 and **p<0.01.

Table 4.9: Summary of physiopathological gastric indices in landfill exposed rats

Exposure duration	Exposure site	Gastric indices		
		Parietal cell count (Cells/HPF)	Mucous cell count (Cells/HPF)	Gastric mucus secretion (mg/g)
4 Weeks	Olodi	193.0±4.04	853.7±59.7	0.68±0.06
	Olusosun	282.7±16.65**	1576.0±87.5**	0.99±0.08*
8 Weeks	Olodi	152.7±3.18	597.3±44.5	1.05±0.07
	Olusosun	194.7±10.71*	270.2±46.4**	0.79±0.04**
12 Weeks	Olodi	199.0±7.23	751.3±48.5	0.68±0.04
	Olusosun	220.8±9.31	499.2±29.8**	0.66±0.05
16 Weeks	Olodi	198.0±1.73	881.0±30.9	0.58±0.05
	Olusosun	218.7±9.04	1046.0±209.7	0.62±0.06
20 Weeks	Olodi	184.3±6.64	905.0±46.5	0.84±0.03
	Olusosun	215.0±12.44	834.3±56.3	0.83±0.05
24 Weeks	Olodi	92.0±10.39	281.0±25.4	0.36±0.03
	Olusosun	133.7±9.83*	559.8±78.3*	0.66±0.06*

Values are mean ± SE. Using Student's t-test mean values of exposed groups (Olusosun) are significantly different from control groups (Olodi) at *$p<0.05$; **$p<0.01$ and ***$p<0.001$.

positive correlation with both mucus cell count (p=0.001; corr-coeff=0.533) and gastric mucus secretion (p=0.047; corr-coeff=0.334); and mucus cell count significantly correlated positively with gastric mucus secretion (p=0.000; corr-coeff=0.554).

The combined data of rat exposed *in situ* to Olusosun landfill, parietal cell count showed negative correlations with blood concentrations of Cd (p=0.700; corr-coeff =-0.067), Cr (p=0.832; corr-coeff =-0.040) and Zn (p=0.856; corr-coeff =-0.031); and positive correlations with Pb (p=0.913; corr-coeff =0.020) and Cu (p=0.977; corr-coeff =0.005), however, these were not statistically significant (p>0.05).

Combined data of mucus cell count showed negative correlations with blood concentrations of Pb (p=0.277; corr-coeff = -0.195), Cd (p=0.251; corr-coeff =-0.199), Cu (p=0.410; corr-coeff =-0.148) and Zn (p=0.728; corr-coeff =-0.060); and positive correlation with Cr (p=0.986; corr-coeff =0.003), however, these were insignificant (p>0.05).

Similarly, mucus secretion showed negative correlations with blood concentrations of Pb (p=0.345; corr-coeff =-0.167), Cd (p=0.320; corr-coeff =-0.173), Cu (p=0.006; corr-coeff =-0.471) and Zn (p=0.162; corr-coeff =-0.238); and positive correlation with Cr (p=0.721; corr-coeff =0.067), however, this was only significant (p>0.05) with Cu concentration in blood.

4.3.9 Histological alterations in viscera of landfill exposed rats

Histological alterations in the liver of rat

The histological slides of the hepatic tissues of control rats showed normal morphology, which is composed of hexagonal or pentagonal lobules and peripheral hepatic triads or tetrads embedded in connective tissues. Hepatocytes are arranged in trabecules which are separated by sinusoids containing kupffer cells. They are regular and contain a large spheroidal nucleus. Some cells have two nuclei (Figure 4.35 A and B). However, morphological alteration seen in exposed rats ranged from moderate to severe focal areas of periportal inflammation, hepatic cell necrosis, fibrosis, centrilobular steatosis, infiltration of inflammatory cells into the portal triad, sinusoidal

dilation and congestion, hepatic portal and vein congestion, and proliferation of bile ducts (Figure 4.35 C and D; Figure 4.36 A to D).

Histological alterations in the kidney of rat

Normal histological architecture of the cortex and medulla was seen in the kidney of the control rats with renal glomerular tightly filling the Bowman's capsule. Cortical tubules showed normal tubular brush borders with distinct lumen. Sinusoids were observed to be normal without congestion or inflammation (Figure 4.37A). However, some morphological nephrotoxic alterations were seen in the exposed rats. These lesions include mild/moderate perivascular inflammation, periglomerular inflammations, peritubular inflammation, congestion of blood vessels, fibrosis, shrinking/degeneration of the glomeruli, focal area of tubules with vesicular nuclei, focal area of eosinophilic granule (renal cast) and focal area of sloughing of tubules (Figure 4.37 B to D).

Histological alterations in the lungs of rat

The histological presentation of the pulmonary tissues and alveolar of the control rats showed normal architecture with thin alveolar wall and sacs (Figure 4.38A). However, morphological alterations seen in exposed rats ranged from moderate to severe alveolar cell hyperplasia, oedema, peribronchiolar inflammation, perivascular inflammation, fibrosis, congestion, fibroblast aggregates, thickened arteries and alveolar septa, air space enlargement, focal areas of haemorrhage, adipocyte infiltration, pulmonary atelectasis and desquamation and distortion of alveolar architecture (Figure 4.38 B to F).

Histological alterations in the spleen of rat

The histology of the splenic tissues of control rats showed a normal architecture with normal distribution of the red and white pulps (Figure 4.36A and B). However, morphological alterations seen in exposed rats ranged from moderate to severe focal areas of fatty infiltration, fusion of white pulps, congestion of splenic blood vessels, reduction of white pulps and predominance of red pulps, thickened and irregular trabeculae, haemosiderin deposition, fibrosis, presence of lymphoid follicles, macrophages, and megaryocytes (Figure 4.39C to F).

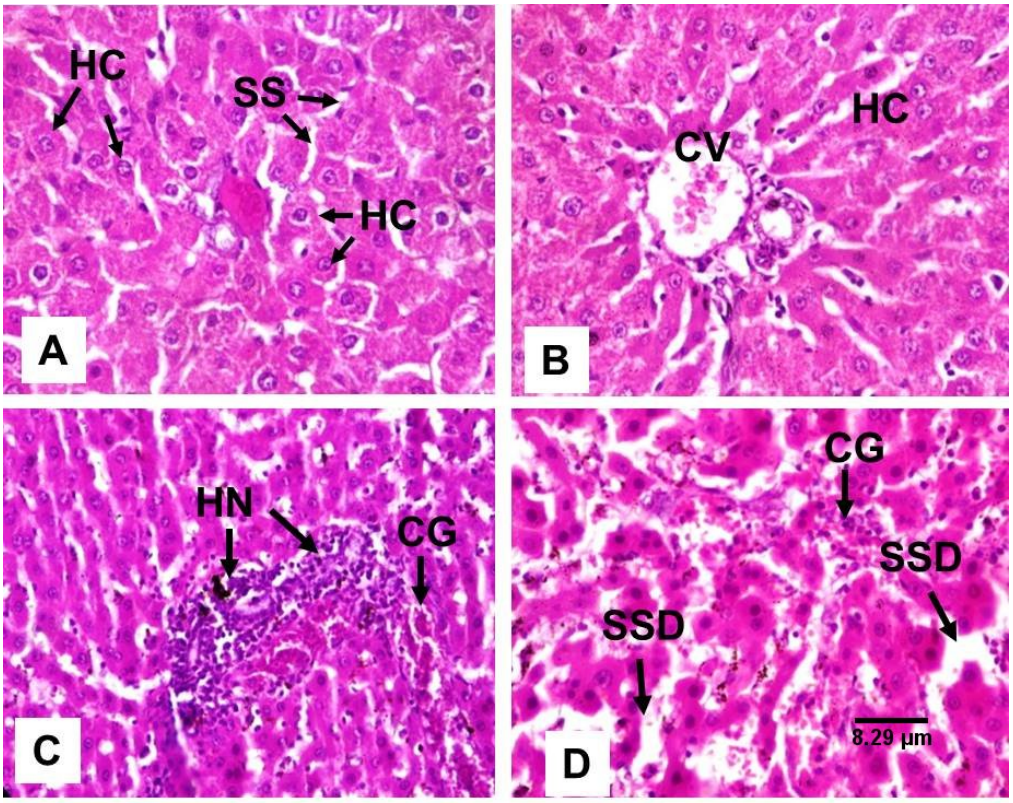

Figure 4.35: Hepatic tissue of rats exposed to borehole water and ambient air at OL and control. H&E, X400.

(A - B) Normal organisation of hepatocyte (HC), sinusoids (SS), portal triads and central veins (CV) as observed in the control rats.

(C) Hepatic cell necrosis (HN) and congestion (CG) - Observed in some rats exposed for 20 and 24 weeks (Pts 1, 2 and 3).

(D) Sinusoid dilation (SSD) cum congestion (CG) - Observed in some rats exposed for 4 weeks (Pt2), 20 weeks (Pt1) and 24 weeks (Pts 2 and 3).

Pts - points

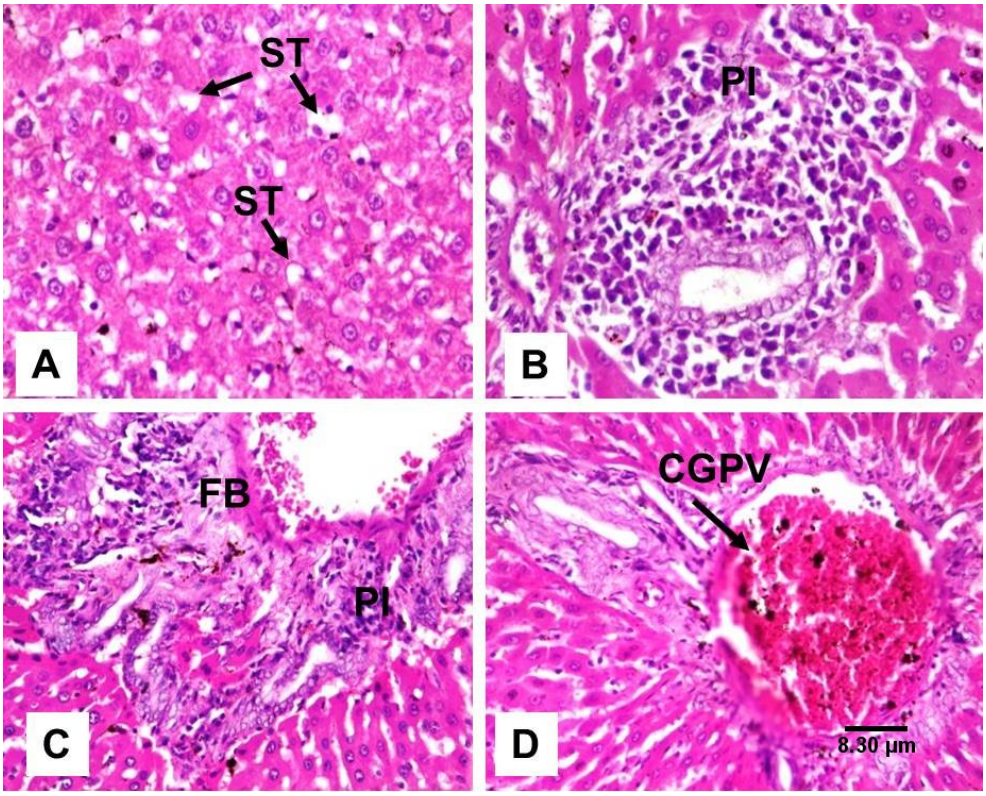

Figure 4.36: Hepatic tissue of rats exposed to borehole water and ambient air at OL. H&E, X400.

(A) Focal areas of steatosis (ST) – Observed in rats exposed for 8 weeks (Pts 1 and 2), 12 weeks (Pt1) and 16 weeks (Pt2).

(B) Moderate periportal inflammation (PI) – Observed in rats exposed for 4 and 8 weeks (Pt2), 12 and 24 weeks (Pts 2 and 3), and 20 weeks (Pt3).

(C) Severe periportal inflammation with fibrosis (FB) – Observed in rats exposed for 20 and 24 weeks (Pt3).

(D) Congestion of hepatic portal vein (CGPV) – Observed in rats exposed for 16 weeks (Pt1) and 20 weeks (Pt3).

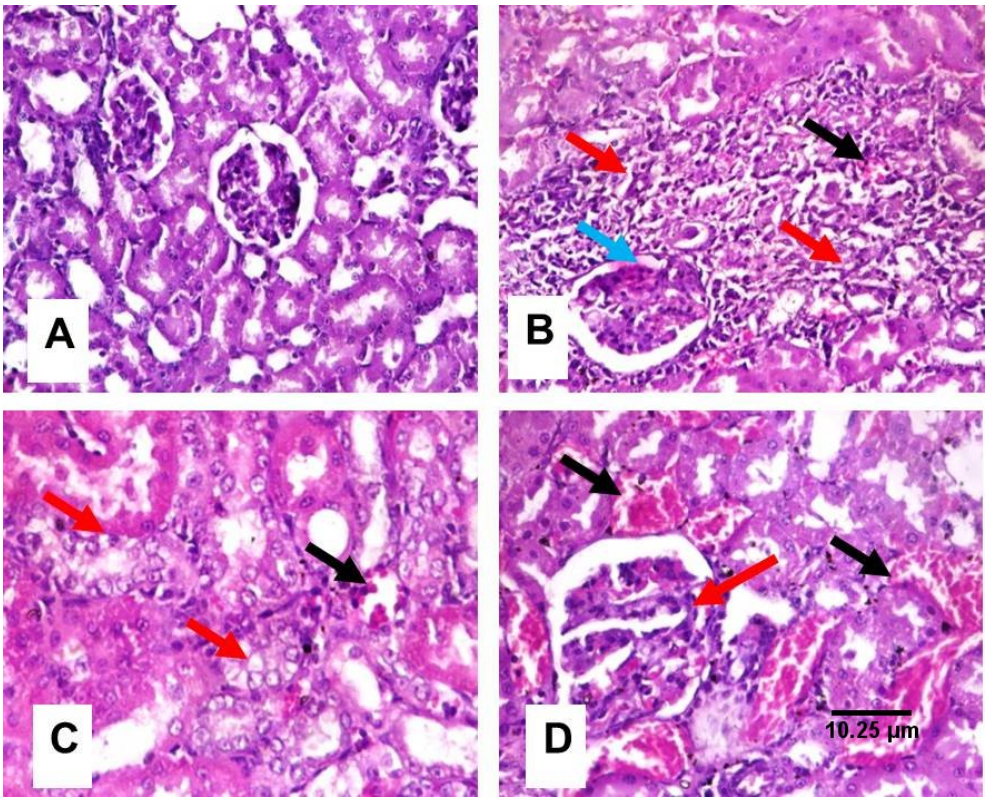

Figure 4.37: Renal tissue of rats exposed to borehole water and ambient air at OL and control. H&E, X400.

