DNA AND SYSTEMIC DAMAGE INDUCED BY LANDFILL LEACHATES, AND HEALTH IMPACTS OF HUMAN EXPOSURE TO LANDFILLS IN LAGOS AND IBADAN, NIGERIA

BY

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A thesis in the Department of ZOOLOGY

Submitted to the Faculty of Science in partial fulfilment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the
UNIVERSITY OF IBADAN

Department of Zoology
University of Ibadan
Ibadan

January 2013

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ABSTRACT

Municipal solid waste landfills in Nigeria are unsanitary. The release of hazardous chemicals via leachates from these landfills may have grievous consequences on the environment and biota. However, there is limited information on leachate induced DNA and systemic damage in vertebrates from different ecological habitats, and human health associated with living around landfills. This study was undertaken to evaluate the cytogenetic and systemic toxicity of leachates and human health impacts of exposure to landfills in Lagos and Ibadan, Nigeria.

Olusosun and Aba-Eku landfills in Lagos and Ibadan respectively were purposively selected. *Clarias gariepinus* (mud catfish), *Coturnix japonica* (Japanese quail) and *Mus musculus* (mouse) were exposed to leachates from Olusosun (OSL) and Aba-Eku (AEL) landfills at different concentrations (0 - 50%) for genotoxicity evaluation using the micronucleus assay. Blood collected from Wistar rats (*Rattus novergicus*) exposed to the leachates was analysed for biochemical parameters (Alanine Aminotransferase, ALT; Aspartate Aminotransferase, AST; and albumin) using standard methods. Liver, kidney and thymus tissues excised from the rats were processed for histopathology. Biochemical Oxygen Demand (BOD), copper, manganese, lead, chromium and cadmium concentrations in the leachates were determined by APHA methods. The health status of residents within 2 and 6 km radius of the landfills was assessed using pre-tested and structured questionnaire. Data were analysed using descriptive statistics, ANOVA and Chi square at $p = 0.05$.

There was concentration dependent, significant induction of micronucleus in the erythrocytes of *C. gariepinus* (OSL = 1.4±0.0 – 9.6±0.2; AEL = 0.8±0.4 – 8.6±0.6), bone marrow cells of *C. japonica* (OSL = 1.0±0.2 – 2.1±0.6; AEL = 0.7±0.1 – 3.2±0.6) and *M. musculus* (OSL = 5.5±0.6 – 18.5±0.0; AEL = 6.5±0.3 – 18.1±1.3). There was significant increase in ALT (OSL= 31.1±2.1 – 52.7±1.6 IU/L; AEL = 30.7±1.5 – 49.5±1.3 IU/L) and AST (OSL = 86.9±13.2 – 168.4±1.0 IU/L; AEL = 84.5±1.5 – 161.9±1.2 IU/L), but significant decrease in albumin level (OSL = 2.6±0.2 – 5.11±0.3 g/dL; AEL = 2.7±0.2 – 5.2±1.2 g/dL) in serum of exposed rats compared to the negative control. Necrosis and vacuolation of the hepatocytes; cortical congestion and haemorrhage in the kidney; and infiltration of macrophages, inflammation and apoptotic

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lymphocytes in the thymus were observed in exposed rats. The frequencies of these anomalies were higher in OSL than AEL exposed animals. The concentrations (mg/L) of BOD (306.0 – 601.0), copper (0.9 – 3.9), manganese (0.6 – 3.9), lead (0.8 – 2.1), chromium (1.4 – 2.4) and cadmium (0.3 – 2.2) were above NESREA wastewater limits. The frequency of complaints of bad odour (OSL, 95.4%; AEL, 90.1%) and dermal contacts with vermin (OSL, 80.8%; AEL, 54.2%); respiratory (OR=9.7, 95% CI=6.6 – 14.6), dermal (OR=7.2, 95% CI=4.8 – 10.8) and gastrointestinal (OR=7.9, 95% CI=6.2 – 12.1) anomalies were significantly higher among residents nearer (2 km) the landfills.

Leachates from Olusosun and Aba-Eku landfills were potential sources of genetic and systemic toxins. Human exposure to toxins from the landfills was associated with adverse health effects. There is need for proper solid waste management to enhance environmental and public health safety.

Keywords: Cytogenotoxicity, Landfill leachate, Systemic toxicity, Human health impact.

Word count: 480

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DEDICATION

This project is dedicated to my beautiful wife, Edith Uju and our lovely baby Ngozi Silvia for their prayers, supports and being the source of my happiness during the assemblage of this thesis.

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CERTIFICATION

I certify that this work was carried out by ALIMBA, Chibuisi Gideon in the Cell Biology and Genetics Unit of the Department of Zoology, University of Ibadan.

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ACKNOWLEDGEMENTS

My heartfelt gratitude goes to my amiable supervisor, Dr A. A. Bakare. Your patience, tolerance, commitment and intellectual criticisms and input in this research is thankfully acknowledged and appreciated. Sir, you motivated and inspired me in the field of cytogenotoxicity and environmental toxicology. I am indeed a "hybrid" from your wealth of "true breed" experience. You are indeed a standard mentor and my good God will keep you and your family in good health to enjoy the fruits of your good labours (Amen). I am grateful to Dr. G. Ana of the Department of Environmental Health Sciences, University of Ibadan for invaluable contributions and corrections made into the public health aspect of this research.

I thank Prof. Odeigah P.G. and Drs Adekoya K.O., Ogunkanmi L.A. and Njoku, K.L. of the Department of Cell Biology and Genetics, University of Lagos for their motivations and contributions towards the completion of this thesis. I am indebted to Prof. (Mrs) Oboh B.O. for her inspirations, motivations and supports that facilitated the award of a Doctorial grant from the University of Lagos which enhanced my travelling to Australia for the *in vitro* aspect of this study. May God perfect all that concerns you (Amen). I want to appreciate members of staff of the Department of Cell Biology and Genetics and Faculty of Science, University of Lagos for their cooperations and supports that enhanced the completion of this thesis.

I am grateful to Prof. Fenech Michael, Dr. Varinderpal Dhillon and Mrs Carolyn of CSIRO Food and Nutritional Sciences, Adelaide, Australia, for the provision of materials, training and supervisions in the field of *in vitro* cytogenotoxicity. I will always appreciate the acquired skill. I appreciate the assistance of Drs Ozegbe, P.C. and Aina, O.O. of the Department of Veterinary Anatomy, University of Ibadan, in the organ histopathology and provision of Japanese quail used for the avian micronucleus assay.

I acknowledge the efforts and contributions of my teachers in the Department of Zoology, University of Ibadan: Prof and Dr (Mrs) Ugwumba, Profs. A.B. Odaibo and A.T. Hassan, Drs. Morenikeji A.O., Nwuba R. I, Anumudu C.I., Adeogun A.O, Popoola K.O.K, Awobode H. Ala, A. Sowunmi A.A., Okorie A. and Mr. Latunji C. A. I thank you all for the knowledge transferred in the field of animal Biology. I also want to appreciate the contributions of the technical and

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administrative staff of the department towards this thesis. May God reward your efforts accordingly (Amen).

I acknowledge the contributions of Mrs. Shote of Orthopaedic Hospital, Igbobi Yaba, Lagos state, Nigeria in the biochemical analysis; Mr Ladipo of Jaja Clinic, University of Ibadan and Mr Adelabu of the Department of Cell Biology and Genetics, University of Lagos for their assistance in the haematological analysis.

I appreciate the physical, spiritual, moral and financial assistance from my parents; Mazi Alimba N.S. and Madam Alimba C. and sibs; Chinyere, Ifechimere Ekwere, Chioma, Chukwuemeka, Amarachi and Esther towards the completion of this thesis. May God bless you all (Amen). I thank Reverends Odeleye, O.J. and Onyinrimba I.M., and Pastors Enoch E. and Gbolagade J. of Assemblies of God Church, Nigeria for their spiritual and moral supports. I also appreciate Mr. Tony Amune and Mrs Ugbo for their prayers and moral supports that enhanced the completion of this thesis. May God reward you accordingly, Amen.

I appreciate my wife, Edith Uju, for her cooperation, love, patience, prayers and moral supports that facilitated the completion of this thesis. Also, my little angel, Silvia Ngozi; your arrival created the joy that enhanced the completion of the thesis.

Finally, to my redeemer and saviour, Jesus Christ; you favoured me by providing good health, financial support, knowledge and wisdom to put this work together. May you alone receive all glory and honour (Amen).

Chibuisi Gideon ALIMBA

January 2013

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CHAPTER ONE

1.0 INTRODUCTION

World Health Organization (WHO) estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (Kimani, 2007). This implies that there are close links between the environment and human well-being. A quality environment may enable humans to live longer in good health while environmental pollution, can pose significant risks to human health and the ecosystems if not properly monitored. Anthropogenic activities in industries, agriculture, medicine, municipality and educational institutions generate wastes, which are capable of releasing several thousands of chemicals into the environment (Esakku *et al*., 2003). These chemicals may significantly impact on human health and contaminate the ecosystems.

In recent time, solid wastes generation from anthropogenic activities and their disposal into the environment around the world is increasing at alarming rate. This may be attributed largely to accelerated industrialisation, unplanned urbanisation, low environmental awareness or ignorance and population growth. The unsanitary disposal of solid wastes into the environment has elicited strong national and international concerns about the possible environmental contamination and health effects of living within the vicinity of these wastes (Coker and Sridhar, 2010; Porta *et al*., 2009). The struggle against pollution from increase in solid waste generation had led to the proliferation of different methods of managing these wastes. Common methods of solid waste management worldwide include incineration, landfilling, recycling, composting, pyrolysis, disposal into sea and water ways, burning along major roads and surface dumping. Among these, landfilling is the most common, accounting for the management of about 95% of the total solid waste collection worldwide (Kurniawan *et al*., 2006).

In Nigeria, the disposal of solid wastes into unsanitary landfills and or open dumping is the most common method of solid waste management. These landfills are unlined and are located in public places surrounded by residential quarters and in wetland or other places with seasonally high water tables. Sometimes solid wastes are disposed into water bodies and around river banks, in gullies excavated by erosions and human activities, in gutters and channels constructed for flooding and burning on major roads. These disposal methods are capable of releasing chemicals in the forms of gases and or liquid solutions into the environment and can endanger the survival of living organisms including human.

Solid wastes decompose in landfills through complex and highly variable biological and chemical processes leading to landfill gas and leachate production. Landfill gases and leachates

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are complex mixtures of hazardous and deleterious chemicals which can be released in large quantities to nearby ground and surface water, surrounding land and air (Esakku *et al*., 2003; Oyeku and Eludoyin, 2010; Saha *et al*., 2003). Human and other biotic components of the ecosystem may be exposed to these toxic chemicals through inhaling gases and particles (from odours, smoke and dust) during the dispersion of landfill gases or leachates in air or soil; ingestion of chemicals from leachate contaminated water and food, and dermal contact with these chemicals (Coker and Sridhar, 2010; Hertzman *et al*., 1987; Oyeku and Eludoyin, 2010; Wards *et al*., 1996). Chemicals like heavy metals and persistent organochlorine pollutants readily found in landfill leachates can bioaccumulate in lower aquatic and terrestrial forms and wildlife; fish, birds and mammals and biomagnify in human (Cuadra *et al*., 2006; Sanchez-Chardi *et al*., 2007; Sanchez-Chardi and Nadal, 2007). Xenobiotics present in landfill gases and leachates may pose threats to the environment, ecosystem and health of individuals residing and earning their livelihood in the vicinities of the disposal sites.

The incidence of cancers among residents of Niagara, United States who were exposed to chemicals leaching out of Love canal landfill (Janerich *et al*., 1981), along with the reports of Meyer (1983) and Vianna and Polan (1984) on liver dysfunctions and incidence of low birth weight respectively among residents exposed to hazardous chemicals in landfill leachate, increased the awareness about the adverse human health effects that might be associated with landfills. These reports drew the attention of researchers to landfill toxicity studies, with the identification of specific constituents of these chemicals as the most commonly used approach to show the hazardousness of chemicals in landfill wastes (Christensen *et al*., 1998; Ikem *et al*., 2002; Laniyan *et al*., 2011; Oyeku and Eludoyin, 2010; Paxeus, 2000). This method has a major limitation of the inability to provide information about all the toxic chemicals present in the waste mixture and the potential synergistic and antagonistic interactions of these chemicals in living organisms.

Epidemiological studies, which usually evaluate the distribution or pattern of diseases and their association with possible sources of exposure to agents that could potentially cause the disease, have been used to demonstrate elevated incidence of birth defects like congenital anomalies, low birth weight, stillbirth, and spontaneous abortion among residents within 2-3km away from landfills (Elliott *et al*., 2001; Fielder *et al*., 2000; Geschwind *et al*., 1992; Palmer *et al*., 2005). The incidence of diseases like immune and respiratory system disorders (Kudyakov *et al*., 2004; Vine *et al*., 2000; Williamson *et al*., 2006), degenerative neurologic and endocrine disorders, cancer, stroke, diabetes and liver dysfunctions were also evaluated among resident

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closer to landfills (Meyer, 1983; Porta *et al*., 2009; Shcherbatykh *et al*., 2005). In recent times, the most frequently reported human health effects due to exposure to hazardous chemicals from landfills include rhinitis, pharyngitis, conjunctivitis, allergic pulmonary alveolitis, dermal infections, diarrhea and other infections of the gastrointestinal system (Abdou, 2007; Kimani, 2007; Porta *et al*., 2009; Ray *et al*., 2005; Schrapp and Al-Mutairi, 2010). Marsh and Caplan, (1987) classified all observed health outcomes among landfill workers and residents around landfills into reproductive impairment outcomes, chromosomal damage and neurotoxic effects. Among the human health studies, no particular landfill chemicals were definitely fingered as the cause of the observed health anomalies since no actual chemical exposure were measured. Hence, proximity to landfills was used as surrogate for exposure to landfill chemicals.

Epidemiological data from Nigeria on health impacts of solid waste disposal into unsanitary landfills is relatively scarce despite higher risk of human exposure to landfill chemicals and microorganisms (Coker and Shridar, 2010; Efuntoye *et al*., 2011; Laniyan *et al*., 2011; Oshode *et al*., 2008; Oyeku and Eludoyin, 2010). Moreso, there is poor or lack of stringent regulations that could protect human and wildlife from exposures. Furthermore, reports from recent studies showed that groundwater resources from communities around landfills contained higher concentrations of heavy metals which are deleterious to living organisms (Laniyan *et al*., 2011; Oyeku and Eludoyin, 2010). These studies are supported by the reports of the United Nations Children's Fund that about 52% of Nigerians make use of unprotected underground and surface water for their domestic and commercial activities (Adeyeye, 2006). This may indicate increase risk of exposure to landfill leachate contaminated water in communities around landfills. There was fear of epidemic looming among human population living and working around Ojota area of Lagos State due to exposure to thick smoke which caused poor visibility to these populations, storm runoff contamination of sources of their water supply, mosquito and housefly (vectors of diseases) proliferations and offensive odour oozing out of Olusosun landfill (Nwogu, 2010). Moreover, there was incidence of poor yields of farm produce and death of domestic animals around Ona Ara local government area, Oyo state when Aba-Eku landfill leachate contaminated Omi river was used as sources of drinking water for animals and irrigation of crops (Bakare and Wale-Adeyemo, 2004). This suggests a higher health risk among communities residing around these landfills; thus the need to investigate the health status of populations around the landfills.

Scarcity of epidemiological data on health status of residents around landfills in Nigeria may probably be attributed to ignorance of the health risk associated with exposures to landfill

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chemicals and the reluctance by Nigerians to give information to researchers. Though there is paucity of information from epidemiological studies on public health impacts of solid waste disposal in unsanitary landfills in Nigeria, experimental toxicity studies have been widely used to show the acute toxicity, cytotoxicity, mutagenic and genotoxic effects of landfill leachates (Alkassasbeh *et al*., 2009; Amahdar *et al*., 2009; Bakare and Wale-Adeyemo, 2004; Bakare *et al*., 2003; 2005; Bortolotto *et al*., 2009; Iwegbue *et al*., 2007; Li *et al*., 2010; Oni *et al*., 2011; Talorete *et al*., 2008). However, despite that landfill chemicals are capable of contaminating all ecological habitats (arboreal, aquatic and terrestrial), information on landfill leachates induced cytotoxicity and genotoxicity in representative members of these habitats is relatively scarce. Thus, there is a need to evaluate the cytogenotoxic potentials of landfill leachates in representative animals from different ecological habitats.

So far no information exist on the use of avian species despite that leachates seem to be the available source of drinking water to wild and free ranging birds around landfills and they constitute aerial vertebrate species commonly found foraging around landfills/dumpsites. Information is also limited on the genotoxicity of landfill leachates in aquatic species from Nigeria despite the fact that fish has been identified as a major route for human exposure to xenobiotics from aquatic environment (Cuadra *et al*., 2006; WHO, 1990). Moreso, *in vivo* genotoxicity studies in these species may be significant in assessing health risk to higher vertebrates, especially human beings (Powers, 1989). Information is also scarce on the cytogenotoxic evaluation of landfill leachates in rodents (terrestrial mammals) using the micronucleus assay. There is need for adequate information on the probable mechanisms of landfill leachates induced toxicity and cytogenotoxicity in biological systems. Available studies have suggested heavy metals induced free radical formation in mammalian systems *in vivo* (Bakare *et al*., 2012; Li *et al*., 2006a,b; Li *et al*., 2010) and *in vitro* (Talorete *et al*., 2008) as possible mechanism of landfill leachates induced genotoxicity. The evaluation of cytogenotoxic effects of individual and mixture of heavy metals may contribute to the knowledge on possible mechanisms of leachate induced toxicity and cytogenotoxicity.

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, excretion, secretion 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 chemical induced toxicity and cytogenotoxicity. Therefore, it may be suggested that evaluation of systemic toxicity of landfill leachates using different biomarkers; such as histopathology,

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biochemistry and haematology, and cytogenotoxicity evaluation of heavy metals will enhance the understanding of the probable mechanisms of landfill leachates induced toxicity and cytogenotoxicity.

The results from this study will present information on the cytogenetic and systemic damage induced by landfill leachates and health impacts of human exposure to landfill emissions in Nigeria. It will also suggest some possible mechanisms of landfill leachates induce toxicity and cytogenotoxicity. The findings will serve as reference material for further studies in the field of landfill toxicology and genotoxicology. It will also be useful to policy makers and environmental managers in promulgating rules against illegal waste disposal and instituting better waste management methods. It will be useful in effective health education on the hazardous nature of emissions from unsanitary landfills.

1.1 AIM OF THE STUDY

This study aims at investigating the health impacts of residents around Olusosun (Lagos) and Aba-Eku (Ibadan) landfills and DNA and systemic damage induced by leachates from these landfills in vertebrates from different ecological habitats.

1.2 OBJECTIVES OF THE STUDY

The objectives are to:

(i) Evaluate the genotoxic effects of Olusosun and Aba-Eku landfill leachates in *Clarias gariepinus* (mud catfish) and *Coturnix japonica* (Japanese quail) using the micronucleus assay.

(ii) Evaluate the cytotoxic and genotoxic effects of Olusosun and Aba-Eku landfill leachates in *Mus musculus* (Albino mice) using the micronucleus assay.

(iii) Investigate the effects of leachates from these landfills on the histopathology of the kidney, liver, thymus, spleen, heart and lungs; body weight and organ weight indices; haematological parameters and serum biochemistry of *Rattus novergicus* (Wistar rats).

(iv) Assess the causal relationship between exposure to landfills and morbidity pattern in communities around Olusosun (Lagos) and Aba-Eku (Ibadan) landfill sites in southwestern, Nigeria.

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(v) Investigate the genotoxic and cytotoxic effects of heavy metals (chromium, lead, manganese, copper) with higher concentrations in the landfill leachates, singly and in their combined form using *in vitro* Cytokinesis Block MicroNucleus cytome (CBMN–cytome) assay in WIL2-NS lymphoblastoid cell line.

(vi) Analyse the leachate samples for some physical and chemical parameters.

1.3 HYPOTHESES OF THE STUDY

The following research hypotheses were developed to guide the study:

H0: 1 There is no significant relationship between the genetic damage induced in Olusosun and Aba-Eku landfill leachate treated *Clarias gariepinus*, *Coturnix japonica* and *Mus musculus* compared to the corresponding negative controls.

H0: 2 There is no significant relationship between systemic damage (haematology, histopathology and biochemistry) in *Rattus novergicus* exposed to Olusosun and Aba-Eku landfill leachates compared to the corresponding negative controls.

H0: 3 There is no significant relationship between health impacts of residents within 2 km radius of the landfills (exposed) and those within 6 km (control populations).

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CHAPTER TWO

2.0 LITERATURE REVIEW

Wastes have been the products of human activities since the dawn of human history. With advancements in science and technology from the beginning of the 20th century, the production of consumables for the sake of satisfying the ever-growing human population proceeded at unprecedented rate. Due to this unreserved production rate, waste generation has become almost uncontrollable and leading to waste accumulation in the environment. This is often observed in most developing countries where the priority of the government is mainly centered on political and economic agenda than environmental protection (Sha'Ato *et al*., 2007).

Wastes are mostly in the forms of solids, liquids and gases. Liquid wastes are mainly wastewater or effluents from industrial, agricultural, municipalities and medical activities, requiring the use of large volume of water for their finished products. Solid wastes are mainly paper and paperboard products, yard trimmings and other vegetations, glass, metals, plastic, textile, rubber, wood, tires, food wastes and other materials in the solid states which are no longer useful to the person(s) generating them. Gaseous wastes may include radiations and toxic emissions in the gaseous forms from industrial, medical, agricultural and domestic activities like burning and the use of industrial machines like X rays and generators (Odunaike *et al*., 2008; Oygard and Gjengedal, 2009).

2.1 Classification of wastes

Wastes have been classified into different categories based mainly on the sources of their production and degree of their hazardousness. The following classes of wastes exist:

(i) Domestic wastes: These are wastes produced from domestic activities mainly from home kitchen, cooking centres, human raw faeces, urine and wastewater from bathrooms and septic tanks.

(ii) Agricultural wastes: These are produced from animal husbandries including animal beddings and feeds, excreta and urine, carcasses, slaughtering and farming activities like pesticide, herbicides and fertilizer applications, pre – and post – harvesting activities.

(iii) Industrial wastes: These are mainly generated during manufacturing activities that lead to finished goods and services. They may include metal scraps, chips and grits from machine shops, sawdust, waste papers, pieces of glasses, tyres, abandoned vehicles, spent liquids from machine operations, heavy metals, waste water from paper and pulp industries, iron and steel industries, petroleum refining, non-ferrous smelting, synthetic textile dyeing and great amounts of heat discharged into water bodies from power generation.

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(iv) Radioactive wastes: These include wastes from nuclear weapons during warfare or accidental discharge or illegal dumping. It also includes; X-ray from machines, oil prospecting and mining activities. Radionuclide can be integrated into cellular and tissue components of living organisms and induced mutation or cause deleterious effects on the organisms. Traces of radionuclide have been detected in stable food consumed in Nigeria (Jibiri *et al*., 2007), vegetations and environmental fields (Akinloye and Olomo, 2005). These materials are dump indiscriminately on open fields, along major roads, river banks and into unsanitary landfills, where they emit radiations. Oygard and Gjengedal (2009) recently reported high quantities of Uranium (radioactive element) in fourteen solid waste landfill leachates situated in western, Norway.

(v) Medical (hospital) wastes: also known as health care or clinical wastes, are solid or liquid wastes generated from human and /or animal health care activities including research, diagnosis, therapeutic and rehabilitative services or prevention of diseases in humans and animals (Bassey *et al*., 2005). Medical activities are among the largest generators of solid wastes on per capital basis (Coker and Sridhar, 2010). In Nigeria, medical waste is classified under infectious wastes (FEPA, 1991). Within the category of medical wastes are culture and stock of infectious agents, pathological waste, wastes from surgery or autopsy, sharp objects (hypodermal needles, syringes with needles and scalpel blades), blood, carcasses and tissue related swabs. They may also include broken glassware, post mortems, research laboratories, pharmaceutical products, bedpans, incontinence pads, sanitary towels, animal drugs etc. these wastes are usually co-disposed with municipal solid wastes in unsanitary landfills in most developing countries, including Nigeria. Heavy metals, toxic chemicals, pathogenic viruses and bacteria which can cause dysfunction to human organs have been reported in medical wastes (Muhlich *et al*., 2003).

Pharmaceuticals and Personal Care Products (PPCPs) are another source of medical wastes. These products are designed to cure and treat diseases, improved health, and increase life span. They find their usefulness in agriculture, industries and households. Examples include both human and veterinary drugs, cosmetics, detergents, disinfectants, insecticides (Daughton and Ternes, 1999). However, associated with the PPCPs is the discharge of their solid wastes, active ingredients or metabolites into the terrestrial environment. Also, wastewater from the production process of PPCPs is discharged into the aquatic environment. Human exposure could occur from the consumption of water or consumption of aquatic organisms such as fishes that bioaccumulated the pharmaceutical residues (Cunningham *et al*., 2010).

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(vi) Waste Electrical and Electronic Equipments (WEEE or E-wastes) describe all secondary computers, entertainment device electronics, mobile phones, and other items such as television sets and refrigerators, unwanted by their original owners since they have exceeded their shelf life. This includes used electronics which are destined for reuse, resale, salvage, recycling, or disposal (Ogungbuyi *et al*., 2012). Other examples of this class of wastes may include; monitors, batteries and stereos. Toxic substances in e-waste include polyvinyl chloride (PVC plastics), copper, lead, mercury, arsenic (in older models), cadmium, manganese, cobalt, gold, and iron. E-wastes are mostly generated in developed countries, where their disposal is regulated due to the level of toxic substances they generated. A lot of these developed countries, instead of investing money to develop recycling process(es) for the management of the generated e-wastes, will for economic reasons, ship these wastes out to third world countries where laws regulating the disposal of these wastes are less stringent (Gaidajis *et al*., 2010). Majorities of these e-wastes in developing countries eventually end up being disposed off in landfills. Due to their toxic nature, toxins (heavy metals and organic compounds) are released from these wastes during burning, into underground water reservoirs, soil and air presenting environmental hazard to the ecosystems.

2.1.1 Hazardous waste

Hazardous wastes are generally described as wastes that pose substantial or potential threats to public health or the environment. These wastes may be found in different physical states like gaseous, liquids, or solids and are known or tested to exhibit the following characteristics: ignitability (flammability), reactivity, corrosivity and toxicity (carcinogenicity, mutagenicity, teratogenicity and systemic toxicity) to physical or biological systems. Hazardous waste materials are specifically listed by regulatory authorities under hazardous substances. United Nations Environmental Programme (UNEP) estimated that more than 400 million tons of these wastes are produced universally each year, mostly by industrialised countries (Coleman and Schultes, 1996). About 1% of the total production is shipped across international boundaries, with the majority of the transfers occurring between countries in the Organisation for the Economic Cooperation and Development (OECD) (Blankers, 1996). Some of the reasons for shipping these wastes by industrialised countries to developing countries for disposal are the rising cost of disposal and or treatment in their home countries and the degree of hazardousness of these wastes (Blankers, 1996). Different businesses can generate hazardous waste. For example, dry cleaners, automobile, repair shops, hospitals, and photographic processing centers

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and some larger companies such as chemical manufacturers, electroplating companies and oil refineries.

Household Hazardous Waste (HHW) also referred to as domestic hazardous waste or retail hazardous waste is generated from residential households or post-consumer waste. HHW only applies to wastes that are the products of the use of materials that are labeled for and sold for "home use". It includes household chemicals and other substances for which the owners no longer put into use, such wastes are consumer products sold for home care, personal care, automotive care, pest control and other purposes. These products exhibit many of the same dangerous characteristics as fully regulated hazardous wastes due to their potential for reactivity, ignitability, corrosivity, toxicity, and or persistence. Examples include drain cleaners, oil paint, motor oil, antifreeze, fuel, pesticides, herbicides and rodenticides, fluorescent lamps, aerosols (propane cylinders) lamp ballasts, caustics (cleaning agents), medicines and medical products, mercury containing wastes (thermometers, switches, fluorescent lighting) used at home, some types of cleaning chemicals, and consumer electronics (such as televisions, computers, and cell phones). Wastes generated by a company or at an industrial setting are not generally regarded as HHW, but when put into use at home it becomes HHW. Therefore, post-consumer wastes can be qualified as hazardous waste when discarded (Forester and Skinner, 1987).

Hazardous and household hazardous wastes are usually treated before disposal into hazarous wastes designated sanitary landfills in most developed countries. While in most developing countries these wastes are not usually treated due to high cost and are disposed off in unsanitary landfills. This usually results in unfavorable amounts of hazardous materials being released into the environment.

2.2 Solid waste management methods

The production of huge amounts of solid wastes requires appropriate management to prevent or reduce harmful effects on human and pollution of the environment. Solid waste management is a complex process that is beyond just the disposal of the wastes but includes collection, processing and transporting of the generated wastes as well as minimization of wastes at the point of production. Some of the common ways of managing solid wastes around the world include:

(i) Reduction at source: This describes waste minimization at the point of generation and it is the most sustainable form of waste management but it cannot be easily implemented by waste management authorities in isolation from the rest of the society. It involves every individual and sector of the society and all stages of the life cycle of every product. These stages are extraction

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of raw materials, transportation, design, manufacturing, purchasing, packaging, consumption and post consumption fate. This method requires a different concept of economic growth based on reduced consumption, re-used and recycle mentality (Forester and Skinner, 1987).

(ii) Re–use and Recycling: Re–use involves making use of products again by not discarding them before the end of their useful life while recycle is the recovery of materials from products after they have been used by consumers. These processes reduce waste disposal cost, conserve resource, create jobs and reduce emission into air and water (UNEP, 1994).

(iii) Composting: This is a process for the recovery of valuable material from biodegradable organic matter in the waste stream. It is an aerobic and biological process of degradation that produces material that can be used as a soil-amendment. It has the advantages of reducing wastes to a minimal volume that can be landfilled (organic biodegradable matter makes up to 60% of municipal solid waste) and recovery of useful organic matter for use as fertilizer in gardening, agriculture and landscape. It can also lead to reduction in the amount of landfill gas and leachate produced. Although it can generate odours, spores, dust and possibly vermin (Schrapp and Al-Mutairi, 2010).

(iv) Incineration: This is a waste processing option which converts waste to energy and reduces the volume of waste before disposal into landfill. It is an interim waste processing function and not the final stage of waste management. The disadvantages of this method may include: it produces hazardous waste that must be disposed of, enhances mobility and bio-availability of toxic metals present in waste, discharges contaminated wastewater, emits toxic pollutants, and produces carbon dioxide (a greenhouse gas) (Dension and Silbergeld, 1988).

(v) Landfilling: It is the dumping of waste on the land. This explains a wide spectrum of sites ranging from managed, engineered, regulated sites to illegal, uncontrolled dumps (Pheby *et al*., 2002). In developed countries like in United Kingdoms, a typical municipal landfill is an engineered sites with base lined with impermeable clay, plastic, rubber or composite layer covered by earth (figure 2.1). At the end of each day, wastes are covered with an inert material like clay soil and when the cell is eventually filled up, it is covered over with a layer of inert material. During operation, a fence is built around the site to prevent the wind from blowing material off sites. A drainage system is built to collect water runoff and leachates. An energy recovery system is constructed to collect gas which can either be used to generate electricity or is flared (Tubb and Iwugo, 2000). Also, landfill operations are regulated to protect human health. These regulations include banning of the disposal of liquids, infectious clinical wastes, tyres and co-disposal of hazardous wastes and municipal wastes into the landfills. The benefits of waste

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disposal into landfills may include; it is a cheap way of disposing wastes by dumping in disused quarries and abandoned industrial sites, waste is used to backfill quarry before reclamation. It also has the disadvantages of water pollution from leachate and runoff, air pollution from anaerobic decomposition of organic matter producing methane, carbon dioxide, nitrogen, sulphur and volatile organic compounds (Pheby *et al*., 2002).

(vi) Pyrolysis/gasification: As the name suggests, this is a multi-stage process. In the pyrolysis stage, the waste materials are heated in the absence of oxygen. Organic materials are converted to simple gases leaving a residue of carbon char which contains inert materials and any heavy metals. In the gasification stage, carbon residues are reacted out with air and steam in the "water-gas" reaction to produce hydrogen and carbon monoxide. Finally, these gases are combusted to produce energy and heat (DEFRA, 2004).

(vii) Anaerobic digestion: is a biological process which produces biogas from organic materials such as the organic component of municipal solid waste (MSW). Biogas comprises methane and carbon dioxide. The process takes place in the absence of oxygen in an airtight container known as a "digester." Decomposition in the absence of oxygen produces biogas, containing mainly methane and carbon dioxide. The biogas produced from anaerobic digestion is normally burnt to provide heat and/or electricity. If the digested material is of suitable quality, it can be spread on land, improving land quality and reducing the need for chemical fertilizers (DEFRA, 2004).

Landfilling remains the most commonly used among the methods of managing solid wastes around the world. In developed countries, sanitary landfills are used for managing generated wastes. The following rules are applied in the use of landfills in most developed countries:

(i) wastes are treated prior to landfilling except for inert wastes
(ii) aftercare of closing of landfills is required.
(iii) in sites receiving biodegradable wastes, landfill gas must be collected and used or flared.
(iv) co-disposal of hazardous waste with municipal waste is not acceptable.

The benefits of landfilling of wastes are:

(i) it has been a cheap way to dispose of waste by dumping it in disused quarries and abandoned industrial sites.
(ii) waste is used to backfill quarry before reclamation.
(iii) landfill gas contributes to renewable energy supply.

The disadvantages of landfill are:

(i) water pollution from leachate and runoff.

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(ii) air pollution from the anaerobic decomposition of organic matter producing methane, carbon dioxide, nitrogen, gases, sulphur, and volatile organic compounds.

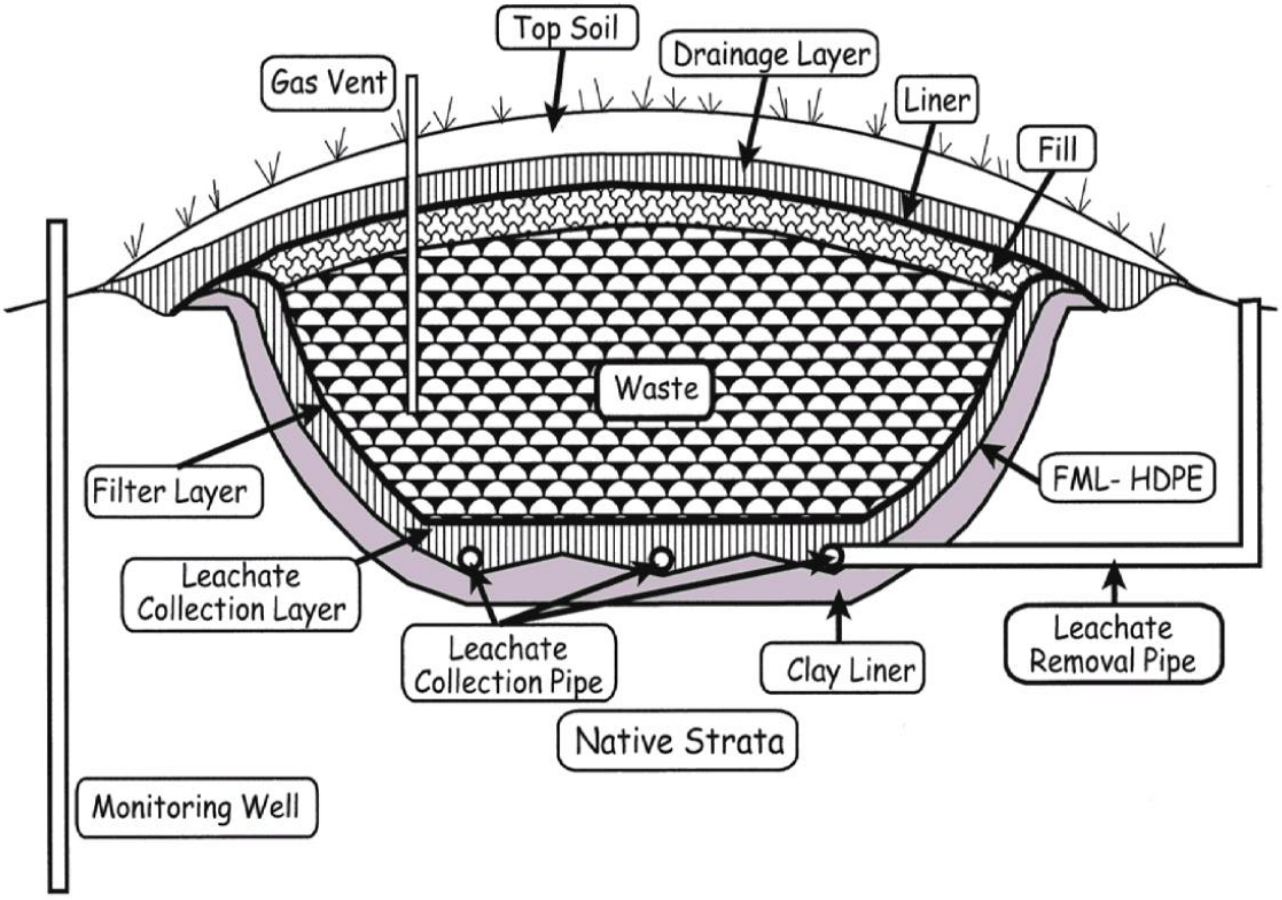

Figure 2.1 A typical sanitary landfill with liner systems at the base and sides to provide barriers that minimise migration of contaminants from the containment site to groundwater. It has engineered capping system, which prevents or controls infiltration of precipitation, minimizing movement of fluids through the wastes. It contains leachate collection systems that removed all leachate generated into leachate wells where they are treated or attenuated.

Source: Lee and Jone (1991).

Contrary to developed countries, unsanitary landfills of open dumping are the preferred method of disposing solid waste in most developing countries including African countries. In open dumps, refuse is simply disposed off in low lying areas on open land. These unsanitary landfills lack engineered measures that prevent the release of landfill gas and leachate into the environment. Also, there are little or no any operational measures like control of the number of tipping fronts and compaction of wastes in the landfills (Zerbock, 2003).

Waste is disposed haphazardly with all kinds, whether municipal, industrial, or clinical/hospital wastes without segregation, into the landfills. This method is neither hygienic nor safe. However, African countries have very little choice but to hang on to this method. Since this uncontrolled waste disposal method is the best that is possible, because of financial and managerial constraints (Remigios, 2010). Most local governments are weak, underfunded, and are faced with growing populations; hence they cannot raise enough funds to construct properly engineered landfills.

Generally in most developing countries, the word landfill is widely misused owing to the fact that there is no rigid definition; hence the operational aspects of the word remain unclear. It is used loosely to refer to any form of dumpsite (Remigios, 2010). It is also used to describe an engineered waste disposal site, which has very little environmental impact (Lee and Jone, 1991). The term open dumping of solid wastes also refers to uncontrolled disposal sites (dumpsites) or unsanitary landfills (Remigios, 2010).

In Nigeria, unsanitary landfills (dumpsites) are common method of solid waste disposal. They are located in public places surrounded by residential quarters and in wetland or other areas with seasonally high water tables. All forms of generated solid waste in the country are indiscriminately disposed off into the dumpsite without any attempt to seclude. Co-disposal of infectious medical wastes, toxic industrial solid wastes, radioactive wastes, agricultural wastes and domestic wastes occur in the dumpsites. These dumpsites are not equipped with landfill gas and leachate collector and they lack landfill liners and caps (figure 2.2).

Other common methods of managing solid wastes in Nigeria include dumping of wastes in open spaces along major roads, river banks and burning of wastes on major roads (figure 2.3). These methods have some disadvantages which may include;

i. water pollution from leachate and runoff solutions.