(A) Renal tissue show normal morphology of glomeruli and tubules, no lesion was observed in all control rats.

(B) Moderate congestion of blood vessels (black arrow), infiltration of inflammatory cells into the interstitial with formation of fibrosis (red arrows) and glomeruli show mild shrinking and degeneration (blue arrow) - Observed in rats exposed for 8, 12 and 20 weeks (Pts 1, 2 and 3).

(C) Renal tissue show slight inflammation (black arrow) and focal area of tubules with vesicular nuclei (red arrows) - Observed in rats exposed for 8 weeks (Pt2).

(D) Renal tissue show severe congestion of blood vessels (black arrows) and glomeruli degeneration (red arrow) – Observed in rats exposed for 16 and 24 weeks (Pts 2 and 3).

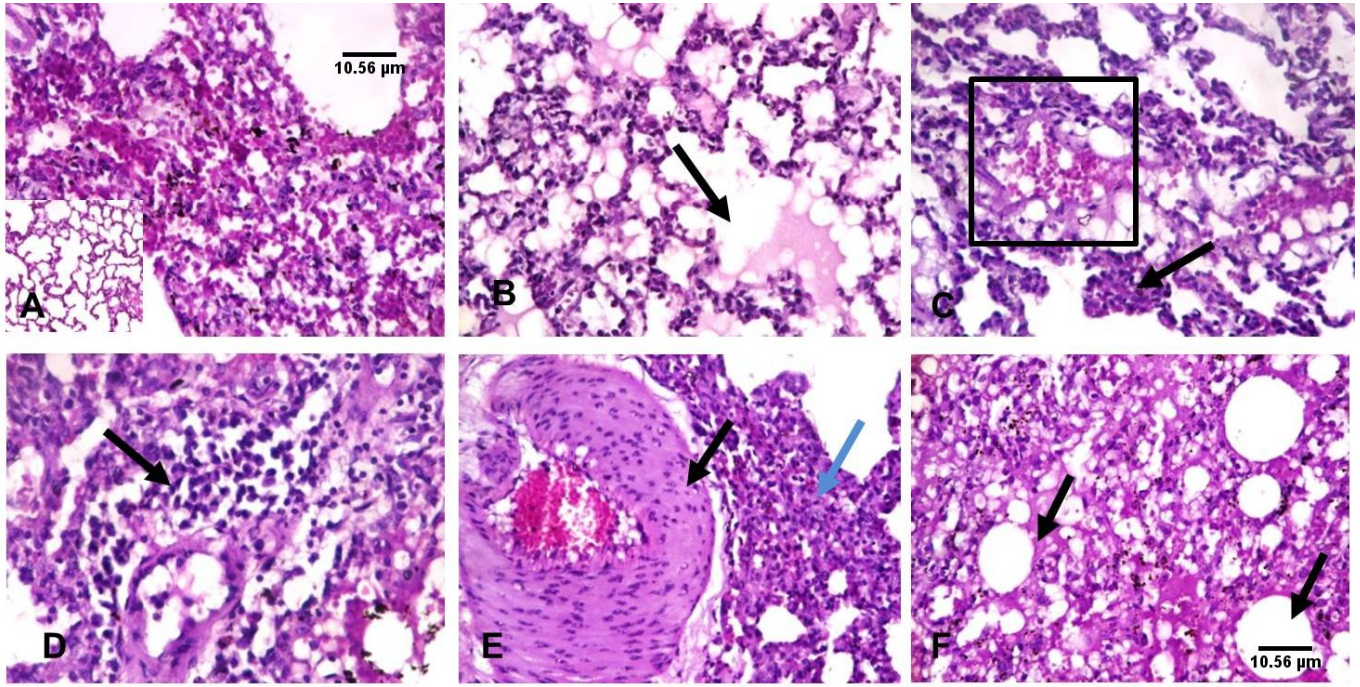

Figure 4.38: Pulmonary tissues of rats exposed to borehole water and ambient air at OL and control. H&E, X400.

(A) Normal organisation of the alveoli and alveolar septa observed in control rats. (B) Moderate oedema (black arrow) – Observed in rats exposed for 4, 8, 12, 16 and 20 (Pts 1, 2 and 3) (C) Alveolar cells hyperplasia (black arrow), thickening of alveolar septa and congestion (square), air space enlargement – Observed in rats exposed for 4, 8, 12, 16, 20 and 24 weeks (Pts 1, 2 and 3) (D) Moderate perivascular inflammation (black arrow) – Observed in rats exposed for 4, 8, 12, 16, 20 and 24 (Pts 1, 2 and 3) (E) Thickened arteries (black arrow) and mild hyperplasia (blue arrow) – Observed in rats exposed for 16 weeks (Pts 2 and 3) and 24 weeks (Pt1) (F) Adipocytes infiltration (black arrow) – Observed in rats exposed for 12 weeks (Pt3) and 20 weeks (Pt1).

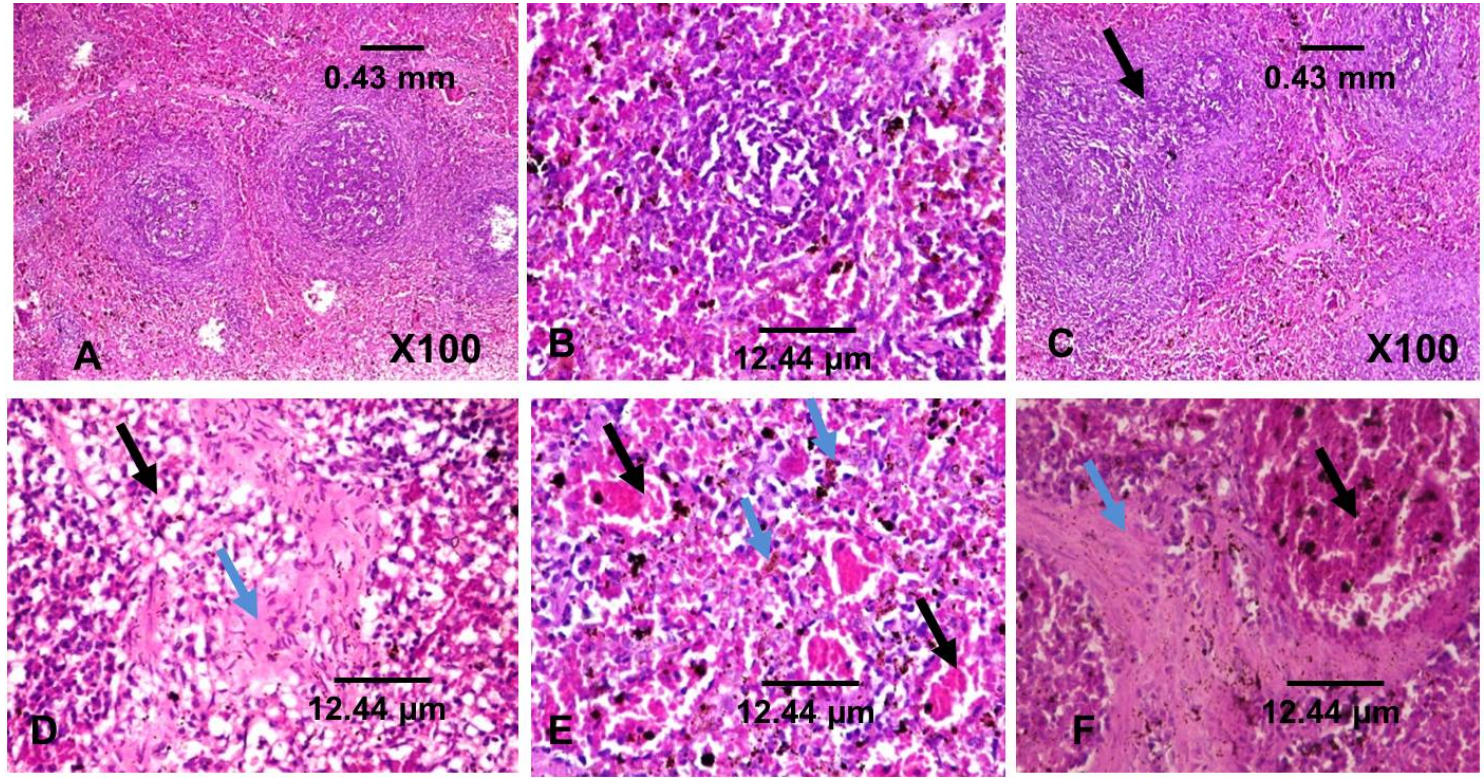

Figure 4.39: Splenic tissue of rats exposed to borehole water and ambient air at OL and control. H&E, X100 and X400.

(A and B) Intact and distinct spleen follicle with clear white pulp, red pulp, and marginal zone in control rats (C) Severe fusion of white pulps (black arrow) (Pt1 – 4, 8, 16 weeks; Pt2 – 4, 20, 24 weeks; Pt3 – 4, 20, 24 weeks exposures) (D) Infiltration of adipocytes into red pulp (black arrow) and focal area of fibrosis (blue arrow) (Pt2 – 8 and 12 weeks exposures) (E) Haemorrhagic lesion in red pulp (black arrow) and prominent heamosiderin depositions (blue arrow) (Pt1 – 4, 8, 12, 24 weeks; Pt2 – 4, 12, 24 weeks; Pt3 – 4 weeks exposures) (F) Mild splenic vessels congestion (black arrow) and thickened and irregular trabeculae (blue arrow) (Pt1 – 4, 8, 12, 16 weeks; Pt2 – 4, 8 and 24 weeks exposures).

Histological alterations in the stomach of rat

The histology of the gastric mucosa of the control rats showed a normal architecture with no lesion; parietal cells with centrally located nuclei, surface mucous cells with mucus staining strong magenta and preserved mucous glands (Figure 4.40 A to C; Figure 4.41 A and E). However, lesions (Figure 4.40 D to F; Figure 4.41 B to D and F) observed in the stomach of the exposed rats ranged from moderate to severe papillary in folding, congestion, vascularization, mucous gland with vesicular nuclei, edema, inflammation of mucosa and submucosa, sloughing of surface epithelium and mild ulceration of surface mucosa. Also, active mucin production in some mucus cells and less/no mucin production in some cells were observed. Also, figure 4.42 to 4.43 showed the histopathological alterations as observed with exposure periods and at different magnifications (X 40, 100 and 400) respectively.

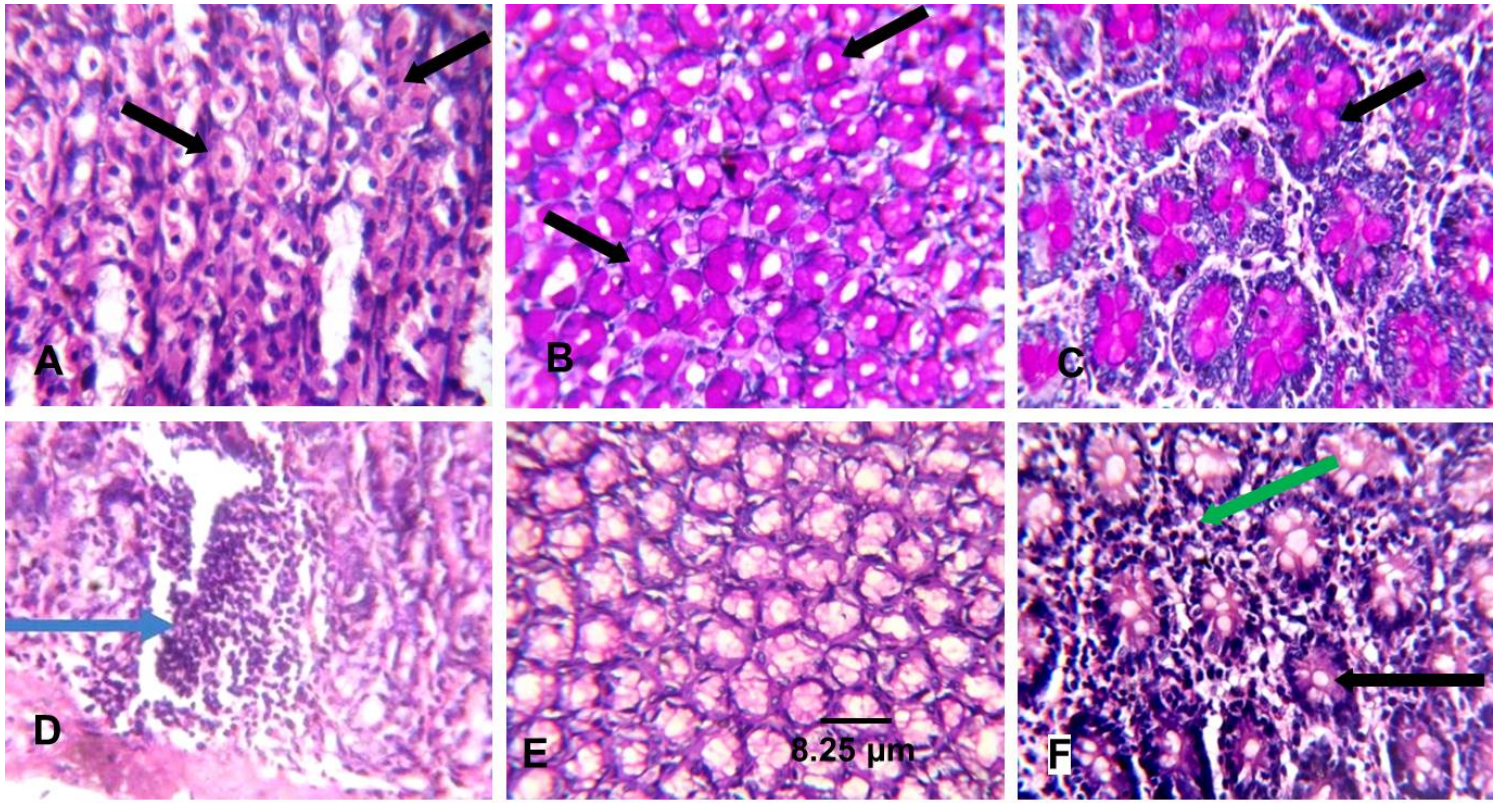

Figure 4.40: Gastric mucosa of rats exposed to borehole water and ambient air at OL and control. H&E, X400.

(A) Normal parietal cells with centrally located nuclei, (B) Normal surface mucous cells with mucus staining strong magenta (C) Preserved mucous glands; as observed in most control (black arrows). (D) Aggregates of chronic inflammatory cells within the mucosa (blue arrow) (E) Numerous mucous glands, but show no mucin production: Pt 1, 2, 3 – 8, 12, 16, 20 weeks exposures (F) Severe inflammation of the mucosa glands, No active mucin production (black arrow) and aggregates of chronic inflammatory cells (green arrow) within the mucosa. (Pt1, 2 and 3 - 8,12,16 weeks exposures).