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ii. air pollution from anaerobic decomposition of organic matter producing methane, carbon dioxide, nitrogen, sulphur, and volatile organic compounds.

iii. not a long term sustainable option for energy generation.

iv. emits toxic chemicals, heavy metals, microbial pathogens.

v. feral animals, birds and vermin (disease vectors like insects) are found around dumpsites.

vi. road traffic hazards.

vii. fire and explosion risks.

viii. Others include psychosocial hazards like nuisance, stress, fear and worry which may affect the quality of life, occupational hazards, drop in property values and resource depletion, landfill gases add to global warming if not utilised, and waste disposal into landfill increases use of fossil fuels (Coker and Sridhar, 2010; DEFRA, 2004; Oshode *et al*., 2008; Pheby *et al*., 2002).

2.3 Landfill leachate

Unsanitary landfills are known for their ability to release large amounts of hazardous and deleterious chemicals to nearby ground water and air, via landfill leachate and gas. When solid waste is disposed off and processed at landfills, it undergoes a combination of physical, chemical and microbial processes (Christensen *et al*., 2001). These processes transform wastes in the landfills into various water-soluble compounds and transfer the pollutants from the refuse to the percolating water. The contaminant-rich water based solution of pollutants is termed "leachate". The sources of percolating water include precipitation, irrigation, surface runoff, groundwater intrusion and the initial moisture content present within the waste (El-Fadel *et al*., 1997). Microbial decomposition of the waste also contributes to the formation of leachate. The rate of generation of leachate in a landfill depends on a number of variables, including waste characteristics, moisture content (amount of liquid in landfilled wastes), temperature, pH and the availability of nutrients and microbes (figure 2.4) (El-Fadel *et al*., 1997). Flowing through the wastes, leachate transports a wide variety of chemicals, particulate matter and microbes to the extremities of a landfill. The quantity of leachate produced by a landfill is highly correlated with the amount of precipitation in or around the landfill (USEPA, 1998). There is also a correlation between landfill size and amount of leachate produced. Large landfills generate greater volumes of leachate than small landfills. For instance, an estimated 100 acre landfill in the northern United States can produce 57 million gallons of leachates every year (USEPA, 1998). Another factor that contributes to leachate volume is ground water. If a landfill is constructed adjacent to a river, in a wetland or in other areas where groundwater is not far beneath the landfill's base,

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groundwater can rise from below a landfill and provide additional liquid to mix landfilled wastes to produce leachates. Since leachate often contains toxic substances, leachate that escapes from a landfill and enters groundwater or surface waters becomes a potential threat to the environment and public health.

Figure 2.2: A typical dumpsite (Unsanitary landfill) in Nigeria without landfill liners, covers and leachate collection systems. It is located in proximity to residential quarters, with regular burning of wastes.

Source: Walling *et al*., (2004)

Figure 2.3. Common methods of solid waste management in Nigeria.

(a) disposal of solid wastes along river bank (arrow) endangering aquatic forms.

(b) burning of solid wastes on major roads (arrow). Smoke produced may lead to decrease visibility and disperse harmful gases into the air.

(c) open dumping (arrow) on a highway constitute eye-sore and environmental pollution risk.

(d) waste collection containers (arrow) are always filled up with wastes and hampering movement as well as oozing out malodour gases to nearby air.

Sources: Walling *et al*., (2004).

2.3.1 Composition of landfill leachates

Numerous studies have classified leachate generated from landfills receiving wastes from different sources as complex mixture of chemicals and microorganisms. Christensen *et al.* (1994) describe landfill leachates as a mixture of four major groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals and xenobiotic organic compounds. Other compounds that may be present, but in minute amounts, in leachate from landfills include: boron, arsenic, selenium, lithium, mercury and cobalt. These compounds are of secondary importance (Christensen *et al.* 2001) and they vary in their compositions depending on the leachate source (Fan *et al.* 2006; Rodriguez *et al.*, 2004). This variation significantly depends on waste composition, waste age, landfill technology, degree of compaction, the hydrology of the site and climate change (Rodriguez *et al.*, 2004). Inorganic macro-components are major inorganic constituents of leachates. They may include: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), ammonium (NH^{4+}), iron (Fe), manganese (Mn), chloride (Cl^{-}), sulphates (SO_4^{2-}) and bicarbonates (HCO_3^{-}). The concentrations of most of the macro components in leachate depend on the stabilisation processes in the landfill (Kjeldsen *et al.* 2002).

Dissolved organic matter describes the soluble organic component of the landfill leachates and is usually expressed as Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) or Biochemical Oxygen Demand (BOD). At early stages of landfilling, leachate usually has high BOD (e.g. 9500 $mg.l^{-1}$) value and even higher COD (e.g. 14 000 $mg.l^{-1}$) content (Kjeldsen *et al.*, 2002). Dissolved organic matter is a bulk parameter covering a wide range of organic degradation products including methane (CH_4), volatile fatty acids and some refractory compounds such as fulvic and humic-like compounds. The decomposition of organic matter gives leachate its colour: yellow, brown, dark or black (Aziz *et al.*, 2007) and also determine to a greater extend the volume and diversities of microorganisms present in such leachates (Donnelly, *et al*., 1990).

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More than 200 organic compounds have been identified in municipal landfill leachates (Paxeus, 2000; Schwarzbauer *et al*., 2002), with about 35 of these compounds having the potential to cause harm to the environment and human health (Paxeus, 2000). Moreso over 1000 organic chemicals have been identified in groundwater contaminated by landfill leachates (Christensen *et al*., 2001; Kjeldsen *et al*., 2002). Majority of these compounds are derived from decomposing vegetation and degradation products of natural materials (Reinhard *et al*., 1984;

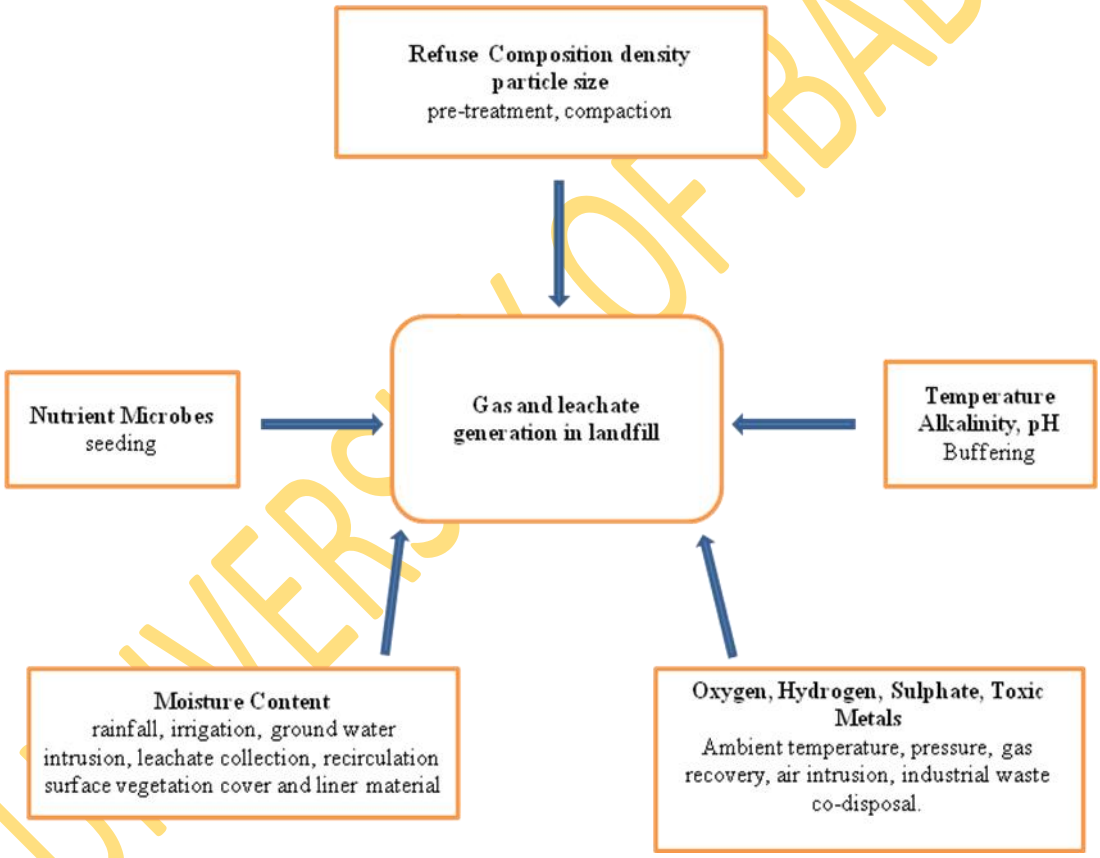

Figure 2.4. Factors influencing the generation of leachates and gases in landfills.
Source: El Fadel *et al*. (1997).

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Schwarzbauer *et al*., 2002), with cellulose and hemicelluloses alone comprising up to 60% of the total dry weight of the MSW (Barlaz *et al*., 1992). Such compounds, aliphatic and aromatic acids, phenols and terpenes, have the tendency to degrade as any leachate plume migrates from a landfill site (Leenheer *et al*., 2003; Reinhard *et al*., 1984). The compounds, widely described as Xenobiotic Organic Compounds (XOCs) are chiefly associated with industrial or conventional hazardous wastes, but a large number occur in municipal or household wastes. Also, paint, garden chemicals, household cleaning agents, human and veterinary medicines, motor vehicle products, waste electrical and electronic equipment and batteries, are all potential sources of xenobiotic organic substances (Slack *et al*., 2005). Slack *et al.* (2007) have shown that municipal landfill leachates from Taiyuan, China contain significant quantities of these hazardous chemicals. Those detected in the leachates include chlorinated and non chlorinated hydrocarbons, carbon tetrachloride, chlorobenzene, toluene, chloromethane, chloroethylene, xylene, phenols, phthalate, camphor, decanoic and nonanoic and cresol. The concentrations of XOCs are higher during the active states of decomposition and gradually decrease as the landfill stabilizes (i.e decreases with time) (Christensen *et al.* 2001) and they continue leaching for decades.

XOCs, organic degradation products, are produced from wastes containing chlorinated aromatics, chlorinated/non-chlorinated hydrocarbons, nitrogen containing compounds, alkyl phenol ethoxylates and alkyl phosphates (Schwarzbauer *et al*., 2002). Types and concentrations of XOCs frequently identified in landfill leachates differ in leachates and the differences is a reflection of the landfill age, waste composition and landfill management processes occurring at the site (Christensen *et al*., 2001). Benzene, toluene, ethylbenzene and xylenes (BTEX compounds) are XOC commonly found in highest concentrations in most landfill leachates. This reflects their common usage as solvents in a large range of products and waste generating processes. Also, next to BTEX are the halogenated hydrocarbons; tetrachloroethylene,

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trichloroethylene and dichloroethanes (Kjeldsen *et al*., 2002). These compounds are almost universal in their occurrence in leachates. They are among the commonly analysed XOCs in leachate samples in most developed countries due to their being designated by US Environmental Protection Agency as priority pollutants based on their aquatic polluting capability (Kjeldsen *et al*., 2002).

Plasticisers are another group of XOC which have been widely reported in landfill leachates. The principal members of XOC among this group of compounds are phthalates, of which di-(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), diisononyl phthalate (DINP) and dibutyl phthalate (DBP) have been extensively used in the manufacture of consumer goods (Jonsson *et al*., 2003). Concern for this group of compounds has increase in recent times since they were being classified as endocrine disruptors and listed as priority pollutants by the US EPA (Schwarzbauer *et al*., 2002). The tendency to persist in the environment and bioaccumulate in organisms are the main contributory factors for their priority status (Schwarzbauer *et al*., 2002). These xenoestrogens are metabolites of various widely used substances that are preferentially adsorbed onto sewage sludge (Marcomini *et al*., 1989), this suggests a possible entry route into landfills.

Gases oozing out from landfill sites due to the decomposition processes of the organic matters in the wastes have been shown to contain toxic organic compounds called volatile organic compounds (VOC). Some of these compounds have clear teratogenic potential or other significant reproductive effects, even at low doses or exposures. Animal and/or human data have proved this (Lee and Jones-Lee, 2003). Health implications of some of the VOCs of the landfill gas at their 'No-Observed-Adverse-Effect-Level' (NOAEL) concentrations is presented in table 1. These numerous organic compounds present in landfill gas may not only have potential health effects on those people working at the landfill site, but also on those living in the local area and may spread for up to mile or more from the landfill site, particularly downwind (Lee and Jones-Lee, 2003).

Some of these gases from landfill have unpleasant odours which can persist for a longer time covering miles or more from the site. Also, the odour can cause unpleasant sensations, by triggering reflexes in the body that may be harmful (Lee and Jone-Lee, 1994). This can lead to the following; nausea, vomiting, headache, upsetting of stomach or appetite, upsetting of sleep, shallow breathing and coughing, decreased heart rate and constriction of blood vessels in skin and muscles, alteration of cells of the olfactory bulbs of the brain, irritation of eyes, nose and throat, annoyance, anger and depression, and a general decrease in well-being and enjoyment

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(Lee and Jone-Lee, 1994). It is also possible that asthma attacks can be triggered by odorous conditions, as bronchial asthma has a hypersusceptibility to odours and are also responsible for exacerbating a number of pre-existing medical conditions, including augmentation of sensitivity to 'morning sickness' in pregnancy (Lee and Jone-Lee, 1994).

The inorganic components of landfill leachates are mostly studied alongside organic constituents. Heavy metals present along with the inorganic fraction are of particular interest due to their hazardous nature (European Council, 2002). The commonly occurring heavy metals in landfill leachates include zinc, copper, cadmium, lead, nickel, chromium and mercury (Reinhart, 1993) and they can form metal colloids or complexes, particularly with organic matter (Christensen *et al*., 2001). As such, the heavy metal content of leachates can be

Table 2.1. Some volatile organic compounds present in landfill gases and their health effects at the No-Observed-Adverse –Effect-Level

Compounds	NOAEL	Effects
Benzene	32mg/m^3	Reduced foetal weight; Retarded ossification
Tetrachloroethylene	2000mg/m^3	Embryolethality; Foetotoxicity
Trichloroethylene	0.2 mg/kg bw	Cardiac defects
Vinyl chloride	130mg/m^3	Retarded ossification; Male testicular effects; Reduced male fertility
1,3-Butadiene	88 mg/m^3	Reduced foetal weight
Carbon disulphide	----------	Uncertain Malformations
Chloroform	147 mg/m^{3*}	Reduced foetal weight; Retarded ossification
1,2-Dichloroethylene	0.025mg/kg bw*	Cardiac defects
Ethylbenzene	430 mg/m^{3*}	Embryolethal; Foetotoxic; Teratogenic
Formaldehyde	12 mg/m^3	Reduced foetal weight
Methyl chloride	525 mg/m^3	Cardiac defects
Alpha-terpinene	30 mg/kg bw	Retarded ossification, Skeletal anomalies
Dichlorobenzenes	1200 mg/m^3	Reduced foetal weight, Skeletal variants
2-Ethyl-1-hexanol	130 mg/kg bw	Embryolethal Foetotoxic
Hydrogen sulphide	140 mg/m^3	No effects observed
Methyl ethyl ketone	1500mg/m^3	Foetotoxic, Teratogenic
Toluene	375mg/m^3	Reduced foetal weight, Retarded ossification, Extra ribs
Xylenes	150mg/m^{3*}	Retarded ossification
Acetone	5200 mg/m^3	Reduced foetal weight
2-Butanol	10,000 mg/m^3	Reduced foetal weight
Carbon tetrachloride	1923 mg/m^3	Reduced foetal weight
Dichloromethane	5,300 mg/m^3	No adverse effect

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Ethanol	40 mg/kg bw	Adverse human reproductive effects
Limonene	1000 mg/kg bw *	Foetotoxic; Rib anomalies
1-Propanol	17500mg/m^3	Reduced foetal weight; Skeletal defects; Reduced male fertility
Styrene	1147mg/kg bw	Maternal effects but no embryofoetal effects
Vinyl acetate	700mg/m^3	Reduced foetal weight; Retarded ossification

Source: ATSDR, (2001).

significantly high. Generally, heavy metals with high level of sorption and precipitation, do not constitute a groundwater pollution threat due to poor migration into the leachate plume and low initial concentrations leached from the solid waste. However, they can reach problematic levels despite their low concentrations in the leachates when they bioaccumulate in tissues of living organisms (Slack *et al*., 2005). Kjeldsen *et al*. (2002) found that heavy metal levels, particularly mercury and cadmium, in domestic waste landfill leachate are barely detectable and pose little threat to groundwater. Zinc, however, is usually recorded at concentrations higher than other heavy metals (Christensen *et al*., 2001). Heavy metals including arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel and zinc occur naturally as ores, in the environment. However, human activities have produced more than are found naturally in the earth crust for different usages. These metals from the various anthropogenic sources are usually deposited in landfills as along with wastes. Metals show varying abilities in their toxicity potencies and these have been linked to the production of reactive oxygen species (Patnaik *et al*., 2011; Slack *et al*., 2005; Stohs and Bagchi, 1995).

Heavy metal contamination of aquifers is of environmental and public health concern. This concern has heightened in recent times due to the concentrations of these metals in most examined leachates which exceeded the legislative permits of the national and international standards (Esakku *et al*., 2003; Ikem *et al*., 2002; Laniyan *et al*., 2011). Particulate matters contaminated with heavy metals are also known primary sources of heavy metal emissions from landfills (Parker *et al*., 2002). Concentrations of heavy metals in landfill leachates are sometimes low and may not pose a great deal to groundwater pollution. However, when they are present in high concentrations, heavy metals can cause a little volume of leachate to be highly toxic

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(Sawaittayothin and Polprasert, 2007). Table 2 presents some of the physical and chemical substances that are commonly analysed in municipal landfill leachates and their concentration ranges.

Dissolved organic matters are less concentrated subgroup xenobiotic organic compounds, but inorganic macrocomponents occur at much higher concentrations even when compared with heavy metals. Iron and manganese belong into inorganic macrocomponents as they are not considered as heavy metals, along with calcium, sodium, potassium, ammonia/ ammonium and others (Stack *et al*., 2005). Ammonical nitrogen (as ammonia and/or ammonium) is recorded at high levels in most studied landfill leachates and they are the dominant pollutants (Kjeldsen *et al*., 2002).

Microorganisms have also been isolated from landfill leachates. Some of these organisms are pathogenic while others are opportunistic. Donnelly *et al*. (1988) observed different species

Table 2.2 Concentration ranges for some components of municipal landfill leachates

Parameter	Typical concentration range	Average
BOD	1,000 – 30,000	10,500
COD	1,000 – 50,000	15,000
TOD	700 – 10,000	3,500
Total volatile acid (as acetic acid)	70 – 28000	NA
Total Kjedahl Nitrogen (as N)	10 – 500	500
Nitrate	0.1 – 10	4
Ammonia	100 – 400	300
Total Phosphate (PO_4)	0.5 – 50	30
Orthophosphate (PO_4)	1.0 – 60	22
Total alkalinity (as $CaCO_3$)	500 – 10,000	3,600
Total hardness (as $CaCO_3$)	500 – 10,000	4,200
Total solids	3,000 – 50,000	16,000
Total dissolved solids	1,000 – 20,000	11,000
Specific conductance (mhos/cm)	2,000 – 8,000	6,700
pH	5 – 7.5	63
Calcium	100 – 3,000	1,000
Magnesium	30 – 500	700
Sodium	200 – 1,500	700
Chloride	100 – 2,000	980
Sulphate	10 – 1,000	380
Chromium (total)	0.05 – 1	0.9

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Cadmium	0.001 – 0.1	0.05
Copper	0.02 – 1	0.5
Lead	0.1 – 1	0.5
Nickel	0.1 – 1	1.2
Iron	10 – 1,000	430
Zinc	0.5 – 30	21
Methane gas	60%	----
Carbon dioxide	40%	----

All values mg/L except as noted
NA- not available
Source: Lee and Jones-Lee (1994).

of gram positive and gram negative bacteria from different bacterial genera in hazardous waste and simulated leachate from experimental landfills. The gram positive species include different species of *Clostridium*, while the gram negative species are mainly rod shaped genera, which include; *Citrobacter*, *Enterobacter*, *Klebsiella* and *Pseudomonas*. Recently, a total of 112 bacteria belonging to about 17 genera were isolated from Aba Eku landfill leachate (Oshode *et al*., 2008). These bacterial species; *Bacillus aureus*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium sordelli*, *Clostridium perfinges* and *Salmomella arizonae* are of public health concern. Bacterial isolates 20 *Staphylococus aureus* and 14 *Clostridium perfringens* from the Aba Eku landfill leachates were investigated for virulence factors and antibiotic resistance. Majority (> 70 %) of the isolates produced enterotoxins. Many of the *S. aureus* isolates tested positive for deoxyribonuclease, haemolysins and slime production. Staphylococcal enterotoxin A was the predominant enterotoxin produced by the *S. aureus.* Six isolates were resistant to methicillin and majority of them were resistant to penicillin, ampicillin and bacitracin. Eleven (78.6 %) of the *C. perfringens* isolates produced enterotoxin and were also beta haemolytic. Except for one strain each of *C. perfringens* which were resistant to penicillin and ampicillin-sulbaltam respectively (Efuntoye *et al*., 2011). The presence of several of the virulence strains investigated and resistance to commonly used antibiotics in many of the *S. aureus* and *C. perfringens* tested raises concern about their dissemination through leachate to the environment. The accumulation of leachate and possible contamination of surface and groundwater sources and soils around the landfill may be of potential risk for public health.

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Some genera in fungi group have also been identified in landfill leachates. They are *Fusarium*, *Monosporium*, *Penicillium*, *Phoma* and *Pleospora* (Donnelly *et al*., 1988), *Aspergillus niger*, *A. flavus*, *A. terreus*, *Fusaruim oxysporium*, *penicillium spp* and *Rhizopus spp* (Oshode *et al*., 2008). These fungi are potential pathogenic and toxin–producing species which can be transported along with leachates into ground or surface water source and contaminating portable water (Daskalopoulos *et al*., 1998). The entry of these pathogenic opportunistic microorganisms can constitute a pollution hazard to all those who may get in contact with the polluted water incidentally. They can also be dispersed in air and settle on food substances during feeding by many live in close proximity to landfills.

Municipal solid waste is a collection of discarded liquid and solid materials that serves as a breeding ground for microorganisms mainly fungi and bacteria. Asides landfill leachates, airborne bacteria, viruses and fungi have been detected in bioaerosols from landfills. A large number of microbial and fungi genera and species were isolated from air samples in residential houses around Jeleeb Al-Shuyoukh landfill. The bacterial genera were *Acinetobacter*, *Pseudomonas*, *Moraxella*, *Pasteurella*, *Streptomyces* and *Bacillus*, while the fungal genera were *Aspergills*, *Circinella*, *Fusarium*, *Candida*, *Mucor*, *Penicillium* and *Scopulariopsis* (Schrapp and Al-Mutairi, 2010). Dusts from landfills have been associated with higher loads of microbial and fungal genera. In Poland, household waste was reported to contain about 14 genera of microbes. These include gram positive bacteria: *Bacillus*, *Micrococcus* and *Streptococcus* and mycological populations: *Aspergillus*, *Fumigates*, *Penicillium*, *Alternaria*, *Rhizopus* and *Mucor*. There bacterial and mycological genera have been implicated with different respiratory, dermal and gastrointestinal diseases in exposed human populations around landfill facilities (Buczynska *et al*., 2011).

2.4 Exposure pathways from landfill site

Exposure is the crucial link between a health hazard and a health outcome. The risk to health depends not only on how much is present but also on whether there is a route by which people may be exposed. A complete exposure pathway starts at the source of contamination and travels through environmental media to the point of exposure and by some route of exposure to an exposed population (WHO, 2000). An exposure pathway has three elements:

i. release from site
ii. transport through environmental media
iii. uptake by people.

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If any of these elements are missing there may be no threat to health (WHO, 2000). Pollutants are released from landfill operations, mainly as gases and liquids. Gaseous releases from landfill migrate off the sites covering about 3–4 km circumference of the landfill sites (Shusterman *et al*., 1991). Organic and inorganic chemicals volatilize from wastes into landfill gases and are disperse in the atmosphere. These chemicals can dissolve in open pools to form a concentrated aqueous solution or may contaminate soil by settling on it (Eduljee, 1992). Landfill leachates are carried off-site by surface runoff. Dust emissions resulting from unloading soild waste, loading temporary landfill cover material, lift construction, vehicle traffic to the site and wind erosion from site can be inhaled. Pollutant levels from landfills vary greatly according to the nature of the wastes deposited and the time period of release.

Pollutants released from landfill site through gases can be inhaled by human populations living in the vicinity or working on the landfill sites. For instance volatile organic chemicals may evaporate into the atmosphere from landfill sites and be inhaled. Pollutants released from the waste site may remain toxic after passing through various environmental media but will only pose a risk to health if they are taken up by people in sufficient quantities and over a sufficient period of time. Potentially exposed populations in this case are mainly waste site workers, on–site trespassers and neighbouring area for recreation. There may be a health risk of inhaling pollutants from combusted gases during landfill flaring (Bridges *et al*., 2000). Health effects have been associated with inhalation of organic dust during the handling of municipal solid waste (Perez *et al*., 2006). Inhalation was the only exposure route considered from Nant–y–Gwyddon landfill sites as the investigation arose out of complaints about odour (Fielder *et al*., 2000).

Other possible routes to landfill exposure are ingestion of particulates chemicals like heavy metals deposited in soils, surface water, food and dust. Lead and cadmium and persistent organic chemicals which contaminated soil may be taken up by crops or eaten by livestock, both of which may be consumed by people (Farmer and Hjerp, 2001). For example, in Love Canal incidence, ingestion was considered to be one pathway by which children were exposed. According to Albert (1987) children were reported to have picked up raw chunks of lindane and phosphorous and threw them around and sloshed through liquid organic wastes. Ingestion by drinking domestic water supply is also considered a major culprit. It was also observed that in Love Canal, chemicals from the landfill polluted neighbourhood creeks were children played. While the Canal was being filled, children swam in the Canal and they could have swallowed the water (Pheby *et al*., 2002).

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Direct skin contact with wastes or with contaminated soils is particularly a threat to landfill workers, and scavengers and children are more likely to be affected since they come in contact with soil than adults. Fire and explosion from combustible materials in the landfill sites may release toxic gases and cause health risk to workers and residents. The ensuing smoke that fills the air from the uncontrolled burning of solid waste during the dry season may constitute serious environmental pollution, adversely affecting landfill workers and pickers. Farmer and Hjerp (2000) reported that fire at a sanitary landfill can arise from hot ashes in a vehicle delivering wastes, a cigarette thrown down by worker or the sun rays through the fragment of glass on the surface.

The incomplete combustion of refuse burning emits particulate matters, carbon monoxide which is a common by-product of the incomplete combustion of fuels such as paper, cardboard and wood, acrolein, formaldehyde and other pollutants depending on the composition of wastes. Landfill fires emit a variety of pollutants that have the potential to affect the health of people exposed to the smoke. Toxic and hazardous wastes when burnt with other solid waste like asbestos fibre may introduce potential carcinogenic fibre to the smoke plume. Fire periodically breaks out in open dumps, generating smoke and contributing to air pollution. Farmer and Hjerp (2000) reported that some dump managers deliberately set periodic fires at the dumps in order to reduce the volume of the wastes, creating room for more wastes and thus extending the life of the dumps. Human scavengers may also cause intentional fires since metals are easier to spot and recover among ashes after the fires than among piles of mixed wastes.

The food and kitchen wastes disposed off in landfills may attract birds, rats, flies and other organisms to the dump sites. It is possible that within some hours in a warm temperature, sterile organic matter can become a potential lethal source of disease producing organisms. This is possible since the landfill environment is normally provided with spores, bacteria, viruses, insects, vermin and other vectors awaiting a favourable site on which to multiply (Tauhid-Ur-Rahman, 2006). These organisms act as vectors or intermediate host in the transmission of diseases to human living or working in the vicinity of the landfill sites. Figures 2.5 and 2.6 showed schematic presentations of possible human exposure pathways from landfill facilities.

2.5 Environmental effects of landfill leachate

Leachate constituents are mostly toxic and may have negative impacts within both surface and groundwater environments. Impacts of leachates on the aquatic environment can affect human, animal and plant lives. During the early (or acetogenic) biodegradation phase of

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the landfill wastes, the leachate is characterised by a high, Chemical oxygen demand (COD), Biochemical oxygen demand (BOD), and sodium, chloride and ammonium contents. These constituents are toxic to aquatic life and can have serious consequences if leachate pollutes surface water sources (Jones *et al.* 2006). Under aerobic conditions, ammonium contained in the leachate can be transformed by nitrification to nitrate which is assimilated by plants. When nitrate is combined with phosphate, the condition can lead to eutrophication of surface water courses (Jones *et al.*, 2006). The high concentrations of ammoniacal nitrogen (NH_3-N) can enhance algal blooms, which can deplete dissolved oxygen and have toxic impacts on aquatic life. Kurniawan *et al*. (2006) reported that at concentrations higher than 100 mg/L, NH_3-N can be highly toxic to aquatic organisms including zebrafish, freshwater fish and luminescent bacteria. Pivato and Gaspari (2006) emphasized that the toxicity of leachate might consistently depend on the ammonia concentration and that leachate toxicity is much lower in old landfills where ammonia had been degraded. Cheung *et al*. (1993) reported acute toxicity of landfill leachate on green algal species and that ammoniacal-nitrogen and organic compounds appear to be the leading factors affecting toxicity of leachate.

Leachate from Olusosun landfill has been reported to pollute water bodies within 2 km radius to the site (Oyeku and Eludoyin, 2010). Accordingly, 20 water samples obtained from randomly selected hand dug wells and boreholes and analysed for selected heavy metals showed that the samples were generally alkaline (8.3 ± 2.77) and contained Cu (0.02 ± 0.04 mg/l), Fe (4.23 ± 6.4 mg/l), Pb (2.4 ± 3.3 mg/l) and Co (1.03 ± 1.1 mg/l) concentrations that are higher than the permissible limits recommended by the World Health Organization (Oyeku and Eludoyin, 2010). Also, Bacud *et al.* (1994) reported that groundwater around dumpsites were acidified and nitrified by chemicals from the sites, while bacteria present in dumpsites wastes had been reported to contaminate drinking water sources of communities around dumpsites (Torres *et al*, 1991) in some cases, causing poisoning, cancer, heart diseases and teratogenic abnormalities among the populations in these communities (Sia Su, 2007).

Soil samples around dumpsites in Port Harcourt, Nigeria were analysed for various physico-chemical characteristics and heavy metal concentrations. It was observed that these soils were moderately acidic with a mean pH range (5.5 - 5.8), with total organic carbon (TOC) levels of 3.41% - 2.90%. The cation exchange capacity (CEC) of the soils ranged from 21.36 – 28.79 meq/100g. The heavy metal concentrations in all the locations of the waste dumpsites were above permissible limits (Ogbonna *et al*., 2009).

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Burning of wastes in landfills, which typically occurs at temperatures between 250 ºC and 700 ºC in oxygen starved conditions, produces air toxins. Hydrocarbons, chlorinated materials and pesticide compounds under these conditions produce wide range of toxic gases harmful to the environment and public health. These gases contain dioxins / furans, volatile organic compounds, particulate matter (PM), hydrogen chloride (HCl), carbon monoxide (CO) and oxides of sulfur and nitrogen and liberate metals including antimony, arsenic, barium, beryllium, cadmium, chromium, lead, manganese, mercury, phosphorus and titanium. Some of which are responsible for odours from landfills (SME, 2006).

In addition, solid waste burning produces large amounts of bottom and fly ash and other debris. Fly ash is made of light particles which is carried out by combustion gas and is laden with toxic metals, dioxin / furan and other products of incomplete combustion. Fly ash can travel thousands of kilometers before it drops back to earth where its chemical load might enter the human food chain (SME, 2006). Table 2.3 presents some inorganic and organic chemicals present in smoke produced during waste burning, their health impacts and environmental effects.

2.6 Toxicity of landfill leachates

The incidence of cancers among residents of Niagara, United States who were exposed to chemicals leaching out of Love canal landfill (Janerich *et al*., 1981), along with the reports of Meyer (1983) and Vianna and Polan (1984) on liver dysfunctions and incidence of low birth weight respectively among residents exposed to hazardous chemicals in landfill leachates,

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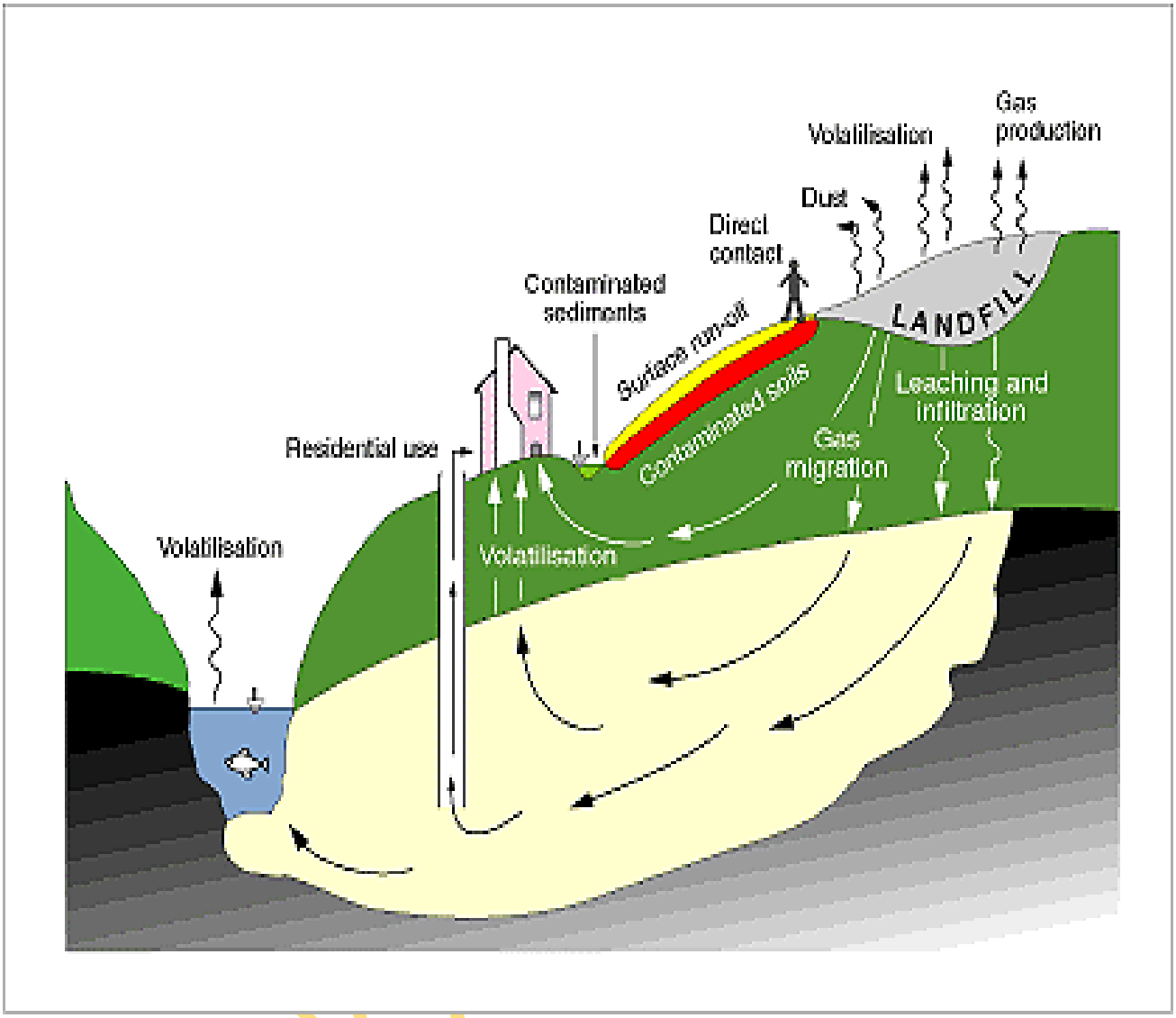

Figure 2.5. Schematic illustrations of possible exposure pathways from a landfill site.

Source: Eduljee, (1992).

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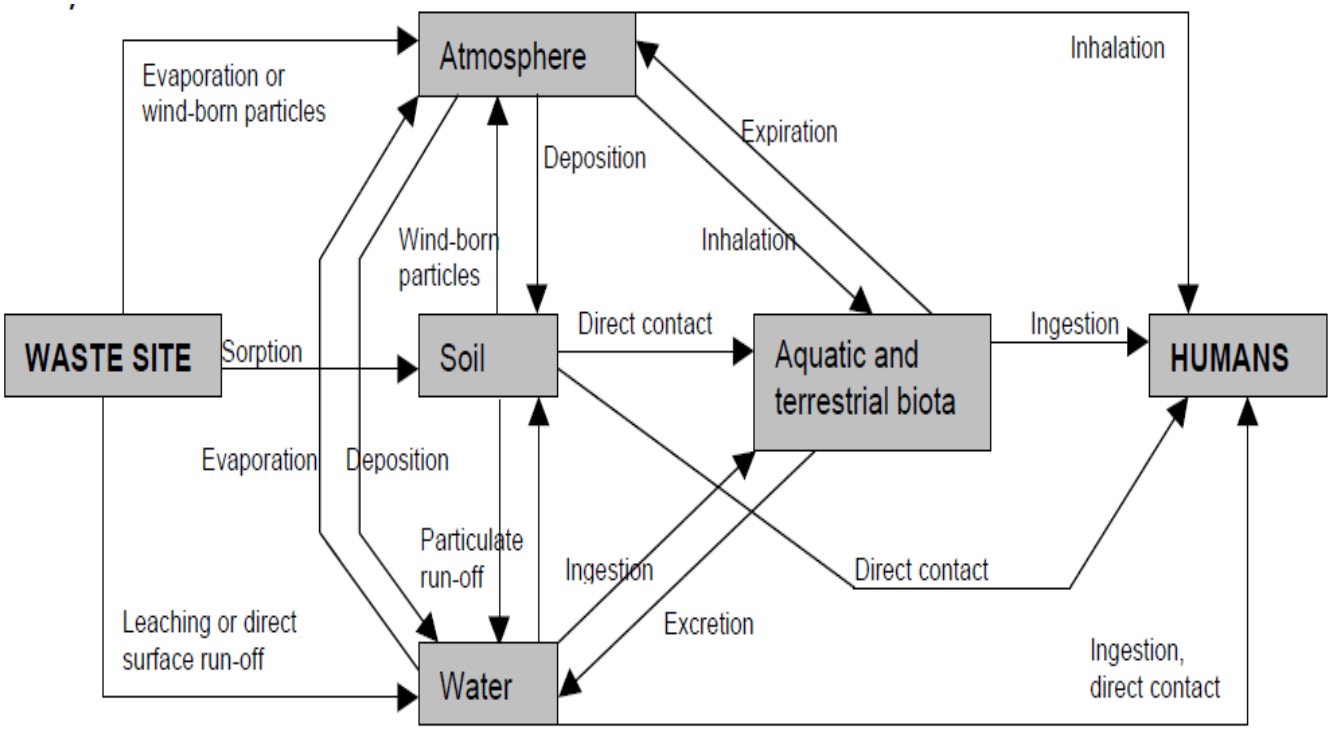

Figure 2.6. Schematic illustrations of potential exposure sources from a landfill site.
Source: National Research Council (1991).

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increased the awareness about the adverse human health effects that might be associated with landfills.

These reports drew the attention of researchers to landfill toxicity studies, with the identification of specific constituents of these chemicals as most commonly used approach to

show the hazardousness of chemicals in landfill wastes (Greer, 1984). This method has a major limitation of inability to provide information about all the toxic chemicals present in the waste mixture and the potential synergistic and antagonistic interactions of these chemicals in living organisms. This led to experimental toxicity studies conducted with the aim of determining the toxic effects of landfill leachates.