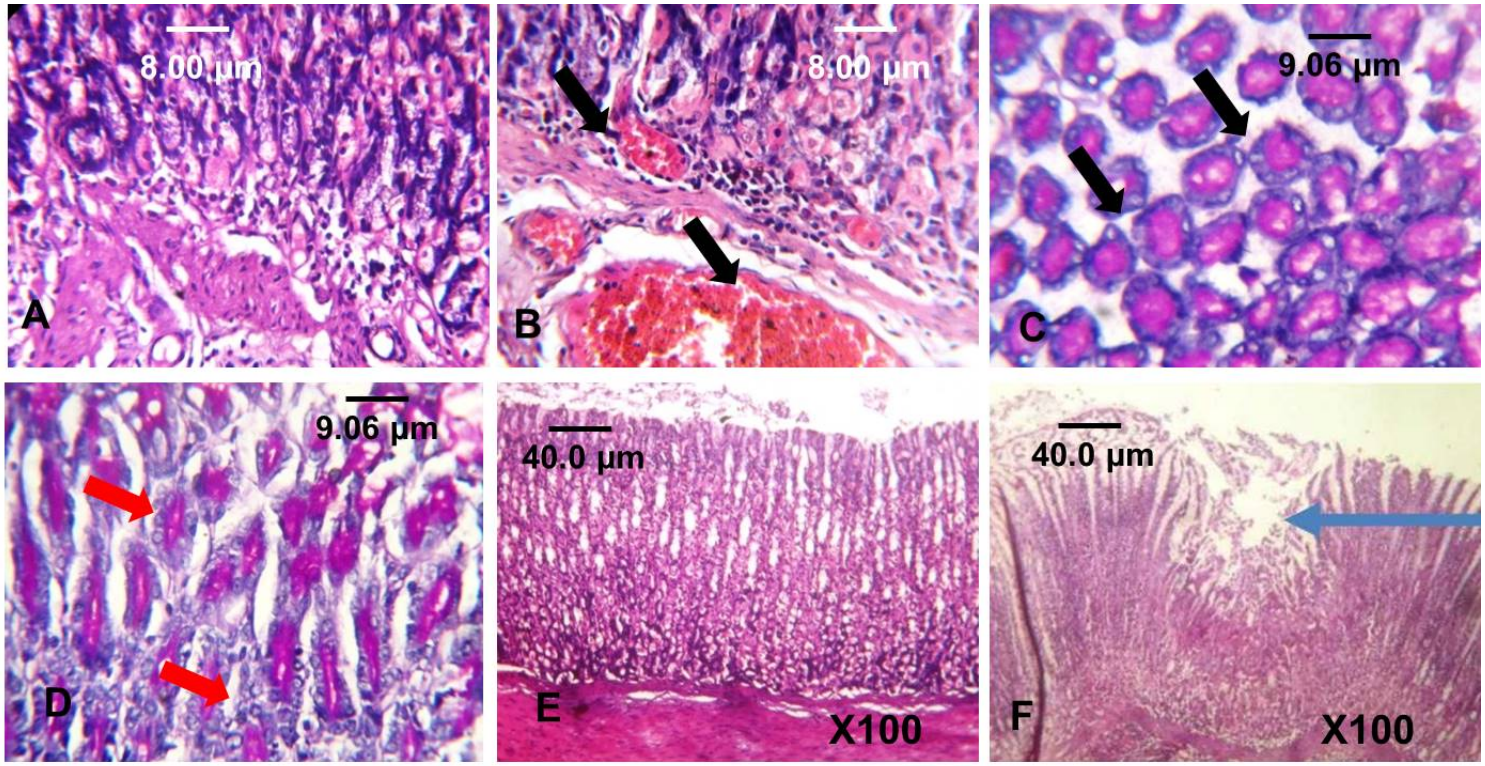

Figure 4.41: Gastric mucosa and submucosa of rats exposed to borehole water and ambient air at OL and control. H&E,X100, X400.

(A) Normal mucosa and submucosa (control) (B) Mild congestion (black arrow) of mucosa and submucosa, increased vascularisation of mucosa, and there is active mucin production (Pt 1, 2, 3 – 4, 20, 24 weeks). (C and D) Numerous mucous glands, but show low mucin production (black arrow) while those producing mucin have vesicular nuclei (red arrow) (Pt 1, 2, 3 – 8, 16, 24 weeks) (E) Moderate papillary infolding, no significant lesion, the surface epithelial is well preserved. No ulcer or erosion seen (control) (F) Mild ulceration (blue arrow) of the surface mucosa (Pt3 – 24 weeks).

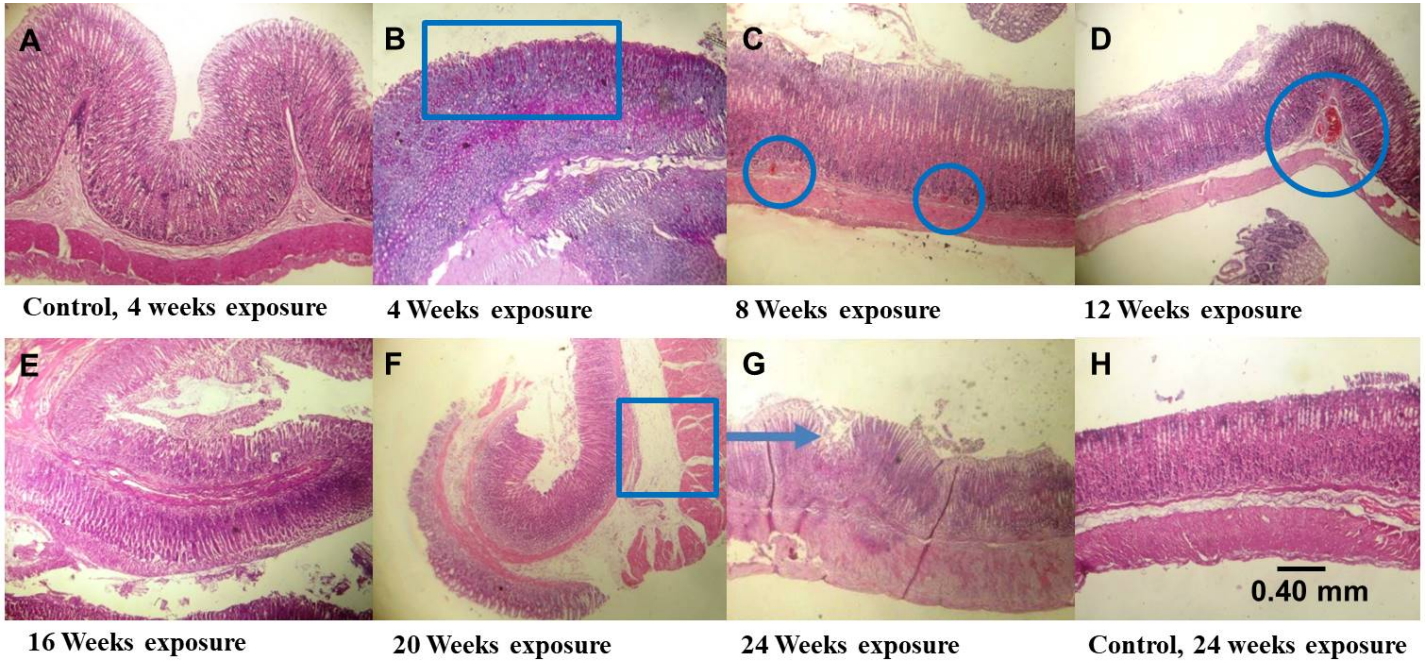

Figure 4.42: Gastric mucosa and submucosa of rats exposed to borehole water and ambient air at OL and control as observed in with exposure periods. H&E, X40.

(A) Gastric tissue show normal papillary infolding of mucosa and submucosa, surface epithelial is well preserved (control rats).
(B) Surface epithelial with active mucin production (blue rectangle).
(C) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(D) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(E) Gastric mucosa show slight papillary infolding and sloughing of surface epithelial.
(F) Mild papillary infolding, mild oedema (blue square), moderate inflammation (black arrow at X400) of mucosa and submucosa.
(G) Mild ulceration (blue arrow) of surface mucosa and moderate inflammation (black arrow at X400) of mucosa and submucosa.
(H) Gastric tissue show moderately persevered mucosa and submucosa.

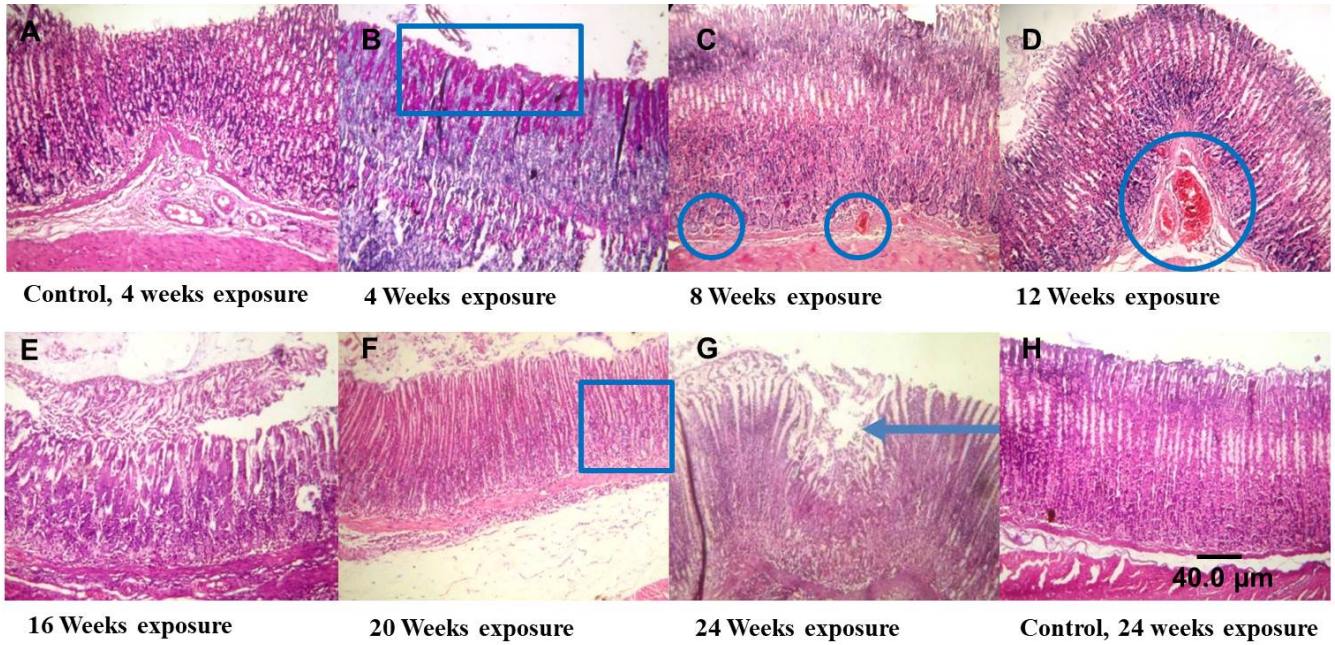

Figure 4.43: Gastric mucosa and submucosa of rats exposed to borehole water and ambient air at OL and control as observed in with exposure periods. H&E, X100.

(A) Gastric tissue show normal papillary infolding of mucosa and submucosa, surface epithelial is well preserved (control rats).
(B) Surface epithelial with active mucin production (blue rectangle).
(C) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(D) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(E) Gastric mucosa show slight papillary infolding and sloughing of surface epithelial.
(F) Mild papillary infolding, mild oedema (blue square), moderate inflammation (black arrow at X400) of mucosa and submucosa.
(G) Mild ulceration (blue arrow) of surface mucosa and moderate inflammation (black arrow at X400) of mucosa and submucosa.
(H) Gastric tissue show moderately persevered mucosa and submucosa.

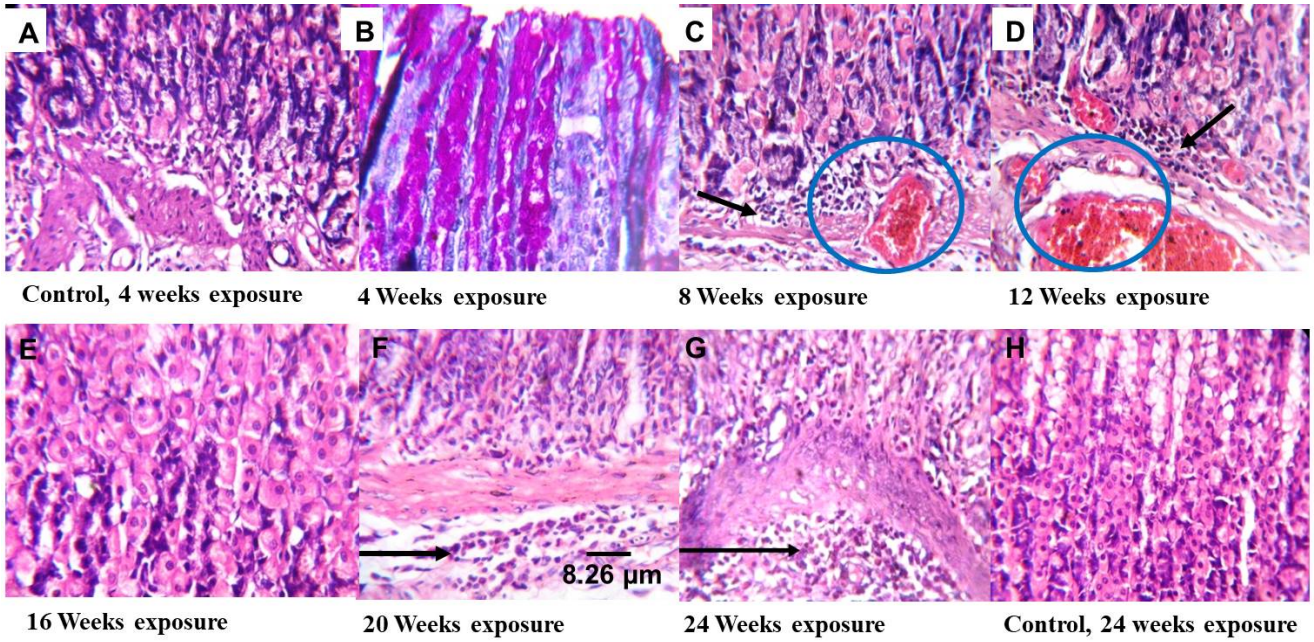

Figure 4.44: Gastric mucosa and submucosa of rats exposed to borehole water and ambient air at OL and control as observed in with exposure periods. H&E, X400.