2.6.1 Acute toxicity of landfill leachates

Different investigators have examined the acute toxicity of landfill leachates using different test organisms ranging from prokaryotes (bacteria and algae) to eukaryotes. Some of these studies examined "natural" (untreated) leachates from several existing landfill operations, "synthetic" (simulated) leachate from lysimeter studies and /or treated leachates. Cheung *et al.* (l993) reported the acute toxicity of leachates from two landfills on four species of algae species; *Chlorella pyrenoidosa, C. vulgaris, Scenedesmus sp.* and *Dunaliella tertiolecta*. They utilised the standard static 96-hour bioassay and observed that Junk Bay leachate was more toxic to the four algal species than the second leachate, Gin Drinkers' Bay. The values of 96 hr-EC_{50} of Junk Bay for all the tested algae were lower than those of Gin Drinkers' Bay. The authors implicated the acute toxicities of the leachates on the high content of ammoniacal-nitrogen and organic compounds analyzed in the leachate samples. Bakare *et al.* (2003) evaluated the acute toxicity of simulated and raw leachates from three different dumpsites in southwest Nigeria using a terrestrial animal (mice; *Mus musculus*). They obtain different LC_{50} values for both simulated and raw leachates from the different dumpsites. These values range from 50 –100 % and they concluded that the toxic chemicals most importantly the heavy metals induced the observed toxic effects.

Bernard *et al*. (1996) examined the acute toxicity of twenty-seven landfill leachates in prokaryotes and lower eukaryotic forms using the conventional biotic static test (microalgae, duckweeds, daphnids) and new microbiotests (rotifers, crustaceans, protozoans, luminescent bacteria). They observed different toxicity values for the different test species used and the various leachates samples. Atwater *et al*. (1983) studied the acute toxicity of leachate from a refuse municipal landfill using *Daphnia pulex*, rainbow trout (*Salmo gairdneri*) and Sockeye salmon (*Oncorhynchus nerka*) as test organisms. They concluded from their study that both Daphnia and fish were suitable test organisms sensitive to the toxicity of combination of chemicals in the leachates.

Cameron and Koch (1980) utilized standard static 96-hour bioassay and the residual oxygen bioassay techniques to evaluate the toxicity of natural and synthetic leachates on rainbow trout (*Salmo gairdneri*). They concluded that leachate toxicity varies widely with a range of more than three orders of magnitude. The Lysimeter leachate was found to be the most highly toxic with average 96 hour LC_{50} value of 0.35% by volume. Raw natural leachates were also highly toxic with 96 hour LC_{50} values being in the range of 4.9 to 7.0 %. Ernst *et al.,* (1994) obtained samples of leachate from four locations in effluent streams from a municipal waste landfill near Halifax, Nova Scotia. These samples were analyzed for a number of physic-chemical parameters and organic chemicals. They carried out a static acute toxicity tests (48hrs to 96hrs) on rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and water fleas (*Daphnia magna*). The observed acute toxicity on the leachate samples on the test organisms was primarily provoked by its ammonia content.

Acute toxicity testing of landfill leachates from different landfills using different species of fish gave different LC_{50} values. Sisinno *et al.* (2006) evaluated the acute toxicity of untreated and treated leachate from Morro do Ceu dumpsite, Brazil using zebrafish (*Brachydanio rerio*) and the 48 hour LC_{50} were 2.2% and 5.7% for the untreated and treated respectively. Osaki *et al*. (2006) reported a 48 hour LC_{50} values of 19.2% and 53.0% for adults and larvae Japanese killer fish Medaka (*Oryzias latipes*) respectively, when they were exposed to leachates from Okayama landfill, Japan. 96 hour LC_{50} value of an acute toxicity testing of Aba Eku landfill leachate from Nigeria, using *Clarias gariepinus* (mud catfish) was 36.6% (Oshode *et al*., 2008). In a recent study, the 96 hour LC_{50} values of leachates from three landfills in Malaysia, using fry of *Cyprinus carpio* are 1.13, 2.00 and 3.82% for the different landfills (Alkassasbeh *et al*., 2009). It was concluded from the different LC_{50} values obtained from the various test organisms that landfill leachates are highly toxic and their toxicities depend on the concentrations of ammonia, heavy metals and organic components of the leachates which can individually or in combinations be responsible for the acute toxicity (Clement *et al*., 1993).

Table 2.3 Pollutants produced during waste burning and their health and environment effects

Pollutants	Health Effects	Environmental Effects
Aldehydes	Causes eye and respiratory illness, headaches animal carcinogen.	Is an Increased toxic loading on environment; leads to contaminated water and land, affects animals health.
Carbon monoxides	Causes dizziness, headaches and reflexes. Affect mental function, visual acuity and alertness.	Oxidized to carbon dioxide (which is a green house gas) in the atmosphere.
Chlorofluorocarbons (CFCs)	Causes dizziness, headaches and slowed reflexes.	The primary contributors to stratospheric ozone level depletion and are involved in the global warming effect.
Dioxins and Furans	May cause cancer; causes growth defects; affects DNA; affects immune and reproductive systems.	Increased toxic loading on environment; leads to contaminated water/land, affects animal health. Very toxic and bioaccumulate in the food chain.
Heavy Metals	Highly toxic; bioaccumulates in living system. Non-lethal effects causes chronic respiratory or intestinal distress, poisoning of the central nervous system, disruption of effects of the body's hormone system, inhibition of growth and development in children.	Increase toxic loading on environment; leads to contaminated water and land, affects animal health.
Hydrochloric Acid	Irritation of respiratory tract, causes respiratory illness; dulls the body's senses.	Increased toxic loading on environment; leads to contaminated water/land, affects animal health.
Hydrogen Sulfide (H_2S)	Toxic, causes respiratory disease. Healthy people experience shortness of breath, sore throats, breath difficulties, irritated eyes.	Contributes to acid rain; may damage vegetation; causes offensive odors.
Ozone (O_3)	Exposure to ozone can injure biological tissues and cells. Reduce lung function, including tightness of the chest, coughing pain and breathing difficulty.	Ground-level ozone damages vegetation and ecosystem, affects animal health.
Nitrogen Oxides	Causes respiratory illness, fluid collection in the lungs and fibrotic changes.	Contributes to acid rain and ozone formation.
Particulate Matter (PM)	Irritation of respiratory tract, aggravated asthma, contributes to chronic obstructive pulmonary diseases.	Increased toxic loading on the environment; leads to contaminated Water/land and affects animal health.
Polynuclear Aromatic Hydrocarbon (PAH's)	Cancer causing agent in most animal species including mammals, fish and birds.	Increased toxic loading on environment; leads to contaminated water/land, affects animal health.
Volatile Organic	Directly toxic including problems ranging from cancer risks to nervous disorders.Causes respiratory irritation/ illness, animals health.	Contributes to low level ozone (smog), Compounds (VOCs) causes vegetative damage. Leads to contaminated water/land, affect animals health.
Sulphur Oxides (SO_2)	Increase in heart/lung disease, acute/ chronic respiratory diseases. Health people experience shortness of breath, sore throats, breathing difficulties.	Causes vegetative damage; corrodes many materials; contributes to acid rain (forests, aquatic and urban environments i.e. structures

Source: Aspinwall, (2001)

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2.6.2 Immunotoxic effects of landfill leachates

The immune system is both a target and a mediator of environment-induced injury. Chemicals and physical stressors such as ionizing radiation can damage the immune system resulting in impaired immunity and impaired auto-surveillance for cancer. The immune system can also mediate the damage produced by exogenous agents through mechanisms such as hypersensitivity and autoimmunity (Bernier *et al*., 1995). Environmental chemical exposures have also resulted in combinations of toxic and allergic responses called "toxic allergic" syndromes. Examples of the latter include toxic oil syndrome in Spain and eosinophilia myalgia syndrome produced by L-tryptophan contaminants in the United States (Shenker *et al*., 1992). The immune system is a complex combination of organs, cells and chemical mediators that act together to identify and sequestor or kill "foreign" substances. Immune organs include the thymus, spleen, and lymphatic system. Immune cells include lymphocytes, other white blood cells, and tissue macrophages (Shenker *et al*., 1992).

There is paucity of information on landfill leachates induced immunotoxic effects in animals. The available information on the immunotoxic effects of leachates examined the organic components of the leachate in different strains of mice. Silkworth *et al*. (1989) orally administered the organic phase of leachates (OPL; over 100 organic compounds including 2,3,7,8- tetrachlorodibenzo-p-dioxin, TCDD) from Love Canal chemical dumpsite, in corn oil to male BALB/cByJ (Ahb/Ahb) mice and DBA/2J (Ahd/Ahd) mice. TCDD was similarly administered to another set of mice. Antibody response (Pfc) and organ weight gain were examined at 4 days post-exposure. OPL produced thymic atrophy and hepatomegaly in both strains at all dose levels. The Pfc/spleen in BALB/cByJ (Ahb/Ahb) was significantly reduced according to the doses in the range 13 to 34% of the control. Serum anti-SRBC antibody levels and relative spleen weight were also reduced. The only immune effect in the DBA/2J mice was a decrease of the PFC/spleen to 58 % of the control at the highest dose. TCDD decrease the relative thymus and spleen weights only in BALB/cByJ mice. However, TCDD produced hepatomegaly, decrease in serum antibody and decrease in PFC/spleen in both BALB/cByJ and DBA/2J mice. The authors suggested that OPL induced immunosuppression in BALB/cByJ mice, was caused primarily by TCDD.

Although, information is relatively scarce on the immunotoxic potentials of landfill leachates among humans, but available information showed that residents in close proximity to a superfund site containing organochlorine pesticides, volatile organic compounds, and metals

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located in Aberdeen, North Carolina, had significantly ($p < 0.05$) lower mitogen-induced lymphoproliferative activity than residents who lived farther away (Vine *et al*., 2000). The authors concluded that the superfund site induced immunosuppression in the exposed population.

2.6.3 Genotoxicity and mutagenicity of landfill leachates

Numerous studies have shown that leachates and leachate contaminated water bodies induced genotoxic and mutagenic effects in microbial, plant and animal systems using different biological assays. This is necessary since many congenital and non congenital diseases are often linked to DNA damage. USEPA (1980) evaluated the mutagenicity of different waste extracts and their XAD-2 concentrates in three mutagenic assays with and without metabolic activation, using *Salmonella/*microsome assay, *Saccharomyces* $can^1/hist^+$ dual assay and *Salmonella* UvrB repair assay respectively. The result showed that except for the arsenic-contaminated groundwater, none of the other wastes or their XAD-2 concentrates displayed mutagenic activity in the first assay. In the second assay only the XAD-2 concentrate of arsenic-contaminated groundwater sample was mutagenic without metabolic activation for a 24 hr exposure. None of the waste extracts displayed mutagenic activity in the third assay, either with or without metabolic activation. Also, Omura *et al.* (1991) reported that leachate from domestic waste landfills in Japan was mutagenic using *Salmonella typhimurium*/mammalian-microsome assay. The study also showed that the acidification of the leachate sample before XAD treatment is effective to disclose high mutagenicity from leachate sample. Results obtained by Donnelly *et al.* (1988) from a study on bacterial mutagenicity of leachate from municipal sewage sludge-amended soils are in concert with those of Omura *et al*. (1991).

In a follow up study, Omura *et al.* (1992) recovered the organic concentrates from the leachate of municipal solid waste landfills using XAD-2/8 resin adsorption. The mutagenic activities were later tested for 8 months using the Ames *Salmonella/*microsome assay. Highly polluted leachate (COD and BOD > 40mg/l) generally had equal or higher mutagenic activities than lightly polluted leachate (COD and BOD < 40mg/l). But there was no clear difference in mutagenicity per amount of concentrate between the two leachate samples. The results suggest that the mutagenic activity of landfill leachate is decided to some degree by the organic concentrates in the leachate. Kamiya *et al.* (1989) studied the mutagenicity of refuse leachate from municipal incinerator by liquid rec-assay and Ames assay. Volatile components were found to be negative and nonvolatile components positive in the Ames assay, and the leachate was found to have DNA-damaging capacity in the liquid rec-assay with S-9 mix. PAHs derived

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from tobacco ash and carbonyl compounds generated by the putrefaction of foods were confirmed to be main contributors to DNA damaging capacity and mutagenicity in refuse leachate.

Schrab *et al*. (1993) assessed the genetic and acute toxicities of four MSW landfill leachates and groundwater samples using the *Salmonella*/microsome (Ames test) mutagenicity bioassay, the *Bacillus subtilis* DNA repair bioassay, the diploid *Aspergillus nidulans* chromosome damage bioassay and the Microtox test. All the leachate samples were acutely toxic, and three of the four leachate samples were genetically toxic. Two of the leachates had mean estimated cumulative cancer risks on the same order of magnitude (10^{-4}) as leachates from co-disposal and hazardous waste landfills. In a comparative study to evaluate the performance of four bacterial short-term genotoxicity assays (*Salmonella*/microsome assay, SOS chromotest, Microscreen phage-induction assay, differential DNA repair test) that are widely used and/or have a promising potential for the genotoxicity testing of water samples, landfill leachate was found to display higher genotoxic activity than other samples tested (Helma *et al.,* 1996). Both landfill leachate samples tested were genotoxic in all assays with the exception of the Microscreen assay, which failed to detect genotoxicity due to viral toxicity.

Singh *et al*. (2007) analysed leachates derived from dry wastes of the metal, tannery, and dye industries of the state of Uttar Pradesh (India) for their mutagenic potential using *Salmonella* reverse mutation assay. Both the spot and plate incorporation assays were conducted with four tester strains of *Salmonella typhimurium* (TA97a, TA98, TA100, and TA102). Their result suggests that leachates derived from metal and tannery wastes possess mutagenic properties. Ten soil samples from a hazardous waste site were compared for their genotoxic activity by the Ames test and a modified SOS colorimetric test. Organic extracts were prepared from the soil sample by soxhlet extraction. Analysis of variance indicated that with S9mix, Ames and SOS results were similar for the same soils and solvent extractions. However, without S-9 mix, the SOS test was significantly more sensitive than the Ames test to the genotoxins extracted from the soils. Both the Ames and SOS test detected lower concentrations of genotoxins in methylene chloride than in cyclohexane extracts (McDaniels *et al.,* 1993).

Plants have also been utilized in assessing the mutagenic and cytogenetic effects of landfill leachates. Sang *et al*. (2006) investigated the genotoxicity of a municipal landfill leachate using *Hordeum vulgare* root-tip cytogenetic bioassay. Chemical oxygen demand (COD Cr) was used as a measure for leachate concentration. Leachate induced decrease in mitotic index (MI) and significant increases in micronucleus (MCN) frequencies in a concentration-dependent and

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time-dependent manner. In addition, pycnotic cells (PNC) occurred in root tips at tested leachate concentrations, and PNC frequencies had a positive relationship with the treatment concentration and the exposure time. The results suggests that components of leachate was genotoxic in plant systems and imply that long term exposure to leachate at low concentrations in the aquatic environment may pose a potential genotoxic risk to organisms. Helma *et al.* (1994) also used the *Tradescantia* micronucleus (Trad-MCN) assay to evaluate the clastogenic effects of contaminated groundwater collected near a hazardous waste landfill. Maximal values (6.1± 4.7) of micronuclei (MCN/100 tetrads) observed in the treated plant was six-fold higher than the control group.

Recently, the *Allium cepa* (2n = 16) assay was used to evaluate the cytotoxic, genotoxic and mutagenic activities of landfill leachates. Results from these studies have shown that solid waste leachates induced different types of chromatid and chromosome aberrations, as well as micronuclei in apical meristematic cells of *A. cepa*. (Bakare, 2001; Bakare *et al*., 1999; 2000a,b; Bakare and Wale-Adeyemo, 2004; Cabrera and Rodriguez, 1999; Chandra *et al*., 2005; Iwegbue *et al*., 2007; Obidoska and Jasinka, 2008). Leachates from both domestic and industrial solid wastes had also been reported to inhibit mitotic index (MI) and induced concentration-dependent increases in the frequencies of micronuclei and aberrant anaphase cells in the root tip of *Vicia faba* (Chandra *et al.*, 2004; Feng *et al*., 2007; Radetski *et al*., 2004; Sang and Li, 2004). Furthermore, municipal landfill leachate induced cytogenetic damage in the root tip of *Hordeum vulgare* (Sang *et al*., 2006) and caused alterations in the cell cycle along with sister chromatid exchange and induction of micronucleus in the root cells of *Triticum saestivum* (Li *et al*., 2008).

Animal test systems (mostly vertebrates) are often generally accepted than microbes and plant test systems in predicting toxicity and genotoxicity in humans. This is because animals are similar in the way they enzymatically metabolize many carcinogens (Stegeman and Lech, 1991), and respond to oxidative enzyme damage (Washburn and Di Giulio, 1989). Different animal models with varying cytogenetic biomarkers have been utilized in evaluating the genotoxic effects of solid waste leachates. Bakare *et al*. (2005) reported that raw and simulated solid wastes from three dumpsites in Nigeria induce dose-dependent increase in the number of sperms with abnormal morphology in mice. Treated and untreated domestic and industrial solid waste leachates from Japan induced increase frequency of micronuclei in gill cells of goldfish (*Carassius auratus*) (Deguchi *et al*., 2007). Leachate prepared from municipal sludge and industrial wastes induced dose-dependent inhibition in the mitotic index (MI) and increased the frequency of chromosome aberrations in the bone marrow of treated mice compared to control

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(Tewari *et al*., 2005). Also, increased in frequencies of micronucleated polychromatic erythrocytes and DNA damage were observed in mouse bone marrow cells exposed to leachates from municipal solid wastes and municipal sludge (Chandra *et al*., 2006; Li *et al*., 2004; Sang and Li, 2005; Tewari *et al*., 2005). The erythroblasts of the bone marrow cells of albino rat examined post treatment also show dose dependent increase in structural chromosomal abnormalities such as breaks, gaps, rings and acentrics (Alimba *et al.*, 2006). Simulated and raw leachates from electronic wastes have also been reported to induce a dose-dependent increase in the number of sperms with abnormal morphology in mice, inhibited the mitotic index and increased the frequency of chromosome aberrations in the bone marrow cells and spermatogonia and increased frequencies of micronuclei in bone marrow cells of treated mice (Alabi and Bakare, 2011).

The alkaline single-cell electrophoresis assay or Comet assay, a molecular toxicological assay, has also been utilized to evaluate the DNA damaging potentials of leachates. In an *in vitro* study using Comet assay, leachates of solid wastes from a polyfiber factory (PFL), an aeronautical plant (AEL), and a municipal sludge leachate (MSL) induced significant concentration-dependent increases DNA damage in human peripheral blood lymphocytes (Bakare *et al*., 2007). The municipal sludge leachate also induced DNA damage (as assessed by the comet assay) in somatic tissues and organs of the mouse (Bakare *et al*., 2012). *In vivo*, leachates from tannery wastes, metal-based wastes and dye and pigment wastes induced systemic genotoxicity in exposed mouse (Chandra *et al*., 2006). Using same assay, industrial solid waste leachates from flashlight battery, pigment and tannery wastes induced dose-dependent increase in DNA damage in cells of *Drosophila melanogaster* (Siddique *et al*., 2005; 2007); likewise DNA damage in the gill epithelial cells of goldfish (*Carassius auratus*) (Deguchi *et al*., 2007) were reported. Landfill leachates from Settat town in Morocco induced micronucleus and also affected proliferation kinetics of human peripheral blood lymphocytes in *in vitro* (Amahdar *et al*., 2009). Landfill leachates from Tunisia induced cytotoxic effects in MCF-7 cells by inducing necrosis. It also enhanced the expression of HSP and various stress related proteins (heterogeneous nuclear ribonucleoprotein E1, phosphoglycerate mutase and nuclear matrix protein 200) in the MCF-7 cells (Talorete *et al*., 2008).

2.6.4 Systemic toxicity studies of landfill leachates

Living organisms are made up of different organ systems which are coordinated in a manner that bring about the normal functioning of the organisms. Systems, the highest level of

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organization, comprise of organelles, cells, tissues and organs (Taylor *et al*., 1998). The components of the system are involved in modulation, assimilation, metabolism, detoxification, storage, immunity, and elimination of xenobiotics and there metabolites from the body of organisms. Hence, they are vulnerable to direct pathological lesions and oxidative stress from the actions of xenobiotics. This makes them important target organs for xenobiotic induced injuries. Most of these organ systems are on the priority list of Agency for Toxic Substances and Disease Registry (ATSDR) as organs mostly affected by chemicals from landfills (Johnson, 1999).

Xenobiotic induced alterations in the organ system of organisms can be reflected on the total functioning and coordination of the body. These alterations can be easily assessed from changes in biochemical parameters, haematological indices and histopathological observations of these organs (Michael *et al*., 2007; Tayeb *et al*., 2010). Alterations in organ systems by xenobiotics, if affected the functioning of the body, will be observed in the body and organ weight gains of the organisms and in the general behaviour of the organism as clinical signs (Matos *et al*., 2010; Michael *et al*., 2007). Therefore, systemic toxicity offers possible means of understanding the mechanisms of xenobiotic induced organ and or tissue damage.

Information is relatively limited on leachates induced systemic toxicity in mammals considering the evaluation of biochemical parameters, histopathology and organ weight gain of viscera and haematological parameters. Available information showed that leachates from three dumpsites in Nigeria induced hair loss, anorexia, diarrhea and sluggishness in mice intraperitoneally exposed to different concentrations of the leachates (Bakare *et al*., 2003). Xinguo landfill leachate treated mice induced changes in the kidney, liver, heart and spleen relative organ weight gain and increase in protein oxidation levels and DNA-protein crosslink formation in these organs compared to the control (Li *et al*., 2010). Similarly, this leachate induced alterations in the concentrations of thiobarbituric acid reactive substances (TBARS) and activities of Cu, Zn-superoxide dismutase (Cu, Zn-SOD), Se-dependent glutathione peroxide (Se-dependent GPx) and catalase in the tested organs of the treated mice compared to the control group (Li *et al*., 2006a, b). These showed that the observed systemic toxicity in mice was due to chemical substances in the leachate which induced oxidative stress in the tested organs treated mice. Chemicals can also bioaccumulate in these organs and will lead to alterations in the biochemistry, haematology and histology and weight gains of the organs. This assumption is supported by the findings that wild species of woodmouse, *Apodemus sylvaticus*, and greater white-toothed shrew, *Crocidura russula*, exposed to leachates from Garraf landfill, Spain, bioaccumulated heavy metals (Pb, Hg, Cd, Fe, Mg, Zn, Cu, Mn, Mo and Cr) from the leachates

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to a lethal concentration that induced change in organ weight gain and the concentrations of serum Alanine aminotransferase, Aspartate aminotransferase and creatinine (Sanchez-Chardi *et al*., 2007; Sanchez-Chardi and Nadal, 2007). Also, they observed different histopathological alterations like cell cycle arrest (apoptosis and necrosis), inflammation, pre-neoplastic nodules, vacuolation and micro-steatosis in the hepatocytes, tubular necrosis and dilatation, inflammation, and cylinders in the kidney and no pollution-related alterations were observed in the lung, spleen, pancreas, gonads, oesophagus, intestine, or adrenals of the landfill exposed wild mice compared to those from the reference sites (Sanchez-Chardi *et al*., 2008). They concluded that the bioaccumulated metals induced the observed effects in the exposed mice and can be biomagnified along the trophic levels in ecosystems involving humans.

Simmons *et al*. (1985) exposed male F344 rats to 0.5 – 5.0 ml/kg of 10 different complex mixtures of organic and inorganic components from wastes by oral gavaging and after 24 hours of exposure, 9 of these samples caused increase in relative liver weight gain and 8 samples caused increase in serum ALT and AST with concomitant induction of necrosis and degeneration in the centrolobular region of the liver. They concluded that these wastes contained hepatotoxicants, which interacted in a combination of synergies, antagonisms and or additively to cause the observed effects. Hepatic necrosis and cellular infiltrations with inflammatory cells were reported in workers exposed to hepatotoxic chemicals from industrial wastes (Cheong *et al*., 2007). Oshode *et al*. (2008) implicated histopathological leisions observed in the liver, kidney and gills, and changes in the haematological parameters of *Clarias gariepinus* exposed to different concentrations of Aba Eku landfill leachates, to heavy metals and physico-chemical compositions and microbial load of the tested leachates. They suggested that the leachate induced immune response and increase toxicity in the treated *C. gariepinus*.

Alterations in red blood cells and platelet counts, percentage haematocrit and haemoglobin concentration in organisms have been reported in rats exposed to treated and untreated textile dye wastewater for 15 days (Sharma *et al*., 2007a), mice (*Mus musculus*) exposed to different concentrations of distillery soil leachate for 30 days (Sharma *et al*., 2007b) and in mud catfish (*Clarias gariepinus*) exposed to Aba Eku landfill leachates for 7, 14 and 21 days. The alterations observed in the studies, may be an indicator of anaemia, stress, failure in oxygen carrying capacities of red blood cells and poor health status in the exposed animals (Larsson *et al*., 1985). MCV, MCH and MCHC are erythrocytic indices which depend on red blood corpuscle count, haemoglobin concentration and packed cell volume. Alterations in the concentrations of these indices have been implicated with macrocytic and hypochromic anaemia

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(Barger, 2003). MCHC is an expression of the average concentration of haemoglobin in red blood cells. Its value shows the ratio of the weight of haemoglobin to the volume of red blood cells. Its 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). Increase in MCV suggests an intensified compensating activity of the haemopoietic system, which may be in response to haemolytic action of the leachate constituents (Sharma *et al*., 2007a). Increase in MCH was linked to macrocytic anaemia that was probably caused by hepatic or pulmonary diseases in Algerian mice inhabiting an area contaminated with heavy metals (Nunes *et al*., 2001), and Swiss albino mice exposed to distillery soil leachate (Sharma *et al*., 2007a) and textile dye wastewater (Mathur *et al*., 2003). Sanchez-Chardi *et al*. (2008) reported that bioaccumulation of heavy metals (Pb, Cu and Cr) from landfills correlated well with significantly alterations in the haematological parameters observed in small mammals (Shrew) than those of reference sites.

2.6.5 Mechanism of landfill leacheate induced toxicity

Since landfill leachates contain inorganic (including harmful heavy metals), organic, microbial populations and soluble particulate matters (hence complex mixture of chemicals), understanding its mechanism of toxicity in living cells poses difficulty. The toxicity of leachates was believed to be due to the actions of the individual components, their synergy that may lead to potentiation of the individual components, and or antagonistic interactions among the individual components. Simmons *et al*. (1985) orally exposed male Fischer-344 rats to 10 different complex waste mixtures for 14 days and observed that 8 among these waste mixtures caused alterations in enzyme biochemistry, histopathology and body and organ weight gain of exposed rats compared to the control. Their conclusion was in support that the interactions among the many chemicals in these mixtures, which may include a combination of synergies and antagonisms as well as additives, caused the observed effects.

Heavy metals commonly analyzed in most leachates have been reported to induce toxicity and genotoxicity in several biological systems via reactive oxygen production, either by autoxidation or enzyme catalyzed oxidation of chemicals (Galaris and Evangelou, 2002; Stohs and Bagchi, 1995). The genotoxic activities of heavy metals were reported to be the result of formation of DNA and/or protein cross-links (Costa *et al*., 1994; De Flora *et al*., 1990). Studies probing into finding out the possible mechanisms of leachate induced toxicity and genotoxicity supported the reactive oxygen species

formation by autoxidation or enzyme catalyzed oxidation of chemicals present in the leachates. The oxidative stress induction by leachates caused alterations in the antioxidant and lipid peroxidation status in leachate exposed mice, induction in protein cross-link in leachate exposed mice compared to the control (Bakare, 2009; Bakare *et al*., 2012; Li *et al*., 2006a,b; 2010).

2.7 Health impacts associated with landfill management

The epidemiological evidence of health effects associated with exposure to substances from landfill sites has been the subject of a number of recent reviews (Johnson, 1997; Johnson, 1999; Pheby *et al*., 2002; Porta *et al*., 2009). Most of these studies on landfill sites and human health impacts, focused on hazardous waste sites with relatively high emissions, while very few information exist on household or domestic waste sites (Mochungong *et al*., 2011; Perez *et al*., 2006; Schrapp and Al-Mutairi, 2010).

Public concern in the vicinity of landfills has prompted a number of single-site studies (Berry and Bove, 1997; Kharrazi *et al.*, 1997; Kilburn, 1999; Williams and Jalaludin, 1998). A number of ecological (geographic comparison) studies have also been undertaken which have examined rates of adverse health outcomes in counties containing waste sites and compared these to state or national rates (Harmon and Coe, 1993; Schwartz *et al*., 1998). Retrospective case control studies have also been undertaken (Marshall *et al.*, 1997; Polednak and Janerich, 1989). In these studies, exposures of people with disease (i.e. cases) are compared to exposures of people without disease (i.e. controls). A collaborative European study ('EUROHAZCON') examined the association of non-chromosomal congenital anomalies with 21 hazardous waste landfill sites. In this study, a 'proximate' zone of 3 km radius from the site (within which it was assumed that most exposure to chemical contaminants would occur) was compared to a zone of radius 3-7 km from the site. A 33% increase in the risk of non-chromosomal anomalies for residents living within 3 km of the sites was reported (Dolk *et al*., 1998). Recently, a similar analysis for chromosomal anomalies suggested a comparable level of risk to that found for non-chromosomal anomalies (Vrijheid *et al.*, 2002).

In Great Britain, risks of adverse birth outcomes in populations living within 2 km of 9,565 landfill sites which were each operational at some time between 1982 and 1997 were compared with those in a reference population who resided more than 2 km from all known landfill sites. For all congenital anomalies combined, the relative risk for residence near landfill

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sites adjusted for confounders (e.g. social deprivation) was 1.01 (Elliot *et al.*, 2001). In this study it was noted that the confidence interval (CI) of the relative risk estimation is important and as it defines (at a specified level of probability) the range in which the estimate of the relative risk lied. For all congenital anomalies combined, 99% confidence interval for relative risk is in the range 1.005 to 1.023. Some higher relative risks were also found for specific anomalies: neural tube defects 1.05 (99% CI 1.01 – 1.10); abdominal wall defects 1.08 (99% CI 1.01 to 1.15); and hypospadias and epispadias 1.07 (99% CI 1.04 – 1.10). The authors also compared different types of sites i.e. 7,803 sites for non-special waste and 774 sites for special waste. For special waste sites, relative risks increased when compared to non-special waste sites: all anomalies 1.07 (99% CI 1.04 to 1.09) compared to 1.02 (99% CI 1.01 to 1.03); neural tube defects 1.07 (99% CI 0.95 to 1.20) compared to 1.06 (99% CI 1.01 to 1.12); hypospadias and epispadias 1.11 (99% CI 1.03 to 1.21) compared to 1.07 (99% CI 1.04 to 1.11). For abdominal wall defects and for cardiovascular defects, the comparison could not be made as results were not statistically significant.

In the US, a 12% increase in congenital malformations was reported for women residing within 1 mile of 590 hazardous waste sites in New York State (Geschwind *et al.*, 1992). A follow-up study, with improved study design, found no association between potential exposures from hazardous waste sites and risks of musculoskeletal and central nervous system birth defects (Marshall *et al.*, 1997). In a larger US study of women living within 1 mile of 1,281 sites over the entire United States, no increase in congenital malformations was observed (Sosniak *et al.*, 1994). The lack of information on alternative pollution sources hampers the interpretation of all such multi-site studies. Most multi-site investigations have concentrated upon congenital malformations, but increased bladder cancers and leukaemias have been reported in women residing in areas likely to be exposed to landfill gas (Lewis *et al*., 1998). Renal disease was investigated in people residing within 1 mile of 37 sites in New York State, but the evidence for increased incidence of kidney disease did not achieve statistical significance (Hall *et al*., 1996).

There have also been a large number of health surveys which have relied upon residents reporting symptoms through questionnaires (Vrijheid, 2000). Many of these studies have reported increase prevalence of dermal, respiratory and gastrointestinal disorder (Mochungong *et al*., 2011; Perez *et al*., 2006; Schrapp and Al-Mutairi, 2010), stroke, cancers, still birth, low birth weight and congenital abnormalities (Perez *et al*., 2006). These studies used 2-3 km proximity to the landfill as surrogate to exposure and increased incidence of reported symptoms in exposed

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areas compared to unexposed areas may indicates the risk associated with exposure to landfill emissions.

2.8 Review of methodology

Toxicological studies have undergone a significant evolution in the past few decades with more emphasis placed on systemic toxicity, carcinogenesis, mutagenesis, immunotoxicity and cytogenotoxicity. Hence, the increasing interest in the use of biological markers in biomonitoring and identification of exposure to environmental toxicants. The ultimate goal of this assessment was in the use of biological data to indicate symptoms of early diseases and to probably predict risk for development of long-term health consequences.

Genetic toxicology assays are used to identify germ cell mutagens, somatic cell mutagens and potential carcinogens. They detect diverse kinds of genetic alterations that are relevant for human healths. Over the last three decades, hundreds of chemicals have been evaluated for genotoxic effects (Preston, 1996). Genetic toxicology assays serve two interrelated but distinct purposes in the toxicologic evaluation of chemicals;

1. identifying mutagens for purposes of hazard identification, and
2. characterizing dose-response relationships and mutagenic mechanisms, both of which contribute to an understanding of genetic and carcinogenic risks (Preston *et al*., 1981).

Among the mammalian cytogenetic assays; metaphase chromosome aberration analysis, sister chromatid exchange analysis and aneuploidy in mitotic cells are time consuming and requires considerable skill (Preston, 1996). There is need for the development of simpler cytogenetic assays, micronucleus assay evolved as a standard battery for genotoxicity testing of chemicals and radiations.

Micronuclei were described in the cytoplasm of erythrocytes more than a century ago and called "fragment of nuclear material" by Howell or "corpuscules intraglobulaires" in the terminology of Jolly in the late 1800s and early 1900s. These structures are known to the hematologist as "Howell-Jolly bodies". Similar structures were described in other cell types (e.g. in mouse and rat embryos by Brenneke (1937) or in *Vicia faba* by Thoday (1951) and called "fragment nuclei" or "micronuclei". These micronuclei were consistently found after radiation exposure of cells, and it was assumed that they originated from acentric fragments, which were excluded from the two daughter nuclei at the late stages of mitosis (Evans *et al*., 1959). The authors also discovered its usefulness as markers for cytogenetic damage, when they compared the efficiency of neutrons to that of gamma-rays in *Vicia faba* roots. The decisive breakthrough

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of micronuclei as assay system for the genotoxic potential of agents came with the work of Boller and Schmid (1970), when they suggested the term micronucleus test for the first time and Heddle (1973) by adapting the assay to bone marrow erythrocytes. Shortly thereafter, Countryman and Heddle (1976) introduced lymphocytes as another useful cellular system for the detection of chromosomal damage by determination of micronucleus production, and recommended using micronuclei as a biomarker in testing schemes. Micronuclei result from lesions/adducts at the level of DNA or chromosomes, or at the level of proteins directly or indirectly involved in chromosome segregation (e.g. tubulin) (Schmid, 1975). The formation of micronuclei originating from chromosome fragments or chromosome loss events requires a mitotic or meiotic division. Figure 2.7 and 2.8 show schematic presentation of micronucleus formation during cell division in both mononucleated and binucleated cells. The simplicity of scoring and the wide applicability of the *in vitro* micronucleus test in different cell types make it an attractive tool to assess cytogenetic abnormality. It is well adapted in virtually all invertebrate and vertebrate cells both *in vivo* and *in vitro*.

However, the limited understanding of the induction mechanisms of micronuclei and the absence of adequate methods to assess concurrently cell proliferation and micronucleus induction delayed the validation of the *in vitro* micronucleus test for genotoxicity testing. Development of the cytokinesis-block methodology by Fenech and Morley (1986) by addition of the actin inhibitor cytochalasin-B during the targeted *in vitro* mitosis allowed the identification of once-divided nuclei as binucleates and provided an efficient approach to study the mechanism leading to the induction of micronuclei. By restricting scoring of micronuclei in once-divided cells, the cytokinesis-block micronucleus assay solved the problem of variation in micronucleus frequency caused by alterations in the proportion of dividing cells which may occur either when cells are exposed to genotoxic/cytostatic agents or due to suboptimal culture conditions. In this regard, micronucleus assay has been widely used to measure genotoxicity, both *in vitro* and *in vivo*. Moreso, micronucleus assay is a relatively simple assay since micronucleated cells are easily identified and scored microscopically.

Mammalian *in vivo* micronucleus test is especially relevant to assessing mutagenic hazard in that it allows consideration of factors of *in vivo* metabolism, pharmacokinetics and DNA-repair processes although these may vary among species, among tissues and among genetic endpoints. An *in vivo* assay is also useful for further investigation of a mutagenic effect detected by an *in vitro* system (Viktram *et al*., 2007). Micronucleated cells formed in the bone marrow during exposure to clastogens can be detected easily in peripheral blood of mouse, but in case of adult

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rats, spleen selectively removes micronucleated RBC, hence decreasing its sensitivity (Krishna and Hayashi, 2000). The assay, when performed appropriately, detects both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to dysfunction of mitotic apparatus). In the same micronucleus assay, the PCE-NCE ratio between test agent-treated animals and vehicle-control animals provides a cytotoxicity index (Krishna and Hayashi, 2000). The use of mammalian bone marrow and peripheral blood erythrocytes micronucleus assay and kidney, gill and peripheral blood erythrocytes from piscine system in evaluating the cytogenotoxic effects of landfill leachates is limited and none exist on the use of bone marrow and peripheral blood erythrocytes from avian system.

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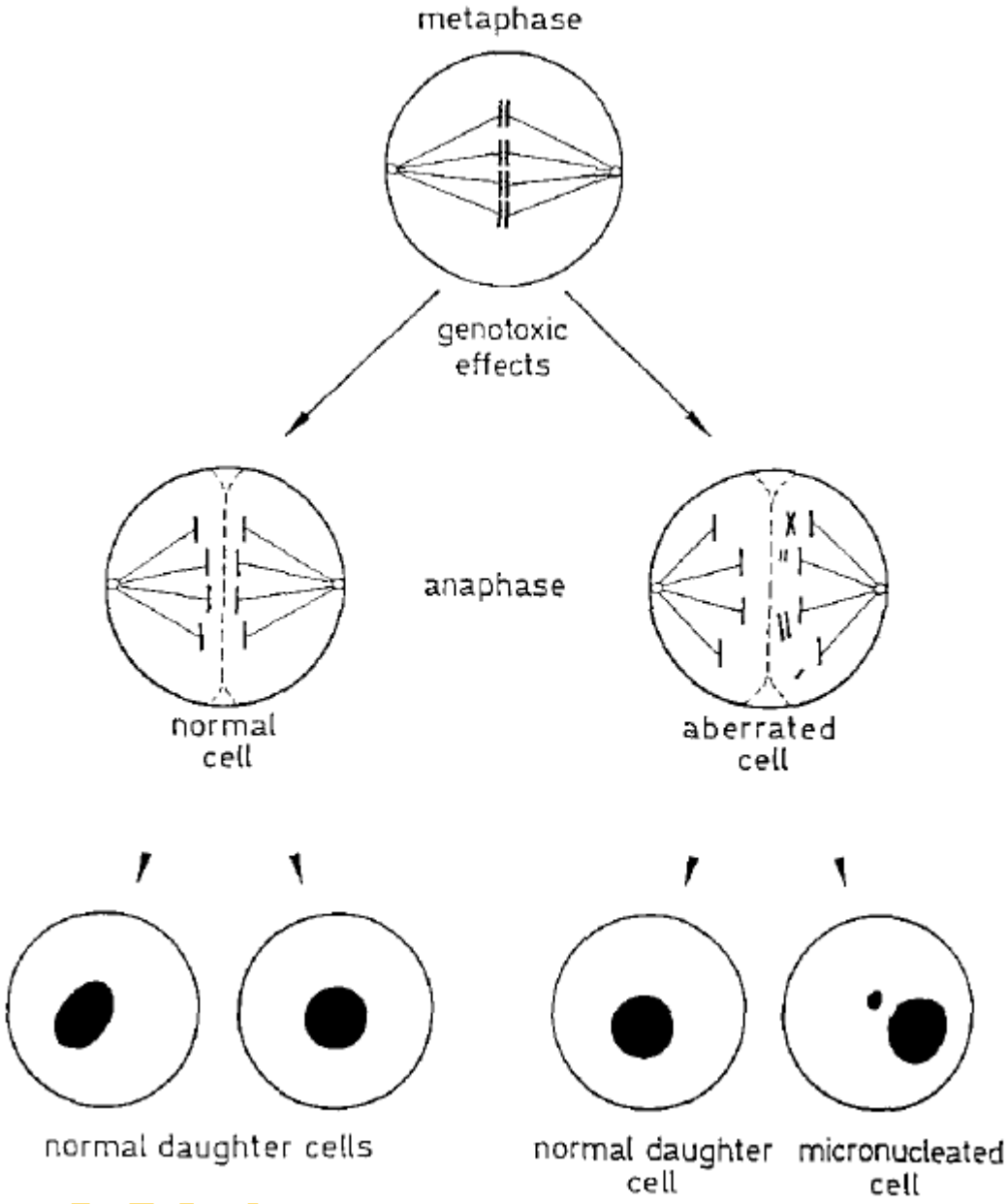

Figure 2.7 shows the schematic illustration of the mechanism of micronucleus formation in mononucleated cells.