(A) Gastric tissue show normal papillary infolding of mucosa and submucosa, surface epithelial is well preserved (control rats).
(B) Surface epithelial with active mucin production (blue rectangle).
(C) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(D) Mild congestion (blue cycles) and inflammation (black arrow at X400) of mucosa and submucosa.
(E) Gastric mucosa show slight papillary infolding and sloughing of surface epithelial.
(F) Mild papillary infolding, mild oedema (blue square), moderate inflammation (black arrow at X400) of mucosa and submucosa.
(G) Mild ulceration (blue arrow) of surface mucosa and moderate inflammation (black arrow at X400) of mucosa and submucosa.
(H) Gastric tissue show moderately persevered mucosa and submucosa.

CHAPTER FIVE

DISCUSSION

Landfilling and/or open dumping is the most widely used method of municipal waste management and in recent years, there is growing concerns about landfill capacity and consequent environmental and health impacts. Heavy metals such as Pb, Cd, Hg, As, Cr, Cu, Ni, Zn, Fe, etc. are major contaminants from landfills and had been identified in environmental compartments such as air emissions, soils and groundwater and in leachates sampled around landfills. This is due to metal leachability during burning or decomposition of waste which takes place in the landfills. These heavy metal contaminants and other organic compounds may either individually or in combination induce cytogenotoxic and system toxicity in exposed plant, animal and human population (Christensen *et al.*, 2001; Ikem *et al.*, 2002; Alimba *et al.*, 2006; Karthikeyan *et al.*, 2011; Bakare *et al.*, 2012b).

In most developing countries such as Nigeria, inadequate settlement planning, rapidly growing population and poverty have increased the population of people who derive their livelihood from waste picking/recycling and this has made living in shacks and shanty towns around waste dumpsite a common scene. In addition, residential building and estates are now sited on waste-reclaimed lands and around landfills in urban cities. Therefore, there is the potential risk of human exposure to deleterious chemicals emanating from landfills either through inhalation, ingestion, dermal contact or combination of these. In this study, the heavy metal concentrations in underground water at Olusosun landfill, concentrations of selected metals in tissue and organs (blood, liver, kidney and lungs) of rats exposed *in situ* to the borehole water and ambient air at the landfill were investigated. In addition, systemic and cytogenetic toxicity were assessed in the exposed rats in order to gain insight into the potential genetic and systemic toxicity effects such exposures might be having on animal and human populations who are exposed *in situ* to toxicants from the landfill.

5.1 Heavy metal concentrations in underground water at the landfill

In this study, concentrations of Pb, Cd, Cr, Cu, Zn and Fe observed in the borehole water at Olusosun landfill were significantly higher than those in the control borehole water at Olodi-Apapa and also higher than permissible limits for drinking water quality (NESREA, 2011; USEPA, 2017). This observation suggests that there must have been leachate interaction and contamination of the groundwater at Olusosun landfill site and environs. This is also possible given the fact that the groundwater used in this study is situated right on the landfill.

The Olusosun landfill is an unlined landfill/dumpsite and the waste placed on this landfill is prone to either groundwater under flow or being infiltrated with rainfall. As water flows through the heaps of waste, together with biological decomposition and chemical reactions, leachates are generated and this accumulates at the bottom or flow out of the landfill (Mor *et al.*, 2006). These leachates had been reported to contaminate underground water sources within the vicinity of landfills. Previously, Oyeku and Eludoyin (2010) had observed that the concentrations of copper, iron, lead and cobalt in the wells and boreholes around Ojota residential areas and Olusosun landfill area were above USEPA permissible limits and they noted that 2 km radius is a proximal distance for groundwater around major landfill to be vulnerable to contamination from the landfill.

Also, these levels of groundwater contamination with heavy metals observed in this study must have been aided by the background geology and nature of underground aquifer in Lagos State. Longe *et al.* (1987) noted that the Lagos underground aquifer consist of loose, medium to coarse sand and the subsurface geology reveals a lateritic cover, reddish brown in colour with sand and clay portions. Also, due to predominant lateral water flow, the groundwater in Lagos might be prone to contamination.

The toxicity of lead (Pb) in both plants and animal system has been confirmed and there is no safe level of lead (Canfield *et al.*, 2003). Longe *et al.* (1987) ascerted that the movement of Pb in clay soil is low. However, in this study, the concentration of Pb in the underground water at Olusosun were significantly higher than the background level at Olodi-Apapa. The possible explanation for this increased Pb concentration in

the groundwater could be due to long term build up in the soil; which is also supported by the close proximity of the borehole to the contaminant source.

Although, high iron (Fe) concentration is characteristic of groundwater in Lagos State (WHO/UNICEF, 2006) as observed in the control borehole water, which were 3.0 to 4.2 times the USEPA/WHO limits (0.3 mg/L) for drinking water quality. However, the concentrations of Fe measured in the borehole water from the Olusosun landfill far more exceeded this, and were 24.8 to 208.4 times the USEPA (2017) limits. Similarly, Longe and Enekwechi (2007) measured high Fe concentrations (Range: 0.40 – 4.19 mg/L) in groundwater at the vicinity of the Olusosun landfill with no discernable attenuation trend down gradient of the landfill. This suggests impact of leachate from the waste dumpsite.

The heavy metal contamination and impact on groundwater observed in this study is in agreement with the reports of several authors (Ikem *et al.*, 2002; Akinbile, 2012; Afolayan *et al.*, 2012; Oni and Hassan, 2013; Oyiboka, 2014). Akinbile (2012) and Oyiboka (2014) asserted that the impairment of groundwater quality parameters and elevated concentrations of heavy metals (Fe, Zn, Pb and Cu) in the water makes them unsafe for consumption and agricultural purposes. The implication of this is that the underground water at Olusosun landfill and within the vicinity of the landfill is unsafe for human consumption.

5.2 Cytogenotoxicity in landfill exposed rats

The results of the micronucleus assay in this study showed that exposing rat to contaminated borehole water and ambient air at OL has genotoxic and cytotoxic effects. *In vivo* rodent micronucleus (MN) assay has been widely used to detect genotoxicity. The assay is sensitive to both clastogenic and aneugenic effects induced by mixtures of contaminants (Krishna and Hayashi, 2000). The statistically significant elevation in the frequencies of MNPCE and MNNCE formation; and significant decreases in the PCE to NCE ratios in the rats' bone marrow erythropoietic cells as compared to the control rat groups, is an indication of the clastogenic and cytotoxic effects respectively, of the constituents in Olusosun underground water and air emissions. MN usually results from chromosome breakage (clastogenic process) or from lagging or vagrant chromosomes lacking centromere, which will not migrate

together with other chromosomes during mitotic process. These broken fragments are not usually integrated in the daughter nuclei, forming a micronucleus in the cytoplasm.

Heavy metals such as Pb, Cd, Cr, Cu and Zn and other unidentified toxic constituents in the groundwater and air emission at Olusosun; and their interactions are capable of inducing mutagenic effects on the genetic materials of exposed animals and human through different mechanisms. Copper (Cu) has high affinity for G:C base pairs and can bind directly to the nitrogenous bases and phosphate group in DNA, thus causing disruption of the stacking structure of the DNA (Buttke and Sandstrom, 1994). High levels of Cd, Cr and Pb observed in the groundwater are similar to those reported in e-waste contaminated groundwater and landfill leachates (Alabi and Bakare, 2011; 2014; Alimba and Bakare, 2016) and in the liver of White footed mice (*Peromuscus leucopus*) dwelling in an hazardous waste site, and these metals have been suggested to be responsible for the increased chromosomal aberration found in the small mammals (Tull-Singleton *et al.*, 1994). In the study herein, no strong correlation ($p>0.05$) were found between metal levels in blood of landfill exposed rats and genetic damages (frequencies of MNPCE and MNNCE) during the exposure periods. Tull-Singleton *et al.* (1994) similarly reported that, despite the significantly increased levels of Pb and Cr in tissues of landfill exposed mice (*Peromuscus leucopus*), their correlation results indicated that the metals cannot be considered totally responsible for the increased levels of genetic aberrations. They explained that a number of other toxic compounds present at the landfill site such as partially combusted hydrocarbons and polynuclear aromatic hydrocarbons may have contributed to or caused the induction of genetic damage.

Also, constituents in the groundwater and air emissions can be absorbed into the cells and this can induce change in the pH of the intra- and intercellular environment. This change in pH can affect enzyme activities and change the structure of DNA (Meng *et al.*, 2002). The constituents of the groundwater and air emissions can also cause DNA damage/breakage by inducing free radical species (ROS) formation either via autoxidation or enzyme catalyzed oxidation (Li *et al.*, 2006). The decreased PCE/NCE ratios at the three points (Pt1, Pt2 and Pt3) of exposure and throughout each duration (4, 8, 12, 16, 20 and 24 weeks) of exposure suggest that the underground water and air constituents elevated the rate of aging of the erythrocytes from PCE to NCE, thereby

decreasing their normal life span and increasing the risk of genotoxicity (Alabi and Bakare, 2011).

5.3 Systemic toxicity in rats exposed to landfill site

5.3.1 Clinical signs of toxicity

The clinical signs of toxicity observed in the rats exposed to the groundwater and ambient air at Olusosun dumpsite were more apparent during the first 4 weeks of the exposure. Obvious weight loss (during the first 4 weeks of exposure), loss of appetite (reduced feeding), diarrhea, abscess, reduced activities, poor grooming, hair loss and discolouration compared to the control were evidence that the exposure stimulated a central nervous, physiologic and immunologic response in the rats (Benlahcen *et al.*, 2009). Similar observations of hair loss, anorexia, diarrhea, sluggishness, labored breathing and sneezing were reported by Bakare *et al.* (2003) and Alimba *et al.* (2012) in mice and rats exposed to different concentrations of landfill leachates. These signs and symptoms may have been triggered by sudden change in environment, environmental exposures and infection, resulting from increased load of pathogenic microorganism and their associated vectors (houseflies, roaches, mosquitoes) and the presence of xenobiotics (in air and water) which the animals were exposed to in the landfill environment. This assertion is supported by the report of Oshode *et al.* (2008), Karthikeyan *et al.* (2011) and Alimba (2013), where they observed potentially pathogenic and toxin producing microorganism, disease vectors and vermin and toxic contaminants (including heavy metals) in leachates, water and air from municipal landfill sites. Also, anorexia observed during the 1 – 3 weeks of exposure to the landfill together with reduced body weight gain is consistent with previous reports, and this is a notable symptom of liver injury connected with exposure to chemical contaminant from waste dumpsites (Weingand *et al.*, 1996; Alimba *et al.*, 2012).

5.3.2 Weight gain in body and organ in landfill exposed rats

Body and organ weight changes in chemical testing toxicology have long been accepted as a sensitive indicator of chemical induced changes to the animal body and organs (Michael *et al.*, 2007). Significant increase in body weight and percentage change in body weight gain in most exposed rats and throughout each periods of exposure (except at 4 weeks exposure) could be a toxic effect manifesting with

excessive weight gain. In contrast, previous studies reported weight loss after exposing mice and rats to contaminated groundwater and leachate from landfills (Bakare *et al.*, 2003; Alimba *et al.*, 2012; Bakare *et al.*, 2013a). These studies are laboratory controlled experiments with little or no influence from miscellaneous environmental chemical contaminants order than the treatments.

The possible explanation for the observed weight gain as a toxicological sign could be the presence of endocrine disrupting chemicals [EDCs – e.g. PCBs, PAHs, OCPs (though not analyzed) and heavy metals] in the landfill air emissions and groundwater. Certain EDCs which have been identified in landfill air, particulate matters (PMs), contaminated groundwater and leachates are capable of altering the pathway of adipose tissue production in the body of animals (Kelishadi *et al.*, 2013). In further support of this observation, Baillie-Hamilton (2002) noted that in *in vivo* and *in vitro* animal experimentations, low concentrations of environmental toxic chemicals and some synthetic industrial agents stimulate weight gain. However, at high levels, they may be catabolic to organisms. Also, Takahama *et al.* (1972) noted that, one of the toxic effects of these chemicals appears to be weight gain. Unlike the well-known weight loss resulting from high exposure to toxins, the weight gain tends to occur at much lower levels of exposure, which fail to make animals or humans obviously ill. Recently, in a Nigerian aquatic environment, Adeogun *et al.* (2016) observed that fish (*Sarotherodon melanotheron*) sampled from a water reservoir contaminated with EDCs (such as PCBs, PAHs, OCPs and heavy metals) exhibited good growth (higher condition factor - CF) which paralleled increases in transcriptional activation of *ppar* and *cyp*1 isoforms as compared to fish sampled from an unpolluted river. They concluded that the contaminants in the water could be inducing an obesogenic effects. These assertions possibly give credence to the observation on weight gain observed in the study herein.

A possible mechanism suggested for EDCs leading to excess weight gain is that exposure to obesogen chemicals from the environment may influence the steroid hormone receptors or may change serum levels of metabolic hormones or may influence nuclear receptor signaling pathways in preadipocytes, which would result in adipocyte differentiation and a tendency to excess weight (Grun and Blumberg, 2007).

Similarly, the absolute and relative liver, kidney, lungs and spleen weight gain were higher in most exposed rats and at most exposure periods compared to their corresponding control. For the landfill exposed groups, absolute liver, kidney, lungs and spleen weights showed significant ($p<0.05$) positive correlations with exposure periods, however, relative liver, kidney, lungs and spleen weights showed significant ($p<0.05$) inverse relationships with exposure periods. Increase in organ absolute and relative weight has been associated with nephrotoxicity, hepatotoxicity and immunotoxicity (Alimba *et al.,* 2012). In support of this assertion, chemical treatment has been related to liver weight gain; and this is associated with hepatocellular hypertrophy (e.g. enzyme induction of peroxisome proliferation) (Juberg *et al.*, 2006). Also, heavy metal and landfill chemical induced kidney weight gain in exposed rats may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Graeves, 2000). While splenic weight changes have been associated with immunotoxicity, stress and physiological perturbations (Michael *et al.*, 2007).

The increase in organ weight observed herein, suggests the obstructions of these organs by the underground water and air constituents, likely the heavy metals, and this is because of the duty of these organs in mopping away metals immediately they enter the body systems (Barbier *et al.*, 2005). Alimba *et al.* (2012) noted that the increased organ weight gain could possibly be due to heavy metal bioaccumulation in the organs. We observed significant higher concentrations of lead, cadmium, chromium, copper, and zinc in the liver, kidney and lungs of exposed rats compared to their corresponding controls, though not duration dependent. In further support, in this study for the landfill exposed rats, the absolute liver weight showed positive correlations with the accumulations of Pb, Cu and Zn in the liver; the absolute kidney weight showed positive correlations with the accumulations of Cu and Zn in the kidney; and the absolute lung weight showed positive correlations with the accumulations of Cr and Cu in the lungs. However, the relative liver, kidney and lung weights showed negative/inverse relationships with the accumulations of Pb, Cd, Cr, Cu and Zn in the respective organs. The inverse relationships between the relative organ weight with exposure periods and organ-metal bioaccumulations respectively, suggest a potential hepatocellular, nephrotubular and pulmonary degeneration/atrophy, resulting from the toxic effects of the bioaccumulated heavy metals.