Source: Al-Sabti and MetCalfe, (1995).

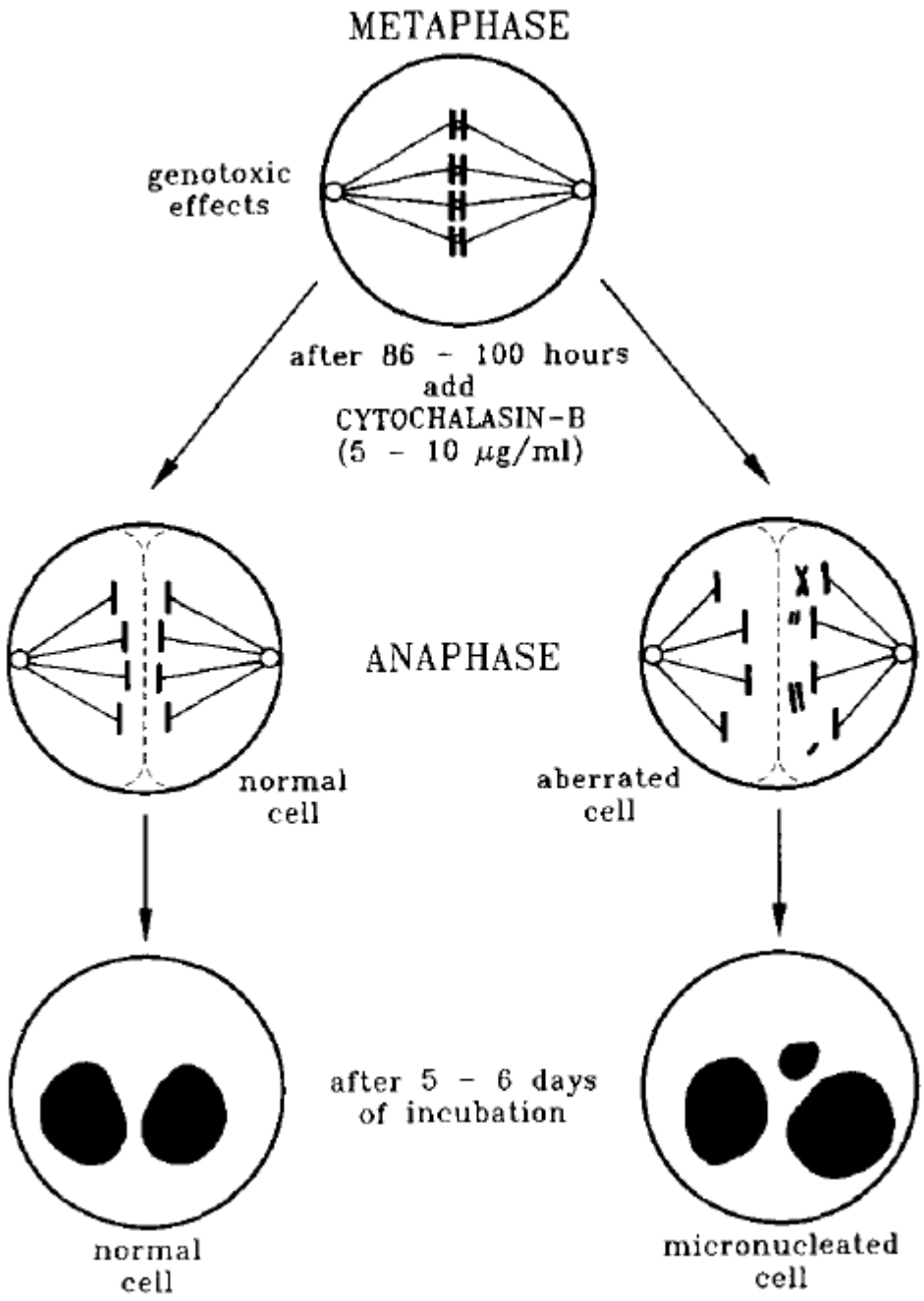

Figure 2.8 shows the schematic illustration of the mechanism of micronucleus formation in binucleated cells.

Source: Al-Sabti and MetCalfe, (1995).

The CBMN Cyt assay is a technique that allows rapid and accurate measurement of the genotoxicity of xenobiotics at chromosomal level using three biomarkers: micronuclei, MN, (biomarker of chromosome breakage and/or loss), nucleoplasmic bridge, NPB MN, (biomarker of chromosome rearrangements, DNA stand break misrepair and /or telomere end fusion) and nuclear bud, Nbud, (biomarker of gene amplication and / or elimination of DNA repair complexes). In the same assay, the frequencies of necrotic and apoptotic cells (cytotoxicity) and nuclear division index (cytostatic or cell proliferation index) are measured (Fenech, 2007; Fenech and Crott, 2002). Figure 2.9 shows the schematic presentation of the use of CBMN Cyt assay in the determination of necrotic and apoptotic (cytotoxicity) and nuclear division index (cytostatic or cell proliferation index). According to figure 2.9, Apoptosis may occur either before the first nuclear division or after nuclear division.

(A). Presents the stage of apoptosis when chromatin condensation occurs without disintegration of nuclear membrane in either mononuclear or binucleated cells.

(B). Presents the late stage of apoptosis when the nucleus disintegrates into smaller nuclei with condensed DNA, without cytoplasmic division due to inhibition by cytochalasin-B.

(C). Shows the early stage of necrosis when the cytoplasmic membrane is damage, cytoplasmic boundaries are not clearly defined cytologically, nuclear material is not condensed but nuclear shape becomes atypical and extensive vacuolization is evident. While

(D). shows the late stage of necrosis when the cytoplasm is lost but the nucleus, with abnormal shape and disintegrating nuclear boundary, is still evident.

Similarly, figure 2.10 shows the schematic illustration of the use of CBMN Cyt assay in the measure of nucleoplasmic bridge as biomarkers of genotoxicity.

(A). Initiating events in the formation of NPB. Double-strand DNA break (DSB) is induced in one chromosome but remains unrepaired until after replication (Top). Misjoining of the broken ends after replication leads to the formation of a dicentric chromatid and an acentric fragment. Two DSBs on either side of the centromere of one chromosome are induced (Middle). The broken ends are misrepaired producing a ring chromatid and two acentric fragments which are subsequently replicated. DSBs are induced in two homologous or non-homologous chromosomes (Bottom). Misjoining of the broken ends leads to the formation of a dicentric chromatid and acentric fragment which are subsequently replicated.

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(B). Centromeres of a dicentric chromatid are pulled to opposite poles of the cell at anaphase to form NPB and the acentric chromatid fragment lags behind to form an MN (Top). Centromeres of a dicentric chromatid are both pulled to the same pole of the cell and no NPB is formed, however, the lagging acentric chromatid fragment results in the formation of a MN (Bottom).
(C). The ring chromatids are segregated normally but the acentric fragments lag at anaphase to form two MNi (Top). The two ring chromatids, after completing one SCE, are transformed into one large dicentric ring chromatid which leads to the formation of a NPB when the centromeres are pulled to the opposite poles of the cell at anaphase (Middle). The accompanying acentric fragments lag at anaphase to form two micronuclei. The two ring chromatids, after completing two SCEs, become concatenated, which leads to the formation of a NPB when the centromeres are pulled to the opposite poles of the cell at anaphase (Bottom). The accompanying acentric fragments lag at anaphase to form two MNi.
(D). Centromeres of dicentric chromatids travel to the same pole of the cell and no NPB are formed (Top). Centromeres of one of the dicentric chromatids are pulled to opposite poles of the cell leading to the formation of one NPB (Middle). Centromeres of both dicentric chromatids are pulled to opposite poles of the cell leading to the formation of two NPBs (Bottom). In each of the above cases one MN is produced from the lagging acentric chromosome fragment accompanying the dicentric chromosome

In systemic toxicity, body and organ weight changes had long been known as sensitive indicator of chemical induced changes to the organs and are widely accepted in the evaluation of test article associated toxicities (Michael *et al*., 2007; Seelers *et al*., 2007). Alterations in body weight may lead to increases or decreases in some organ-to-body weight ratios (Seelers *et al*., 2007) and in toxicological studies the comparison of the absolute and relative organ weight changes of the treated animals with untreated groups (the negative control) is an accepted index (Michael *et al*., 2007; Seelers *et al*., 2007). Moreso, the society for toxicologic pathology (STP) advocated that organ weights be incorporated into rodent toxicity studies lasting longer than 7 days (Seelers *et al*., 2007). Since changes in the weight of these organs may also be considered as sensitive indicators of immunotoxicities (immune stimulation or depletion), stress and physiological perturbations. Moreso, histopathological changes in these organs correlates well with organ weight change (Michael *et al*., 2007).

Histopathological evaluation of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most sensitive end point for detecting organ toxicity (Lanning *et al*., 2002). It is also useful in providing information about acute or chronic effects of

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toxic substances that may not be detected by other biomarkers and correlates well with biochemical analysis and change in organ weight (Amacher *et al*., 2006).

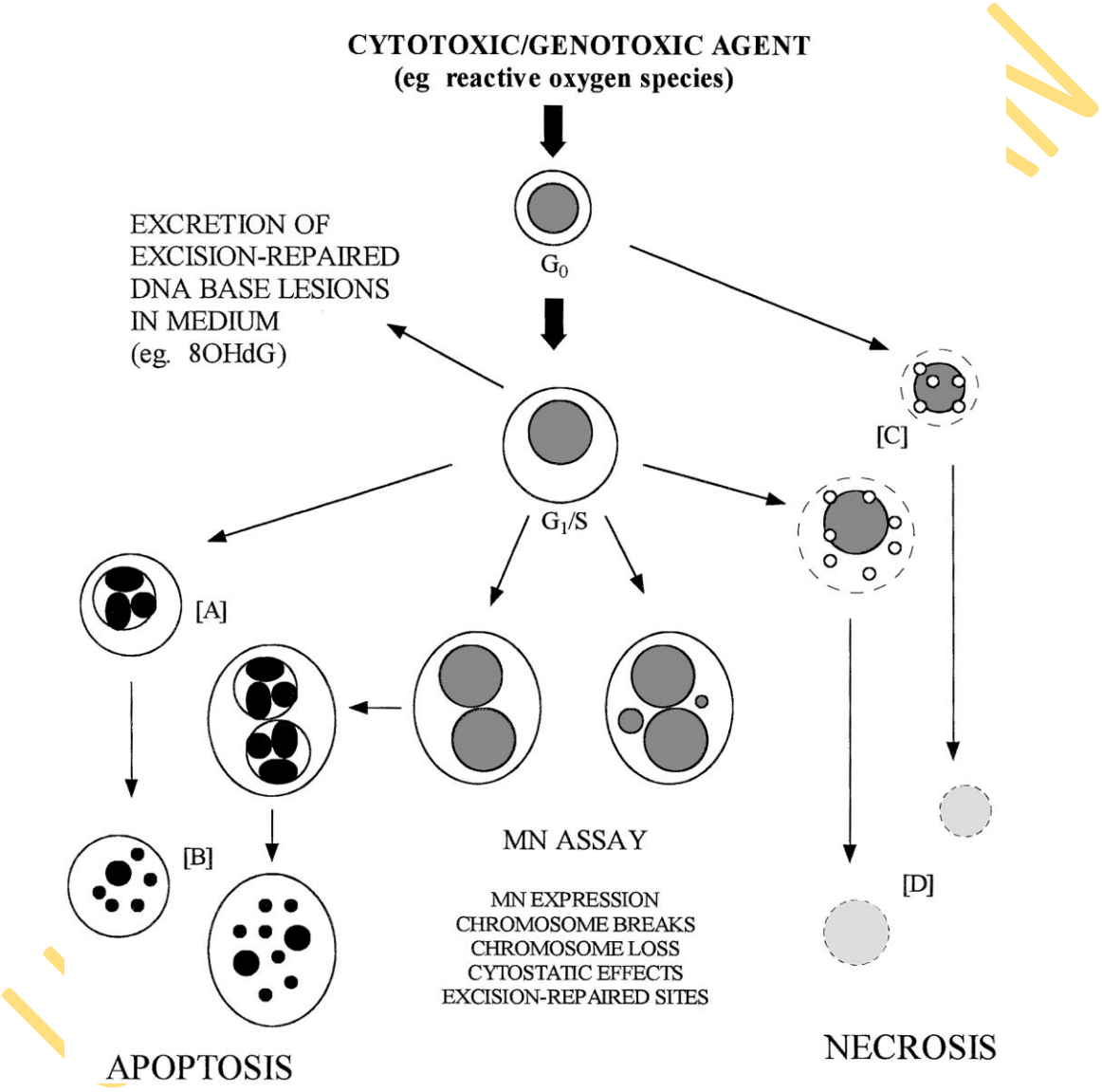

Figure 2.9. Schematic diagram of normal, apoptotic and necrotic pathways for cultured cells in the cytokinesis-blocked assay.

Source: Fenech *et al*. (1999).

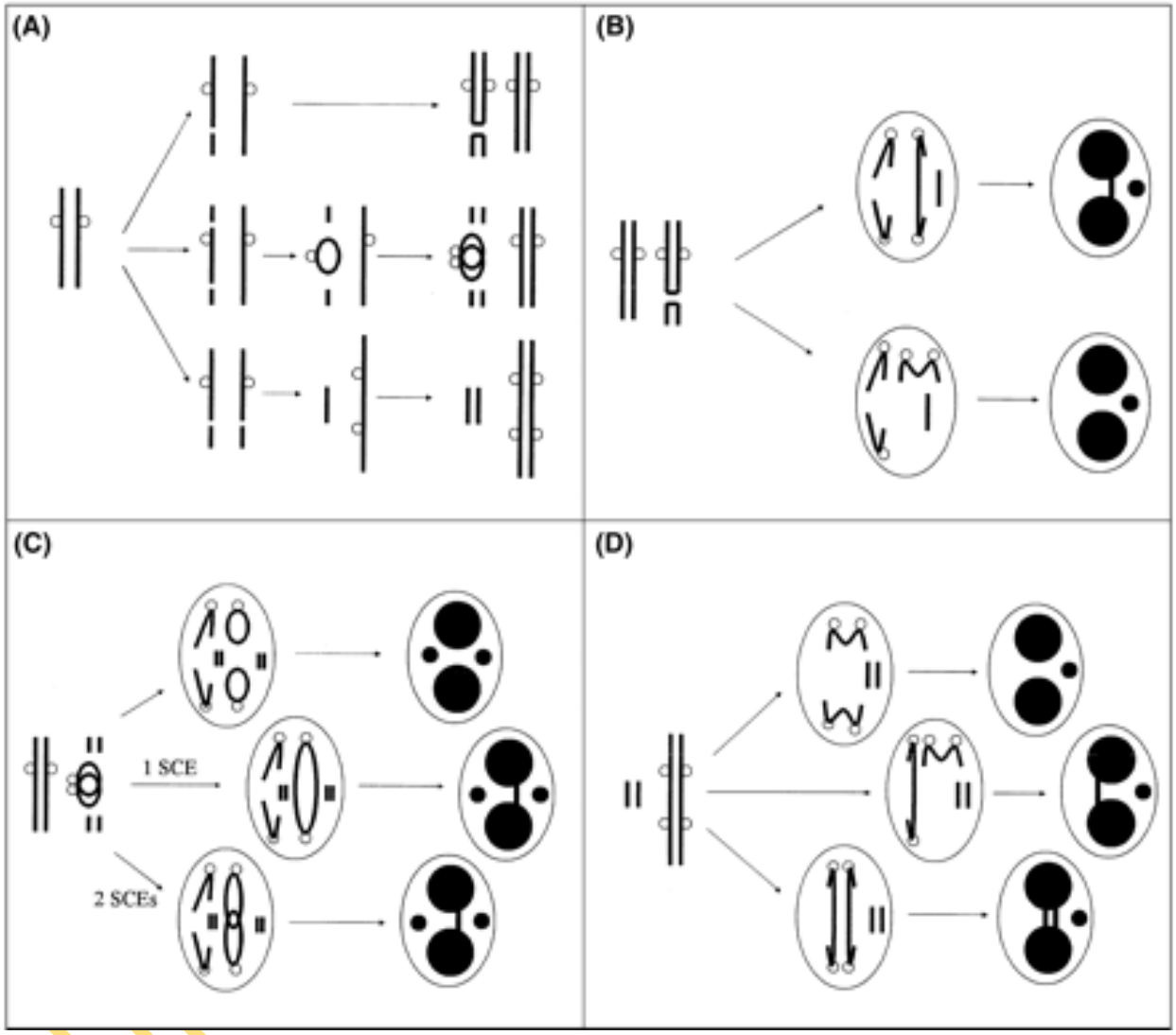

Figure 2.10. Schematic diagram showing the formation of nucleoplasmic bridge, a sensitive measure of chromosome rearrangement in the cytokinesis-blocked micronucleus assay.
Source: Thomas *et al*. (2003).

A large number of biochemical analyses from blood serum have been used in *in vivo* studies using laboratory animals to evaluate the toxic potentials of chemical compounds. Some of these biochemical analyses have been validated as organ-specific biomarkers and may be useful in determining possible mechanisms of toxicity (Travlos, 1996). Moreso biochemical analysis correlates well with histological leisions to confirm the induction of organ-specific effects of xenobiotics. In several organs, cell damage is followed by the release of a number of cytoplasmic enzymes into the circulatory system, this provides the basis for clinical diagnosis of these enzymes and biochemical substances released into the circulation (Sundberg *et al*., 1994).

Haematologic characteristics have been widely used in diagnosing diseases and pathologies of humans and domestic animals. Blood cells are exposed to any agent absorbed or injected into the bloodstream, even those that are rapidly metabolized and excreted. This is because blood is an important liquid connective tissue participating in all vital functions in the animals' body. Haematological data from *in vivo* toxicological studies is one of the most predictive measures for human risk (Evans, 2008) since changes in blood parameters are mainly due to injuries and or infections of some tissues and organs. Changes in blood parameters may be an adaptive response of bone marrow or peripheral blood cells to physiological and immunological changes due to exposure to xenobiotics, stress, diseases and hypoxia (Blaxhall, 1972; Duthie and Tort, 1985).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study sites

Two landfills were selected for this study. They were Aba Eku landfill, located in Ona Ara Local Government Area of Ibadan (longitude 3^{o} 5^{1} East and latitude $7^{o}23^{1}$ North), Oyo State (Figure 3.1) and Olusosun landfill Ojota, located in Oregun area of Kosofe Local Government Area (longitude 3^{o} 24^{1}E and latitude 6^{o} 34^{1} N), Lagos State (Figure 3.2). Ibadan and Lagos are megacities located in southwestern Nigeria. They are notable for their high population growth with many light and heavy industries which make them more urbanize than other cities and towns in Southwestern, Nigeria. These features are responsible for the *daily per capita* generations of 0.33kg solid wastes in Ibadan (Ayininuola and Muibi, 2008) and 0.50kg solid wastes in Lagos (Lasisi, 2002). The selected landfills are co-disposal landfills receiving all categories of wastes, ranging from domestic, Industrial, Agricultural, Commercial, medicals to Institutional wastes from the public and private sectors. They have no landfill covers, liners and leachate collecting system. Burning of wastes and presence of flies and rodents were common features on the landfills.

Aba-Eku landfill in Ibadan has been in use as a landfill for over 17 years. It covers about 6 hectares of land and depth of about 1.5 meters. Leachates produced from the landfill are discharged into Omi stream. This stream is the major source of water supply to about 16 villages in Ona Ara Local Government Area. The dumpsite is surrounded by residential quarters within 1 km radius and increase human activities like farming, scavenging and sites for foraging of domesticated animals like goats and poultry (Figure 3.3).

Olusosun landfill in Lagos state was a swampy area prior to the landfilling activities. It covers about 42 hectares of land and with an excavation of about 18m deep into the landfill area, which makes it the largest landfill in the nation (Aderibigbe, 2010). It has no means of leachate collection 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. People live on and work in the dumpsite as scavengers. There are shops, bars, restaurants, a barber and temporary residential quarters on the dumpsite (Figure 3.4)

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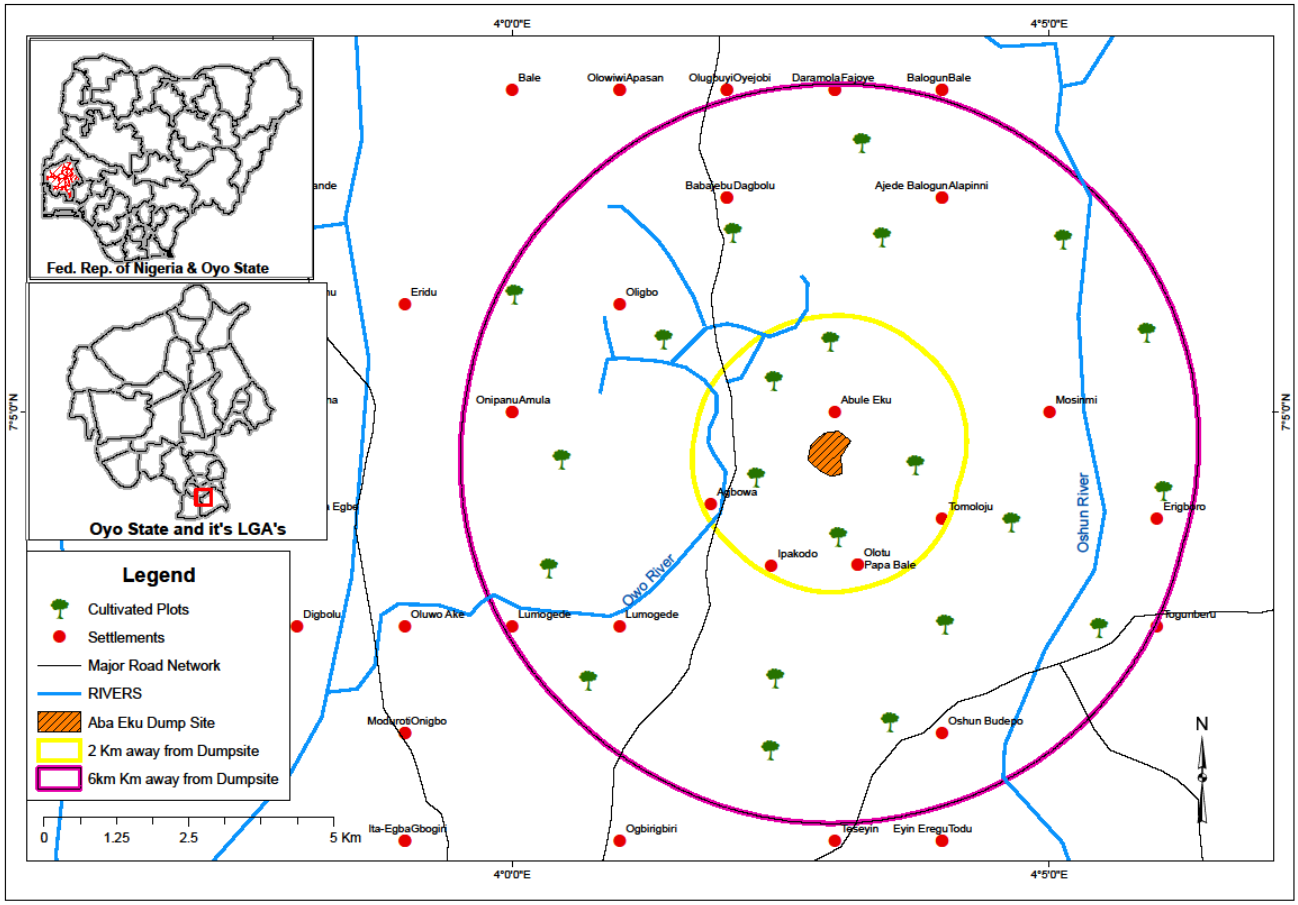

Figure 3.1Ibadan study area. Showing Aba Eku landfill site and study locations for the epidemiological study (settlements within 2 km and 6 km away from landfill) with questionnaire administrations.

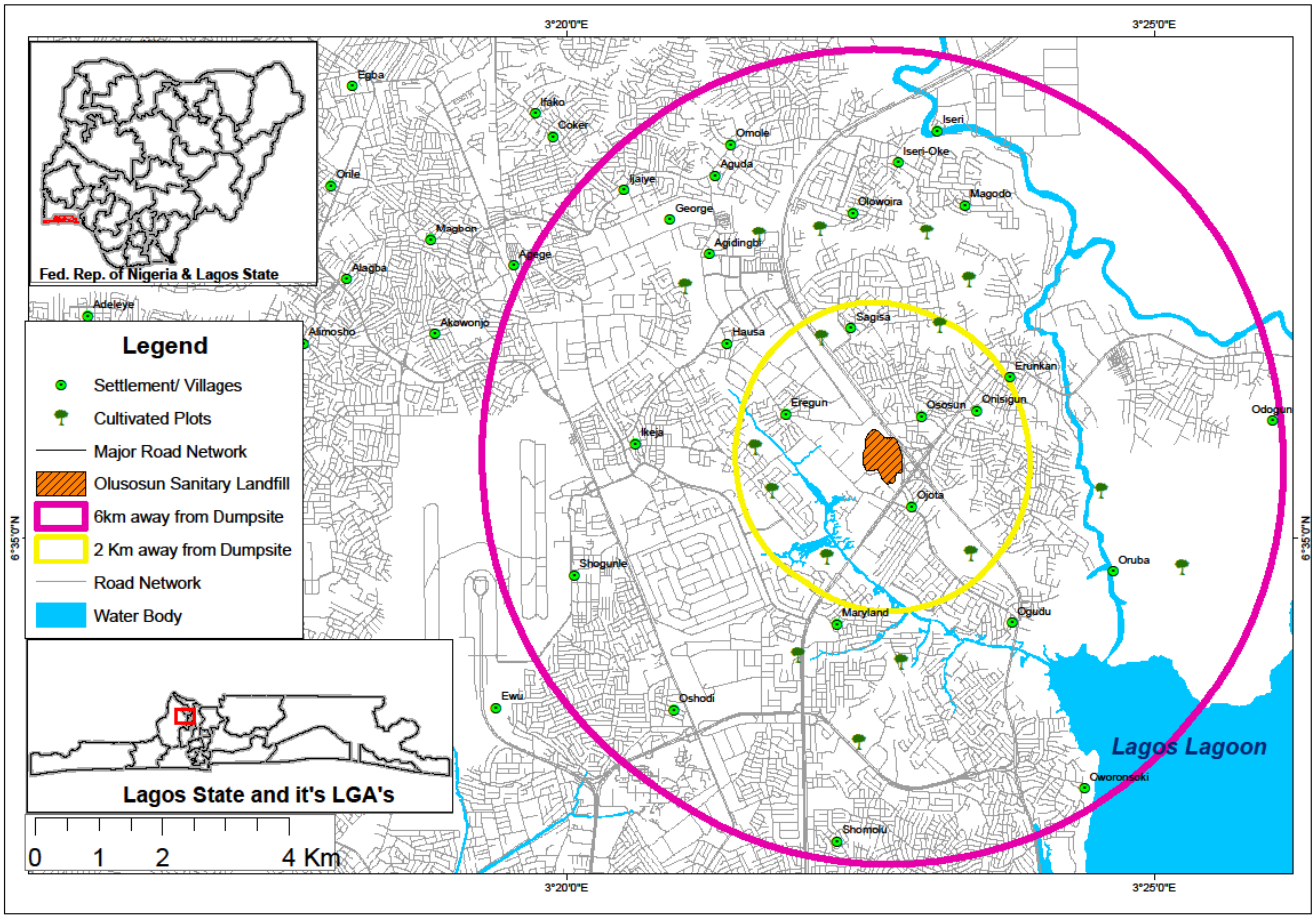

Figure 3.2 Lagos study area. Showing Olusosun landfill site and study locations (the settlements within 2 km and 6 km away from the landfill) for the epidemiological study with questionnaire administrations.

3.2 Leachate sampling and simulation from decomposed wastes

Raw leachates collected from 20 different leachate wells (holes in the ground where leachate seeps out) were thoroughly mixed to provide a representative sample for each of the landfills. Leachate simulation from decomposed solid wastes was done by randomly collecting municipal soil samples from 10 different spots on the landfills and were mixed together to make a single representative sample for each site. The raw leachates and soil samples were transported to the laboratory. The soil samples were air-dried for a period of 2 weeks, after which the coarse particles were finely ground with a pestle and mortar, and sifted through a 63-µm (pore size) sieve to obtain homogeneous mixtures for each landfill. These mixtures were used to simulate (25 %) the leachate in accordance with ASTM (1992) and Ferrari *et al*. (1999) as modified by Bakare *et al*. (2007). 250 g of the solid waste soil samples from each landfill was weighed out into a 2 L flask and 1,000 ml of distilled water (w/v) was added to form soil solution. This solution was mechanically stirred at an hour interval using glass rod for 24 hours at 26.7°C. After 24 hours, the suspensions were allowed to settle for at least an hour and the supernatant was filtered with 2.5-µm filters (Whatman® No. 42) to remove suspended particles (Appendix I).

The filtrate from decomposed solid waste simulation and the raw leachate samples were centrifuged (UNISCOPE Refrigerated Centrifuge machine, Model SM902B, Surgifield Medicals, England) at 3000 revolution per minute (rpm) for 10 minutes at room temperature. The supernatants from the leachate samples were decanted into 1.5 L sterile sample bottles, their pH measured and were labeled as Olusosun Raw Leachate (OSRL), Olusosun Simulated Leachate (OSSL), Aba-Eku Raw Leachate (AERL) and Aba-Eku Simulated Leachate (AESL). The leachates considered as the stock samples were stored at 4^{o}C until they were used for the experiments.

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Fig. 3.3 (a) Burning and residential quarters (red arrow) within Aba-Eku landfill.

(b) Leachate well (red arrow) meant to receive leachate from the dumpsite for subsequent treatment but no longer functional, hence discharges leachates into Omi stream.

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(c) A tributary of Omi stream (red arrow) used for both domestic and commercial water supply to the villages in Ona Ara LGA.

Figure 3.4 (a) Temporary residential structures and selling and buying of food items (red arrow) on Olusosun dumpsite.

(b) Borehole construction (red arrow) in the dumpsite with burning of wastes.

(c) Burning of wastes (rd arrow) increases human exposure to landfill chemicals.

3.3 Physicochemical parameters and heavy metal analysis

Physical and chemical components of the leachates were analysed according to standard methods by American Public Health Association (APHA, 1998). Nitrate, ammonia, chloride, phosphate, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total solids (TS) were determined. Heavy metal concentration: Iron (Fe), Lead (Pb), Copper (Cu), Manganese (Mn), Arsenate (As), Cadmium (Cd), Chromium (Cr) and Nickel (Ni), were determined in accordance with USEPA (1996a) and APHA (2005). 100 ml of each leachate was digested by heating with concentrated HNO_3. The resulting volume of about 2–3 ml of the leachate solution was made up to 10 ml with 0.1N HNO_3 and the concentrations of the metals were then estimated using PerkinElmer® A3100 atomic absorption spectrophotometer.

3.4. Biological materials

The animals and cell lines used as biological models were; *Clarias gariepinus* (mud catfish), Japanese quail (*Coturnix japonica*), mouse (*Mus musculus*), Albino rat (*Rattus novergicus*) and WIL2–NS lymphoblastoid cell line.

Juveniles of *C. gariepinus* of similar sizes with mean ± SD total length of 6.6 ± 0.74 cm and weight of 9.76 ± 0.85 g obtained from the aquaculture unit of the Department of Marine Sciences, University of Lagos, Nigeria were used. The fish were kept in plastic tanks (34 × 27 × 48.5 cm) which were half filled with dechlorinated water obtained by aerating tap water in a plastic tank with an aerator (Cosmo Aquarium, air pump 11,000) for 24 hours. This was done to allow for rapid evaporation of chlorine gas from the water. They were fed 5 % of their body weight with Coppens fish feed which contains 35 % crude protein twice daily. The water was changed once every 24 hours to prevent the accumulation of waste metabolites and food particles.

Male Japanese quails (*Coturnix japonica*) 6 – 7 weeks old weighing 132 – 140 g (mean ± SD of 136. 68 ± 2.75 g) were obtained from the Nigerian Veterinary Research Institute, Ekiti State. They were placed in plastic cages with metallic wire in the animal house unit of the Department of Veterinary Anatomy, University of Ibadan, Nigeria. They were fed with corn

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soyabean meal-based feed (total protein 23 %) and water given *ad libitum* during acclimatization and experimentation.

Apparently healthy male and female mice (4 – 6 weeks old) which had been bred for several generations, were obtained from the animal breeding unit of the Cell Biology and Genetics unit, Department of Zoology, University of Ibadan. The animals were transported to the Animal house unit, Department of Cell Biology and Genetics, University of Lagos, until they were 7–8 weeks old and with mean ± SD weight of 23.71 ± 3.65g.

Male and female Wistar albino rats (6–7 weeks old), which had been bred for several generations, were obtained from the animal house of the College of Medicine, University of Ibadan, Nigeria. They were transported to the animal house unit of the Department of Cell Biology and Genetics, University of Lagos, until they were about 8–9 weeks old with mean ± SD weight of 80.37 ± 2.14g. The *Mus musculus* and *Rattus novergicus* had access to drinking water and were feed standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. 40 mg/kg of cyclophosphamide (CYP) (Endoxan™ Mfg Lic. No. 186. Frankfurt am Main, Germany) and distilled water (0.0 %) were used as positive and negative controls respectively for the Japanese quail, mouse and rats experiments.

All animals were free from any ailment and maintained in an apparently clean laboratory with conditions of 12 hours dark and light cycle, temperature of 26.7 ± 4^{o}C, relative humidity of 70 ± 20 % for a minimum of 14 days to allow them acclimatize to their new environments before the commencement of the cytogenetic and systemic toxicity experiments. These conditions were in accordance with laws and regulations governing animal care and use in research (AFS, 2004; Gad, 2007).

WIL2-NS lymphoblastoid cell line (ATCC no. CRL8155) obtained from Prof. A. A. Morley's laboratory (Flinders Medical centre, Bedford Park, Australia) was used for the *in vitro* cytokinesis block micronucleus assay.

3.5 *IN VIVO* MICRONUCLEUS ASSAY METHODOLOGY

3.5.1 96 hours LC_{50} Acute toxicity testing of Leachates and piscine micronucleus assay

In order to determine the leachate concentrations for the sub-lethal piscine micronucleus assay, a static renewal bioassay technique was adopted in which the test media were renewed at the same concentration once every 24 hours. Preliminary screening was carried out to determine the appropriate concentration range for testing the leachate samples as describe by Solbe (1995). Depending on the test concentrations, a given volume of dechlorinated tap water was measured

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into a plastic tank (34 × 27 × 48.5 cm) and a predetermined volume of either OSRL or AERL was added into the tank to make it up to 1,000 ml (Total volume of test medium) to achieve the desired test concentration. Ten (10) active fish were used in triplicates for each concentration of the raw leachates. Each concentration of the leachates was organised in triplicate so that a total of thirty (30) fishes per concentration including the control were used for both the range finding test and the LC_{50} determination. Fish were assumed to be dead when body or opercular movement was not observed even when prodded with a glass rod.

Five concentrations: 5, 10, 15, 20 and 25 % (leachate / tapwater, v/v), of OSRL and AERL were used for the acute toxicity test according to Cavas and Ergene-Gozukara (2003), for the 14 days sub-lethal toxicity study. Cyclophosphamide was used as the positive control. 20 mg of cyclophosphamide was dissolved in 1litre of dechlorinated tap water (Ali *et al*., 2008). 35 fish were exposed to each of the sub-lethal concentrations of the leachates and controls in 40 litre transparent plastic tank for a period of 14 days. During the period of exposure each leachate concentration and control solutions were renewed every 24 hours to ensure the continuous exposure of fish to the test leachates. At the end of 3^{rd}, 6^{th}, 9^{th} and 14^{th} day, peripheral blood was collected from the caudal veins of five fish per concentration into Ethylene Diamine Tetraacetic Acid (EDTA) bottles using sterile syringe and needle and the fish sacrifice. Gills and kidneys of the sacrificed fish were dissected out into petri-dish containing normal saline (8.5g of NaCl / L distilled water) using scissors and forceps. This ensures that blood stains were removed from the organs prior to genotoxicity study.

A drop of blood from the EDTA bottle was used to make thin smear of peripheral blood on precleaned grease free slides. Three slides, prepared per fish / concentration, were left for 24 hours to air dry at room temperature. Dried slides were fixed in absolute methanol for 20 minutes. Each slide was then stained with 2 % May-Grunwald's solution for 10 minutes, rinsed in distilled water and counter stained in 5 % Giemsa solution for 30 minutes. Slides were thoroughly rinsed in distilled water to wash off excess stain and allowed to air dry.

Gill was transferred from the petri-dish in normal saline into a vial containing 45 % acetic acid where it was broken up with forceps after 5 min, allowing for the gill epithelial cells to be scraped off the gill arch. Likewise, the kidney was transferred into a vial containing 45 % acetic acid where the tissue was minced to isolate the kidney cells. Tissue clumps and gill arches were removed and discarded. The solution was then centrifuged at 1000 rpm for 5 minutes to collect the gill epithelial cells and kidney cells into eppendoff tubes. Pelleted cells were then resuspended in 5 ml of hypotonic solution (0.075 mol/dm^3 of KCl or 0.56 % KCl) for 5 minutes

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at room temperature to rupture the cell membrane. The solution was then centrifuge at 1000 rpm for 5 minutes to obtain cell suspension. The cell suspensions obtained were fixed in two successive changes of freshly prepared cold ice Carnoy fixative (acetic acid and methanol, 1:3). Thin smear of fixed cells were made on precleaned grease free slides, allowed to air-dry at room temperature and stained with 2 % May-Grunwald's solution for 10 minutes. Rinsed in distilled water and counter stained in 5 % Giemsa solution for 30 minutes. Only the gill epithelial cells and kidney cells isolated from surrounding cells were scored. From each fish 2000 cells were scored for micronucleus using x100 objective (oil immersion). Nuclear abnormalities (NAs) were also scored along with MN as genotoxic parameters in the peripheral blood according to Carrasca *et al*. (1990). Briefly, cells with two nuclei were considered as binucleated (BN). Blebbed nuclei (BL) present a relatively small evagination of the nuclear membrane, which contains euchromatin. When the evaginations are larger than the blebbed nuclei and containing several lobes, it is considered as lobe nucleus (LB), while Notched nucleus (NT) contains vacuoles and appreciable depth into the nucleus that does not contain nuclear materials.

3.5.2 Japanese quail exposure and avian micronucleus assay

A total of 40 male Japanese quails (*C. japonica*) were randomly assigned to three different concentrations (10, 25 and 50 % leachate/distilled water, v/v) of OSRL and AERL. Five (5) quails/cage/concentration were administered 0.5 ml of leachates and control solutions per quail per day by oral gavaging (*per os*) for 14 consecutive days using oral canula. At 24 hours post exposure, quails were anesthetized by injecting 0.2 ml ketamin hydrochloride and 1ml of blood was collected by cardiac puncture into EDTA bottles. Heparinised blood was diluted with Fetal Bovine Serum (FBS) in the ratio 1:1 (peripheral blood / FBS, v/v). Thin smear of the resultant solution was made on precleaned microscope slides and allowed to air dry overnight in dust free room at ambient temperature. Air dried slides were fixed in absolute methanol for 20 minutes and allowed to air dry. Six (6) hours after fixation, slides were stained with 2 % May-Grunwald's solution for 10 minutes and counter stained in 10 % Giemsa solution for 30 minutes and thoroughly rinsed after each staining with distilled water. Rinsed slides were air dried overnight and scored with light microscope using x100 objectives (oil immersion). Three slides were made per quail and a total of 2000 peripheral erythrocytes per slide were scored for micronucleus and nuclear abnormalities. During scoring, only cells with intact cellular and nuclear membrane were scored for micronucleus and nuclear abnormalities. Although, analysing micronucleated erythrocytes has been generally recommended as the major criterion for genetic

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damage; but in lower vertebrates including birds and fish other nuclear abnormalities (NAs) have been shown to augment the scoring criteria for genotoxicity. Therefore, nuclear anomalies were scored along with micronucleated cells. These nuclear abnormalities were classified into four as binucleated (BN), budding nucleus (BudN), two–lobe nucleus (TLN) and tail nuclei (TN) (Kursa and Bezrukov, 2007).

Avian bone marrow micronucleus test as described by Bhunya and Jena (1992) with slight modifications was used for the bone marrow cell micronucleus preparation. The two femoral bones were dissected out from the sacrificed quails and freed of adherent tissues using a pair of scissors and forceps. The proximal and distal ends of the femur were carefully cut off with scissors. Using a 1 ml syringe and gauge 25 needles, 1 ml of 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 into eppendoff tube. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. The eppendoff tube was gently agitated on a vortex mixer to disperse cell clumps. Cells were then resuspended in 0.5 ml of FBS and four slides were made per animal by evenly spreading a drop of the cell suspension over each slide. Air-dried slides were fixed in absolute methanol for 30 min and allowed to air dry. Fixed slides were stained in 2 % May and Grunwald's solution for 5 minutes in coplar jars and rinse thoroughly in distilled water. Rinsed slides were counter stained in 5 % Giemsa for 30 minutes, thoroughly rinsed with distilled water and allowed to air dry overnight at room temperature. Coverslips were mounted on the air dried slide preparations by clearing and mounting in xylene and Diphenyl Xylene (DPX) respectively. Slides were scored with light microscope using x100 objectives (oil immersion). Four slides were made per quail and a total of 2000 bone marrow erythrocytes per slide were scored for the presence of micronucleus.

3.5.3 *Mus musculus* exposure and mammalian micronucleus assay

Three (3) mice/concentration/sex were randomly assigned to five different concentrations of 1.0, 2.5, 5.0, 10.0 and 25.0 % (leachate/distilled water, v/v) of OSRL, AERL, OSSL and AESL. The animals were intraperitoneally (i.p.) exposed to 0.5 ml of the leachate samples and control solutions for 5 consecutive days. At 24 hours post exposure, mice were sacrifice for micronucleus analysis.

Mouse-Erythrocyte-Micronucleus (Mus-EMN) assay was carried out according to MacGregor *et al*., (1980) with slight modifications. About 0.8 ml of peripheral blood was collected from the retro–orbital venous plexus of the mice using 70 ml micro–haematocrit

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capillary tube into EDTA bottles and was diluted with FBS (Zayo-Sigma chemicals Ltd. 33106 Paderborn, Germany) in the ratio 1:1 (peripheral blood / FBS, v/v). Thin smear of the resultant solution was made on precleaned microscope slides. 5 slides were made per animal and allowed to air dry overnight in dust free room at ambient temperature. Air dried slides were fixed in absolute methanol for 20 minutes and allowed to air dry. Fixed slides were counter stained with 2 % May-Grunwald's solution for 10 minutes and 5 % Giemsa solution for 30 minutes. They were thoroughly rinsed after each staining with distilled water. Micronucleated polychromatic erythrocytes (MN PCE) in 2,000 polychromatic erythrocytes (PCE) were scored per animal.

Mouse bone marrow erythrocyte micronucleus test as described by Schmid (1975) with slight modifications was used for the bone marrow cell micronucleus preparations. Mice were sacrificed by cervical dislocation and the two femoral bones harvested and freed of adherent tissues using a pair of scissors and forceps. By gentle traction the distal epiphyseal portion was torn off together with the rest of the tibia and the surrounding muscles. The proximal end of the femur was carefully shortened with scissors until a small opening to the marrow canal became visible. Using 1 ml syringe and gauge 25 needles, 1 ml of 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 into eppendoff tube. It was then centrifuged at 1000 rpm for 10 minutes after which the supernatant was discarded. The Eppendoff tube was briefly agitated on a vortex mixer to disperse cell clumps. 100µl of FBS was added, mixed properly and centrifuged at 1000 rpm for 10 mins. The supernatant was decanted and the residue in eppendolf tube was briefly agitated on a vortex mixer to disperse cell clumps. Cells were then resuspended in 80 µl of FBS and four slides were made per animal by evenly spreading a drop of the cell suspension over each slide. The slides were fixed in absolute methanol for 30 minutes and allowed to air dry. Fixed slides were stained in 100 % May-Grunwald's solution for 5 minutes in coplar jars, rinse thoroughly in distilled water. Then stained again in 1:1 May-Grunwald /distilled water for 5 minutes and rinse thoroughly in distilled water to remove excess stain. Slides were double stained in 5 % Giemsa for 30 minutes, rinsed thoroughly with distilled water and allowed to air dry overnight at room temperature. Coverslips were mounted on the dried slide by clearing and mounting in xylene and DPX respectively. 2000 cells of polychromatic erythrocytes (PCE) were scored for micronucleated PCE as index of genotoxicity. Also from each slide, on the same microscope field 1000 cells were scored to determine PCE / NCE ratio, an index of cytotoxicity (Krishna and Hayashi, 2000; Vilar *et al*., 2008).

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3.6 Systemic toxicity assays

Five (5) male and female *Rattus novergicus*/cage/sex were randomly assigned to a concentration each of the leachate samples. Each animal in a group was gavaged with 0.5 ml of 1.0, 2.5, 5.0, 10.0 and 25.0 % (leachate/distilled water, v/v) leachate concentrations for 30 consecutive days (USEPA, 1996b). Similar treatments were concurrently given to the negative and positive groups.

3.6.1 Clinical observations and body weight measurement

Each rat was observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural patterns, respiratory system, morbidity and mortality. The body weights of each animal in the treatment groups and concurrent controls were measured at the beginning of exposure and once weekly during the exposure period using Acculab® USA, Model-vic-303 electronic analytical weighing balance.

3.6.2 Blood collection and organ weight measurement.

At 30 day post exposure, survivors were fasted overnight and weighed prior to blood collection and sacrificed to determine the terminal body weight. Blood was collected from the orbital plexus using heparinized 70 ml micro – haematocrit capillary tubes into lithium coated serum separator tubes (for serum biochemical analysis) and an aliquot of the blood into EDTA coated bottles (for haematological analysis). The blood samples in lithium coated tubes were allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate the serum (supernatant) and stored at –70^{o}C prior to biochemical analysis within 48 hours. Liver, kidneys, spleen, thymus, heart and lungs of the animals were surgically removed using a pair of scissors and forceps, rinsed with ice – cold normal saline, blotted dry with whatman filter paper and weighed to determine the absolute weight of the organs. The relative organ weight (absolute organ weight / body weight x 100 g) was determined for all the viscera.

3.6.3 Serum biochemical analysis

Serum clinical chemistry biomarkers of importance which have been established and utilized in occupational and environmental epidemiological studies as tools for detecting early

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and reversible clinical effects during exposure to effluents from waste landfill (Indulski and Lutz, 1995) were evaluated. These biomarkers were: creatinine, urea, total proteins, transaminases and albumins. They were assessed using diagnostic kits (Randox Lab, Ardmore, Northern Ireland, UK) in the Chemical pathology laboratory unit, Orthopaedic hospital, Igbobi Yaba, Lagos.

Aspartate aminotransferase (AST) and Alanine aminotranferase (ALT) enzyme activities in the serum were determined according to Reitman and Frankel, (1957). AST analysis is based on the principle that oxaloacetate formed from the aspartate aminotransferase catalysed reaction between alpha ketoglutarate and aspartate is coupled with chromogen (2, 4 – dinitrophenyl hydrazine) in alkaline medium to form coloured hydrazone. The intensity of the coloured hydrozone measured with colorimeter is proportional to the aspartate aminotransferase activities. Using kit protocol, 0.25 ml of buffer (substrate) solution was added to 0.05 ml of each serum sample in test tube. The content was incubated at 37^{o}C for 60 minutes in a water bath followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20 minutes at room temperature after which 2.5 ml of 0.4 mol/dm^{3} sodium hydroxide ($NaOH_{aq}$) was added and mixed together. The absorbance was read after 5 min against blank at 540 nm using a colorimeter (AE-11D, ERMA Inc). The blanks were treated as the samples but without the addition of chromogen solution used to stop all enzymatic reactions. AST activity (IU/L) was determined from the standard curve.

Serum ALT activity determination was based on the principle that pyruvate was formed from alanine aminotrasferase catalysed reaction between α-ketoglutarate (oxaloglutarate) and L-alanine. The pyruvate formed couples with chromogen solution in an alkaline medium to form coloured hydrozone, the intensity of the hydrozone as measured with colorimeter, is proportional to alanine aminotransferase activity. Using the kit protocol, 0.25 ml of buffer (substrate) solution was added to 0.05 ml of each serum sample in a test tube. The solution was incubated in a water bath at 37^{o}C for 30 minutes followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20 minutes at room temperature. Then 2.5 ml of 0.4 mol/dm^{3} sodium hydroxide solution was added and the compound formed properly mixed. The absorbance was read after 5 minutes against the blank at 540 nm. ALT activity (IU/L) was read off from the standard curve.

Serum urea concentration was determined according to Weatherburn (1967) based on the principle that ammonia (from the urease catalyzed hydrolysis of urea to ammonia and CO_2) is converted to indophenol blue in the presence of sodium nitroferricyanidephenol and hypochlorite reagents. The absorbance was read with spectrophotometer set at 625nm.

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Serum creatinine concentration was measured according to Henry (1974). Briefly, 0.8 mL of acid tungstate was added to 0.1 ml of serum sample in a test tube. The resultant solution was thoroughly mixed and centrifuged at 2000 rpm for 10 minutes. The supernatant was separated into a clean test tube containing 0.2 ml picric acid. 0.1 ml of 1.4 mol/dm^3 sodium hydroxide was added to the resultant supernatant and the solution read at 500 nm absorbance.

Serum total protein was measured according to Treitz (1970). Briefly the interaction of copper ions with serum protein peptide bonds in an alkaline medium forms a coloured complex. The intensity of the colour is measured spectrophotometrically at 546nm against a reagent blank and total protein concentration (g/dl) determined from a standard calibration graph.

Serum albumin was measured according to Doumas *et al*. (1982). Briefly the interaction between BromoCresol Green (BCG) and serum albumin forms a colour complex, in which the intensity measured at 578 nm is directly proportional to the albumin concentration (g/dl) in the serum sample. The absorbance of all the tests were measured spectrophotometrically (HAICE®, DR 3000, Germany) (Appendix II).

3.6.4 Histopathological analysis

The viscera excised from exposed and control animals were cut into thin slices and fixed in 10 % neutral buffered formalin solution. After 48 hours of fixation, tissues were dehydrated by passing through different concentrations of ethyl alcohol – water (50 %, 70 %, 90 % and 100 %), cleared in xylene, infiltrated with paraffin, and sequentially embedded in fresh paraffin wax blocks using rotary microtome. Tissue sections of 3 – 5 µm thick were cut and routinely stained with Haematoxylin-Eosin (H-E), mounted in neutral DPX medium for morphological examination. Trained pathologist evaluated slides for histopathological alterations at x 400 magnification using (Olympus, Tokyo) light microscope. Photomicrographs were produced using Sony digital camera (DSC-S730).

3.6.5 Haematological analysis

The blood sample collected into EDTA bottle was used to determine the recommended haematological indices including: Red Blood Cell count (RBC), Haemoglobin content (Hb), Haematocrit (Ht), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), platelets, total white blood cell count (WBC) and differentials [lymphocytes, neutrophils and Mixed Cell Count, (MXD; eosinophils, basophils and monocytes)] (Brown, 1992), using automated analyser machine, CELL-DYN 1700

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counter, (Abbott Haematology Analyser Cell-Dyn 1700, Abbott Laboratories, Abbott Park, Illinois, USA) in the haematology unit of the Lagos State University teaching hospital (Appendix III).

An aliquot of the blood samples were used to make thin blood films on pre-cleaned slides (three slides per rat), air-dried, fixed in absolute methanol for 20 min and stained in 5% Giemsa solution for 20 min (Dacie and Lewis, 1984). 500 erythrocytes were scored per rat at x 1000 to determine poikilocytosis (variations in red blood cell shapes) and acanthocytosis (variation in red blood cell sizes) according to Christopher (2004) and Cheesbrough (2005).

3.7 *IN VITRO* CYTOKINESIS–BLOCK MICRONUCLEUS CYTOME ASSAY USING WIL2–NS LYMPHOBLASTOID CELL LINE

WIL2-NS human lymphoblastoid cell were exposed to six different concentrations (0.01, 0.10, 1.00, 10.00, 100.00 and 1000.00 µM) of Potassium chromate (K_2CrO_4), Copper (II) chloride dihydrate ($CuCl_2.2H_2O$), Manganese (II) chloride tetrahydrate ($MnCl_2.4H_2O$), and Lead acetate [$Pb(CH_3COO)_2$] salts (Sigma St Louis, MO) and their mixtures in two different folic acid (FA) concentrations (20 and 2000 nM) of RPMI 1640 medium. The vehicular solvent (Milli Q) for the dissolution of the metallic salts, was used as control (0.00 µM). The experiment was conducted with three replicates of each concentration. 2000 nM is a replete FA concentration for which WIL2-NS human lymphoblastoid cell line has been reported to yield optimal viable cell growth and minimal genome damage during exposure to xenobiotics (Teo and Fenech, 2008).

Viability test using trypan blue exclusion (Sigma, Australia) and cell counts using a Coulter counter (Z1 Coulter® Particle Counter, Beckman coulter, USA) were performed to achieve a seeding density of 0.30 x 10^6 viable cells/ml of WIL2-NS cells line. The cells were added to folate – free RPMI 1640 medium to which folic acid (FA) was added to achieve concentrations of either 20 or 2000 nM. The medium was supplemented with 5% (v/v) foetal bovine serum (FBS), 1 % (v/v) penicillin / streptomycin and 1 % L – glutamine (Trace Biosciences, Australia). Using a 48 well culturing plate, 490µl of either 20 nm FA or 2000 nM FA was added to each well plus 10µl of metal solution of the different concentrations (0.01, 0.10, 1.00, 10.00, 100.00 and 1000.00µM) and Milli Q (0.00µM control) to achieve a 0.3 x 10^6 viable cells/ ml in 500µl per well. Three replica per 20 nM and 2000 nM FA per metallic salt concentration were prepared. The cells were incubated for 48 hours. At the end of 48 hours

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incubation, cells were centrifuged at 100 g for 10 minutes to wash off the metal solution followed by careful aspiration of 300 µl supernatant. 500µl of fresh pre-warmed (37^{o}C) complete medium of 20 or 2000 nM FA was added and centrifuge as above and this was repeated for proper washing of the metal. After this second washing, 300µl of the supernatant was carefully removed avoiding the disturbance of the cell layers. A final 300µl of pre-warmed complete medium of 20 or 2000 nM FA was added to the appropriate wells followed by 37.5µl of Cytochalasin – B (Cyto – B) (Sigma St Louis, MO) and incubated for another 24 hours at 37^{o}C. After 24 hours of post incubation, the cells were harvested. Slides were prepared using cytocentrifugation. Dimethylsulfoxide (DMSO) (Sigma St Louis, MO) was added at a final concentration of 5% to minimize clumping of the cells and thus optimizing recognition of cytoplasmic boundaries. One slide with two cytospots on each per replicate per metal was prepared, air dried for 10 minutes, fixed in absolute methanol and stained using Diff – Quick (Lab Aids, Australia). Slides were rinsed to remove excess stains and allowed to air dry overnight. Cells were covered with slide coverslips and were scored according to Fenech (2007), briefly one thousand binucleated (BN) cells were scored for the frequencies of Micronucleus in Binucleated cells (MN-BN cell), Nucleoplasmic bridge in Binucleated cells (NPB-BN cells) and nuclear bud in binucleated cells (Nbud–BN) and five hundred cells scored for mononucleated (M_1), binucleated (M_2) and multinucleated (M_3) and number of necrotic cells was scored along with the 500 viable cells. The nuclear division index (NDI) was calculated from the formular [NDI = $M_1 + M_2 + M_3 / T$] where T is the total number of viable cells scored. Necrosis was expressed as percentage of the total scored cells.

3.8 Human health impact assessment

Health survey was carried out on populations inhabiting 2 km radius of the landfills (exposed) and 6 km away from the landfills (unexposed or control population).

The survey instrument, a pre-tested and well structured questionnaire, Self Reported Health Impact Assessment Questionnaire (SRHIAQ), described the general background; lifestyle or habits; health outcomes (respiratory, dermal, gastrointestinal, neuromuscular and psychological symptoms, reproductive and cancer status); self-rated views about health; beliefs about exposure routes to noxious substances, site characterisation and possible confounding factors (Appendix IV).

The questionnaire was administered by face-to-face interviewing of participants who volunteered to participate after they were well informed about the study. A total of two hundred

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and eighty seven (287) of the exposed and two hundred and seventeen (217) of the unexposed (control) groups from Olusosun landfill locality and two hundred and forty eight (248) of the targeted (exposed) and one hundred and sixty two (162) of the unexposed (control) groups from Aba-Eku landfill locality, who willingly indicated their interest to participate, were administered the SRHIAQ.

3.9 Statistical analysis

Data obtained from the micronucleus test and systemic toxicity were presented as mean ± SE of the sample size and /or percentage (%) change from control. One Way Analysis of Variance (ANOVA) was used to determine the significant ($p < 0.05$) differences among the treated and control group and when the corresponding F test for difference in means among the treated groups was significant pair wise, the treated groups were compared with corresponding negative controls using Multiple Comparison Procedure of the Dunnett Post-hoc Test at $p < 0.05$ levels of significance (Dunnett, 1964). Two-way ANOVA was also used to determine the significance of the independent effects of leachates, sex and the interactions between the effects of leachates and sex on the cytogenotoxicity and systemic toxicity.

Probit analysis was used to determine the LC_{50} of the landfill leachates. Data obtained from the health impact survey were analysed using descriptive statistics and percentages to describe the demographic and socio-economic, potential exposure pathways and possible confounding factors. Chi square test was used to show the association between health symptoms observed in exposed and unexposed populations. Odd Ratio (OR) and 95 % confidence interval (CI) were used to indicate the magnitude of likelihood of the health impacts between the studied populations. All statistical analyses were conducted with Graphpad prism 5.0® computer programs and statistical package for the social scientist, SPSS–16®.

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CHAPTER FOUR

4.0 RESULTS

4.1. Physico–chemical characteristics and heavy metal analysis

The physico-chemical parameters and heavy metals analysed in the tested leachate samples are shown in Table 4.1. Olusosun and Aba Eku raw leachates had strong foul smelling odour than the simulated leachates and they were dark brown in colour, while OSSL and AESL were brown and pale brown in colour respectively. The pH values range from neutral to slightly alkaline and are within the standard permissible limits of wastewater discharge into the environment (NESREA, 2009) in Nigeria. The values of COD, BOD, Total solid, hardness, alkalinity, chloride, phosphate, sulphate, nitrate, ammonia, Cr, Ni, Mn, Cd, Pb, Fe, As and Cu detected in the leachate samples, were higher compared to standard permissible limits. The concentrations of these parameters in the raw samples (OSRL and AERL) were higher than in the simulated samples (OSSL and AESL).

4.2. LC_{50} determination OF OSRL and AERL using *Clarias gariepinus*

Table 4.2 shows the 96 hours acute toxicity of OSRL and AERL using *Clarias gariepinus*. The LC_{50} for OSRL ranged from 46.07 % (24 hours) to 34.52 (96 hours), while for AERL ranged from 63.50 % (24 hours) to 43.29 (96 hours). The LC_{50} of the leachates decreased with exposure duration. The toxicity factor (TF) for the 96 hours LC_{50} showed that OSRL was 1.25 times more toxic to *Clarias gariepinus* than AERL (1.00).

4.3. CYTOGENOTOXICITY

4.3.1 Micronuclei induced by OSRL and AERL in *Clarias gariepinus*

Tables 4.3 – 4.7 show the frequencies of MN induced in peripheral erythrocytes, gill epithelial cells and kidney cells of mud catfish exposed to 5 – 25 % concentrations of OSRL and AERL at different time intervals. The MN frequencies in peripheral blood erythrocytes, at the different time interval, showed a concentration dependent significant ($p < 0.05$, One–way ANOVA) increase compared to the corresponding negative controls. There was a positive correlation between the MN formation and leachate exposure during the different days [$r = 0.8341$, $F = 23.46$ (3^{rd} day), $r = 0.9110$, $F = 47.74$ (6^{th} day), $r = 0.8778$, $F = 33.52$ (9^{th} day) and $r = 0.8748$, $F = 32.60$ (14^{th} day)] for OSRL and [$r = 0.8493$, $F = 26.30$ (3^{rd} day), $r = 0.8009$, $F = 18.77$ (6^{th} day), $r = 0.8407$, $F = 24.64$ (9^{th} day) and $r = 0.8845$, $F = 35.75$] for AERL.

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Table 4.1. Physico-chemical parameters analysed in Olusosun and Aba-Eku landfill leachates

Parameters*	AERL	AESL	OSRL	OSSL	NESREA[a]	USEPA[b]
Colour	Dark brown	Pale brown	Dark brown	Brown	-	-
pH	7.8	7.2	8.1	7.6	6.0 – 9.0	6.5 – 8.5
Nitrate	54.4	38.6	72.3	43.2	10	10
Ammonia	86.40	28.4	122.10	32.8	10	0.02
BOD[#]	601	397	594	306	50	-
COD[**]	512	410	487	398	90	-
Phosphate	122.02	98.86	215.70	106.13	2.0	-
Chloride	1106	1003	1,099	1012	250	250
Sulphate	114.34	109.35	218.12	122.51	250	250
Hardness	805	793	1,200	809	150	0 - 75
Alkalinity	502	439	623	456	-	20
TS[##]	3116.67	2300.50	4100.30	2200.60	-	-
Copper	2.44	0.92	3.86	1.02	0.5	1.3
Iron	3.20	1.87	4.71	2.03	-	0.3
Lead	2.08	0.81	2.00	1.18	0.05	0.015
Cadmium	1.44	0.31	2.20	0.65	0.2	0.05
Manganese	2.90	0.61	3.10	1.04	0.2	0.05
Arsenic	1.50	0.90	2.60	1.20	-	0.01
Nickel	1.88	1.96	2.51	2.12	0.05	-
Chromium	2.32	1.40	2.43	1.80	0.05	0.1

*All values are in mg/L except pH,
[#]BOD – Biochemical Oxygen Demand;
[##]TS – Total Solid;
**COD – Chemical Oxygen Demand,
[a]National Environmental Standards and Regulation Enforcement Agency (2009) (Nigeria) maximum permissible limits for wastewater.
[b]United State Environmental Protection Agency (2006) (www.epa.gov/safewater/mcl.html).

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Although, the MN frequencies showed time dependent increase but there was decrease in the induction of MN on the 6th day of exposure compared to other days (Table 4.3).

The frequencies of MN induction in gill epithelial cells increased with leachate concentrations and duration of exposure. One way ANOVA showed varying degrees of significance in the MN induction in the gill epithelial cells at the different days of leachate treatments [$p < 0.0295$ (3rd day), $p < 0.0269$ (6th day), $p < 0.0053$ (9th day) and $p < 0.0064$ (14th day)] for OSRL and [$p < 0.0013$ (3rd day), $p < 0.0082$ (6th day), $p < 0.0008$ (9th day) and $p < 0.0206$ (14th day)] for AERL. Comparison of the MN induction at different leachate treatments with corresponding negative controls for the different days using Dunnett's multiple comparison posthoc test was significant ($p < 0.05$). Different correlation coefficient values were obtained for the frequencies of MN induction in the gill epithelial cells during the days of leachate exposure [$r = 0.3743$, $F = 2.792$ (3rd day); $r = 0.3794$, $F = 2.853$ (6th day); $r = 0.4604$, $F = 3.982$ (9th day) and $r = 0.4516$, $F = 3.843$ (14th day)] for OSRL and [$r = 0.5182$, $F = 5.020$ (3rd day); $r = 0.4398$, $F = 3.662$ (6th day); $r = 0.5365$, $F = 5.401$ (9th day) and $r = 0.3938$, $F = 3.032$ (14th day)] for AERL. The correlation coefficient between the MN formation in the gill epithelial cells and leachate exposure was lower than in peripheral erythrocytes. Frequencies of MN induction in the gill cells increased with days of exposure except for the 6th day (Table 4.4).

The frequencies of MN induction in the kidney cells increased with leachate concentration and days of exposure. One way ANOVA showed that MN formation in the kidney cells at the different days of OSRL and AERL treatment was significant ($p< 0.05$). Comparing the MN induction at the different concentrations of leachate treatments with the corresponding negative controls for the different days of exposure using Dunnett's multiple comparison posthoc test was significant ($p < 0.05$). Correlation coefficients obtained for the MN induction in kidney cells by OSRL and AERL during the different sample days [$r = 0.8170$, $F = 20.83$ (3rd day); $r = 0.6233$, $F = 7.722$ (6th day); $r = 0.6017$, $F = 7.051$ (9th day) and $r = 0.6908$, $F = 10.42$ (14th day)] for OSRL and [$r = 0.8089$, $F = 19.75$ (3rd day); $r = 0.6723$, $F = 9.573$ (6th day); $r = 0.6810$, $F = 9.963$ (9th day) and $r = 0.7170$, $F = 11.82$] for AERL showed positive correlation between leachate treatments and MN induction in the kidney cells. Also, the correlation coefficients were higher than those obtained for gill epithelial cells. The frequencies of MN induction increased with leachate treatments and days of exposure except for day 6 of exposure (Table 4.5). Figure 4.1 showed micronucleated erythrocytes, gill epithelial cells and kidney cells in the mud catfish.

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Table 4.2. 96 hours acute toxicity determination of AERL and OSRL using *Clarias gariepinus*

Exposure Time (Hrs)	LC_{50} (95% CI; %,v/v)	LC_5 (95% CI %,v/v)	LC_{95} (95% CI %,v/v)	Slope ± S.E	Probit line equation	DF	TF
ABU-EKU RAW LEACHATE (AERL)							
24	63.50 (111.27 – 55.46)	40.55 (46.34 – 23.54)	99.44 (470.24 – 74.23)	8.45 ± 1.60	Y = -10.22 + 8.45x	10	
48	57.70 (55.02 – 47.44)	33.19 (36.85 – 27.58)	100.28 (144.01 – 82.86`)	6.85 ± 1.12	Y = -7.07 + 6.85x	10	
72	55.93 (154.05 – 45.59)	29.34 (37.62 – 4.79)	106.61 (3883.37 – 70.37)	5.87 ± 0.87	Y = -5.260 + 5.87x	10	
96	43.29 (57.48 – 35.47)	22.87 (29.80 – 8.38)	81.96 (276.17 – 60.29)	5.94 ± 0.69	Y = -4.71 + 5.94x	10	1.00
OLUSOSUN RAW LEACHATE (OSRL)							
24	46.07 (48.27 – 44.03)	31.86 (34.46 – 28.27)	66.62 (75.63 – 61.33)	10.27 ± 1.22	Y = -12.08 + 10.27x	10	
48	49.96 (67.05 – 42.83)	25.23 (31.67 – 12.46)	98.94 (292.39 – 71.47)	5.54 ± 0.75	Y = -4.42 + 5.54x	10	
72	39.14 (56.58 – 28.24)	21.19 (27.37 – 2.80)	72.26 (514.39 – 52.27)	6.18 ± 0.67	Y = -4.84 + 6.18x	10	
96	34.52 (46.48 – 23.08)	19.71 (26.96 – 3.10)	60.46 (302.99 – 45.40)	6.76 ± 0.69	Y = -5.39 + 6.76x	10	1.25

[a]TF = toxicity factor (96h LC_{50} value of Aba-Eku Raw Leachate/96hLC_{50} value of Olusosun Raw Leachate)

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Table 4.3 Mean (± SE) of MN frequencies in peripheral blood erythrocytes of *Clarias gariepinus* exposed to varying concentrations of OSRL and AERL.

Treatment	conc (%)	3 days	6 days	9 days	14 days
Control	0	0.82 ± 0.02	0.61 ± 0.04	0.70 ± 0.40	1.02 ± 0.03
CYP	20	5.60 ± 0.11[b]	4.42 ± 0.12[b]	6.25 ± 0.49[c]	7.03 ± 0.12[c]
OSRL	5	1.81 ± 0.18	1.40 ± 0.04	1.62 ± 0.41	2.01 ± 0.06
	10	2.20 ± 0.21[a]	2.03 ± 0.12[a]	2.40 ± 0.60[a]	2.83 ± 0.21[a]
	15	4.03 ± 0.04[b]	3.20 ± 0.20[a]	3.61 ± 0.37[a]	3.87 ± 0.35[a]
	20	6.21 ± 0.13[b]	5.62 ± 0.24[b]	7.03 ± 0.55[c]	8.20 ± 0.13[c]
	25	9.05 ± 0.42[c]	6.81 ± 0.48[b]	8.20 ± 0.73[c]	9.62 ± 0.15[c]
AERL	5	1.20 ± 0.23	0.83 ± 0.37	1.41 ± 0.20	1.81 ± 0.20
	10	1.83 ± 0.14	1.60 ± 0.51	2.05 ± 0.14[a]	2.20 ± 0.31
	15	3.02 ± 0.35[a]	2.65 ± 0.33[a]	2.03 ± 0.02[a]	3.43 ± 0.23[a]
	20	5.00 ± 0.24[b]	4.80 ± 0.12[b]	7.20 ± 0.17[c]	7.53 ± 0.71[c]
	25	8.11 ± 0.71[c]	6.47 ± 0.63[b]	7.81 ± 0.26[c]	8.62 ± 0.63[c]

CYP (cyclophosphamide, mg/L), OSRL (Olusosun raw leachate), AERL (Aba Eku raw leachate), superscripts significantly ($p < 0.05$) different from corresponding negative control using Dunnett's multiple post hoc comparison test.

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Table 4.4. Mean (± SE) of MN frequencies in gill epithelial cells of *Clarias gariepinus* exposed to OSRL and AERL.

Treatment	Conc (%)	3 days	6 days	9 days	14 days
Control	0	1.11 ± 0.18	1.08 ± 0.18	1.04 ± 0.84	1.15 ± 0.66
CYP	20	8.42 ± 0.27[c]	7.21 ± 0.47[c]	8.40 ± 0.50[c]	9.02 ± 0.32[c]
OSRL	5	2.40 ± 0.41	2.20 ± 0.28	2.46 ± 0.32	2.82 ± 0.49
	10	2.93 ± 0.13	2.53 ± 0.55	2.91 ± 0.21	3.25 ± 0.92
	15	3.41 ± 0.03[a]	2.85 ± 0.97	3.20 ± 0.49[a]	3.81 ± 1.59[a]
	20	7.12 ± 0.63[c]	6.60 ± 0.21[b]	7.02 ± 2.35[c]	7.60 ± 1.33[c]
	25	9.63 ± 0.32[c]	8.64 ± 0.04[c]	10.60 ± 2.86[c]	11.82 ± 3.02[c]
AERL	5	2.24 ± 0.25	2.06 ± 0.51	2.17 ± 0.37	2.41 ± 0.37
	10	2.53 ± 0.32[a]	2.31 ± 0.66	2.45 ± 0.05	2.76 ± 1.03[a]
	15	3.27 ± 0.55[a]	2.78 ± 0.51	2.86 ± 0.51	3.31 ± 1.32[a]
	20	6.81 ± 0.69[b]	6.44 ± 0.73[b]	6.82 ± 0.43[b]	7.22 ± 2.20[c]
	25	9.48 ± 0.23[c]	8.21 ± 0.58[c]	8.44 ± 0.50[c]	9.81 ± 2.06[c]

CYP (cyclophosphamide, mg/L), OSRL (Olusosun raw leachate), AERL (Aba Eku raw leachate), superscripts significantly ($p < 0.05$) different from corresponding negative control using Dunnett's multiple post hoc comparison test.

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Table 4.5 Mean (± SE) of MN induction in kidney cells of *Clarias gariepinus* exposed to OSRL and AERL.

Treatment	Conc (%)	3 days	6 days	9 days	14 days
Control	0	1.21 ± 0.04	1.10 ± 0.24	1.08 ± 0.18	1.24 ± 0.11
CYP	20	11.83 ± 1.50[c]	11.21 ± 1.86[c]	12.03 ± 0.09[c]	12.52 ± 1.16[c]
OSRL	5	2.83 ± 0.37	2.40 ± 0.68	2.95 ± 0.58	3.22 ± 0.86
	10	3.43 ± 0.87	3.07 ± 0.15[a]	3.20 ± 0.86	4.06 ± 0.63[a]
	15	3.85 ± 0.32[a]	3.40 ± 0.86	3.72 ± 0.36[a]	5.29 ± 0.32[a]
	20	8.20 ± 0.66[b]	6.98 ± 0.99[b]	7.87 ± 0.47[b]	8.91 ± 0.21[b]
	25	12.61 ± 1.03[c]	13.04 ± 1.12[c]	12.20 ± 1.18[c]	13.61 ± 1.28[c]
AERL	5	2.71 ± 0.51	2.32 ± 0.73	2.60 ± 0.51	3.06 ± 0.95
	10	3.29 ± 0.71	2.80 ± 0.58	3.03 ± 0.98	3.43 ± 0.87
	15	3.61 ± 1.52	3.25 ± 0.73	3.47 ± 0.93	4.41 ± 1.47[a]
	20	7.82 ± 1.02[b]	7.27 ± 1.63[b]	7.68 ± 1.12[b]	8.24 ± 1.24[b]
	25	12.09 ± 1.73[c]	11.22 ± 1.60[c]	12.09 ± 1.38[c]	12.60 ± 1.69[c]

CYP (cyclophosphamide, mg/L), OSRL (Olusosun raw leachate), AERL (Aba Eku raw leachate), superscripts significantly ($p < 0.05$) different from corresponding negative control using Dunnett's multiple post hoc comparison test.

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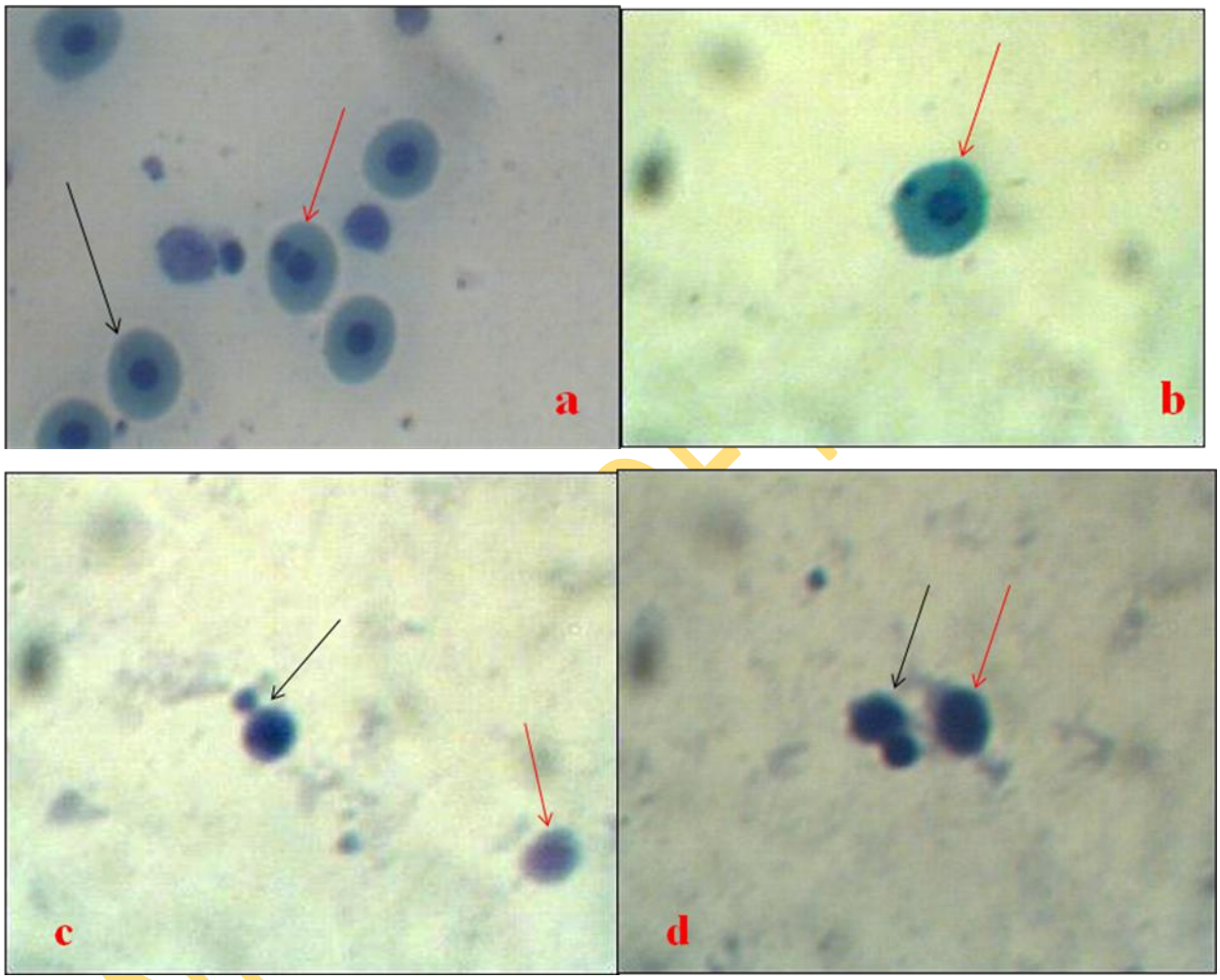

Figure 4.1 Micronucleated peripheral erythrocytes, kidney cell and gill epithelial cell (arrows) observed in *Clarias gariepinus* exposed to Olusosun and Aba-Eku landfill raw leachates:

(a) normal (black arrow) and micronucleated (red arrow) peripheral erythrocytes.

(b) micronucleated peripheral erythrocytes (red arrow).

(c) normal (red arrow) and micronucleated (black arrow) kidney cell.