Similar observations by Sánchez-Chardi and Nadal (2007) and Sánchez-Chardi *et al.* (2007) on small mammals; white-toothed shrew (*Crocidura russula*) and wood mouse (*Apodemus sylvaticus*) inhabiting Garraf landfill. They reported significant bioaccumulation of heavy metals in liver and kidney of these rodents, however, with increased relative organ weight. Pereira *et al.* (2006) also observed significant metal (Pb, Cd, Cr, As, Zn, Fe, and Ni) bioaccumulation in the hair and tissues (liver, kidney and spleen) of wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandon landfill site in Portugal. Also, organ weight change correlate well with histopathological changes in chemical exposed animals (Michael *et al.*, 2007).

In summary, the observations herein together with other reports mentioned above suggest that heavy metal bioaccumulation in the organs may be associated with altered relative liver, kidney, lungs and spleen weight gain in the landfill exposed rats.

5.3.3 Metal bioaccumulation and interactions in organ-system of exposed rats at OL

In this study, contaminated borehole water at OL was the routes of metal and other unidentified pollutant exposure. The results herein showed significant elevation of the analysed metals in the liver, kidney, lungs and blood of rats exposed to borehole water. Following exposure to heavy metal contaminated water, PM, and other air emissions, soft tissues such as liver, kidney, lung and brain are usually where metals are strongly accumulated (Li *et al.*, 2015).

The liver is the main detoxification organ primarily exposed to toxic and essential substances that enter the animal through inhalation and ingestion. Its main blood supply comes from the intestine (Pereira *et al.*, 2006). The liver extracts metals from plasma, metabolizes, stores and redistributes them in various forms either into the bile or back into the blood stream (Bortey-Sam *et al.*, 2016). These statements give credence to the consistent higher metal concentrations measured in the liver of landfill exposed rats throughout the study periods as compared to the corresponding controls.

In this study for landfill exposed rats, there was inverse relationship between Cd accumulation in liver and Zn accumulation in liver, though not significant. In further support of this observation, at each exposure periods and points significant increase in Cd concentrations in the liver corresponds with insignificant increase in Zn

concentrations in the liver and vice versa. Steep reductions and non-significant increase in concentrations of Cd in the liver at the 12 weeks exposure period were concomitant with a significant elevation in the concentrations of Zn in the liver at the 12 weeks exposure period. Anetor *et al.* (2008) and Ugwuja *et al.* (2015) observed similar inverse relationships between Cd and Zn in the blood of environmentally exposed human populations in Nigeria. A higher Cd:Zn ratio was observed in cigarette smokers, which was 4.5-fold the level in non-smokers (Anetor *et al.*, 2008). Zinc and cadmium invariable occur together in nature because of their similar chemical properties, and while Zn is an important micronutrient involved in numerous physiological and molecular activities, Cd is an extremely toxic element to biological system (Anetor, 2012). Zinc and Cd are metabolic antagonist to each other, and usually compete for binding sites on metallothionine (MT), however, Cd has higher affinity for MT than Zn (Klaassen *et al.*, 2009). Anetor (2012) noted that small repeated low doses of Cd could accumulate and mimic Zn and increase in Cd levels diminishes Zn levels in the system leading to adverse health effects. Invariably, pre-treatment with Zn was found to be protective against Cd toxicity (Jemai *et al.*, 2010). These observations support the inverse relationships in the concentrations of Cd and Zn observed in the liver of landfill exposed rat in the study herein.

Similarly, lower and non-significant level of Cd measured in liver at 12 weeks exposure could be the result of the natural detoxification action of the liver via biliary excretion, which will then be passed out through the feaces and urine (Klaassen *et al.*, 2009). However, on continuous/chronic exposure to the Cd sources (in this case, water and air), the Cd accumulation in the liver were further significantly increased. A possible reason for this subsequent elevation in liver-Cd level could be a protective and adaptive increase in metallothionein (MT) synthesis in the liver, which in turn increase the hepatic uptake of Cd out of the systems into the liver (Klaassen *et al.*, 2009) for subsequent excretion.

It has been mentioned that the liver of rats and mice is the main target organ for some essential elements (such as Zn, Cu and Cr) and this organ helps to maintain homeostasis of these elements in the body (Pereira *et al.*, 2006). In this study, Zn and Cu levels in the liver reached their peaks during the chronic 20 and 24 weeks (5 and 6 months) exposures on the landfill. This also follows with reduction in liver-Cd

concentration during this period. A possible explanation for this could be that Zn and Cu may have interacted with the Cd in the liver, because these metals (Zn and Cu) are known to participate in detoxification processes, and as part of the enzymes of the protective antioxidant systems, such as superoxide dismutase (CuZnSOD) and in metallothioneins (Sánchez-Chardi and Nadal 2007). The significant strong positive correlation of Cu and Zn accumulations in liver with exposure periods; the significant strong positive relationship between Cu and Zn in the liver; and inverse relationships of both Cu and Zn with Cd respectively in the liver of landfill exposed rats supports this assertion of a potential physiological protective function from the toxic effect of Cd. This is in concordance with the finding of Sánchez-Chardi and Nadal (2007) on Liver-Cu level in the juvenile shrews inhabiting metal-polluted site.

It is also worth noting that herein, despite the observed potential protective functions of Zn and Cu in the exposed rat liver and kidney, they could pose toxic effects at extreme high concentrations. Continuous loading of the animal system with essential elements (which are needed in minute quantities) could be toxic to the system. The concentration of these essential elements reported herein are higher than previously reported (Sánchez-Chardi *et al.*, 2007; Sánchez-Chardi and Nadal, 2007; Pereira *et al.*, 2006). These metals too may have bioaccumulated and may have contributed to the observed toxicity in the liver and other system. In giving credence to this observation, Ma and Talmage (2001) noted that, in mammalian systems, the uptake, bioaccumulation and excretion of essential elements are effectively controlled physiologically and they bioaccumulate only in cases of extreme high intake or disrupted metal metabolism.

The main organ responsible for excretion in mammalian systems is the kidney, and the kidney has been said to have a faster metabolism than any other organ (Li *et al.*, 2015). However, kidney has been mentioned as one of the preferred organ for metal bioaccumulation (Pereira *et al.* 2006). In the study herein, going by the average range of Pb, Cd and Cu concentrations measured in the blood and soft tissues, the kidney may be said to be the most preferential organ of toxic metal bioaccumulation. The possible reason for this could be that the metal-metallothionein complex that is formed in the kidney and liver is eventually stored in the kidney before reabsorption. Sánchez-Chardi *et al.* (2007) noted that, bioaccumulation in organs is associated with

detoxification mechanisms, namely the formation of metal-metallothionein in liver, which is transported by blood and then stored mainly in the renal cortex. Similar mechanism was agreed to explain the age-dependent accumulation of Hg and Pb in kidney as reported for other small mammal species (Sánchez-Chardi *et al.*, 2009). Also, Sánchez-Chardi and Nadal (2007) observed that the distribution of Pb reached higher concentrations in kidney when compared with liver in the shrews from Garraf landfill. Furthermore, Onifade *et al.* (2016) reported higher Pb accumulation in kidney compared to liver of rats fed with lead contaminated mushroom (*Pleurotus pulmonarius*). They explained that, due to regular renewal of the hepatocytes during intoxication in the liver, the Pb^{2+} were released from the metallothioneins and glutathione conjugates back into the systemic circulation and are subsequently sent to the kidney via blood. On the contrary, the distribution of metals to the kidney was not consistent with the observation of Sánchez-Chardi *et al.* (2007b) for *Crocidura* species inhabiting an abandon pyrite mine site.

However, this phenomenon of preferential tissue distribution of metals may be related to physiological mechanisms to decrease toxicity (e.g. metal-MT complex), type and time of exposure, and/or the half-life of metals in soft tissues (Sánchez-Chardi and Nadal 2007). In the study herein, though kidney-Cd concentrations in landfill exposed rats showed an insignificant weak inverse relationship with exposure periods, the accumulation of Cd in kidney remained consistently and significantly higher than those of the control throughout the study periods. The possible explanation for this could be due to the long biologic half-life (6 – 38 years) and low excretory rate of Cd in the kidney. ATSDR (2012) reported that once Cd enters the kidney, it remains tightly bound to metallothionein and is almost completely reabsorbed in the renal tubules, making the rate of excretion to be very low and bioaccumulation in the kidney high. Anetor *et al.* (2008) also noted that due to the extremely long biological half-life of Cd, ex-smokers still have higher Cd levels in their blood compared to non-smokers.

Lead levels in blood, liver, kidney and lungs in this study decreased subsequently over the periods of chronic landfill exposure after attaining a certain accumulation maximum. This observation is supported by the findings of Moore (1975) on the assessment of chronic lead exposure in the heart and other organs in rats. They observed that rats given 1 mg Pb/L in drinking water attained a maximum

accumulation in blood, bone and soft tissues after 6 months (24 weeks). Cikrt *et al.* (1983) noted that Pb is excreted by biliary clearance; and other part of the accumulated Pb is being mineralized in the rats' bone. These various processes of lead removal and detoxification could be the reasons for the subsequent decreases in Pb level in blood and soft tissues after attaining an accumulation maximum as observed herein. The control groups never get to these Pb accumulation maximum in blood and tissues, so this patterned decreases in Pb levels was not observed. This observation further affirmed that the Pb accumulation in landfill exposed rats could only have been from environmental exposure.

In the absence of known exposure, whole blood chromium concentrations in human are in the range of 0.02 to 0.03 mg/L. Lower levels occur in rural areas and higher levels occur in large urban centres. Values above background levels are considered potentially toxic. However, Cr rapidly clears from the blood and measurement in blood only reflects recent exposure (ATSDR, 2012). The upper range of Cr concentrations in blood of control rats possibly reflect exposure to environmental Cr from urban centre (Lagos metropolis), while the upper range of Cr concentrations in blood of landfill exposed rats possibly reflect recent exposure from the landfill contaminated groundwater and air emissions. Also, in this study, chromium was almost not detected in the liver in the 4 and 8 weeks exposures of rats to the landfill. However, Cr was significantly detected in the blood, lungs and kidney at the 8 weeks exposure. This observation suggests inhalation as the main route of exposure to Cr during this period of the study. Chromium is known to show lower intestinal absorption (Outridge and Scheuhammer, 1993) and intestinal absorption is the direct route to the liver. Thus, the non-detection of Cr in the liver and presence in the blood and lungs during the same period possibly showed that the route of exposure to the chromium source during this period is via inhalation.

Subsequently, in this work, significant elevation of Cr in the blood and liver of rats exposed for 12 weeks, corresponded with a decrease in the level of Cd in the blood and liver at the same period. A report by ATSDR (2012) supports this observation. They noted that the presence of elevated Cr or Zn in the diet (contaminated water in this case) decreases Cd uptake. This observation gives credence to the fact that oral exposure to Cr may have also taken place. In summary, given the significant

accumulation of Cr in blood, lungs and liver observed herein, it may be said that exposed animals could be at risk of chromium toxicity. Arita and Costa (2009) noted that though Cr is an essential element but too much and long term exposure to Cr compounds produce adverse health effect which may include cancer of the lungs and liver.

5.3.4 Haematological alterations in landfill exposed rats

In the study herein, there was an initial significant decrease in the RBC with concomitant significant decrease in PCV, Hb, MCV and MCH in rats exposed for four weeks to the landfill as compared to the control groups. Decrease in RBC and RBC parameters could be due to destruction of the blood cells resulting from the toxic constituents of the landfill such as heavy metals. It is known that the haematopoietic system in mammals is highly sensitive to inflammations due to their rapid synthesis and destruction of cells (Pietras, 2017). The observed decrease in RBC and RBC parameters may be due to toxic metals and pathogens induced inflammation which resulted in disturbance in the haem biosynthesis pathway. Wachukwu and Eleanya (2007) and Odewabi *et al.* (2012) observed similar decrease in RBC, PCV, Hb and MCV in waste management workers. Their findings corroborate with the deleterious influence of systemic inflammation on the haematopoietic system. Also, Alimba and Bakare (2012) reported similar significant reduction in reb blood cell, haemoglobin, haematocrit and MCHC in Wistar albino rats orally gavaged for 30 consecutive days with 0.5 ml different concentrations (1 to 25 %) of leachate from Olusosun and Aba-Eku landfills, both in Southwestern Nigeria.

Heavy metals identified in the groundwater and in the blood of exposed rats are potential culprits in inducing inflammation and eventual oxidative stress in the animal system. In support of this assertion Flora *et al.* (2008) noted that lead (Pb) exposure targets haem synthetic pathway, inhibiting haem and haemoglobin synthesis. It also induced damage in haematopoietic systems. Similar observation was made by Hounkpatin *et al.* (2013), where they exposed Wistar rats orally to chronic (28 days) doses of Cd, Hg and their combinations. They reported significant decrease in RBC, Hct, Hb and MCHC in metal treated rats. MCHC is an expression of the average concentration of haemoglobin in red blood cells. It value shows the ratio of the weight

of haemoglobin to the volume of red blood cells. It decrease signifies that a unit-volume of packed red blood corpuscles contains less haemoglobin than normal or that haemoglobin has been replaced by erythrocytic stomal materials as in iron deficiency (Fischbach, 1984). A decrease in haemoglobin and concomitant decrease in MCHC indicate a tendency to macrocytosis and hypochromic haematopoiesis (Hounkpatin *et al.*, 2013).

However, in this study, rats exposed to the landfill pollutants beyond four weeks show a general tendency towards increase in the RBC and RBC parameters. In other words, with continuous and prolong exposure, there may have been an adaptive and compensatory response to haematotoxicity by the erythropoietic cells, which tends to produce more blood cells to make up for what is being destroyed directly due to xenobiotics and/or hypoxic condition (decreased RBC oxygen carrying capacity) induced by the xenobiotics. Similar observation of increased RBC, Hct and Hb was made by Oshode *et al.* (2008) on fish exposed to a landfill leachate as the concentrations of exposure increases. They noted that, this might indicate an adaptive/protective compensatory erythropoiesis, which resulted in production of RBC to recompense the older ones that are rapidly destroyed due to decrease in blood's oxygen carrying capacity induced by environmental toxicants. Furthermore, in support, Soivio and Nikinmaa (1981) noted that increase in haematocrit (PCV) is an indication of a stress response, causing RBC swelling, or haemoconcentration due to plamatic volume reduction. These assertions are possible explanations to the findings in the study herein.

Moreso, in the study herein, significant positive correlation between RBC, PCV and Hb and between MCV and MCH in the landfill exposed rats as the duration of exposure increase (4 to 24 weeks) further strengthen this assertion. These correlations were not observed in the control animals. Similar tendency was observed by Sanchez-Chardi *et al.* (2008) in shrew *Crocidura russula* living in a metal polluted site.

White blood cells (WBCs) play an important role in the immune system of living organisms. An unusually high WBC count can indicate hypersplenism, inflammation, trauma and stress (Oshode *et al.*, 2008). In this study, increase in WBC both at acute and continuous/chronic exposures of rats to the landfill contaminants was observed (as

compared to corresponding controls). This observation suggests an immune response by the exposed rats due to the environmental contaminants and pathogens. It also suggest that there are underlying chronic inflammations which is eliciting immune response (Odewabi *et al.*, 2012). Odewabi *et al.* (2012) similarly observed significant increase in WBC count in municipal solid waste management workers when compared with the reference participants.

Neutrophils, basophils and eosinophils are different types of WBC commonly release during immune response. Neutrophils or granulocytes are the most common immune cells in the body. With an infection, their number increases rapidly. They are the major components of pus and are found around most common inflammations. Their job is to phagocytize and destroy foreign materials (Cheesbrough, 2005). Observed increase in neutrophils in some rats with continuous and chronic exposures (16 and 20 weeks) in this study could be an immune response against foreign bodies (pathogens and xenobiotics) and/or response toward induced inflammations by toxic constituents of the groundwater and air emissions.

Previous studies (Oshode *et al.*, 2008; Efuntoye *et al.*, 2011) showed the presence of pathogenic feacal coliforms which have been implicated in gastroenteritis, in leachates from landfill sites in Southwestern Nigeria. Enterotoxin produced by these diarrhea-causing bacteria may be implicated for the elevated phagocytic neutrophils. In the same vein, recurrent abscesses observed in the exposed animals further strengthen the presence of foreign bodies and infections in exposed rats. Odewabi *et al.* (2012) observed similar increase in neutrophil in the blood of individuals occupationally exposed to municipal solid waste in Ogun state, Nigeria. They noted that, inflammation and phagocytic cells (such as neutrophils) associated with inflammation are the major source of oxidative stress in the system of exposed waste workers. Decreases in neutrophils in rat at most other exposure periods (4, 8, 12 and 24 weeks) as compared to their corresponding controls could be due to rapid cell death and loss resulting from direct interaction of xenobiotics (such as heavy metals) and their metabolites with these phagocytic cells. This is possible in a situation where the toxicants overwhelm the defensive system, especially from continuous exposure or dosage, and thus poisoning the cells (Don-Pedro, 2009).

The increase in lymphocyte observed in the study herein may be due to the presence of bacterial infections, protozoan infections and granulomatous process like hypersensitivity pneumonitis affecting the health of the exposed rats (Cheesbrough, 2005). Wachukwu and Eleanya (2007) reported similar observation in the blood of solid waste disposal workers in Port Harcourt, Nigeria. Also, Oshode *et al.* (2008) reported increase WBC and lymphocyte numbers in leachate treated mud cat fish. However, decreased lymphocytes observed in some rats with chronic exposure (16 and 20 weeks) to the landfill may indicate tendencies toward lymphocytopenia and immune-suppression. This reduction in lymphocyte may also be attributed to the toxic effects of xenobiotic contaminants from the landfill on the lymphoid tissues, hence reduction in the quantity of lymphocytes released into the circulation (Alimba and Bakare, 2012). It is also possible that cortisol secreted during contaminant induced stress in treated rats shortened the life span of the lymphocytes and promotes their apoptosis or reduce their proliferation rate, hence leading to compromised immune response (Pietras, 2017).

The observed alteration in haematological parameters in landfill exposed rats may have been due to bioaccumulation of heavy metals from the groundwater and air emission. This assumption is supported by the findings of Sanchez-Chardi *et al.* (2008), that bioaccumulation of heavy metals (Pb, Cr and Cu) from landfills significantly negatively correlated with altered haematological parameters in a small mammal. Similar negative correlations were observed between blood concentrations of Pb and PCV, RBC, Hb, MCV, MCH, MCHC, WBC and lymphocyte; between Cd and MCV, MCH and neutrophils; and between Cr and MCV, MCH and neutrophils in landfill exposed rats in the study herein. Conversely, blood concentrations of Cu and Zn indicated positive correlations with PCV, RBC, Hb, MCV, MCH and neutrophil.

These assertions and findings are consistent with the significant DNA damage/chromosome breakage observed via bone marrow micronuclei (MN) formation and oxidative stress (increased CAT, GSH and MDA; and reduced SOD) induced in the study herein and suggests direct damage to blood cells or impairment of bone marrow erythropoietic cells by landfill xenobiotics. Furthermore, the observed positive correlations between blood concentrations of Cu and Zn and red blood cells and RBC parameters also suggest the role of these micronutrients in cell damage repair

and antioxidant systems. Anetor *et al.* (2008) noted that Pb may interfere with Ca and Zn level possibly by substitution, however, careful manipulation to increase the levels of Ca and Zn ions for instance in the body system may modulate Pb toxicity.

5.3.5 Serum biochemical changes in rats exposed *in situ* to landfill

Serum biochemistry has been validated in *in vivo* studies in assessing the toxic potentials of chemical compounds. Some serum biochemical analyses are organ-specific biomarkers and are used in detecting possible mechanisms of chemical toxicity (Travlos *et al*., 1996). The liver and kidney function serum biochemistry were analysed in this study because the liver and kidney are important organs involved in metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and these organs are particularly vulnerable to damage (WHO, 1992). Also, biochemical analysis correlates well with histological alterations in affirming organ-specific induction of toxicity by xenobiotics.

In organs, cell and membrane destruction is accomplished with the release of diverse cytoplasmic enzymes into the circulatory system. Alanine aminotransferase (ALT) is found in greatest concentration (highly specific) in the liver of rats and dogs; aspartate aminotransferase (AST) can be found in various concentrations in muscles, heart, liver, kidney and intestine; and alkaline phosphatase (ALP) is present in the greatest amounts in tissues that have an absorptive or transport function, such as intestine, kidney, placenta and liver. ALP is also found in bone and human neutrophils and could indicate bone and hepatobiliary diseases. However, concomitant increase in the activities of these enzymes in the serum denotes acute hepatocellular injuries and xenobiotic induced necrosis/cell death (Davies, 1992). Serum ALT, AST and ALP are the most used markers of hepatocellular necrosis and are considered sensitive indicators of hepatic injury and cell membrane damage and leakage (Friedman *et al.*, 1996).

The significant and progressive increase in the activities of serum AST, ALT and ALP observed in this study compared to the control groups indicate a progressive chronic hepatocellular injury, which was induced by the toxic constituents of the contaminated underground water and ambient air emissions at the waste landfill site. The heavy metals and other unanalyzed deleterious constituents (organic, inorganic and

mycotoxins) present in the contaminated water, particulate matters and air emissions and their metabolites may induce oxidative tissue damage to the hepatocytes, leading to increasing cell membrane permeability and eventual cell death. This finding was supported by the surgical detections of hepatic cyst and tumor which were consistent and progressive in severity in rats liver exposed for 8, 12, 16, 20 and 24 weeks on the landfill. Whereas, this observation of hepatic cyst and tumor were completely absent in the liver of the control groups.

Similar increase in serum AST and ALP were observed in residents consuming leachate contaminated groundwater in Hardeman County, Tennessee, USA (Meyer, 1983). Meyer (1983) noted that, there must have been a subclinical transitory liver injury. Sanchez-Chardi *et al.* (2007) observed elevated activities of ALT in the blood of wood mouse (*Apodemus sylvaticus*) inhabiting Garraf landfill (NE Spain) and exposed to leachate from the site; and this was concomitant with structural alterations in the liver and kidney of the exposed animal. Bakare *et al.* (2013a) reported significant increase in the activities of ALT and AST in mice given e-waste contaminated well water to drink for up to 5 weeks and another group IP injected with 0.5mL (1 – 50 %) e-waste leachate for 5 consecutive days. Also, Alimba *et al.* (2012) observed elevated ALT and AST activities with multifocal degeneration of hepatocyte, congestion and hepatocellular necrosis in liver of male and female rats exposed orally for 30 consecutive days to 0.5 – 25 % leachates obtained from two landfills in Southwest Nigeria. They concluded that the observed alterations in serum biochemistry and lesions may be due to oxygen deficiency and/or the presence of ROS induced by metals or other leachate components.

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme which is involve in catalyzing the interconversion of pyruvate and lactate, with the concomitant interconversion of NADH and NAD^+. LDH is predominantly found in skeletal muscles, heart, brain, liver and all tissues/cells where cellular respiration takes place (Valvona *et al.*, 2016). The normal cell membrane is impermeable to LDH and it is only release in case of plasma membrane damage and necrosis; thus LDH can be measured as an index of cytotoxicity in living tissues (Chan *et al.*, 2013).

In the study herein, LDH activities significantly increase in the serum of rats exposed *in situ* to borehole water and ambient air at OL as compared to the control rats throughout the exposure periods. When disease or injury damages tissue, cells release LDH into the blood stream (Chan *et al.*, 2013). Heavy metals and other unanalyzed chemicals present in the groundwater could be responsible for this cell/tissue damage. Karthikeyan and Bavani (2009) reported that prolonged Cd exposure in rat (*Rattus norvegicus*) led to significant increases in LDH_1 and LDH_2 (LDH-isoenzymes) in the liver tissue. They suggest that LDH might be a marker enzyme for liver injury. Mekkawy and Lashein (2003) observed that sub-lethal doses of Pb and Cd induced increases and variability in LDH-isoenzymes in developing teleost fish *Ctenopharyngodon idellus* and this was associated with necrosis of the caudal fin margins, spinal and vertebral curvature and pale body pigmentation.

Mitochondria have long been known to be sensitive to lead (Pb) and other toxic metals (Oskarsson and Fowler, 1985) with both morphological and biochemical alterations and with decreased respiratory functions (reduced ATP/energy production via anaerobic glycolysis). Mitochondria impairment and/or oxygen scarcity conditions induced by metals can cause a switch in cellular respiration to anaerobic respiration; this usually involves increase activity of the LDH enzyme (Valvona *et al.*, 2016). In support of this assertion, Parveen *et al.* (2017) reported increase in the activities of serum LDH in fish *Channa punctatus* exposed to different concentrations of tannery waste water. Alterations in physico-chemical quality and higher chromium (Cr) concentrations in the waste water were believed to induce cellular hypoxia, creating anaerobic condition and cellular damage. Thus, rise in serum LDH activities in this study indicates cytotoxicity to different tissues/organs of the body induced by heavy metals and other contaminants from the landfill environment.

Serum total protein (TP) and albumin (ALB) are supplementary indicators of hepatic synthetic functions (Meyer and Harvey, 2004) and nephrotoxicity (Gowda *et al.*, 2010). In this study, concentrations of serum TP and ALB generally decreased in the exposed rats as compared to their corresponding controls. The decrease in serum total proteins and albumin in landfill exposed rats may indicate disorders in protein synthesis, metabolism and necrosis as a result of individual actions or interactions of the complex chemicals, such as heavy metals and other unidentified constituents of the

leachate contaminated groundwater and air emissions. Metals in their ionic forms, bind to albumin and plasma proteins thereby affecting their metabolic processes and/or transport to the kidney tubules (Barbier *et al.*, 2005). It has been similarly reported that rats exposed to Cd showed reduced serum protein with increased serum urea and creatinine due to functional damage to kidney (Gounden *et al.*, 2020) and concomitant decrease in serum albumin and serum total protein has been associated with hepatocellular damage (Ekam and Udosen, 2012).

Furthermore, strengthening the observations of potential hepatocellular and cytological alterations in the exposed rats, though there was no strong correlation between exposure periods and serum activities of AST, ALT, ALP and LDH; AST and LDH activities indicated insignificant directly proportional relationship with exposure periods. Also, serum levels of ALB and TP indicated inverse relationships with exposure periods, and this was significant with serum ALB levels. Also, despite the higher accumulations of lead, cadmium, chromium, copper and zinc in the liver of landfill exposed rats, significant, strong directly proportional relationship were only observed between liver-Cr concentrations and AST, ALT, ALP and LDH activities; and similarly, significant strong inverse relationship were observed between liver-Cr concentrations and ALB levels; and between liver - Pb and Cd concentrations and TP levels.

These results imply that Pb, Cd and Cr are the potential culprit in the observed cytotoxicity and hepatocellular damages. However, apart from Cr which indicated obvious significant strong correlation with altered liver function parameters, the weak correlations observed with other toxic metals suggest that the observed cytotoxicity and hepatocellular damages could have been the effects of additive/synergistic interactions of the heavy metals and also contributions from unanalyzed organic pollutants present at landfill sites cannot be overlooked. This is similar to the observation and assertion of Tull-Singleton *et al.* (1994), where they correlated the higher concentrations of Pb and Cr in liver-tissues of landfill exposed white footed mouse (*Peromyscus leucopus*) to genetic and systemic damages. Parveen *et al.* (2017) observed similar systemic damage in fish (*Channa punctatus*) and associated this to the altered physico-chemical parameters and higher Cr concentrations in the waste water.

Serum creatinine and urea concentrations are biomarkers of renal injury (Travlos *et al.*, 1996; Gowda *et al.*, 2010). Their elevation is associated with impairment of renal function (Gowda *et al.*, 2010). Renal function may be impaired for instance, when there is decrease in glomerular filtration rate resulting from restriction of renal blood supply, or from damage to nephrons by physical or chemical agents. This usually leads to retention of waste products of metabolism (including urea) in the blood.

The observations from the study herein showed possible progressive impairment of renal functions resulting from progression in the severity of kidney injury. Serum creatinine and urea were elevated in all the rats exposed to Olusosun landfill contaminants via the underground water and air emissions, with more significant elevation at each points of exposure with longer exposure periods as compared to the corresponding control. In further support of these observations, correlation analysis for the pooled data on landfill exposed rat revealed that there was significant strong positive relationship between exposure periods and serum urea levels, although, serum creatinine indicated a significant inverse relationship with exposure periods. Brzoska *et al.* (2003) similarly observed elevated urea level and histopathological lesions such as degeneration and hypertrophy of epithelial cells and dilation in glomeruli in rats exposed to Cd, ethanol and their mixture for 12 weeks.

Creatinine and urea levels were similarly elevated in serum of rats orally exposed to leachates from two landfills in Nigeria. This was equally accompanied with degenerative epithelia of renal tubules and necrosis (Alimba *et al.*, 2012). Heavy metals and anions present in the leachates were said to have possibly induced kidney dysfunction in rat via free radical formation and/or direct chemical disruption of the organs. Also, drinking water contaminated with metals such as Pb, Cd, Cu, Mo, Ni and Cr resulted in elevated creatinine and urea levels in blood of residents in Hail, KSA, and this was related to increased incidence of kidney disease observed in the study area (Shokr *et al.*, 2016).