(d) normal (red arrow) and micronucleated (black arrow) gill epithelial cell.

4.3.2 Micronuclei induced by OSRL and AERL in *Coturnix japonica*

The results of the MN studies in *C. japonica* are shown on Table 4.6. The incidence of MN induction in peripheral erythrocytes of male Japanese quails exposed to 10, 25 and 50 % concentrations of OSRL and AERL showed concentration dependent significant ($p < 0.05$) increase in MN formation in both leachate samples. Only the 25 and 50 % concentrations of the leachates were significantly different from the negative control ($p < 0.05$) as determined using Dunnett's multiple posthoc analysis and a positive correlation was obtained between the incidence of MN induction and leachate exposure. Also, the frequency of micronucleated (MNed) erythrocytes in the bone marrow of leachate treated *C. japonica* was concentration dependent and significantly ($p < 0.05$) increased. Dunnett's multiple posthoc test analysis showed that only 50 % concentration of both leachate samples were significant ($p < 0.05$). There is a positive correlation between leachate treatments and MN formation in bone marrow cells. The results also showed that more MNed erythrocytes were induced in peripheral erythrocytes than bone marrow cells from the same *C. japonica*. OSRL induced more MN in the peripheral erythrocytes and bone marrow cells than AERL at the same concentrations. Figure 4.2 shows MNed erythrocyte in the peripheral blood and bone marrow of Japanese quail.

4.3.3 Nuclear Abnormalities induced by OSRL and AERL in *Clarias gariepinus* and *Coturnix japonica*

Nuclear abnormality (NA) and total nuclear abnormality (TNA) frequencies in peripheral erythrocytes of *C. gariepinus* and *C. japonica* treated with different concentrations of OSRL and AERL and the corresponding negative and positive controls are summarised in tables 4.7 – 4.9. Tables 4.7 and 4.8 show that NA and TNA frequencies in *C. gariepinus* erythrocytes increased with leachate concentrations and time of exposure. Binucleated cells (BN) were most common among the NAs scored in leachate exposed *C. gariepinus*. One way Analysis of variance showed that BN formation was significant ($p < 0.05$) and comparing individual concentrations of the leachates with negative control showed that except for 5 % concentration, BN formation was significant at the different concentrations of the leachate samples ($p < 0.05$) and was also time dependent. Other observed nuclear abnormalities were not significantly ($p > 0.05$) different from the negative control, but the total nuclear abnormalities (TNA) was significant ($p < 0.05$) and

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increased with leachate concentrations and time of exposure. The frequencies of NAs occurred in the order: BN > BL > LB > NT.

Table 4.6 Mean (± SE) of MN induction in peripheral erythrocytes and bone marrow cells of *Coturnix japonica* exposed to OSRL and AERL.

Treatment	Conc (%,v/v)	Mean ± SE	Lower	Upper	
		PERIPHERAL ERYTHROCYTES			
Dist. water	0	0.40 ± 0.24	-0.2801	1.080	
CYP	40	9.60 ± 1.40[c]	5.713	13.49	
OSRL	10	2.20 ± 0.80	-0.0211	4.421	p = 0.0002
	25	3.60 ± 0.81[a]	1.344	5.865	r^2 = 0.6616
	50	5.60 ± 1.75[a]	0.7432	10.46	F = 9.774
AERL	10	1.60 ± 0.75	-0.477	3.678	p < 0.0001
	25	2.80 ± 1.16[a]	-0.4140	6.014	r^2 = 0.6836
	50	4.60 ± 1.44[a]	0.6150	8.585	F = 10.80
		BONE MARROW ERYTHROCYTES			
Dist. water	0	0.20 ± 0.08	-0.0267	0.4261	
CYP	40	3.19 ± 0.59[c]	1.528	4.850	
OSRL	10	1.00 ± 0.15	0.5855	1.409	p = 0.0006
	25	1.73 ± 0.32	0.8360	2.630	r^2 = 0.6106
	50	2.13 ± 0.57[a]	0.5411	3.724	F = 7.839
AERL	10	0.73 ± 0.12	0.3857	1.079	p = 0.0004
	25	1.40 ± 0.71	0.4561	2.342	r^2 = 0.6231
	50	3.19 ± 0.60[a]	0.2881	3.303	F = 8.265

Superscripts are significant ($p < 0.05$) different from corresponding negative control using Dunnett's multiple post hoc comparison test.

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CYP (40 mg/kg/bw) = cyclophosphamide.

OSRL = Olusosun raw leachate.

AERL = Aba Eku raw leachate.

Dist. water = Distilled water.

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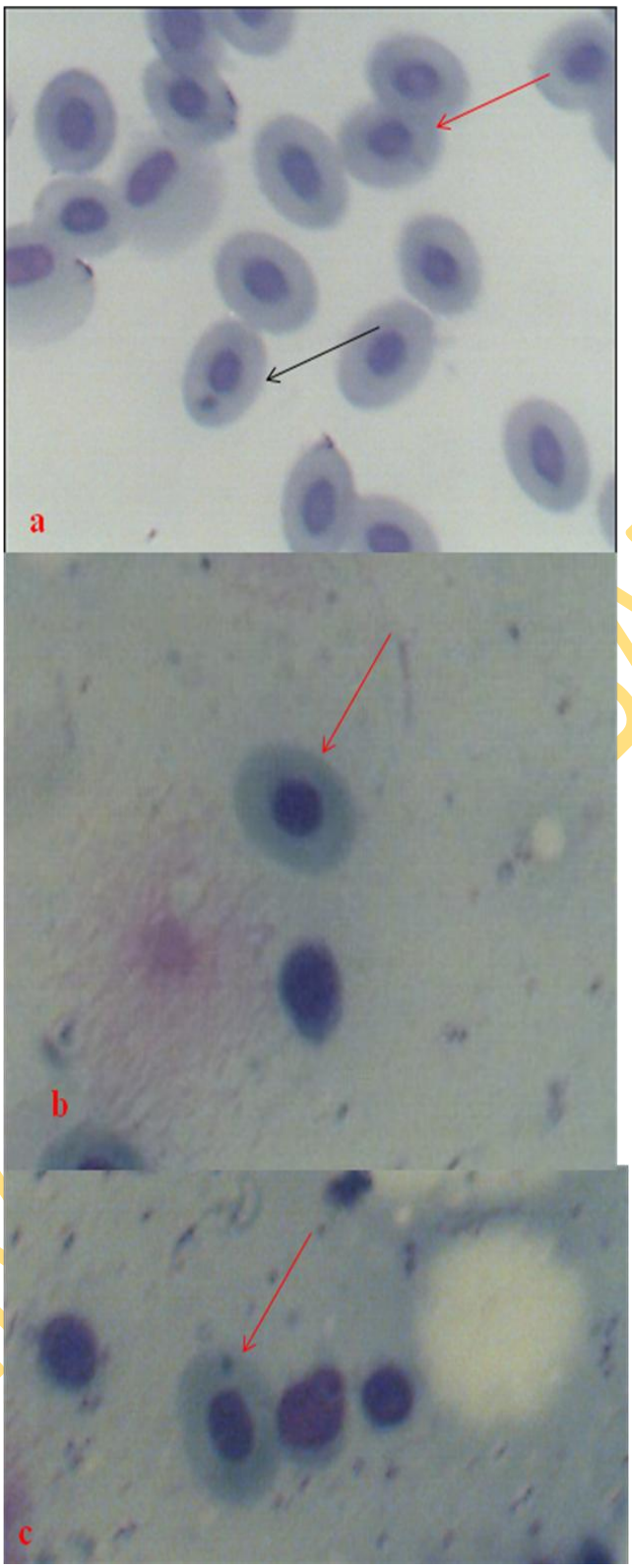

Figure 4.2 Peripheral and bone marrow erythrocytes of leachate treated Japanese quail:

(a) normal peripheral (black arrow) and micronucleated (red arrow) erythrocytes.

(b) normal bone marrow erythrocytes (red arrow).

(c) micronucleated bone marrow erythrocytes (red arrow).

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The different nuclear abnormalities observed in the peripheral erythrocytes of OSRL and AERL treated *Coturnix japonica* are binucleated cell (BN), cell nucleus with bud (BudN), cell with two lobe nucleus (TLN) and cell with tail nucleus (TN) induced different types of nuclear abnormalities in peripheral erythrocytes. The frequencies of these NAs including the TNA were significantly higher than their corresponding negative controls ($p < 0.05$). Correlation between OSRL and AERL exposure and the induction of TNA was high ($p < 0.0001$, $F = 8.69$, $r = 0.71$).

4.3.4 Micronuclei induced by Olusosun and Aba Eku landfill leachates in *Mus musculus*

The results of the micronucleus test in Olusosun and Aba Eku landfill leachates treated *M. musculus* are presented in Table 4.10. The frequencies of the micronucleated polychromatic erythrocyte (MNPCE) induced in the bone marrow cells of male and female mice exposed to 1 – 25 % concentrations of the leachates were significantly ($p < 0.05$) higher compared to the negative control. Comparing the different treatment groups with the negative control using Dunnett's Multiple Posthoc Test was significant ($p < 0.05$). Mned PCE induced in the bone marrow cells was higher in females than males; analysis using two way ANOVA showed that the effect of sex in the induction of MNPCE was significant ($p < 0.05$), Although, the percentage contributions of leachates in the induction of MNPCE (97.07 %; $p<0.0001$ for the raw leachates and 96.46 %; $p<0.0001$ for the simulated leachates, of the total variance) was rather higher than sex (0.62 %; $p = 0.0010$ for raw leachates and 1.04 %; $p = 0.0001$ for simulated leachates) and the interactions of leachate and sex (0.44 %; $p = 0.7701$ for raw leachates and 0.72 %; $p = 0.2447$ for simulated leachates).

The frequencies of MNPCE in the peripheral blood cells of exposed mice were higher compared to the negative control group. One way ANOVA showed that increase in MNPCE induction in the peripheral erythrocytes of leachate treated *Mus musculus* is significant ($p < 0.05$). Comparing MNPCE formation in the different concentrations of the leachate treatment with the negative control using Dunnett Multiple Posthoc Test was significant ($p < 0.05$). MN PCE induction in peripheral blood of leachate treated mice was higher in females than males and using two way ANOVA showed that the effect of sex in the induction of MNPCE was significant (1.39 %; $p = 0.0011$ for raw leachates, 0.63 %; $p = 0.7158$ for simulated leachates) only for the raw leachates. Although, the percentage contribution of leachates in the induction of the MNPCE

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Table 4.7 Mean (± SE) of nuclear abnormalities and total nuclear abnormality induced in peripheral blood of *Clarias gariepinus* exposed to OSRL

NA	Duration (days)	Concentrations (%, v/v)						
		Tap water	CYP	5	10	15	20	25
BN	3	1.20 ± 0.58	3.80 ± 0.58[a]	2.40 ± 0.81	3.40 ± 1.08[a]	3.80 ± 1.80[a]	4.20 ± 1.39[a]	6.21 ± 1.56[b]
	6	1.40 ± 0.51	4.60 ± 1.60[a]	3.00 ± 0.55[a]	3.40 ± 0.68[a]	4.20 ± 1.39[a]	4.80 ± 1.07[a]	6.60 ± 1.75[b]
	9	1.20 ± 0.49	5.40 ± 1.57[a]	2.40 ± 0.60	3.20 ± 0.97[a]	4.20 ± 1.87[a]	5.00 ± 1.27[a]	6.00 ± 1.14[b]
	14	1.80 ± 0.66	5.20 ± 1.66[a]	3.40 ± 0.81[a]	3.80 ± 0.86[a]	4.40 ± 0.81[a]	5.60 ± 1.44[a]	6.80 ± 1.46[b]
LB	3	0.40 ± 0.24	3.00 ± 0.32[a]	1.80 ± 0.58	2.40 ± 0.68	2.00 ± 0.32	3.00 ± 0.45[a]	3.40 ± 0.68[a]
	6	1.00 ± 0.45	3.40 ± 0.40[a]	2.00 ± 0.55	2.00 ± 0.32	2.40 ± 0.51	2.80 ± 0.37	3.80 ± 0.86[a]
	9	0.80 ± 0.37	3.20 ± 0.20[a]	1.40 ± 0.24	2.20 ± 0.20	2.00 ± 0.55	3.20 ± 0.66[a]	3.80 ± 0.66[a]
	14	1.20 ± 0.37	3.60 ± 0.60[a]	2.20 ± 0.66	2.60 ± 0.4	2.80 ± 0.73	3.40 ± 0.68[a]	4.00 ± 0.63[a]
NT	3	0.60 ± 0.24	1.80 ± 0.20	0.80 ± 0.20	0.60 ± 0.24	1.60 ± 1.24	2.00 ± 0.32	3.00 ± 0.32[a]
	6	0.60 ± 0.24	1.80 ± 0.37	0.60 ± 0.24	1.00 ± 0.32	1.20 ± 0.37	1.20 ± 0.20	2.40 ± 0.40[a]
	9	0.40 ± 0.24	1.80 ± 0.37	0.40 ± 0.24	1.20 ± 0.20	1.60 ± 0.24	2.00 ± 0.32[a]	2.20 ± 0.20[a]
	14	0.40 ± 0.24	1.80 ± 0.24	1.00 ± 0.32	1.80 ± 0.20	2.40 ± 0.24[a]	3.00 ± 0.32[a]	3.20 ± 0.37[a]
BL	3	0.30 ± 0.20	3.60 ± 0.51[a]	1.60 ± 0.24	1.60 ± 0.24	2.20 ± 0.20[a]	3.20 ± 0.37[a]	4.00 ± 0.45[b]
	6	0.60 ± 0.40	3.20 ± 0.37[a]	1.80 ± 0.37	1.80 ± 0.37	2.40 ± 0.60	3.00 ± 0.84[a]	4.20 ± 0.58[b]
	9	0.60 ± 0.24	3.80 ± 0.73[a]	1.20 ± 0.37	1.80 ± 0.49	1.80 ± 0.37	2.60 ± 0.24[a]	3.40 ± 0.81[a]
	14	0.80 ± 0.37	4.40 ± 0.81[a]	2.20 ± 0.34	2.60 ± 0.24	3.20 ± 0.20[a]	4.20 ± 0.37[a]	4.80 ± 0.86[b]
TNA	3	3.00 ± 1.08	15.25 ± 2.25[c]	8.25 ± 1.65[b]	9.75 ± 3.04[b]	12.00 ± 2.42[c]	15.50 ± 2.26[c]	20.75 ± 3.57[c]
	6	4.25 ± 0.75	16.25 ± 2.87[c]	9.25 ± 2.46[b]	9.75 ± 2.63[b]	12.75 ± 3.09[c]	15.50 ± 3.12[c]	21.25 ± 4.37[c]
	9	3.75 ± 0.85	17.75 ± 0.85[c]	6.75 ± 2.06[b]	10.50 ± 2.10[b]	12.00 ± 3.03[c]	16.00 ± 3.24[c]	19.25 ± 3.97[c]
	14	5.25 ± 1.49	19.50 ± 2.99[c]	11.00 ± 2.45[b]	12.50 ± 2.50[b]	16.00 ± 2.16[c]	19.75 ± 2.87[c]	23.50 ± 3.86[c]

Superscripts significantly ($p < 0.05$) different from the corresponding negative control using Dunnett's multiple posthoc comparison test.

CYP = Cyclophosphamide (20 mg/L).

BN = Binucleated cells.

NT = Notch nucleus.

BL = Blebb nucleus.

LB = Lobe nucleus.

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TNA = total nuclear abnormality.

Table 4.8 Mean (± SE) of nuclear abnormalities and total nuclear abnormality induced in peripheral blood of *Clarias gariepinus* exposed to AERL

NA	Duration (days)	Concentrations (%, v/v) Tap water	CYP	5	10	15	20	25
BN	3	1.20 ± 0.58	3.80 ± 0.58[a]	2.40 ± 0.87	3.40 ± 0.51[a]	3.80 ± 1.83[a]	4.00 ± 0.71[a]	5.60 ± 2.23[b]
	6	1.40 ± 0.51	4.60 ± 1.60[a]	3.20 ± 0.86	3.60 ± 0.98[a]	4.00 ± 1.41[a]	4.60 ± 1.75[a]	6.20 ± 1.56[b]
	9	1.20 ± 0.49	5.40 ± 1.57[b]	3.60 ± 1.47	3.60 ± 1.83[a]	4.40 ± 1.60[a]	4.80 ± 1.20[a]	6.00 ± 1.58[b]
	14	1.80 ± 0.66	5.20 ± 1.66[b]	3.40 ± 1.08	3.80 ± 1.02[a]	4.60 ± 2.38[a]	5.00 ± 1.52[b]	6.60 ± 2.52[b]
LB	3	0.40 ± 0.24	3.00 ± 0.32[a]	1.40 ± 0.51	2.00 ± 0.32	2.20 ± 0.49	2.60 ± 0.51[a]	3.00 ± 0.71[a]
	6	1.00 ± 0.45	3.40 ± 0.40[a]	2.20 ± 0.20	1.80 ± 0.37	2.40 ± 0.40	2.80 ± 0.37	3.40 ± 0.51[a]
	9	0.80 ± 0.37	3.20 ± 0.20[a]	1.90 ± 0.37	2.00 ± 0.44	2.40 ± 0.68	2.40 ± 0.24	4.00 ± 0.55[b]
	14	1.20 ± 0.37	3.60 ± 0.60[a]	1.80 ± 0.17	2.20 ± 0.37	2.60 ± 1.03	3.00 ± 0.32[a]	3.80 ± 0.37[a]
NT	3	0.60 ± 0.24	1.80 ± 0.20	0.60 ± 0.24	0.80 ± 0.37	1.20 ± 0.15	1.60 ± 0.40	2.40 ± 0.40
	6	0.60 ± 0.24	1.80 ± 0.14	0.40 ± 0.24	1.00 ± 0.32	1.20 ± 0.37	1.20 ± 0.20	1.80 ± 0.48
	9	0.40 ± 0.24	1.80 ± 0.37	0.40 ± 0.24	1.00 ± 0.32	1.40 ± 0.24	1.80 ± 0.49	2.20 ± 0.37
	14	0.40 ± 0.24	1.80 ± 0.24	0.80 ± 0.24	1.60 ± 0.51	2.00 ± 0.32	2.60 ± 0.40[a]	3.00 ± 0.32[a]
BL	3	0.20 ± 0.20	3.60 ± 0.51[b]	1.40 ± 0.24	1.20 ± 0.37	2.20 ± 0.37[a]	2.80 ± 0.37[a]	3.60 ± 0.51[b]
	6	0.60 ± 0.40	3.20 ± 0.37[b]	1.40 ± 0.51	1.80 ± 0.58	2.60 ± 0.24[a]	3.00 ± 0.55[a]	4.00 ± 0.45[b]
	9	0.60 ± 0.24	3.80 ± 0.73[b]	1.60 ± 0.24	1.80 ± 0.20	2.20 ± 0.20[a]	2.80 ± 0.37[a]	3.60 ± 0.24[a]
	14	0.80 ± 0.37	4.40 ± 0.81[b]	2.40 ± 0.24[a]	2.80 ± 0.49	3.40 ± 0.50[b]	4.00 ± 0.32[b]	4.20 ± 0.58[b]
TNA	3	3.00 ± 1.08	15.25 ± 2.25[c]	7.25 ± 1.84[a]	9.25 ± 2.87[b]	11.75 ± 2.67[b]	13.75 ± 2.46[b]	18.25 ± 3.47[c]
	6	4.25 ± 0.75	16.25 ± 2.87[c]	9.00 ± 2.97[a]	10.25 ± 2.75[b]	12.75 ± 2.87[b]	14.50 ± 3.48[b]	22.67 ± 4.26[c]
	9	3.75 ± 0.85	17.75 ± 0.85[c]	9.25 ± 3.30[a]	10.50 ± 2.72[b]	13.00 ± 3.19[b]	14.75 ± 3.25[b]	19.50 ± 3.93[c]
	14	5.25 ± 1.49	19.50 ± 2.99[c]	10.50 ± 2.72[b]	13.00 ± 2.35[b]	15.75 ± 2.81[b]	18.25 ± 2.69[c]	22.25 ± 3.82[c]

Superscripts significantly ($p < 0.05$) different from corresponding negative controls using Dunnett's multiple post hoc comparison test.

CYP = Cyclophosphamide (20 mg/L).

BN = Binucleated cells.

NT = Notch nucleus.

BL = Blebb nucleus.

LB = Lobe nucleus.

TNA = total nuclear abnormality.

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Table 4.9 Mean (± SE) of nuclear abnormalities (NA) in peripheral erythrocytes of *Coturnix japonica* exposed to OSRL and AERL.

Conc. (%, v/v)		BN	TLN	BudN	TN	TNA
Dist. water	0	0.80 ± 0.49	1.20 ± 0.58	0.80 ± 0.37	1.20 ± 0.37	5.03±0.58
OSRL	10	5.40 ± 1.40[a]	6.60 ± 3.34[a]	5.20 ± 2.60[a]	4.40 ± 2.11[a]	27.14±2.27[a]
	25	7.00 ± 2.81[b]	9.60 ± 2.54[b]	8.40 ± 2.36[b]	5.60 ± 1.54[a]	40.25±5.98[c]
	50	8.60 ± 2.25[b]	11.20 ± 4.33[c]	10.40 ± 3.31[c]	8.20 ± 1.99[b]	46.02 ± 2.48[c]
AERL	10	4.20 ± 0.66[a]	5.60 ± 1.29[a]	5.60 ± 2.09[a]	4.20 ± 1.07[a]	24.50 ± 2.02[a]
	25	6.00 ± 2.07[a]	9.80 ± 1.93[b]	7.00 ± 2.17[b]	6.60 ± 1.69[a]	36. 75 ± 4.21[b]
	50	7.80 ± 1.24[b]	15.80 ± 3.97[c]	9.40 ± 1.47[c]	8.40 ± 1.72[b]	51.50 ± 9.35[c]
CYP	40	8.80± 2.27[b]	16.20 ± 1.96[c]	9.40 ± 1.83[c]	8.40 ± 1.32[b]	53.50 ± 9.22[c]

Superscripts significantly ($p < 0.05$) different from the corresponding negative control.

CYP = cyclophosphamide (40 mg/kg/bw).

BN = Binucleated cells.

TLN = Two-lobe nucleus.

BudN = Budding nucleus.

TN = Tail nucleus.

TNA = total nuclear abnormality.

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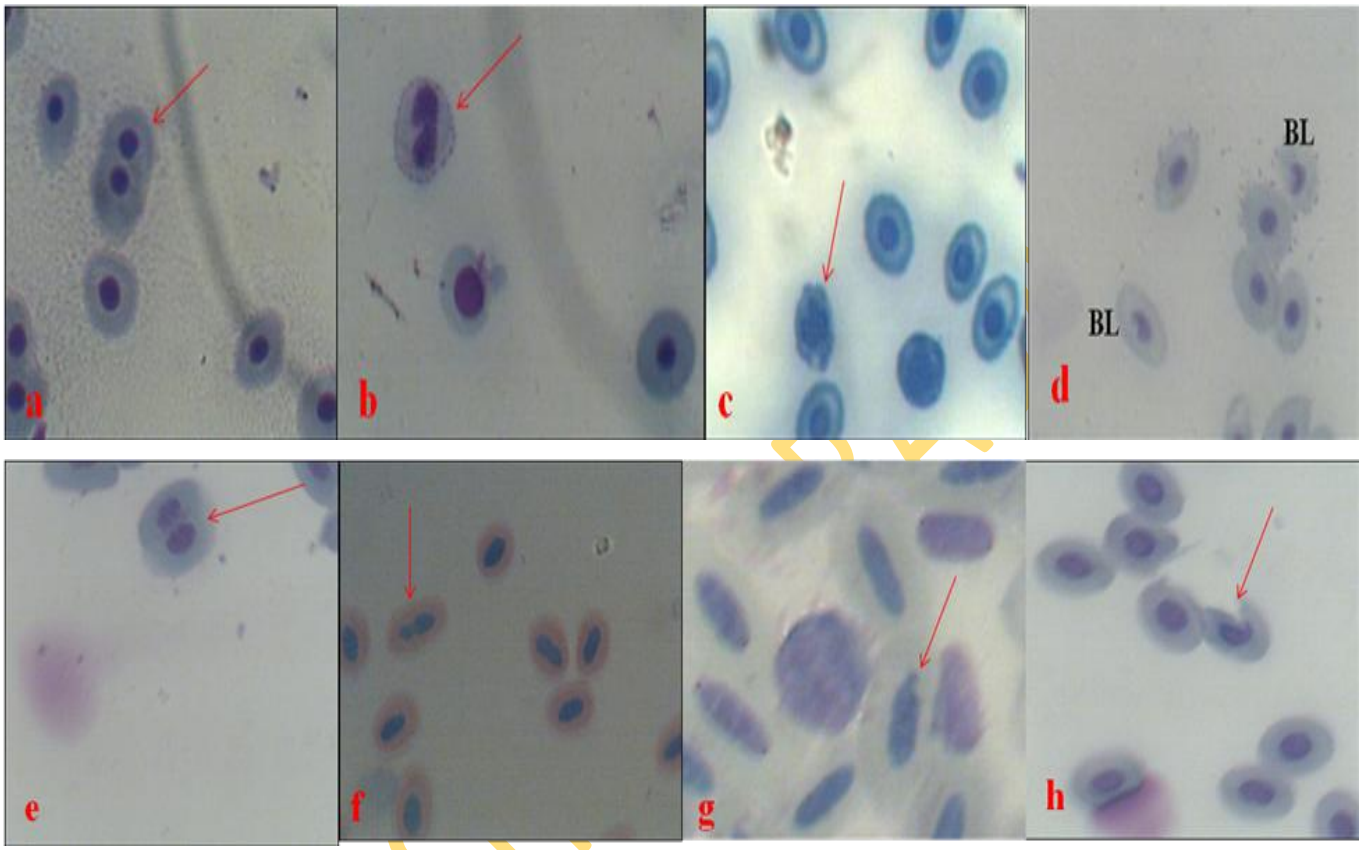

Figure 4.3 Nuclear abnormalities in peripheral erythrocytes (arrowed) observed in *C. gariepinus* (a – d) and Japanese quail (e – h) exposed to Olusosun and Aba-Eku landfill leachates:

(a) Binucleated erythrocyte

(b) Notch nucleus in the erythrocyte

(c) Lobe nucleus in the erythrocyte

(d) Blebb nucleus in the erythrocyte

(e) Binucleated erythrocyte

(f) Bilobe nucleus in the erythrocyte

(g) Bud nucleus in the erythrocyte

(h) Tail nucleus in the erythrocyte

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(93.65 %; p<0.0001 for the raw leachates and 69.97 %; p < 0.0001 for the simulated leachates) of the total variance was higher than sex and the interactions of both leachate concentrations and sex (0.75 %; p = 0.9146 for the raw leachate and 3.45 %; p 0.9794 for the simulated leachate).

The cytotoxic effects of landfill leachate as determined by the PCE / NCE ratio showed a significant (p < 0.05) reduction in all the tested concentrations of the leachates. Dunnett multiple posthoc test analysis for comparing the treated groups with negative control group showed significance (p < 0.05) at higher concentrations of the leachate treatments (Table 4.11). PCE/ NCE ratio was more reduced in males than females.

Increase in the frequencies of MNPCE induction in the bone marrow cells and peripheral blood, and the cytotoxicity induced in the bone marrow cells of Olusosun and Aba Eku landfill leachates treated *Mus musculus* are in the order OSRL > AERL > OSSL > AESL. Figure 4.4 shows polychromatic and normochromatic erythrocytes in the peripheral blood and bone marrow of *Mus musculus* and the observed MNPCE in leachate treated *Mus musculus*.

4.4. Systemic toxicity induced by Olusosun and Aba Eku landfill leachates in rat

4.4.1 Clinical signs of toxicity and mortality

During the 30 days exposure duration, 3 rats died (one male in 25 %; AERL and one female each in 5 and 25 %; OSRL groups) (table 4.12 – 4.13) and the survivors showed clear clinical signs of toxicity. These were bluish discolouration of the skin (at the nasal, ear and genital regions) within 24 hours of exposure, laboured breathing pattern and ungroomed hair. Decreased food consumption and sluggishness were mostly observed among rats exposed to 5, 10 and 25 % of the leachates within the second and third weeks of exposure period. During the fourth week of exposure, frequent sneezing, hair loss (figure 4. 5c), diarrhea, weakness (shown by reduced activities) and decrease food and water consumption were higher among the treated and CYP groups. A male rat in the 25 % AERL group had its eyeball bulged out of the socket, while a female in the 5 % OSRL group had abscess on the neck region (Figure 4.5a) and another male in the 10 % OSRL had abscess which later turned skin lesion on the thigh (figure 4.5b). Another female in the 5 % OSRL and two males, one from 5 % OSRL and the other 25 % AERL groups were unable to move about normally. The female later died in the penultimate day to the termination of the experiment. The reported signs of leachate toxicity seemed not to be sex specific but were more observed in rats exposed to higher concentrations of the leachates. Also

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Table 4.10. Frequencies of MNPCE in bone marrow and peripheral blood cells of *Mus musculus* exposed to Olusosun and Aba-Eku landfill leachates.

Conc (%, v/v)	OSRL	OSSL	AERL	AESL	OSRL	OSSL	AERL	AESL
	BONE MARROW CELL				PERIPHERAL BLOOD CELL			
	MALE							
DW	1.67±0.44	1.67±0.44	1.67±0.44	1.67±0.44	1.00±0.58	1.00±0.58	1.00±0.58	1.00±0.58
1	5.53±0.58	4.50±0.29[a]	6.50±0.29	6.12±0.13	3.67±0.45	2.05±0.14[a]	3.01±0.65	2.63±0.18[a]
2.5	7.10±0.13	6.04±0.16[a]	8.21±0.68[a]	6.00±1.14[a]	4.50±0.69	2.53±0.62[a]	4.00±2.11[a]	2.51±1.01[a]
5	9.91±0.47	6.07±0.76[a]	8.56±0.43[a]	7.65±1.61[a]	7.35±1.02	3.39±0.14[a]	6.12±1.16[a]	3.16±0.61[a]
10	12.21±0.04[a]	7.33±0.33[a]	11.50±1.81[a]	8.65±0.29[a]	10.02±1.21	4.61±0.46[a]	10.53±1.68[a]	4.10±1.12[a]
25	18.50±0.01[a]	9.12±0.16[a]	18.10±1.31[a]	9.67±0.44[a]	15.15±0.58	6.68±0.81[a]	7.01±.0.39[a]	5.21±0.39[a]
CYP	17.61±0.87	17.61±0.87[a]	17.61±0.81[a]	17.61±0.81[a]	12.25±1.32	12.25±1.32[a]	12.25±1.32[a]	12.25±1.32[a]
	FEMALES							
DW	1.93±0.17	1.93±0.17	1.93±0.17	1.93±0.17	1.25±0.36	1.25±0.36	1.25±0.36	1.25±0.36
1	6.00±0.29	5.52±0.11[a]	7.08±0.35[a]	6.17±0.43	5.01±2.01	2.36±1.07[a]	3.00±2.65[a]	3.01±1.06[a]
2.5	7.67±0.73	7.06±0.61[a]	8.16± 0.29[a]	7.26±1.97[a]	6.21±1.16	2.58±0.31[a]	6.02±1.16[a]	3.52±1.16[a]
5	10.32±0.31	6.50±0.72[a]	9.50±0.58[a]	7.83±0.33[a]	8.53±1.77	3.16±0.15[a]	7.06±1.30[a]	3.54±1.00[a]
10	13.01±0.58[a]	7.17±0.93[a]	12.50±1.61[a]	9.61±0.36[a]	12.03±2.31[a]	5.19±1.03[a]	11.51±2.11[a]	5.56±0.31[a]
25	20.25±0.06[a]	10.53±1.17[a]	19.50±2.01[a]	10.17±0.47[a]	18.21±1.96[a]	8.01±0.61[a]	18.26±1.71[a]	6.21±1.11[a]
CYP	18.71±1.44	18.71±1.44[a]	18.71±1.44[a]	18.71±1.44[a]	13.00±1.12	13.00±1.12	13.00±1.12	13.00±1.12

Superscripts significantly ($p < 0.05$) different from the corresponding negative controls using Dunnett's multiple post hoc comparison test. CYP = Cyclophosphamide (40 mg/kg/bw). DW = distilled water.

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Table 4.11 Mean (± SE) of PCE to NCE ratio (PCE/NCE) in 1000 bone marrow cells of male and female mice exposed to OSRL, AERL, OSSL and AESL.

Concentrations	OSRL	AERL	OSSL	AESL
		MALE		
Dist. Water	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01
1.0%	0.94±0.11*	0.96±0.02*	0.97±0.73*	0.97±0.01*
2.5%	0.91±0.22*	0.93±0.01*	0.96±0.02*	0.97±0.02*
5.0%	0.66±0.01a	0.69±0.12a	0.71±0.12a	0.72±0.41a
10.0%	0.52±0.40a	0.56±0.47a	0.63±0.01b	0.66±0.56a
25.0%	0.33±0.91c	0.41±0.21c	0.47±0.52b	0.51±0.01a
CYP	0.40±0.38c	0.40±0.38c	0.40±0.38c	0.40±0.38c
		FEMALE		
Dist. Water	1.06±0.71	1.06±0.71	1.06±0.71	1.06±0.71
1.0%	0.97±0.01*	0.97±0.23*	0.94±0.02*	0.98±0.01*
2.5%	0.97±0.02*	0.99±0.02*	0.96±0.01*	0.97±0.21*
5.0%	0.74±0.91*	0.78±0.01*	0.86±0.03*	0.87±0.13*
10.0%	0.61±0.19a	0.64±0.57a	0.74±0.71a	0.76±0.02a
25.0%	0.42±0.01b	0.48±0.10b	0.53±0.01a	0.54±0.14a
CYP	0.43±0.01b	0.43±0.01b	0.43±0.01b	0.43±0.01b

Superscripts are significantly ($p < 0.05$) different from the corresponding negative control.

CYP = Cyclophosphamide (40 mg/kg/bw) (positive control).

Dist. Water = Distilled water (negative control)

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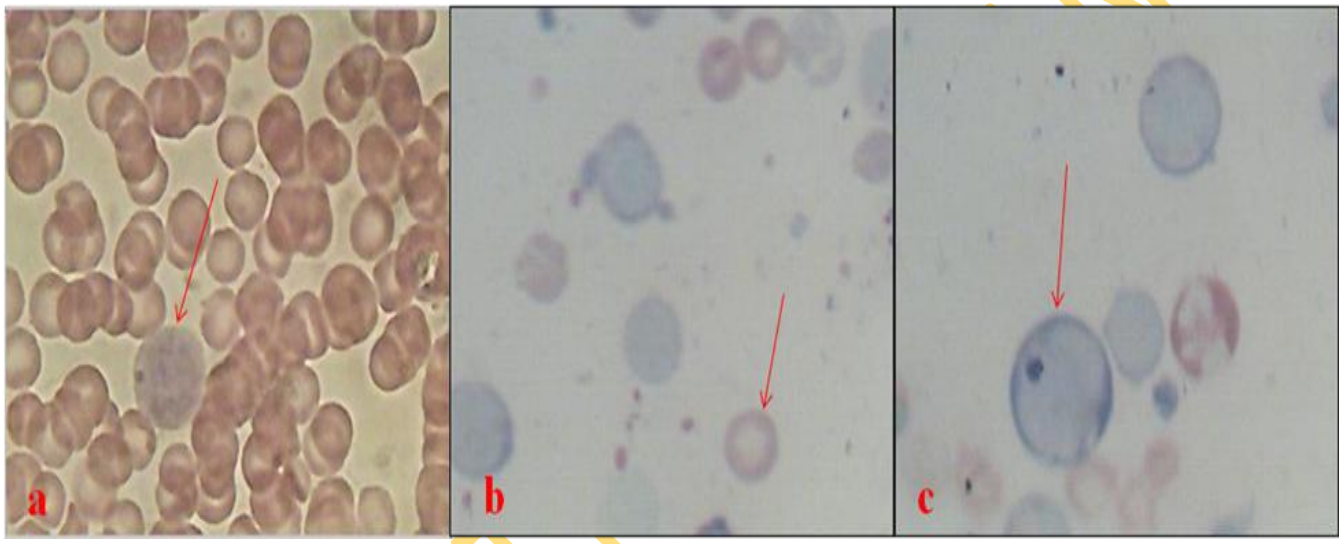

Figure 4.4 Micronucleated polychromatic erythrocytes (PCE) in peripheral blood cell and bone marrow cells (arrows) in *Mus musculus* exposed to Olusosun and Aba-Eku landfill leachates:

(a) micronucleated PCE in peripheral blood cell

(b) normochromatic erythrocyte (NCE).

(c) micronucleated PCE in bone marrow cell.

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raw leachates (OSRL and AERL) were more toxic than the simulated leachates (OSSL and AESL) as shown by the different clinical symptoms expressed by the exposed rats.

4.4.2. Body weight and terminal body weight gain of leachate treated rats

The terminal body weight gain in leachate treated rats at the end of 30 days exposure showed concentration dependent and significant ($p < 0.05$) decrease compared to the negative control for OSRL and AERL exposed rats (table 4.12 and 4.13). While for OSSL and AESL treated rats, a concentration independent decrease in the terminal body weight gain compared to the negative control was observed (table 4.12 and 4.13). Considering that the terminal body weight gain of corresponding negative control rats is 100 %, the percentage terminal body weight gain of male rats exposed to 1.0 % concentrations of OSRL (33.35 %) was higher than that of AERL (32.54 %). But at the highest concentrations of the leachates, male rats of AERL (38.94 %) had a lower percentage terminal body weight gain than that of OSRL (39.13 %). Also considering female rats, at the lowest concentration of 1.0 %, female rats exposed to OSRL (31.13 %) had a lower percentage decrease than AERL (29.98 %) treated rats. And for the highest concentration of 25 %, female rats treated with OSRL (36.17 %) had decreased terminal body weight gain than AERL (35.25 %). These values showed that female rats were more affected by the raw leachates than the males. Similar trend of decrease in terminal body weight gain was observed in rats exposed to the simulated leachates.

The weekly body weight gain in rats exposed to the different concentrations of OSRL, OSSL, AERL and AESL are presented in tables 4.14 – 4.17. There was a constant decrease in the body weight gain of rats exposed to OSRL and AERL when compared to the negative control during each week of the exposure duration. The decrease was significant ($p < 0.05$) from the second week of exposure and with rats exposed to higher concentrations of the leachates (mostly 10 and 25 %) (tables 4.14 and 4.15). Body weight gain of OSRL exposed rats was lower than those of AERL and female rats were more affected than the corresponding males. Two–way ANOVA showed the effects of the tested leachate samples (52.51%, $F = 21.15$, $p<0.0001$) to be significant in the causation of decrease body weight gain than sex (0.73%, $F = 0.5888$, $p = 0.6236$) and interaction between sex and leachates (0.41%, $F = 0.0544$, $p = 1.0000$). Similarly, there was decrease in body weight gain in rats exposed to OSSL and AESL rats for the four weeks exposure durations but this decrease was concentration independent unlike in OSRL and AERL treated rats. Also decrease in body weight of rats treated with OSSL and AESL was

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significantly ($p < 0.05$) different from the corresponding negative controls only at the fourth week of exposure and the female rats more affected than the males (tables 4.16 and 4.17).

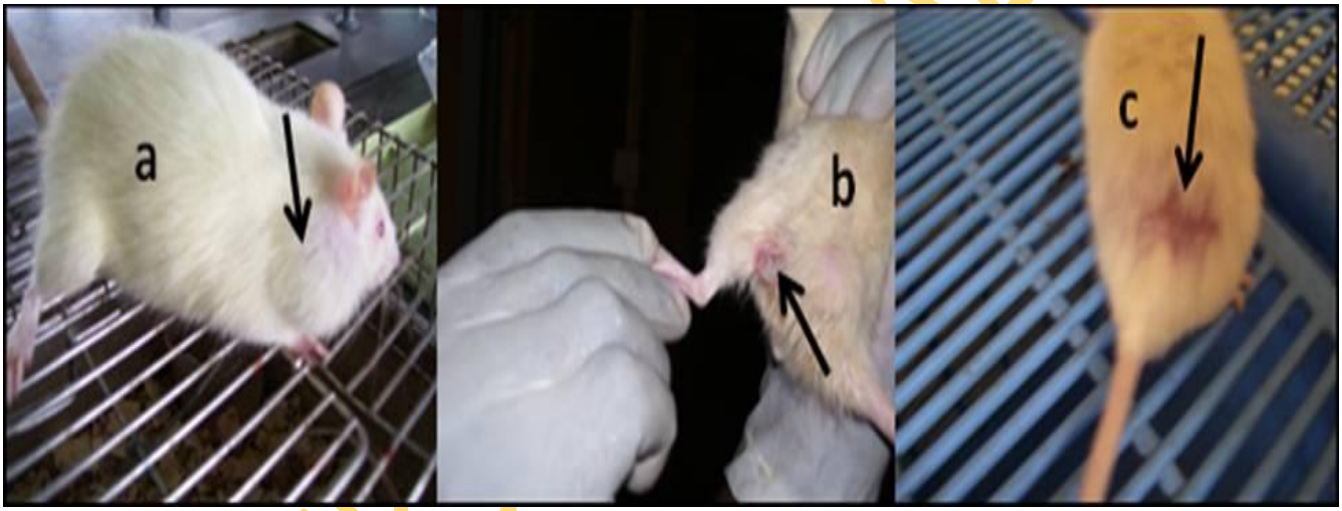

Figure 4.5. Some clinical signs of toxicity observed in Olusosun and Aba-Eku leachate treated rats:

(a) Abscess on the neck of a female rat exposed to 5% OSRL.

(b) Sore on the thigh of the right leg of a male rat exposed to 10 % AERL.

(c) Hair loss in rat treated with 25 % AERL.

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4.4.3 Absolute and relative organ weight gain in leachate treated rats

Thymus: Tables 4.18 and 4.19 show the absolute and relative thymus weight gain in rats exposed to different concentrations of OSRL, OSSL, AERL and AESL. There was concentration dependent and significant ($p < 0.05$) decrease in absolute and relative thymus weight gain in both male and female leachate treated rats compared with negative control rats. Comparing the absolute and thymus weight gain of rats exposed to the different concentrations of the leachates with the corresponding negative control groups using Dunnett's multiple posthoc test showed that the absolute weight gain of male and female rats exposed to OSRL and AERL were significantly ($p < 0.05$) different from the control while only 25 % (male) of AESL and 10 and 25 % (female) of OSSL treated rats were significantly ($p < 0.05$) different from the corresponding negative control.

The relative thymus weight gain in leachate treated rats were insignificantly ($p > 0.05$) different from the corresponding negative control group. Absolute and relative thymus weight gains are lower in leachate treated female rats than the male rats in all the tested leachate samples. Also, absolute and relative weight gain in OSRL and AERL treated rats were lower than those of OSSL and AESL treated rats.

Spleen weight gain

Tables 4.20 and 4.21 show the absolute and relative spleen weight gain of rats exposed to different concentrations of OSRL, OSSL, AERL and AESL. There was significant ($p < 0.05$) increase in both the absolute and relative spleen weight gain of leachate treated rats. Increase in absolute spleen weight gain was only significant ($p < 0.05$) at the 5, 10 and 25 % (OSRL), 25 % (AERL) and 25 % (OSSL) treated male rats and 1 – 25 % (OSRL), 5 – 25 % (AERL) treated female rats, when compared with the corresponding negative control groups,. While for the relative spleen weight gain 5 – 25 % (OSRL), 1 – 25 % (AERL) and 25 % (OSSL) treated male rats and 2.5 – 25 % OSRL and AERL treated female rats were significant ($p < 0.05$), compared to the corresponding negative control groups. Male rats showed higher value for absolute and

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relative spleen weight gain in leachate treated rats than female rats. Also absolute and relative spleen weight gains were higher in OSRL and AERL treated rats than in OSSL and AESL.

Table 4.12. Survival and terminal body weight gain in OSRL and OSSL treated rats for 30 days

Concentration (0.5ml /rat/day)	Survival		Terminal body weight (g)		Body weight gain (%)	
	OSRL	OSSL	OSRL	OSSL	OSRL	OSSL
			MALES			
Dist. water	5/5	5/5	196.70 ± 3.30	174.90 ± 4.30	100.00	100.00
1.0%	5/5	5/5	131.10 ± 1.75^{b}	155.80 ± 2.65^{a}	66.65	89.08
2.5%	5/5	5/5	132.40 ± 2.12^{b}	161.70 ± 3.45	67.31	92.45
5.0%	5/5	5/5	129.50 ± 17.22^{b}	160.91 ± 17.22	65.84	92.00
10.0%	5/5	5/5	126.60 ± 10.47^{b}	152.80 ± 12.33^{a}	64.36	87.36
25.0%	5/5	5/5	121.70 ± 20.94^{c}	149.76 ± 1.74^{a}	61.87	85.63
CYP	5/5	5/5	137.10 ± 1.62^{b}	159.30 ± 6.34^{a}	69.70	91.08
			FEMALES			
Dist. water	5/5	5/5	185.80 ± 3.12	162.30 ± 0.22	100.00	100.00
1.0%	5/5	5/5	127.90 ± 1.30^{b}	146.80 ± 2.21^{a}	68.83	90.45
2.5%	5/5	5/5	124.00 ± 2.36^{b}	149.20 ± 5.81	66.74	91.93
5.0%	4/5	5/5	125.60 ± 21.36^{b}	152.46 ± 2.41	67.60	93.94
10.0%	5/5	5/5	120.80 ± 24.74^{b}	140.90 ± 4.54^{a}	65.02	86.81
25.0%	4/5	5/5	118.60 ± 0.70^{b}	142.87 ± 1.97^{a}	63.83	88.03
CYP	5/5	5/5	135.50 ± 12.40^{b}	141.72 ± 11.6^{a}	72.93	87.32

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Values are in mean (± SE). Superscripts differ significantly from corresponding control value using (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) Dunnett's multiple comparison posthoc test.

Dist. water = Distilled water (negative control)

CYP = Cyclophosphamide (40 mg/kg/bw) (positive control)

Table 4.13 Survival and terminal body weight gain of rats exposed to AERL and AESL for 30 days.

Concentration (0.5ml /rat/day)	Survival		Terminal body weight (g)		Body weight gain (%)	
	AERL	AESL	AERL	AESL	AERL	AESL
MALES						
Dist. water	5/5	5/5	196.70 ± 3.30	174.90 ± 4.30	100.00	100.00
1.0%	5/5	5/5	132.70 ± 1.47^{b}	150.90 ± 3.22^{a}	67.46	86.28
2.5%	5/5	5/5	133.10 ± 16.55^{b}	151.60 ± 1.71^{a}	67.67	86.68
5.0%	5/5	5/5	134.90 ± 2.54^{b}	152.80 ± 5.11^{a}	68.58	87.36
10.0%	5/5	5/5	130.70 ± 11.34^{b}	150.98 ± 3.40^{a}	66.45	86.32
25.0%	4/5	5/5	120.10 ± 16.75^{c}	150.30 ± 8.26^{a}	61.06	85.93
CYP	5/5	5/5	137.10 ± 1.62^{b}	159.30 ± 6.34^{a}	69.70	91.08
FEMALES						
Dist. water	5/5	5/5	185.80 ± 3.12	162.30 ± 0.22	100.00	100.00
1.0%	5/5	5/5	130.10 ± 4.50^{b}	149.71 ± 1.00	70.02	92.24
2.5%	5/5	5/5	131.50 ± 8.17^{b}	145.83 ± 6.52^{a}	70.78	89.85
5.0%	5/5	5/5	125.40 ± 3.76^{b}	150.60 ± 2.14	67.49	92.79
10.0%	5/5	5/5	123.90 ± 3.43^{b}	139.40 ± 4.12^{a}	66.68	85.89
25.0%	5/5	5/5	120.30 ± 3.58^{b}	148.96 ± 6.12^{a}	64.75	91.78

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CYP	5/5	5/5	135.50 ± 12.4	141.72 ± 11.6[a]	72.93	87.32

Values are in mean (± SE). Superscripts differ significantly from corresponding control value using (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = Cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.14 Weekly body weight gain in rats exposed to OSRL for 30 days.

Concentration 0.5ml/rat/day	Initial body weight (g)	1st week	2nd week	3rd week	4th week
MALES					
Dist. Water	87.81 ± 5.68	129.98 ± 8.05	138.95±11.62	161.10 ± 2.59	191.29±4.73
1.0%	71.60 ± 2.29	106.43 ± 3.51	117.48 ± 1.27[a]	123.32 ± 1.77[a]	132.04±11.76[a]
2.50%	81.72 ± 32.12	113.52 ± 12.24	121.04 ± 8.29[a]	129.84 ± 7.25[a]	133.19±6.01[a]
5.0%	79.36 ± 8.20	99.65 ± 10.54	112.36 ± 3.72[a]	120.53±11.01[a]	127.73±7.84[b]
10.0%	89.26 ± 2.69	101.46 ± 4.95	117.03 ± 8.49[a]	121.41±10.85	125.87±11.43[b]
25.0%	78.33 ± 1.16	102.53 ± 3.93	110.11 ± 1.59[a]	119.12±2.65[b]	121.60±5.05[c]
CYP.	83.27 ± 6.96	106.04 ± 7.23	121.43± 5.62	130.31±6.80[a]	136.23±7.87[b]
FEMALES					
Dist. Water	97.78 ± 7.35	117.44 ± 5.30	139.13 ± 3.34	158.14±10.28	183.26±10.17
1.0%	79.57 ± 4.19	90.75 ± 3.36	108.58 ± 4.35[a]	116.52±6.91[a]	125.11±3.81[a]
2.50%	80.03 ± 5.60	94.07 ± 6.38	106.52 ± 7.81[a]	117.79±8.44[a]	123.46±5.29[a]
5.0%	81.87 ± 10.22	99.37 ± 4.63	111.70 ± 9.69	120.37±5.74[a]	124.01±3.17[a]
10.0%	78.42 ± 9.62	99.93 ± 8.67	109.97 ± 7.02[a]	118.94±8.54[a]	120.98±9.39[a]
25.0%	74.07 ± 8.01	98.97±11.27	105.60 ± 5.74[a]	113.11±7.84[a]	116.19±6.10[b]
CYP.	91.39 ± 1.70	108.70 ± 2.23	119.97 ± 1.75	131.43±3.51	136.63±2.71

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Values are mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) using Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.15 Weekly body weight gain in rats exposed to OSSL for 30 days.

Concentration 0.5ml/rat/day	Initial body weight (g)	1st week	2nd week	3rd week	4th week
MALES					
Dist. Water	150.30 ± 6.12	151.40 ± 8.15	157.10±6.79	167.00 ± 7.36	171.71±8.20
1.0%	147.20 ± 10.23	150.00 ± 11.47	154.80 ± 13.04	151.40 ± 4.78	154.70±1.39
2.5%	149.90 ± 5.76	151.50 ± 4.96	155.00 ± 2.01	158.90 ± 1.74	160.6±5.84
5.0%	144.8 ± 14.04	148.40 ± 15.40	152.60 ± 14.47	157.70±15.31	160.60±11.23
10%	143.30 ± 8.26	146.3 ± 7.90	148.30 ± 9.36	149.80±10.47	152.10±11.09[a]
25%	140.60 ± 9.98	143.30 ± 10.18	146.10 ± 9.63	148.21±8.62	149.62±7.66[a]
CYP.	158.92 ± 9.80	156.14 ± 10.82	159.10± 9.38	161.51±10.99	159.30±11.24
FEMALES					
Dist. Water	146.18 ± 6.16	147.31 ± 10.10	149.52 ± 6.32	153.14±1.89	161.35±5.92
1.0%	133.72 ± 3.20	138.23 ± 5.83	143.31 ± 1.62	144.41±7.31	146.61±9.11[a]
2.5%	138.62 ± 3.10	141.57 ± 9.71	148.11 ± 8.11	147.32±4.14	148.23±6.21[a]
5.0%	143.61 ± 2.33	145.22 ± 5.13	149.41 ± 4.91	151.00±7.14	152.31±8.02
10.0%	132.32 ± 9.62	135.13 ± 1.82	137.61 ±5.11	138.81±8.54	139.19±1.11[a]
25.0%	140.40 ± 8.01	138.21±1.27	141.00 ± 4.61	142.11±3.21[a]	142.41±4.21[a]
CYP.	138.42 ± 2.30	141.14 ± 3.69	143.21 ± 11.66	141.73±5.32	140.91±3.72[a]

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Values are mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p < 0.05$) using Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = Cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.16 Weekly body weight gain of rats treated with AERL for 30 days.

Concentration 0.5ml/rat/day	Initial body weight (g)	1st week	2nd week	3rd week	4th week
MALES					
Dist. Water	87.81±5.68	129.98±8.05	138.95±11.62	161.10±2.59	191.29±4.73
1.0%	79.00± 5.06	97.66± 5.09[a]	114.67± 6.70[b]	122.02±9.75[a]	131.02±12.66[a]
2.5%	82.33±8.09	106.60±4.81	113.64±3.86	122.67±15.47	130.37±16.34[a]
5.0%	85.56±10.18	95.03±8.31[a]	105.59±3.13[a]	125.65±2.36	131.57±2.22[a]
10.0%	92.39±8.06	102.54±11.03	121.86±2.40[b]	127.74±10.31	129.25±8.76[a]
25.0%	78.98±6.51	89.95±6.94[a]	106.46±6.14[b]	114.41±4.69	116.35±16.80[b]
CYP	83.27±6.96	106.04±7.23	121.43±5.62	130.31±6.80	136.23±7.87[a]
FEMALES					
Dist. Water	77.78±7.35	97.44±5.30	112.13±3.34	145.14±10.28	185.26±10.17
1.0%	78.77±5.52	89.64±4.30	117.18±1.59	121.94±6.72	128.01±5.52[b]
2.5%	83.00±6.53	101.43±7.49	115.67±11.28	126.68±6.95	130.86±8.08[a]
5.0%	82.28±8.65	91.04±4.78	110.48±5.60	121.41±5.32	123.07±3.67[b]
10.0%	87.26±8.06	91.82±4.36	110.51±7.79	118.22±1.90[a]	121.03±3.41[b]
25.0%	84.37±3.14	96.51±7.11	95.76±10.82	113.31±4.06[a]	119.56±3.77[c]
CYP.	91.39±1.70	108.70±2.25	119.97±1.75	131.43±3.51	136.63±2.71[a]

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Values are means (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) using Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.17 Weekly body weight gain in rats treated with AESL for 30 days.

Concentration 0.5ml/rat/day	Initial body weight (g)	1st week	2nd week	3rd week	4th week
MALES					
Dist. Water	150.30 ± 6.12	151.40±8.15	157.10±6.79	167.00±7.36	171.71±8.20
1.0%	139.22± 4.25	143.41± 5.78	146.93± 5.79	148.42±6.59	150.72±6.39
2.5%	141.33±12.62	146.70±12.77	149.64±13.53	151.23±13.22	149.60±3.88
5.0%	146.78±4.88	148.63±3.97	151.89±4.54	152.3±4.12	153.17±4.73
10.0%	144.69±4.40	147.24±5.98	149.42±4.44	151.01±6.25	150.45±6.19[a]
25.0%	142.58±3.85	145.05±3.53	145.36±3.18	149.31±1.79	149.95±1.84[a]
CYP	158.92±9.80	156.14±10.82	159.10±9.38	161.51±10.99	159.30±11.24[a]
FEMALES					
Dist. Water	146.18±6.16	147.31±10.10	149.52±6.32	153.14±1.89	161.35±5.92
1.0%	143.07±7.52	145.64±5.30	147.48±9.19	148.34±8.27	149.30±5.62
2.5%	136.72±3.71	138.03±2.19	140.47±6.81	142.98±7.50	144.36±3.08
5.0%	141.18±5.45	143.14±4.78	146.58±9.63	147.71±6.32	149.47±3.72
10.0%	132.8±4.06	134.32±5.26	135.23±6.10	137.12±1.21	138.42±4.26[a]
25.0%	141.94±2.54	143.52±8.31	144.38±31.13	146.91±7.06	148.62±3.77[a]

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CYP.	138.42±2.30	141.14±3.69	143.21±11.66	141.73±5.32	140.91±3.72[a]

Values are in means (± SEM). Superscripts differ significantly from corresponding control value (a = p< 0.05) using Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = Cyclophosphamide (40 mg/kg/bw) (positive control).

Lung weight gain

Tables 4.22 and 4.23 show the absolute and relative lung weight gain of rats exposed to 1 – 25 % concentrations of OSRL, OSSL, AERL and AESL samples. There was increase in both absolute and relative lung weight gain in male and female rats exposed to the leachate samples, except for 5.0 % of AESL treated male rats compared to corresponding negative control and this increase was not significant ($p > 0.05$). Compared with the corresponding negative control groups, absolute and relative weight gain were only significant ($p < 0.05$) at 25 % (absolute; OSRL male and female), 1 – 25 % (relative; OSRL male and female), 5 and 25 % (absolute; AERL female) and 5 – 25 % (relative; AERL male) and 5 % (absolute; AESL male). All other concentrations were insignificant ($p < 0.05$). Rats exposed to OSRL and AERL had higher absolute and relative weight gain than OSSL and AESL exposed rats. Leachate treated male rats recorded higher values than to the females.

Heart weight gain

Tables 4.24 and 4.25 show the absolute and relative heart weight gain of rats exposed to 1–25% concentrations of OSRL, OSSL, AERL and AESL. There is increase in both absolute and relative heart weight gain in rats exposed to different concentrations of OSRL and AERL samples. Except for absolute heart weight gain of 1 – 5 % OSSL treated male rats, 1 – 2.5 % AESL treated male and female rats and relative heart weight gain of 5.0 % AESL treated male rats that were lower than the corresponding negative controls, the absolute and relative heart weight gain of the rest of the concentrations of OSSL and AESL treated rats were higher than the negative control. Using one way ANOVA, the increase in absolute and relative heart weight gain

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of leachates treated rats were insignificant ($p > 0.05$). Absolute and relative heart weight gain was higher in rats treated with OSRL and AERL than OSSL and AESL exposed rats, and in males than female rats.

Liver weight gain

Tables 4.26 and 4.27 show the absolute and relative liver weight gain in rats exposed to 1 – 25 % concentrations of OSRL, AERL, OSSL and AESL. There is an increase in both absolute and relative liver weight gain in rats exposed to the different leachate samples. Increase in absolute and relative liver weight gain in rats treated with the leachate samples were significant ($p < 0.05$). OSRL and AERL treated rats showed higher absolute and relative liver weight gain than OSSL and AESL treated rats. Female rats had lower value in both the absolute and relative liver weight gain change in leachate exposure than males.

Table 4.18. Absolute and relative thymus weight gain in rats exposed to OSRL and OSSL for 30days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. thymus weight (g)	Rel. thymus weight (g)	Abs. thymus weight (g)	Rel. thymus weight (g)
MALES				
Dist. water	0.524±0.012	0.266±0.028	0.364±0.030	0.208±0.010
1.0%	0.325±0.021[b]	0.248±0.006	0.312±0.066	0.205±0.062
2.50%	0.301±0.055[b]	0.227±0.032	0.318±0.025	0.197±0.012
5.0%	0.286±0.067[b]	0.221±0.041	0.307±0.038	0.191±0.007
10.0%	0.261±0.064[c]	0.206±0.060	0.301±0.040	0.197±0.021
25.0%	0.196±0.008[c]	0.161±0.023	0.261±.0.032	0.174±0.033
CYP	0.208±0.006[c]	0.152±0.006	0.178±0.033[a]	0.112±0.063
FEMALES				
Dist. Water	0.458±0.024	0.247±0.004	0.267±0.040	0.165±0.004
1.0%	0.216±0.045[b]	0.169±0.022	0.221±0.044	0.151±0.007

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2.50%	0.203±0.006[c]	0.164±0.026	0.216±0.069	0.145±0.020
5.0%	0.198±0.008[c]	0.158±0.031	0.207±0.060	0.136±0.006
10.0%	0.183±0.038[c]	0.158±0.033	0.181±0.086[a]	0.129±0.047
25.0%	0.166±0.004[c]	0.140±0.000	0.178±0.009[a]	0.125±0.042
CYP	0.194±0.013[c]	0.143±0.006	0.154±0.077[a]	0.118±0.053

Values are in means (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.19. Absolute and relative thymus weight gain in rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. thymus weight (g)	Rel. thymus weight (g)	Abs. thymus weight (g)	Rel. thymus weight (g)
MALES				
Dist. water	0.524±0.012	0.266±0.028	0.364±0.030	0.208±0.010
1.0%	0.322±0.047[b]	0.241±0.040	0.314±0.032	0.208±0.074
2.50%	0.318±0.041[b]	0.239±0.041	0.312±0.057	0.206±0.042
5.0%	0.311±0.072[b]	0.231±0.038	0.311±0.070	0.204±0.206
10.0%	0.289±0.017[b]	0.221±0.008	0.304±0.050	0.206±0.062
25.0%	0.224±0.016[b]	0.187±0.010	0.283±.0.006[a]	0.188±0.005
CYP	0.208±0.006[c]	0.152±0.006	0.178±0.033[a]	0.112±0.063
FEMALES				
Dist. Water	0.458±0.056	0.247±0.004	0.267±0.040	0.165±0.004
1.0%	0.212±0.056[b]	0.163±0.022	0.228±0.048	0.152±0.006

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2.50%	0.207±0.049[b]	0.157±0.036	0.217±0.071	0.150±0.005
5.0%	0.198±0.022[b]	0.158±0.031	0.203±0.062	0.135±0.044
10.0%	0.189±0.065[b]	0.153±0.063	0.195±0.007	0.140±0.042
25.0%	0.172±0.007[c]	0.143±0.020	0.182±0.007	0.122±0.006
CYP	0.194±0.013[c]	0.143±0.006	0.154±0.077	0.118±0.053

Values are in means (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.20. Absolute and relative spleen weight gain in rats exposed to OSRL and OSSL for 30 days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. spleen weight (g)	Rel. spleen weight (g)	Abs. spleen weight (g)	Rel. spleen weight (g)
MALES				
Dist. water	0.458±0.040	0.233±0.048	0.510±0.048	0.292±0.058
1.0%	0.508±0.063	0.387±0.042	0.523±0.052	0.336±0.004
2.50%	0.523±0.061	0.395±0.009	0.526±0.026	0.325±0.050
5.0%	0.606±0.006[a]	0.468±0.019[b]	0.534±0.099	0.332±0.006
10.0%	0.669±0.058[b]	0.484±0.058[b]	0.601±0.063	0.393±0.007
25.0%	0.771±0.020[c]	0.634±0.045[c]	0.613±.0.058	0.409±0.044[a]
CYP	0.658±0.021[b]	0.480±0.033[b]	0.676±0.023	0.425±0.044[a]
FEMALES				
Dist. Water	0.327±0.050	0.176±0.020	0.421±0.054	0.259±0.029

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1.0%	0.372±0.023[b]	0.291±0.013	0.426±0.049	0.262±0.030
2.50%	0.496±0.021[b]	0.400±0.006[b]	0.433±0.016	0.295±0.011
5.0%	0.517±0.031[b]	0.412±0.029[b]	0.451±0.063	0.4296±0.010
10.0%	0.591±0.045[c]	0.489±0.024[c]	0.472±0.024	0.335±0.045
25.0%	0.601±0.006[c]	0.508±0.056[c]	0.567±0.057	0.397±0.009[b]
CYP	0.583±0.018[c]	0.430±0.058[c]	0.628±0.049	0.443±0.012[c]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.
Dist. water = distilled water (negative control),
CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.21. Absolute and relative spleen weight gain in rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. spleen weight (g)	Rel. spleen weight (g)	Abs. spleen weight (g)	Rel. spleen weight (g)
MALES				
Dist. water	0.458±0.040	0.233±0.048	0.510±0.048	0.292±0.058
1.0%	0.504±0.061	0.380±0.020[b]	0.526±0.050	0.349±0.038
2.50%	0.444±0.037	0.390±0.045[b]	0.529±0.022	0.349±0.037
5.0%	0.584±0.016	0.433±0.019[c]	0.531±0.064	0.348±0.063
10.0%	0.612±0.039	0.468±0.026[c]	0.600±0.005	0.397±0.044
25.0%	0.734±0.041[c]	0.611±0.006[c]	0.621±.0.046	0.413±0.056
CYP	0.658±0.021[b]	0.480±0.033[b]	0.676±0.023	0.425±0.044
FEMALES				

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Dist. Water	0.327±0.050	0.176±0.020	0.421±0.054	0.259±0.029
1.0%	0.361±0.030	0.278±0.021	0.427±0.049	0.285±0.015
2.50%	0.426±0.050	0.324±0.022[a]	0.435±0.015	0.298±0.009
5.0%	0.508±0.054[a]	0.405±0.051[b]	0.441±0.041	0.293±0.011
10.0%	0.577±0.020[b]	0.466±0.027[c]	0.468±0.087	0.336±0.073
25.0%	0.594±0.004[c]	0.494±0.020[c]	0.547±0.038	0.367±0.025
CYP	0.583±0.018[c]	0.430±0.058[c]	0.628±0.049	0.443±0.012[c]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.22. Absolute and relative lung weight gain in rats exposed to OSRL and OSSL for 30days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. lung weight (g)	Rel. lung weight (g)	Abs. lung weight (g)	Rel. lung weight (g)
MALES				
Dist. water	0.896±0.012	0.456±0.051	1.214±0.059	0.694±0.022
1.0%	0.917±0.010	0.700±0.058[a]	1.216±0.107	0.781±0.052
2.50%	0.947±0.029	0.753±0.090[b]	1.219±0.047	0.754±0.035
5.0%	0.990±0.149	0.765±0.039[b]	1.221±0.044	0.755±0.038
10.0%	1.189±0.074	0.938±0.018[c]	1.263±0.039	0.827±0.049
25.0%	1.401±0.116[b]	1.151±0.046[c]	1.312±.0.129	0.876±0.056
CYP	1.015±0.125	0.740±0.042[b]	1.294±0.009	0.812±0.068
FEMALES				

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Dist. Water	0.730±0.029	0.393±0.011	1.118±0.117	0.689±0.081
1.0%	0.884±0.049	0.691±0.024[b]	1.121±0.111	0.764±0.021
2.5%	0.955±0.037	0.770±0.005[c]	1.119±0.057	0.750±0.038
5.0%	0.971±0.041	0.773±0.006[c]	1.126±0.051	0.739±0.044
10.0 %	0.992±0.067	0.822±0.051[c]	1.211±0.087	0.860±0.059
25.0%	1.215±0.084[b]	1.024±0.103[c]	1.237±0.103	0.8666±0.043
CYP	1.003±0.172	0.740±0.041[c]	1.208±0.064	0.852±0.037

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4.23 Absolute and relative lung weight gain in rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. lung weight (g)	Rel. lung weight (g)	Abs. lung weight (g)	Rel. lung weight (g)
MALES				
Dist. water	0.896±0.012	0.456±0.051	1.214±0.059	0.694±0.022
1.0%	0.919±0.040	0.693±0.011	1.215±0.047	0.805±0.050
2.50%	0.936±0.020	0.703±0.041	1.217±0.083	0.803±0.063
5.0%	0.986±0.009	0.731±0.058[a]	1.207±0.049	0.790±0.058
10.0%	1.099±0.045	0.871±0.041[b]	1.247±0.025	0.826±0.042
25.0%	1.385±0.016[c]	1.153±0.048[c]	1.324±.0.016	0.881±0.052
CYP	1.015±0.125	0.740±0.042[b]	1.294±0.009	0.812±0.068

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FEMALES				
Dist. Water	0.730±0.029	0.393±0.011	1.118±0.117	0.689±0.081
1.0%	0.897±0.061	0.670±0.013	1.122±0.043	0.749±0.037
2.50%	0.936±0.069	0.712±0.086	1.127±0.108	0.773±0.011
5.0%	1.032±0.096[a]	0.789±0.056	1.132±0.009	0.752±0.038
10.0%	0.994±0.046	0.802±0.003[a]	1.193±0.031	0.856±0.044
25.0%	1.198±0.024[b]	0.996±0.020[b]	1.262±0.074	0.847±0.037
CYP	1.003±0.172	0.740±0.041	1.208±0.064	0.852±0.037

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Kidney weight gain

Tables 4.28 and 4.29 show the absolute and relative kidney weight gain in rats exposed to 1 – 25 % concentrations of OSRL, AERL, OSSL and AESL. There is an increase in both absolute and relative kidney weight gain in rats exposed to the various concentrations of the leachate samples. Increase in relative and absolute kidney weight gain in rats exposed to the leachate samples were significant ($p < 0.05$). OSRL and AERL treated rats showed higher absolute and relative kidney weight gain than OSSL and AESL treated rats. Female rats had lower value in both the absolute and relative liver weight gain change during leachate exposure than males.

4.4.4 Histopathological changes in the viscera of leachate treated rats

Histopathological changes in the liver

The liver of the negative control rats showed normal histology which is composed of hexagonal or pentagonal lobules and peripheral hepatic triads or tetrads embedded in connective

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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 4.6A). Liver histology of leachate treated rat showed hepatic necrosis and periportal and cellular infiltration (25 % AERL), diffused hepatic hydropic degeneration with congestion of the portal and cellular infiltration by mononuclear cells and light blurring of the trabecular structure of the hepatic lobules. The cytoplasm of some cells showed empty vacuolar spaces (10 % OSRL). Severe multifocal hepatic necrosis and periportal cellular infiltration by macrophages and neutrophils were also observed in 25 % OSSL treated rats (Figure 4.6B – D).

Histopathological changes in the kidney

The cortical section of the kidney histology of negative control rat showed renal glomerular tightly filling the Bowman's capsule. Cortical tubules showed normal tubular brush borders with distinct lumen. Sinusoid was observed to be normal without congestion or inflammation (figure 4.7A). Cortical congestions and haemorrhage with tubular necrosis were observed in the kidney histology of rat treated with 25 % of OSRL. Foci of severe congestion, interstitial haemorrhage at the cortical region and tubular necrosis of the epithelium were observed in 10 % AERL treated rats. Mild cortical congestion was observed in the kidney sections of 2.5 % OSRL treated rats (Figure 4.7B – D).

Table 4. 24 Absolute and relative heart weight gain in rats exposed to OSRL and OSSL for 30days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. heart weight (g)	Rel. heart weight (g)	Abs. heart weight (g)	Rel. heart weight (g)
MALES				
Dist. water	0.432±0.046	0.230±0.099	0.921±0.039	0.527±0.041
1.0%	0.485±0.042	0.370±0.030	0.913±0.027	0.586±0.014
2.50%	0.512±0.047	0.387±0.040	0.901±0.049	0.557±0.031
5.0%	0.535±0.011	0.413±0.013	0.917±0.040	0.570±0.025
10.0%	0.688±0.014[c]	0.543±0.051[c]	0.931±0.092	0.609±0.062

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25.0%	0.726±0.017[c]	0.597±0.063[c]	0.946±0.064	0.632±0.046
CYP	0.567±0.014[a]	0.414±0.073	0.929±0.018	0.584±0.044
FEMALES				
Dist. Water	0.361±0.029	0.136±0.094	0.591±0.043	0.364±0.030
1.0%	0.448±0.037	0.350±0.035[c]	0.598±0.019	0.407±0.048
2.50%	0.450±0.024	0.363±0.017[c]	0.596±0.087	0.400±0.048
5.0%	0.460±0.030	0.366±0.018[c]	0.601±0.043	0.394±0.073
10.0%	0.487±0.002	0.403±0.021[c]	0.686±0.042	0.487±0.040
25.0%	0.539±0.024[b]	0.455±0.007[c]	0.844±0.026[c]	0.591±0.041[b]
CYP	0.510±0.053[a]	0.376±0.021[c]	0.731±0.039[a]	0.516±0.048[a]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4. 25 Absolute and relative heart weight gain in rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. heart weight (g)	Rel. heart weight (g)	Abs. heart weight (g)	Rel. heart weight (g)
MALES				
Dist. water	0.432±0.046	0.230±0.099	0.921±0.039	0.527±0.041
1.0%	0.476±0.023	0.359±0.023	0.920±0.029	0.610±0.059
2.50%	0.487±0.002	0.366±0.018	0.911±0.007	0.601±0.064
5.0%	0.540±0.098[a]	0.400±0.057	0.903±0.040	0.591±0.017
10.0%	0.619±0.016[c]	0.474±0.051[a]	0.941±0.024	0.623±0.034

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25.0%	0.696±0.010[c]	0.580±0.030[b]	0.944±0.018	0.628±0.031
CYP	0.567±0.014[a]	0.414±0.073	0.929±0.018	0.584±0.044
FEMALES				
Dist. Water	0.361±0.029	0.136±0.094	0.591±0.043	0.364±0.030
1.0%	0.429±0.038	0.332±0.057[a]	0.552±0.052	0.389±0.013
2.50%	0.462±0.008	0.351±0.027[b]	0.541±0.008	0.371±0.017
5.0%	0.463±0.028	0.369±0.024[b]	0.604±0.008	0.401±0.041
10.0%	0.479±0.047	0.387±0.029[b]	0.631±0.037	0.453±0.037
25.0%	0.526±0.053[a]	0.437±0.033[c]	0.721±0.044[a]	0.484±0.004[a]
CYP	0.510±0.053[a]	0.376±0.021[c]	0.731±0.039[a]	0.516±0.048[a]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$; c= $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4. 26 Absolute and relative liver weight gain in rats exposed to OSRL and OSSL for 30days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. liver weight (g)	Rel. liver weight (g)	Abs. liver weight (g)	Rel. liver weight (g)
MALES				
Dist. water	5.940±0.271	3.027±0.182	7.212±0.157	4.123±0.171
1.0%	5.967±0.074	4.553±0.024[a]	7.463±0.263	4.786±0.084
2.50%	6.124±0.051	4.624±0.083[a]	7.492±0.217	4.633±0.225
5.0%	6.513±0.060	5.030±0.762[a]	7.481±0.249	4.645±0.360

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10.0%	6.662±0.122[a]	5.260±0.372[a]	8.021±0.204	5.249±0.189
25.0%	7.134±0.264[a]	5.862±0.801[a]	8.320±0.203[a]	5.556±0.435[a]
CYP	6.61±0.011	4.824±0.114[a]	7.961±0.374	5.006±0.527
FEMALES				
Dist. Water	5.261±0.183	2.832±0.030	5.011±0.515	3.085±0.085
1.0%	5.343±0.061	4.180±0.024[a]	5.154±0.218	3.397±0.270
2.50%	5.372±0.050	4.330±0.070[a]	5.155±0.109	3.455±0.137
5.0%	5.620±0.043	4.472±1.053[a]	5.201±0.120	3.411±0.297
10.0%	6.102±0.012[a]	5.053±1.060[a]	5.436±0.289	3.856±0.147
25.0%	6.874±0.174[a]	5.193±0.124[a]	6.031±0.151	4.221±0.176[b]
CYP	5.273±0.084	3.964±0.350[a]	5.602±0.288	3.953±0.120[a]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (= $p<0.05$; b = $p<0.01$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

Table 4. 27 Absolute and relative liver weights of rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. liver weight (g)	Rel. liver weight (g)	Abs. liver weight (g)	Rel. liver weight (g)
MALES				
Dist. water	5.940±0.271	3.027±0.182	7.212±0.157	4.123±0.171
1.0%	5.943±0.324	4.461±0.140[a]	7.382±0.305	4.891±0.199
2.50%	5.961±0.082	4.483±0.334[a]	7.423±0.253	4.896±0.122

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5.0%	5.983±0.104	4.481±0.821[a]	7.536±0.308	4.932±0.265
10.0%	6.221±0.054	4.762±0.380[a]	7.947±0.213	5.264±0.172
25.0%	6.584±0.132	5.482±0.113[a]	7.991±0.257	5.317±0.345[a]
CYP	6.612±0.103	4.821±0.114[a]	7.961±0.374	5.006±0.527
FEMALES				
Dist. Water	5.261±0.183	2.832±0.030	5.011±0.515	3.085±0.085
1.0%	5.392±0.112	4.143±0.420[a]	5.211±0.127	3.461±0.323
2.50%	5.801±0.060	4.414±0.420[a]	5.194±0.423	3.562±0.315
5.0%	6.023±0.052	4.801±0.014[a]	5.321±0.241	3.533±0.207
10.0%	6.142±0.044[a]	5.003±0.042[a]	5.571±0.247	4.027±0.064[a]
25.0%	6.321±0.061[a]	5.254±0.063[a]	5.943±0.306	3.986±0.191[a]
CYP	5.273±0.082	3.962±0.351[a]	5.602±0.288	3.953±0.120[a]

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

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Table 4. 28 Absolute and relative kidney weight gain in rats exposed to OSRL and OSSL for 30days.

	OSRL		OSSL	
Concentration 0.5ml/rat/day	Abs. kidney weight (g)	Rel. kidney weight (g)	Abs. kidney weight (g)	Rel. kidney weight (g)
MALES				
Dist. water	0.631±0.032	0.323±0.021	1.410±0.237	0.807±0.009
1.0%	0.783±0.027	0.586±0.014[a]	1.478±0.266	0.949±0.051
2.50%	0.890±0.043[a]	0.672±0.013[a]	1.469±0.229	0.908±0.063
5.0%	0.921±0.024[a]	0.705±0.105[a]	1.513±0.287	0.940±0.029
10.0%	0.982±0.053[a]	0.769±0.036[a]	1.646±0.162	1.076±0.060[a]
25.0%	0.994±0.211[a]	0.812±0.210[a]	1.734±0.076	1.156±0.084[b]
CYP	0.892±0.410[a]	0.654±0.013[a]	1.573±0.033	0.991±0.046
FEMALES				
Dist. Water	0.522±0.130	0.281±0.020	0.964±0.039	0.594±0.004
1.0%	0.728±0.003	0.572±0.014[a]	0.973±0.094	0.663±0.039
2.50%	0.836±0.002[a]	0.680±0.023[a]	0.998±0.094	0.669±0.381
5.0%	0.891±0.004[a]	0.714±0.143[a]	1.010±0.177	0.662±0.029
10.0%	0.968±0.012[a]	0.801±0.170[a]	1.062±0.216	0.754±0.062[a]
25.0%	0.984±0.002[a]	0.832±0.003[a]	1.082±0.225	0.757±0.035[a]
CYP	0.801±0.110[a]	0.592±0.062[a]	1.061±0.172	0.739±0.029

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; b = $p<0.01$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

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Table 4. 29 Absolute and relative kidney weight gain in rats exposed to AERL and AESL for 30days.