Kidney injury may be due to depression of glomerular filtration rate and renal tubular cell injury caused by metals (e.g. Cd, Pb and Cr) analyzed in the underground water (Basile *et al.*, 2012). Heavy metals readily bioaccumulate in the kidney and are responsible for a high number of nephrotoxicity observed in mammals (Stohs and

Bagchi, 1995; Mantos *et al.*, 2010). The higher concentrations of metals in the underground water (above permissible limits) and significant accumulation of lead, cadmium, chromium, copper and zinc in kidney observed in this study support these assertions. This was further strengthened with observations from correlation analysis. In this study, serum urea levels indicated a positive correlation with kidney accumulations of Cr, Cu and Zn and this was significant with copper (Cu).

Also, serum LDH activities indicated a significant positive relationship with Cr accumulations in the kidney of the landfill exposed rats. However, weak and insignificant inverse relationship was observed between serum creatinine and metal accumulations in the kidney. Significant accumulations of Cr and Cu in the kidney tissues and their positive association with urea levels and LDH activities in the exposed animal system could imply that these metals are culprit in inducing the observed nephrotoxicity.

In summary, in this study, we observed significant increase serum AST, ALT, ALP, LDH, creatinine and urea with concomitant decrease in albumin and total protein. Kalra *et al.* (2021) noted that liver and kidney are the most important organs involved in metabolism and detoxification of xenobiotics and their metabolites and this made them vulnerable to damage when exposed to toxicants especially heavy metals. Findings from this present study support this assertion. In this study, significant elevations in the concentrations of Pb, Cd, Cr, Cu and Zn in the administered underground water from Olusosun landfill and evident accumulations of these metals in the blood, liver, kidney and lungs of exposed rats was observed. The observed heavy metals and other unanalyzed xenobiotics that may be present in the water and landfill air emissions account for the observed alterations in hepatic and renal function parameters and general systemic cytotoxicity.

This is in concert with the findings of Alimba *et al.* (2012) on leachate exposed rats, Saleh (2014) on rats drinking lead (Pb) contaminated water and Odewabi *et al.* (2012) in workers exposed to municipal solid waste in Ogun state, Nigeria. Selah (2014) observed that lead administration to rats showed a significant elevation in serum AST, ALT, ALP and LDH activities and increased serum level of total bilirubin, beside reduction in serum level of albumin in rats. Similarly, Osuala *et al.* (2014), in a

toxicological evaluation of some heavy metals in mice revealed a potential liver and kidney damages. They observed increase in the level of ALT, urea, albumin, and total protein, and concluded that these batteries of biomarkers could serve as a good early detection and diagnostic tools for heavy metal pollution in mammalian systems. The observed alterations in serum biomarkers of systemic damage could be the resultant toxic effect of individual metal and/or additive, synergistic or antagonistic interactions of two or more metals.

5.3.6 Oxidative damage and lipid peroxidation in liver of rat exposed *in situ* to landfill

The mechanism by which toxic complex mixtures in contaminated underground water and leachate from waste landfills induced abnormal cellular functions and DNA damage has been suggested to be via oxidative stress (Li *et al.*, 2010; Bakare *et al.*, 2012a; 2013a).

In this study, contaminated borehole water and ambient air at OL induced elevated levels of lipid peroxidation (measured from MDA levels) and alterations in the activities of antioxidant enzymes in liver of exposed rats throughout the periods of exposure (4 to 24 weeks). The results herein showed a significant elevation in the activities of CAT and GSH, and a concomitant decrease in the activities of SOD. MDA, an end product of lipoperoxydation, is considered a biomarker of oxidative stress and cellular damage (Kim *et al.*, 2015). Increase in lipid peroxidation may be attributed to the accumulation of heavy metals in the organs and metals have been documented to catalyze the formation of reactive oxygen species (ROS) which are capable of damaging biomolecules such as DNA, proteins and lipids (Pandey *et al.*, 2003).

Increase in the activity of CAT and SOD is usually observed in the presence of environmental pollutants, while SOD-CAT system represents the first line of defence against oxidative stress (Ighodaro and Akinloye, 2018). However, in this present study, SOD activity decreased in the liver of exposed rats. Superoxide radicals or their transformation product, hydrogen peroxide (H_2O_2), are capable of causing the oxidation of cysteine which will lead to decreased SOD activity (Phaniendra et al., 2015). Activities of SOD were markedly decreased especially with more chronic

exposures (20 and 24 weeks) to the landfill pollutants and this resulted in corresponding significant increase in CAT activities. This implied that as more H_2O_2 was being produced from transformed superoxide radicals, more CAT activities were induced to degrade the highly potent hydrogen peroxide oxidant against increased oxidative stress.

GSH is an important antioxidant and it functions in protecting the cells against oxidative damage (Wu *et al.*, 2004). Glutathione response varies depending on the nature of the pollutant, response duration, and species (Lackner, 1998). In this this study, there was apparent significant increase in GSH levels with longer duration (20 and 24 weeks) of exposure. Farombi *et al.* (2007) noted that increase in GSH levels in organs is an adaptive and protective response of this biomolecule against oxidative stress. Thus, the observed increased in GSH levels in this study; especially with longer-term exposures support this assertion.

The observed concentrations of Pb, Cd, Cr, Cu and Zn and other toxic constituents not analysed in groundwater and air emissions from OL and their accumulations in organ-system in this present study can be implicated as the possible cause of increased ROS in the rat's system, leading to concomitant alterations in the antioxidant system. Increased ROS could induce oxidative stress, and this could be the possible underlying mechanism of the genetic and systemic toxicity observed in this study.

5.3.7 Gastric physiopathological alterations in Olusosun landfill-exposed rats

One major route of exposure to environmental chemicals is the oral route via ingestion, and any substance that pass through this route get to the reservoir and digestive organ called the stomach. The vertebrate stomach performs a variety of functions, including serving as a reservoir for food, exposing ingested food to acid secreted by the parietal cells and pepsin secreted by the chief cells. The stomach also provides a barrier that prevents microorganisms from entering the intestine. Also, the mucous layer contains mucus cells that produce mucin; this protects the mucosal surface from the potential harmful components (HCl and pepsin) in the lumen. It is worth to note that, the diverse physiological functions of the stomach depend on an intact gastric mucosal integrity (Duan *et al.*, 2006).

In the study herein, significant increase in acid (HCl) producing parietal cells with concomitant increase in mucus cell counts and increased production of mucin in the stomach at most exposure periods were observed. These alterations in gastric secretion could have been due to the presence of toxic contaminants in the underground water such as identified metals (Pb, Cd, Cr, Cu, Zn and Fe) and unidentified organic pollutants. Also, excessive production of gastric mucus could be a protective and compensatory stress response of the mucosal epithelium to prevent damage that may be induced by the increased acid production of the parietal cells. In support of this assumption, Newman and Maclean (1974) and Gutierrez *et al.* (1978) reported that loss of structural integrity of mucosal folds, degenerative mucosal epithelium, excessive mucus in gut of lumen, amongst others, are common toxic lesions and response observed in the intestine of different fishes exposed to cadmium chloride ($CdCl_2$).

Similarly, in this study, increase in parietal cell count with concomitant significant decrease in the protective mucus cell count and mucus secretion observed at 8 and 12 weeks exposures could be tendency toward gastric juice imbalance, inflammation and ulceration. Abd El-Rady *et al*. (2021) noted that, imbalance between the protective mechanisms (such as mucin, bicarbonate and prostaglandins) and aggressive factors (such as *Helicobacter pylori*, non-steriodal anti-inflammatory drugs [NSAIDs] and high gastric acid) is the most prominent pathogenesis of peptic ulceration.

In addition, longer exposure periods and higher dose of exposure to toxic chemical from the landfill generally decreased parietal cells, mucus cells and decreased mucus secretion over time. In support of this observation, correlation analysis revealed that exposure period indicated inverse relationships with parietal cell count, mucus cell count and gastric mucus secretion. This could be due to desensitization of the gastric mucus cells involved in gastric mucus secretion (Oluwole *et al.*, 2008) by some contaminant in the underground water including heavy metals. These were further supported by the weak negative/inverse correlations observed between metal (lead, cadmium, copper and zinc) levels in blood of landfill exposed rats and mucus cell counts and gastric mucus secretions. The weak negative correlations suggest that, apart from metals, there could be other unanalyzed organic contaminants in the landfill

underground water that are interacting either additively, synergistically or antagonistically to induced the observed gastric alterations.

Furthermore, in this study, the observed gastric histopathological lesions include moderate to severe papillary infolding, congestion, vascularization, mucous gland with vesicular nuclei, eodema, inflammation of mucosa and submucosa, sloughing of surface epithelium and mild ulceration of surface mucosa. Also, active mucin and less/no mucin production were observed. These histopathological alterations observed herein, further give credence to the results from gastric physiological indices and supports previous reports. Asar *et al.* (2000) observed increased mean blood and mucosa Cd levels with concurrent significant decrease in mucosal thickness and mucin content in rats exposed to high Cd concentration via drinking water. Also, rat orally exposed to ethylene glycol (a potential contaminant in household and food products) showed surface epithelium or mucosal erosions, swollen cells and irregular nuclear shape of the cells of the stomach (Khattab, 2007). Haloi *et al.* (2013) observed similar histopathological alterations such as haemorrhage, swelling of cell, breakage of outer membrane, sloughing of mucosa layer and vacuolarization in the stomach of fish (*Channa punctatus*) exposed to organochloride pesticide which are common pollutant of water bodies. They explained that organochloride in the presence of HCl secreted in the stomach forms organochloride acid, which has highly corrosive properties. This acid could destroy mucous secreting cells of the stomach lining to induced the observed pathologies (Haloi *et al.*, 2013).

A possible mechanism to metal induced pathogenicity of the stomach and intestine could be via metal interference with oxidative metabolism and the generation of free radicals in the stomach (Olaleye *et al.*, 2007). Dai *et al.* (2009) and Abdallah *et al.* (2010) noted that lead (Pb) in the stomach causes the increase in the formation of free radicals, which if not mopped up by free radical scavengers, will expose the stomach to inflammation and gastric mucosal damage. Also, oral administration of single high doses of Cd compounds causes direct desquamation of the gastric epithelium (Tarasenko *et al.*, 1974) and Cd exposure led to a significant decrease in the mucin content and prostaglandin levels (Oner *et al.*, 1995). These adverse effects of Pb and other metals, as well as their inhibitory effects on enzyme activities might be the main inducer of intestinal histopathological damage in exposed animals (Dai *et al.*, 2009;

Abdallah *et al.*, 2010). In summary, the observed alteration in the stomach could cause gastric irritation and destruction of the mucous membrane of the stomach and intestine, thereby hampering absorption (Haloi *et al.*, 2013). This mechanism and health effects possibly support and explains the observed alterations in gastric physiological indices and histopathological damages observed in the study herein.

5.3.8 Histological alterations in organs of rats exposed *in situ* to the landfill

Histopathological examinations of specific tissues are useful as biomarkers that give important qualitative and quantitative information about acute or chronic effects of toxic chemicals, which are not easily predicted by other biomarkers (Gurcan *et al.*, 2009). In the present study, continuous and chronic exposure of rats to landfill toxic contaminants in underground water and air emission such as heavy metals and unanalyzed pollutants (hydrocarbons, pesticides, phenols, phthalates, etc) caused the pathological alterations observed in liver, kidney, lungs and spleen either directly (metals/ions intoxication) or indirectly (generation of ROS). The lesions observed are due to the bioaccumulation of toxic chemicals such as heavy metals in the organs (Sánchez-Chardi *et al.*, 2008). This assertion supports the increased absolute and relative organ weight with concomitant elevations in metal accumulations observed in this study.

The hepatocellular lesions observed in the study herein ranged from mild to severe focal areas of inflammations, necrosis, fibrosis, steatosis, sinusoid dilation and congestion and proliferation of bile ducts. Hepatic fibrosis is driven primarily by the development of inflammation in response to parenchymal injury (Balabaud *et al.*, 2004) and liver fibrosis accompanies progressive liver injury and varies from mild to severe forms (Hernandez-Gea and Friedman, 2010). Injury to hepatocytes possibly caused by accumulated toxic metals may results in the recruitment and stimulation of inflammatory cells, as well as, the activation of resident inflammatory cells known as Kupffer cells (Iredale, 2007). Also, following acute liver injury, necrotic and apoptotic cells can occur, which are usually replaced by the regeneration of parenchymal cells and this is accompanied by limited deposition of extracellular matrix (ECM). However, in case of chronic liver injury, the regenerative response fails and hepatocytes are replaced with abundant ECM. This ECM is referred to as fibrosis. A

more severe stage of fibrosis could lead to cirrhosis and also generate hepatocellular carcinoma (HCC) (Bataller and Brenner, 2005; El-Serag, 2011) and polycystic liver diseases (Borhani *et al.*, 2014). These assertions give credence to how the observed hepatocellular inflammations, fibrosis and necrosis were formed in the study herein. It is also in accordance with the progressive hepatic cyst and cirrhosis seen in the gross anatomy of the liver in the landfill exposed rats.

Hepatic steatosis is a clinicopathological condition characterized by lipid deposition in the hepatocytes of the liver parenchyma (Abd El-Kader and El-Den Ashmawy, 2015). Pereira *et al.* (2006) reported that hepatocyte vacuolation and microsteatosis observed in the liver can be the result of lipids, glycogen or water accumulation, which are indicative of metabolic disturbances such as autophagic processes. This view is in concert with the centrilobular and focal areas of steatosis observed in the study herein and this could indicate an extensive damage to the liver, tending toward steatohepatosis (Sánchez-Chardi *et al.*, 2009).

In the kidney, nephrotoxic lesions observed in the exposed rats include mild/moderate peri-vascular/glomerular/tubular inflammations, congestions, fibrosis, degeneration of the glomeruli, vesicular nuclei, eosinophilic granule and sloughing of renal tubules. Also, no lesion was observed in some exposed rats. No renal lesion in some exposed rats could be due to individual physiological differences and adaption to metal toxicity. Also, it may reflect the kidneys' higher tolerance to heavy metals (A. Sánchez-Chardi *et al.*, 2008). Nephrotoxic lesions observed herein are similar to those reported by Sánchez-Chardi *et al.* (2008) and Alimba *et al.* (2012) in rodents exposed to heavy metals from landfill sites.

The main function of spleen is the destruction of aged or damaged red blood cells and the storage of iron (Fe) as ferritin or haemosiderin for future recycling (Kapila *et al*., 2021) and filtration of antigens circulating in the blood, and subsequent immunological responses. Haemorrhagic lesion, numerous macrophages, fibrosis, haemosiderin deposition, congestion, fusion of white and red pulps, e.t.c. are among the lesions observed in the study herein. The observed splenic tissue alterations in the exposed rat compared to control suggest potential adverse immunotoxic effect and splenic

dysfunction due to significant metal accumulation in the spleen. This observation is consistent with the work of Pereira *et al.* (2006).

The lungs have not been well assessed for histopathological lesions due to toxicity from landfills. However, in the study herein, the observed histopathological changes such as inflammations, oedema, fibroblast aggregation, fibrosis, alveolar cell hyperplasia and other alterations in lungs of rats exposed to the landfill air and water indicates that the lungs are susceptible to damages induced by heavy metals and other xenobiotics via inhalation and oral routes. This is similar to report of Alimba (2013) on leachate fed rats. In support, Odewabi *et al.* (2013) on systemic studies in waste management workers noted that airway inflammation and related respiratory complaints are common symptoms among workers.

Although the precise molecular mechanisms by which metals induced the observed pathological alterations in the organs are not clear, the production of ROS is believed to be involved. This can often occur when detoxifying system such as metallothioneins are not responding efficiently enough to bind all metal ions. Thus, depending on the nature of the injury, the generation of this oxidative stressor damages DNA, thereby producing apoptotic and necrotic cells or carcinogenic processes and altering membranes and lipid and protein metabolism (Pereira *et al.*, 2006; Gurcan *et al*., 2009).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

In this study, heavy metal concentrations in blood, liver, kidney and lungs of rats (*Rattus norvegicus*) exposed *in situ* for 1 to 6 months to borehole water and ambient air at Olusosun landfill in Lagos State, Nigeria; and the potential genetic and systemic damages induced in the exposed rats were investigated. The metal (Pb, Cd, Cr, Cu, Zn and Fe) concentrations observed in the underground water at Olusosun landfill were above national and international permissible limits for drinking water quality. The elevated metal levels in the water were due to landfill leachate percolation, interaction and pollution of the underground water aquifer in the vicinity of the landfill. This implies that the water is unfit for animal and human consumption, unless properly treated.

Evidently, rats exposed *in situ* to the landfill contaminants through drinking water and inhaled air emissions revealed elevated concentrations of Pb, Cd, Cr, Cu and Zn in blood and soft tissues. Accumulation of metals in soft tissues possibly results in increased absolute and relative organ weight especially with the liver and kidney. Also, rats exposed to the landfill contaminants showed higher body weight and higher percentage change in body weight gain over the chronic exposure periods. This suggest that some chemical contaminants from the landfill, likely the heavy metals and other unanalyzed endocrine disrupting chemicals (EDCs), may be inducing adipogenesis and may possess obesogenic effects.

It was observed that *in situ* exposure of rats to the landfill contaminated groundwater and air emissions induced DNA damage in erythropoietic stem cells evidenced from increased frequencies of micronucleus formation. The exposure also induced duration dependent decrease in the polychromatic and normochromatic erythrocyte ratio in bone marrow cells of exposed rats. This suggest that the constituents of the landfill contaminated underground water and air emissions is genotoxic and cytotoxic and

could induce genetic instability in exposed animal and human populations. Genetic instability has been associated with decrease cell survival or transformation and cancer formation.

Clinical sign of toxicity such as anorexia, diarrhea, abscesses, poor grooming and hair discolouration (at early stage of exposure) are all potential pointers to the adverse health effects of the landfill exposure on the rat's physiologic, immune and nervous coordinations. In support of these clinical signs in the study herein, there were significant alterations in the serum clinical biochemical markers of hepatic and renal damages and cytotoxicity, together with histopathological and haematological alterations in the soft tissues (liver, kidney, lungs and spleen) and blood respectively.

In situ exposure to the landfill contaminants via contaminated drinking water and air emissions induced gastric physiopathological responses. Parietal cell counts, mucus cell counts and gastric mucous secretions in the stomach of landfill-exposed rats reveal a significant duration-dependent decrease. This suggest a tendency toward gastric epithelium desquamation/sloughing and ulceration. The high concentrations of heavy metals (Pb, Cd, Cr, Cu, Zn and Fe) in the administered water and the inverse relationship between metal (especially Pb and Cd) concentrations in blood of landfill exposed rat and the gastric indices, implies that the observed physiological alterations must have been induced by heavy metals. This observation was also supported by the various histological alterations such as mucous gland with vesicular nuclei, edema, inflammation of mucosa and submucosa, sloughing of surface epithelium and ulceration of surface mucosa observed in the stomach of landfill exposed rats.

The mechanism by which the observed genetic and systemic damage were induced in the study herein is suggested to be via oxidative stress. Metals and other unidentified deleterious contaminants in the groundwater and ambient air from the landfill accumulated in the organ-system of the exposed rats, generated reactive oxygen species (ROS) and these induces oxidative lipid peroxidation, DNA and cell damage.

These findings put together affirmed that Olusosun municipal waste landfill is an unsanitary landfill which is releasing deleterious chemicals including heavy metals into the surrounding underground water, air and other environmental media. Thus, results herein suggest that there may be serious environmental and public health risk,

especially to populations working and/or living in close proximity (within 2km) to the landfill.

Contributions to knowledge

The following are the contributions to knowledge.

1. This is probably the first evidence-based genetic and systemic toxicity study on chronic *in situ* rat exposure to groundwater and air emissions at a MSW dumpsite.
2. *In situ* exposure of rats to Olusosun landfill underground water and air emission led to elevated metal (Pb, Cd, Cr, Cu and Zn) accumulations in blood, liver and kidney and concurrently increased the absolute and relative organ weight of liver and kidney.
3. Rats exposed *in situ* to landfill contaminants had significantly higher body weight gain, which was also evidenced with duration-dependent higher percentage change in body weight gain.
4. *In situ* exposure of rats to Olusosun landfill groundwater and air emissions significantly increased the frequency of micronuclei formation and concurrently decreased the PCE/NCE ratios.
5. *In situ* exposure of rats to Olusosun landfill groundwater and air emissions induced significant duration-dependent decreases in parietal cell counts, mucus cell counts and gastric mucous secretions in the stomach of exposed rats.
6. The *in situ* exposure of rats led to significant alterations in haematology, serum biochemical markers of liver and kidney damage and tissue histology (liver, kidney, lungs and spleen).
7. The exposure to contaminants from the Olusosun landfill especially the heavy metals induced the observed genetic and systemic damage in rats via disruption of the antioxidant enzymes and lipid peroxidation or via generation of reactive oxygen species (ROS) which led to oxidative stress.

This study therefore has the following recommendations:

1. Since waste generation is inevitable and the volume of waste generated will continue to increase with economic development, government, policy makers and waste managers should advocate for proper waste management practices. Effort and resources should be geared toward construction of engineered sanitary landfill in most cities in Nigeria. Also, already existing waste dumpsites should be upgraded to prevent the potential dangers that unsanitary landfill could imposed on the environment and human health.

2. Ground and underground water sources within the vicinity of the Olusosun landfill and other municipal waste landfill in Nigeria should be subject to adequate chemical and bacteriological treatments before being used for drinking and domestic water supply.

3. There is need for environmental education for scavengers and waste disposal workers. Exposed populations should take adequate precaution and protect themselves through proper vaccination (against hepatitis A and B, tetanus, diphtheria, polio, typhoid, encephalitis and rabies) and adherence to the use of safety gears such as protective clothes, goggles, safety boots, hand gloves and good nose mask.

4. There should be regular monitoring and analysis of air emissions (particulate matters, LGs, bioaerosols) from waste landfills. This is because air pollution from waste landfill constitutes serious threat to waste workers and residents in close proximity to the landfill. Scientific research should be carried out to characterize both the physical and chemical (organic and inorganic) components of air emissions from waste landfill, so as to ascertain the level of chemical threat from landfill air emissions.

5. Extraction and fractionation of specific organic pollutants from landfill air emission, and testing in animal and cell line models to determine the specific environmental contaminant that could be posing systemic and genetic threat to animal and human population.

6. Cytogenotoxicity has been associated with increase cancer formation, congenital and reproductive abnormalities. Cytogenetic and epidemiologic studies should be

carried out in residential area with close proximity to major landfills to determine the level of exposure and potential health risk in the population.

7. Herein, long term exposure of rat to the landfill contaminated media led to increased percentage change in body weight gain. Research should be carried out to ascertain possible association between landfill exposure and obesity and possible mechanism.
8. Molecular and gene expression analysis also need to be carried out on samples from landfill exposed animals to determine the toxicity associated genes that are altered and mechanism of landfill induced genetic and systemic toxicity.

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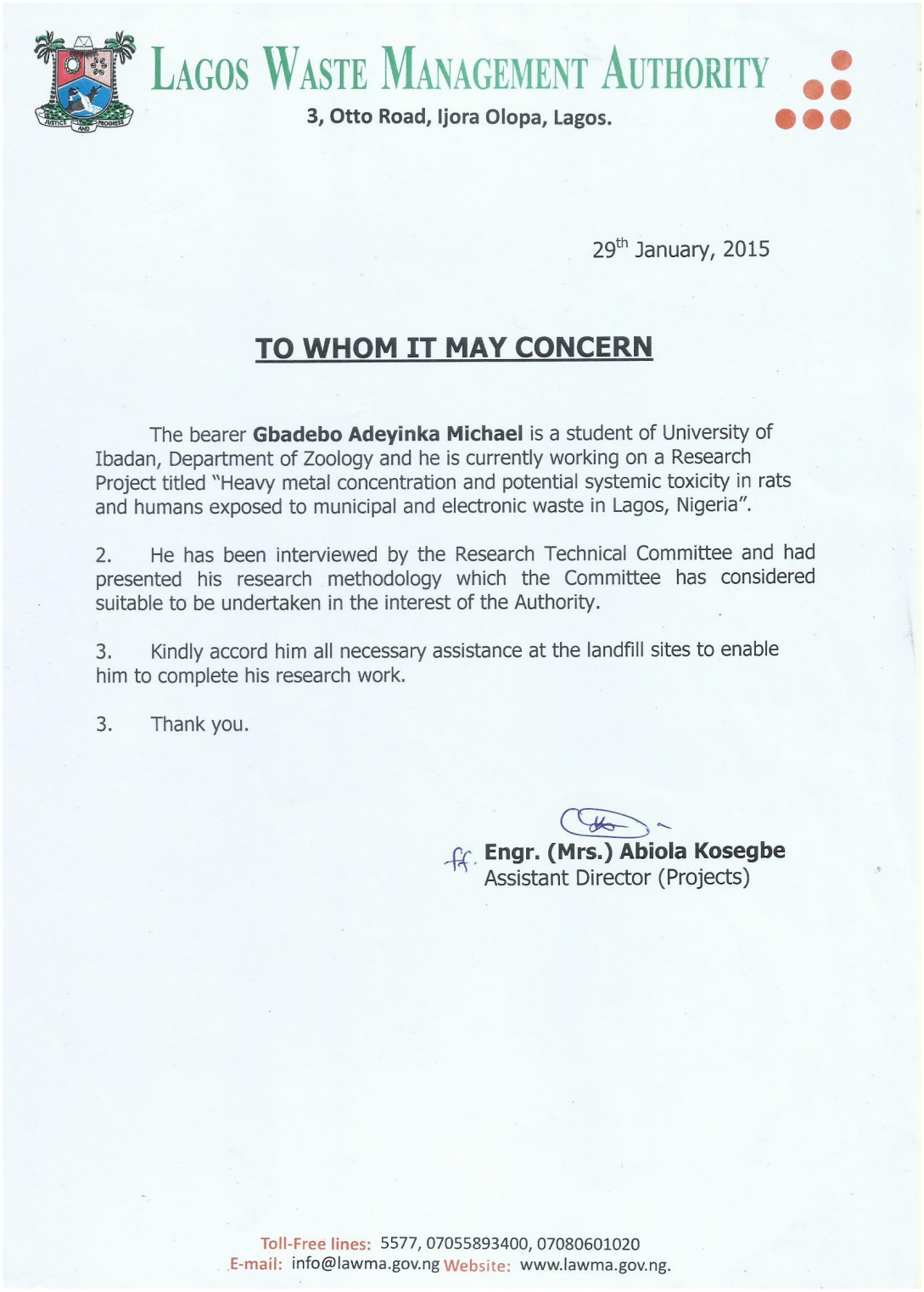
APPENDIX I

Lagos Waste Management Authority (LAWMA) approval for the study



LAGOS WASTE MANAGEMENT AUTHORITY

3, Otto Road, Ijora Olopa, Lagos.

29th January, 2015

TO WHOM IT MAY CONCERN

The bearer **Gbadebo Adeyinka Michael** is a student of University of Ibadan, Department of Zoology and he is currently working on a Research Project titled "Heavy metal concentration and potential systemic toxicity in rats and humans exposed to municipal and electronic waste in Lagos, Nigeria".

2. He has been interviewed by the Research Technical Committee and had presented his research methodology which the Committee has considered suitable to be undertaken in the interest of the Authority.

3. Kindly accord him all necessary assistance at the landfill sites to enable him to complete his research work.

3. Thank you.

ff. **Engr. (Mrs.) Abiola Kosegbe**
Assistant Director (Projects)

Toll-Free lines: 5577, 07055893400, 07080601020
E-mail: info@lawma.gov.ng Website: www.lawma.gov.ng.

APPENDIX II

Ethical approval for the study

UNIVERSITY OF IBADAN
DEPARTMENT OF VETERINARY PATHOLOGY

Head of Department:
PROFESSOR V.O. TAIWO
DVM, MVetSci, Ph.D. (Ibadan), FCVSN

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30th June, 2015

Gbadebo A.M.,
Dept. of Ecology and Environmental Biology Unit,
U.I.

NOTICE OF ETHICAL APPROVAL FOR A PH.D RESEARCH PROJECT PROPOSAL

On behalf of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), I write to grant you an Ethical Approval to carry out your research project work titled: "***Heavy metal concentration and potential systemic toxicity in rats exposed to municipal and electronic wastes in Lagos, Nigeria***" strictly as outlined in your proposal submitted for assessment.

Please quote **UI-ACUREC/App/2015/037** as reference for this approval.

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

Thank you.

30/06/2015

Prof. V.O. Taiwo
Chairperson, UI-ACUREC

Cc: Dean, FVM
Director, Research Management Office.

APPENDIX III

Calibration of alanine aminotransferase (ALT) and aspartase aminotransferase (AST) standard curve

ALT		AST	
Absorbance	U/I	Absorbance	U/I
0.025	4	0.020	7
0.050	8	0.030	10
0.075	12	0.040	13
0.100	17	0.050	16
0.125	21	0.060	19
0.150	25	0.070	23
0.175	29	0.080	27
0.200	34	0.090	31
0.225	39	0.100	36
0.250	43	0.110	41
0.275	48	0.120	47
0.300	52	0.130	52
0.325	57	0.140	59
0.350	62	0.150	67
0.375	67	0.160	76
0.400	72	0.170	89
0.425	77		
0.450	83		
0.475	88		
0.500	94		