	AERL		AESL	
Concentration 0.5ml/rat/day	Abs. kidney weight (g)	Rel. kidney weight (g)	Abs. kidney weight (g)	Rel. kidney weight (g)
MALES				
Dist. water	0.631±0.032	0.323±0.021	1.410±0.237	0.807±0.009
1.0%	0.788±0.011	0.601±0.002[a]	1.456±0.024	0.965±0.043
2.50%	0.883±0.003[a]	0.663±0.054[a]	1.464±0.288	0.973±0.037
5.0%	0.930±0.002[a]	0.691±0.124[a]	1.497±0.138	0.980±0.048
10.0%	0.941±0.204[a]	0.716±0.054[a]	1.534±0.023	1.016±0.005[a]
25.0%	0.948±0.013[a]	0.786±0.231[a]	1.683±0.278	1.120±0.070[c]
CYP	0.892±0.411[a]	0.645±0.014[a]	1.573±0.033	0.991±0.046
FEMALES				
Dist. Water	0.522±0.130	0.281±0.020	0.964±0.039	0.594±0.004
1.0%	0.761±0.012[a]	0.582±0.071[a]	0.976±0.084	0.652±0.017
2.50%	0.783±0.023[a]	0.593±0.064[a]	0.986±0.049	0.676±0.048
5.0%	0.794±0.124[a]	0.631±0.323[a]	0.994±0.162	0.660±0.034
10.0%	0.812±0.323[a]	0.653±0.013[a]	1.124±0.065	0.808±0.013[c]
25.0%	0.863±0.014[a]	0.714±0.214[a]	1.038±0.163	0.697±0.003
CYP	0.801±0.113[a]	0.585±0.058[a]	1.061±0.172	0.739±0.029

Values are in mean (± SEM). Superscripts differ significantly from corresponding control value (a = $p<0.05$; c = $p<0.001$) by Dunnett's multiple comparison posthoc test.

Dist. water = distilled water (negative control),

CYP = cyclophosphamide (40 mg/kg/bw) (positive control).

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Histopathological changes in the heart

The histology of the heart of negative control rat showed normal structure of cardiomyocytes with a nucleus in each of the myocytes (figure 4.8A). Leachate treatment in rats for 30 days did induced necrosis, inflammations, congestion and haemorrhage in the cardiomyocytes of treated rats except mild local vacuolar degeneration of the cardiomyocytes observed in rats treated with 25 % OSRL and OSSL (figure 4.8C and D).

Histopathological changes in the thymus

The histology of the thymic cortex from negative control rat showing normal distribution of lymphocytes and macrophages without inflammatory cell (figure 4.9A). Thymic histology of rats treated with 10 % OSRL showed severe depletion of the thymic lobules, cellular infiltration in the adipose tissues and increase in the number of apoptotic lymphocytes and macrophages (figure 4. 9B). Thymic histology of 25 % AERL treated rat depletion, blurring of the cortico-medullary margins of the thymic lobules and decreased cellularity in the medulla due to lymphocyte apoptosis (figure 4.9C). Severe necrosis and inflammation which resulted in the lymphoid depletion of the cortical tissues were observed in the thymic histology of 25 % OSRL treated rats (figure 4.9D).

Histopathological changes in the spleen

Spleen histology showing normal follicles around the arterioles and normal red pulp was observed in the negative control rat (figure 4.10A). Lymphoid depletions especially of the periarteriolar lymphoid sheath (PALS), haemosiderin deposits, severe congestion of the sinuses and the cords, degenerative, necrosis and inflammatory were observed in the spleen histology of rats treated with varying concentrations of the landfill leachates (figure 4.10B – D) exposed to higher concentrations of the leachates mostly with OSL and AEL. OSSL and AESL show mild lesions in the spleen compared to OSL and AEL. The lesions were also not sex specific.

Histopathological changes in the lungs

Histology of the lungs of negative control rats showed normal alveoli with thin alveolar wall and sacs (figure 4.11A). The histology of the lungs of rats treated with landfill leachates

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showed spreads of proliferative thickened alveolar wall, scattered foci of macrophages with intracellular accumulated black pigment carbon particles. Also observed were localized severe thickening of the alveolar wall, increase histiocytes within the bronchial associated lymphoid

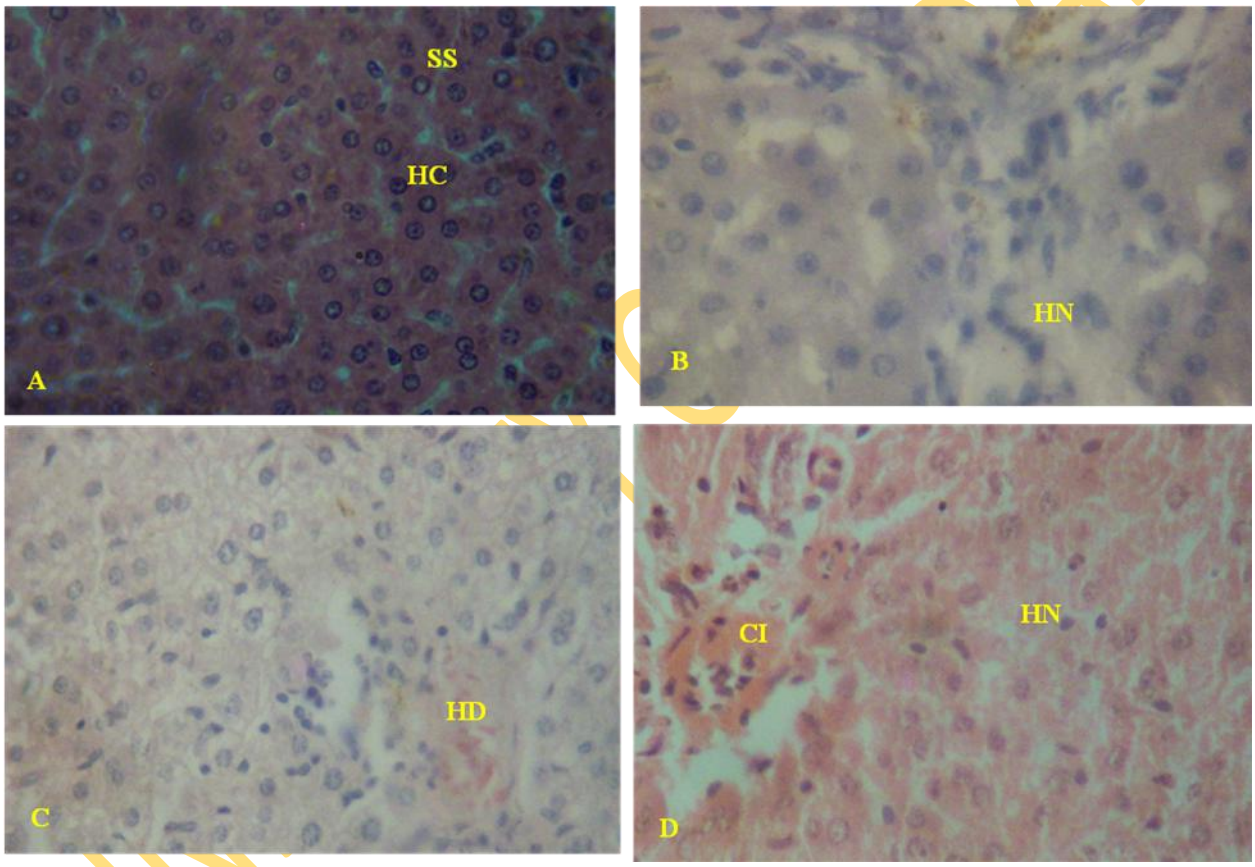

Figure 4.6. Histological sections of liver from leachate treated rats and the negative control:

a. Histopathology of the liver from a distilled water treated rat (negative control).

b. Liver histopathology of 25 % AERL treated rat.

c. Liver histopathology of 10 % OSRL treated rat.

d. Liver histopathology of 25 % OSSL treated rat.

Various leisions observed in liver histology leachate treated rats: HN =Hepatic Necrosis, HD = Hepatic Degeneration, CI = Cellular Infiltration, SS = Sinusoids, HC = Hepatic cord. H&E, x 400.

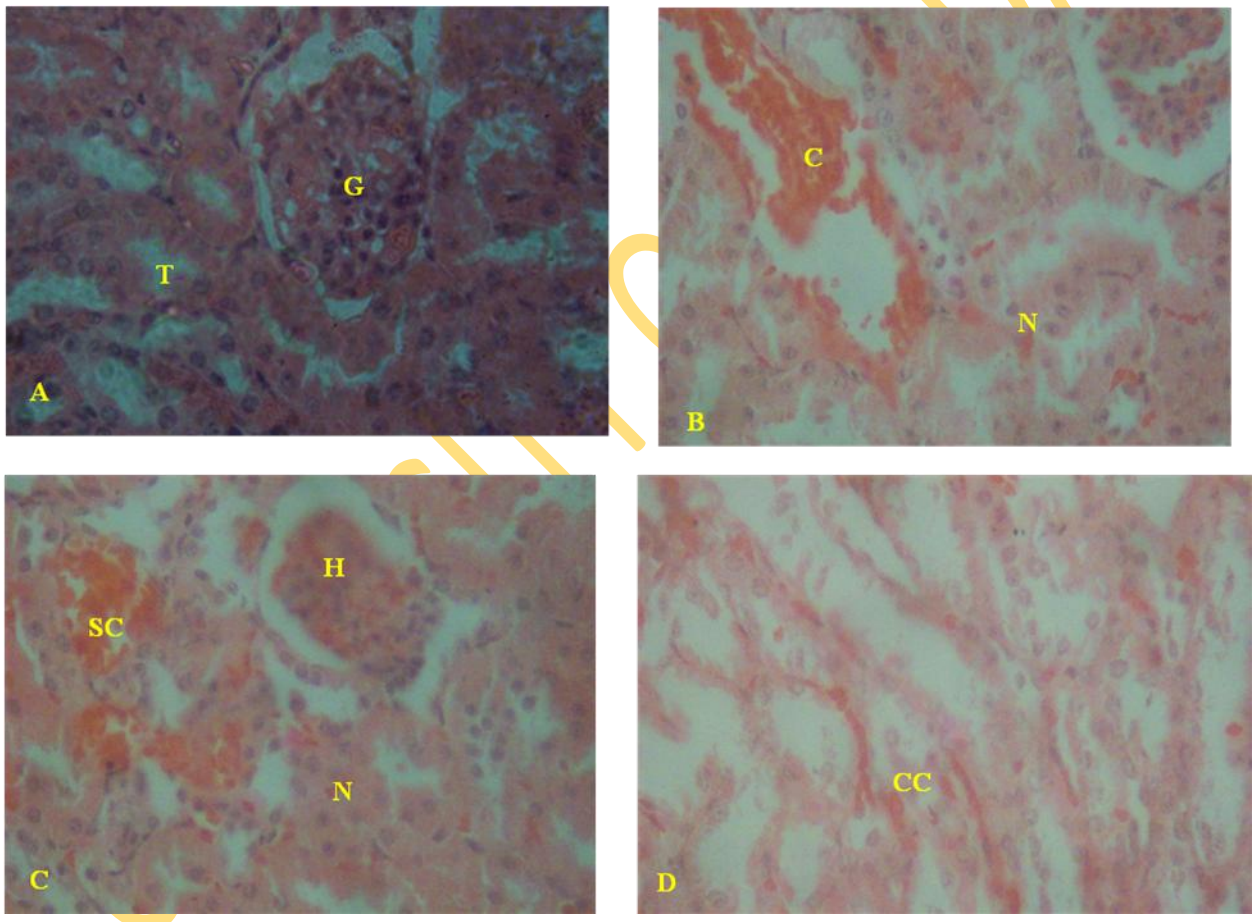

Figure 4.7. Histological sections of kidney from leachate treated rats and the negative control:

(a) Cortical section of kidney histopathology of a negative control rat (distilled water).

(b) Cortical section of kidney histology of rat treated with 25 % of OSRL.

(c) Cortical section of kidney histology of rat treated with 10 % of AERL.

(d) Medullary section of kidney histology of rat treated with 2.5 % of OSRL.

Various leisions observed in kidney histology leachate treated rats: G = Glomerular, T = Tubular brush border, N = Necrosis, SC = Severe Congestion, CC = Cortical Congestion and H = interstitial Haemorrhage. H&E, x 400.

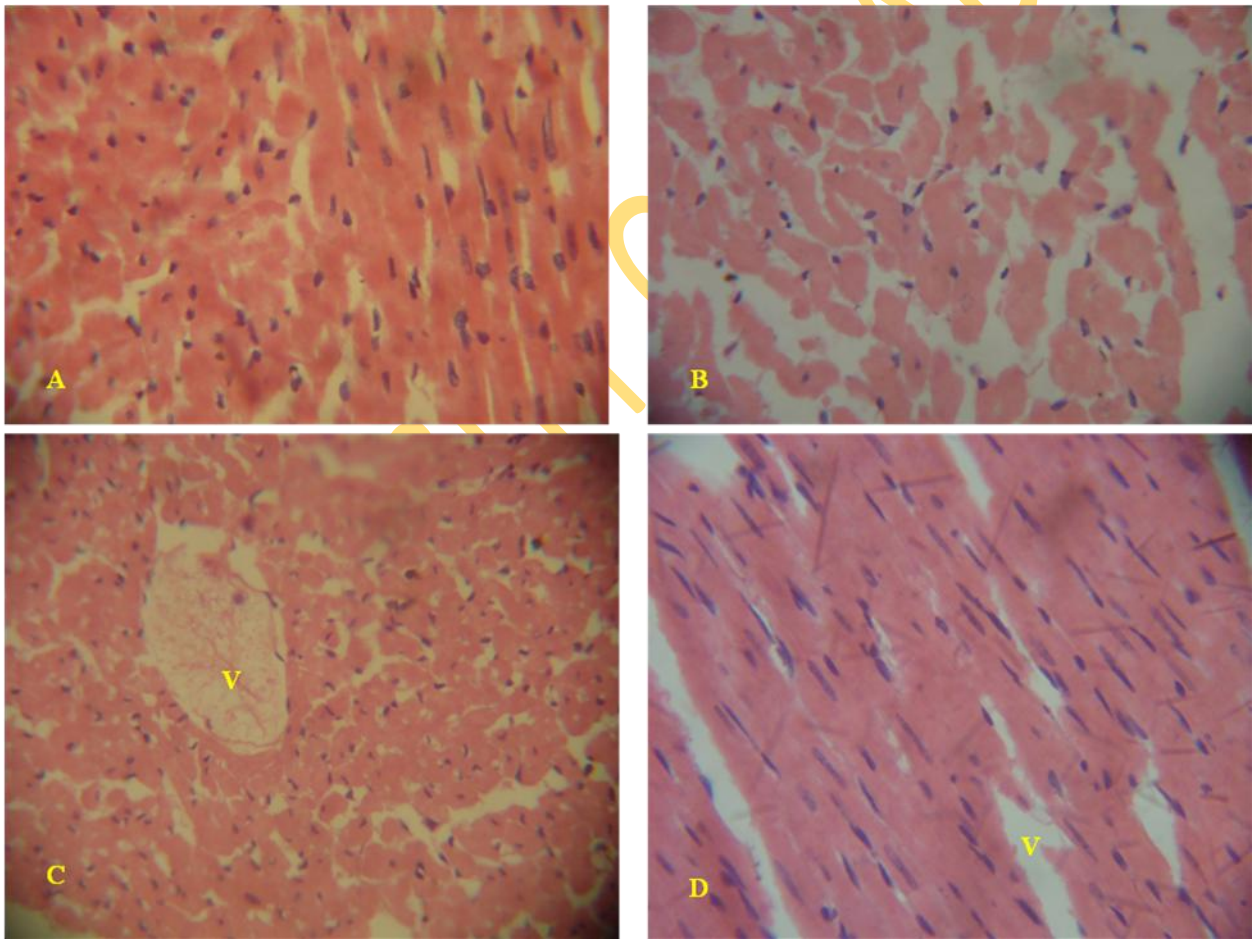

Figure 4.8. Histological sections of heart from leachate treated rats and the negative control: (a) Histopathology of the heart from negative control rat (distilled water) with normal cardiomyocytes.

(b) Histology of the heart from a 10 % OSRL exposed rat showing normal architecture of the heart

(c) Histology of the heart from a 25 % OSRL exposed rat showing mild vacuolar degeneration of the cardiomyocytes (V).

(d) Histology of the heart from a 25 % OSSL exposed rat showing slight extensions of vacuolar degeneration of the cardiomyocytes (V). H&E, x 400.

tissue (BALT), presence of lymphocytic perivascular cuff and congestion of the pulmonary vessels (figure 4.11b – d)

Severity of the histopathological leisions for all tissues increased in rats treated with the higher concentrations of the leachate samples and was not sex specific.

4.4.5 Serum biochemical changes in leachate treated rats

Tables 4.30 – 4.32 show the results of serum biochemical analysis of leachate treated and control rats. There is concentration dependent and significant increase in the activities of serum AST and ALT enzymes in leachate exposed rats than the negative control. The tested concentrations of the leachates and CYP were significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from the negative control,. Although, AST and ALT activities were higher in male rats than females; the significant increase in the activities of these enzymes compared with the negative control was mostly due to leachate concentrations (60.48 % for ALT and 72.29 % for AST of the total variance) rather than rat sex (0.40 % for ALT and 0.42 % for AST of the total variance) and the interactions of leachate concentrations and sex (35.38 % for ALT and 26.94 % for AST) Table 4.30.

The intensities of creatinine and urea in the serum of leachate exposed rats were significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) higher than the negative control except for creatinine where the values at 1% concentration of both AERL and OSRL (male and female) and 5 % of OSRL (male) were lower than the negative control. Although the intensity of serum urea was higher in females than males and the intensity of creatinine higher in males than females; the interactive effects of leachate on the sex was more prominent in the intensity of creatinine (48.78 %, $p<0.0001$) rather than the effects of leachate concentration and rat sex alone (table 4.32). The leachate concentrations affected the intensity of urea most (51.37 %, $p<0.0001$) than sex and the interactions of leachate and sex (table 4.33).

The intensities of serum albumin and total proteins from leachate and CYP treated rats

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significantly ($p < 0.05$) decreased in a concentration dependent pattern. Although, the values of albumin and total protein were higher in females than males; the interactive effects of leachate on the sex was prominent (57.29 % for Albumin and 62.77 % for protein; $p < 0.0001$) than the effects of leachate (30.85 % for albumin and 31.98 % for protein; $p < 0.0001$) and sex (1.48 % for albumin and 1.15 % for protein; $p < 0.0001$) alone (tables 4.34 and 4.35).

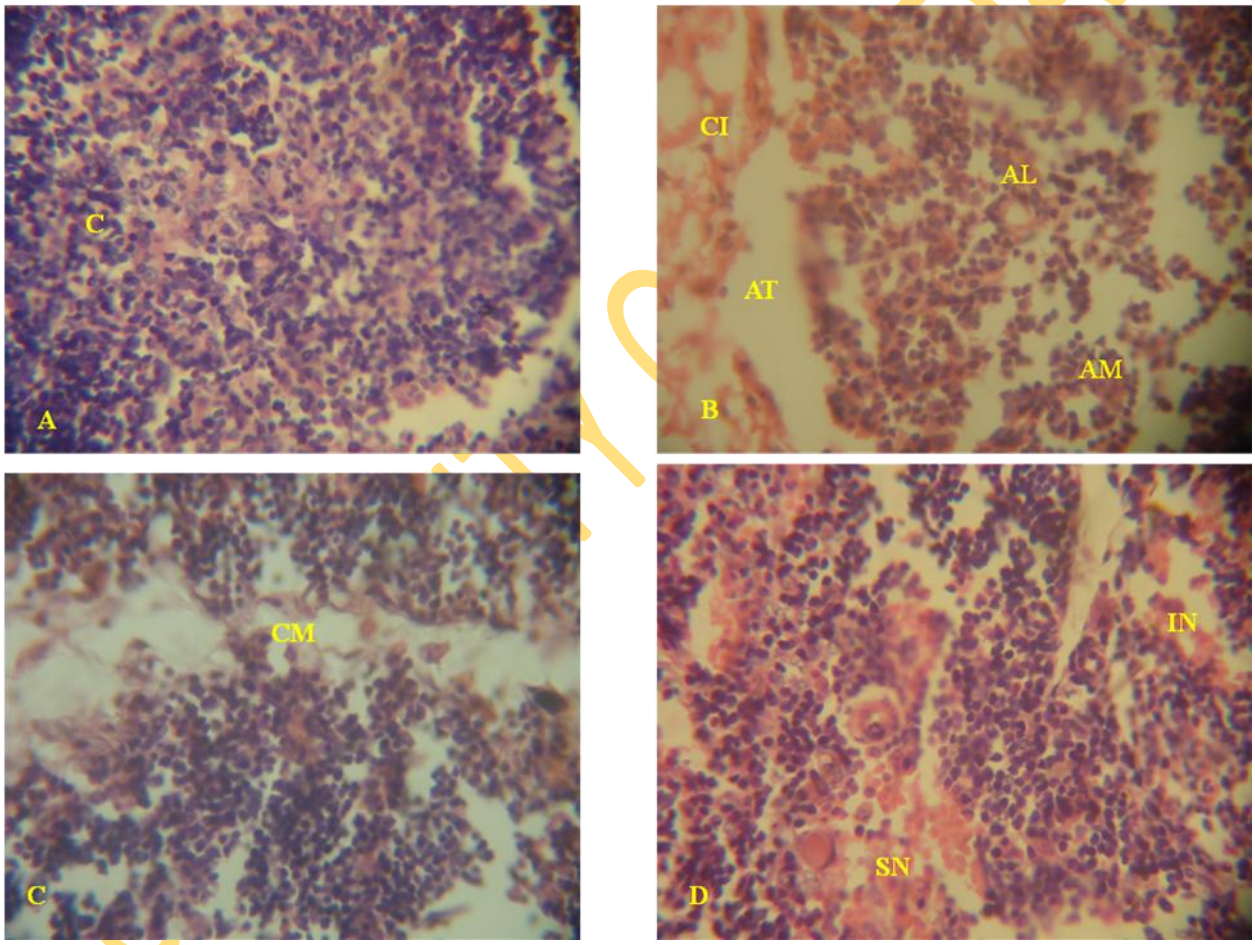

Figure 4.9. Histological of thymus from leachate treated rats and the negative control: (a) Histopathology of the cortical section of the thymus from a negative control rat showing normal distribution of lymphocytes and macrophages.

(b) Thymic histology from a 10 % OSRL treated rat showing severe depletion of the thymic lobules and increase number of apoptotic lymphocytes (AL) and macrophages (AM). Also there is cellular infiltration (CI) in the adipose tissues (AT).

(c) Thymic histology from a 25 % OSRL exposed rat showing indistinct cortico-medullary boundary (CM) with cellular infiltration.

(d) Thymic histology from a 25 % AERL exposed rat showing severe necrosis (SN), infiltrations of macrophages (IN) and lymphoid depletion. H & E, x 400.

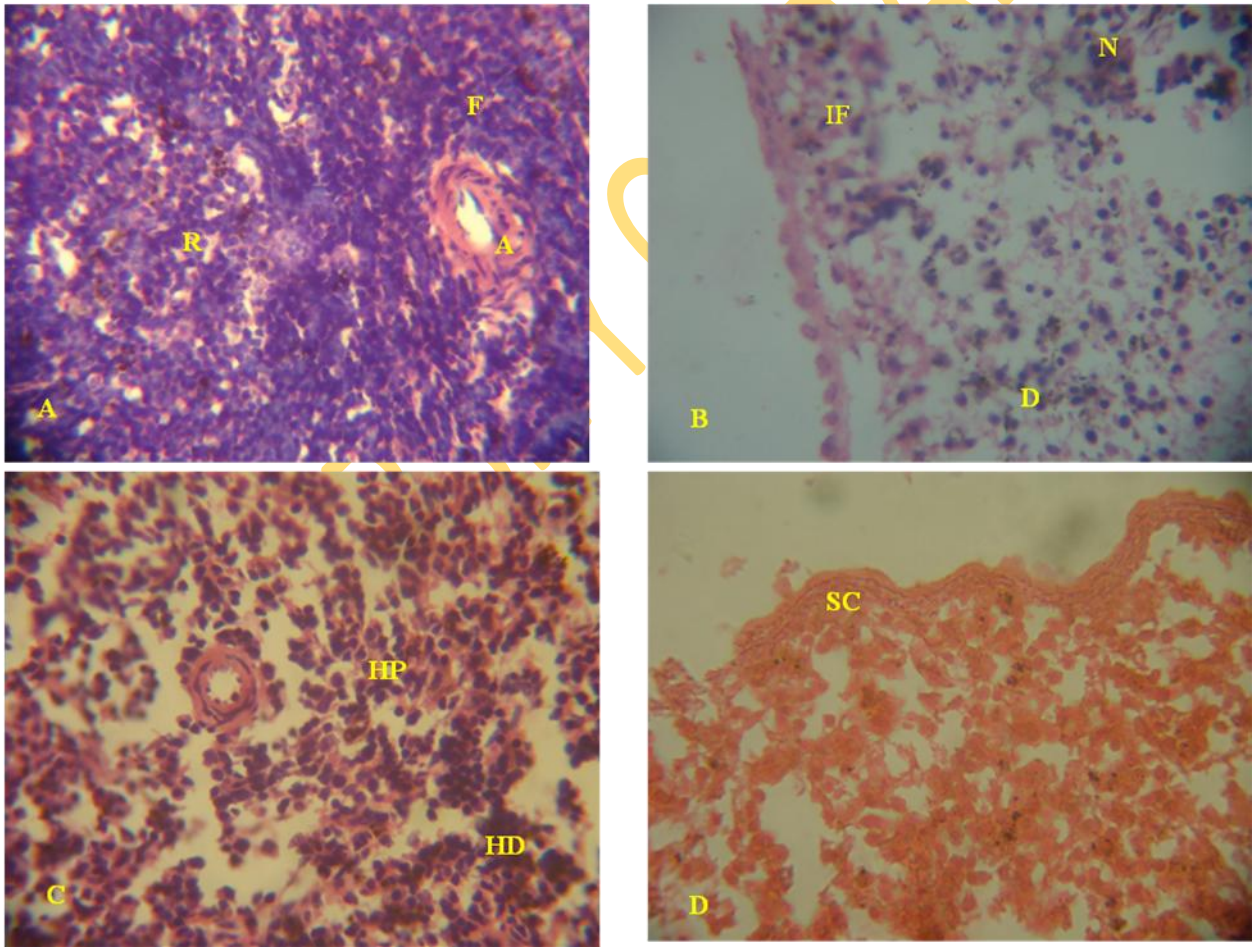

Figure 4.10. Histological section of spleen from leachate treated rats and the negative control:

a. Histopathology of the spleen from a negative control rat showing normal follicle (F) around the arterioles (A) and normal red pulp (R).

b. Spleen histology from a 25 % OSRL exposed rat showing marked lymphoid depletion characterised by degeneration (D), necrosis (N) and inflammatory (IF) change.

c. Spleen histology from a 10 % OSRL exposed rat showing red pulp containing foci of haemosiderin ladened macrophages (HD) and hyperplasia of the periarterial (HP) splenic follicle.

d. Spleen histology from a 25 % AERL exposed rat showing severe necrosis and congestions of the sinuses and the cords. H&E, x 400.

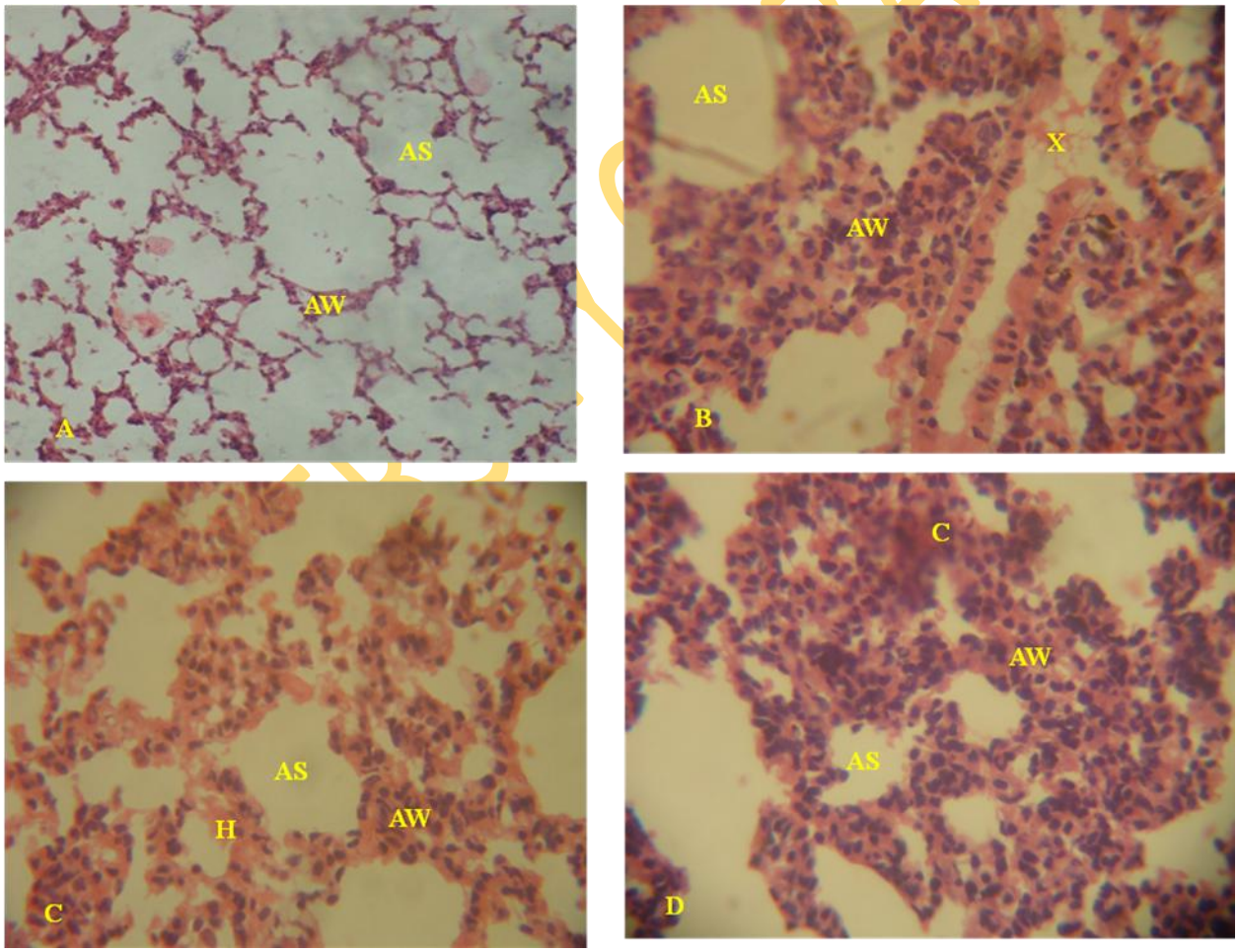

Figure 4.11. Histological sections of lungs from leachate treated rats and the negative control:

a. Histopathology of the lung from a negative control rat showing normal alveoli with thin alveolar wall (AW) and normal alveolar sac (AS).

b. Lung histology from a 25 % OSRL exposed rat showing moderate to severe wide spread proliferative thickening of the alveolar wall (AW). There are scattered foci of macrophages (X) with intracellular accumulation of black pigment (carbon particles).

c. Lung histology from a 10 % OSRL exposed rat showing localized severe thickening of the alveolar wall (AW). There are moderate increase histiocytes (H) within the bronchial associated lymphoid tissue (BALT) and moderate perivascular cuff.

d. Lung histology from a 25 % AERL exposed rat showing moderate widespread proliferative thickening of the alveolar wall (AW). The pulmonary vessels are moderately congested (C) and there were lymphocytic perivascular cuff. H&E, x 400.

4.4.6 Haematological alterations in leachate treated rats

Rat exposed to different concentrations of OSRL, OSSL, AERL and AESL samples showed changes in the production of white blood cell and its differentials (tables 4.33 and 4.34). The leachates at the different concentrations induced concentration dependent and significant ($p < 0.05$) decrease in the total leucocyte counts (leucopenia). When compared with the negative control group, the decrease was significant (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) only at higher concentrations (5 – 25 %) of the leachate samples and CYP. Female rat showed lower values for the white blood cell count than males and raw leachates also show more decrease in white blood cell production than simulated leachates. There was decrease in lymphocyte and MXD production in a concentration dependent manner in leachates exposed rats compared to the negative control and this was significant ($p < 0.05$) at higher concentrations (5 – 25 %) of the leachates. Also, percentage lymphocyte and MXD production was lower for the raw leachates (OSL and AEL) than the simulated leachates (OSSL and AESL) and in female exposed rats than in males. There was increase in the percentage neutrophil production in leachate exposed rat compared to control in a concentration dependent pattern and was significant ($p < 0.05$) at the higher concentrations (5 – 25 %) of the leachate samples. This increase was more in female rat than males and in raw leachates (OSL and AEL) than the simulated leachates (OSSL and AESL).

Figures 4.12 – 4.25 show the results of the erythrocyte and its indices, percentage haematocrits, haemoglobin concentrations and platelets counts in male and female rats exposed to the different concentrations of the leachate samples and CYP for 30 days. There was concentration dependent, significant ($p < 0.05$) decrease in the erythrocyte count in rats exposed to the leachate samples compared to the negative control. Comparing the level of significance of

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the concentrations of the leachates with the negative control using Dunnett's multiple posthoc test showed that all tested concentrations were significant ($p < 0.05$) except for OSRL female rats exposed to the 1% concentration that was insignificant ($p > 0.05$). Significant correlation exist between leachate exposure and decrease in the red blood cell count of the OSRL male rats ($F = 285.7$, $r = 0.9919$, $p < 0.0001$), OSRL female rats ($F = 669.5$, $r = 0.9965$, $p < 0.0001$), AERL male rats ($F = 379.4$, $r = 0.9939$, $p < 0.0001$) and AERL female rats ($F = 1494$, $r = 0.9984$, $p < 0.0001$). Similar trend exist for AESL and OSSL exposed rats. The total red blood cell counts were higher in AESL and OSSL than in OSRL and AERL exposed rats. RBC count of female rats exposed to the 1.0 % concentration of AESL was insignificant ($p > 0.05$).

Table 4.30. Effects of Olusosun and Aba-Eku landfill leachates on serum AST and ALT activities in treated rats

Concentration 0.5ml/rat/day	Serum AST (IU/L)		Serum ALT (IU/L)	
	OSRL	AERL	OSRL	AERL
MALES				
Dist. water	73.54±1.87	73.54±1.87	29.46±1.74	29.46±1.74
1.0%	91.42±1.45^{b}	89.35±2.14^{b}	31.73±1.49	31.58±0.74
2.5%	113.40±0.97^{c}	101.60±1.75^{c}	33.61±1.22	34.21±1.11
5.0%	129.50±1.12^{c}	125.10±2.85^{c}	37.54±1.35^{b}	36.76±1.10
10.0%	144.90±2.03^{c}	139.30±1.08^{c}	44.30±0.38^{c}	45.35±1.88^{c}
25.0%	168.40±0.95^{c}	161.90±1.18^{c}	52.74±1.63^{c}	49.52±1.34^{c}
CYP	114.10±0.78^{c}	114.10±0.78^{c}	39.59±3.33^{b}	39.59±3.33^{b}
FEMALES				
Dist. Water	68.35±2.23	68.35±2.23	26.53±1.18	26.53±1.1
1.0%	86.88±1.64^{c}	84.48±1.46^{b}	31.05±2.10	30.66±1.45
2.50%	108.30±0.91^{c}	98.00±1.81^{c}	32.90±1.96	33.32±1.38^{a}

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5.0%	122.10±1.35[c]	115.10±1.88[c]	37.40±1.64[b]	35.40±1.01[b]
10.0%	127.90±1.29[c]	26.20±1.13[c]	40.41±1.34[c]	44.07±1.79[c]
25.0%	162.68±1.47[c]	158.70±1.37[c]	48.05±1.93[c]	48.60±0.99[c]
CYP	109.40±0.75[c]	109.40±0.75[c]	37.35±1.47[b]	37.35±1.47[b]

End point represents mean (± SE) for 5 rats/sex. Superscripts are significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from corresponding negative water.

Dist water = distilled water (negative control)

CYP = Cyclophosphamide (40 mg/Kg bwt; positive control)

AST = Aspartate aminotransferase.

ALT = Alanine aminotransferase.

Table 4.31. Effects of Olusosun and Aba-Eku landfill raw leachates on serum creatinine and urea intensities in exposed rats

	Serum Creatinine (mg/dL)		Serum Urea (mg/dL)	
Concentration 0.5ml/rat/day	OSRL	AERL	OSRL	AERL
MALES				
Dist. water	2.96±0.07	2.96±0.07	38.99±0.93	38.99±0.93
1.0%	2.87±0.06	2.84±0.14	42.79±1.31	41.42±0.36
2.5%	3.04±0.10	2.97±0.06	45.11±1.26[a]	44.38±0.16
5.0%	2.95±0.22	3.28±0.14	45.93±0.64[a]	45.64±0.15[a]
10.0%	3.87±0.10[b]	3.81±0.18[b]	51.05±0.21[b]	49.77±0.21[a]
25.0%	4.32±0.18[c]	4.30±0.19[c]	56.36±0.24[b]	54.97±0.34[b]
CYP	4.11±0.07[c]	4.11±0.07[c]	48.59±0.96a	48.59±0.96[a]
FEMALES				
Dist. Water	2.76±0.13	2.76±0.13	43.69±0.45	43.69±0.45
1.0%	2.69±0.10	2.68±0.12	44.62±0.99	44.15±0.51
2.50%	2.89±0.28	2.85±0.11	46.04±1.03	45.75±0.14

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5.0%	3.07±0.04	3.02±0.06	46.71±0.39	46.46±0.62
10.0%	3.54±0.16[a]	3.50±0.19[a]	57.13±0.13[b]	54.82±0.27[b]
25.0%	3.89±0.11[a]	3.87±0.14[a]	58.66±0.27[b]	57.17±0.13[b]
CYP	3.31±0.18[a]	3.31±0.18[a]	50.42±0.43[a]	50.42±0.43[a]

End point represents mean (± SE) for 5 rats/sex. Superscripts are significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from corresponding negative water.

Dist water = distilled water (negative control)

CYP = cyclophosphamide (40 mg/Kg bwt; positive control)

Table 4.32. Effects of Olusosun and Aba-Eku landfill raw leachates on serum albumin and total proteins in exposed rats

	Serum Albumin (g/dL)		Serum Total proteins (g/100 ml)	
Concentration 0.5ml/rat/day	OSRL	AERL	OSRL	AERL
MALES				
Dist. water	4.57±0.13	4.57±0.13	8.31±0.51	8.31±0.51
1.0%	4.21±0.22	4.25±0.34	7.57±0.19	7.82±0.36
2.5%	4.10±0.15	4.13±0.08	7.04±0.09[a]	7.47±0.51
5.0%	3.76±0.09[a]	3.97±0.13[a]	6.38±0.31[b]	7.05±0.10[a]
10.0%	3.28±0.05[b]	3.26±0.11[b]	5.06±0.27[c]	5.39±0.34[c]
25.0%	2.62±0.18[c]	2.70±0.15[c]	3.62±0.24[c]	3.97±0.10[c]
CYP	3.97±0.10	3.97±0.10	7.59±0.96	7.32±0.18
FEMALES				
Dist. Water	5.44±0.14	5.44±0.14	10.34±0.52	10.34±0.52
1.0%	5.11±0.31	5.16±1.17	8.24±0.48[a]	8.49±0.40[a]
2.50%	4.41±0.07[a]	4.42±0.26[a]	7.55±0.29[b]	8.11±0.31[a]

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5.0%	3.96±0.09[c]	3.93±0.07[c]	7.14±0.12[b]	7.51±0.34[b]
10.0%	3.69±0.92[c]	3.50±0.19[c]	5.73±0.15[c]	6.02±0.17[c]
25.0%	2.92±0.11[c]	2.87±0.14[c]	4.21±0.23[c]	4.49±0.13[c]
CYP	4.14±0.18[a]	4.14±0.18[a]	7.45±0.43[b]	7.45±0.17[a]

End point represents mean (± SE) for 5 rats/sex. Superscripts are significantly (a = $p<0.05$; b =$p<0.01$; c = $p<0.001$) different from corresponding negative water.

Dist water = distilled water (negative control)

CYP = cyclophosphamide (40 mg/Kg bwt; positive control)

The correlation coefficients between leachate exposure and decrease in the red blood cell count are ($F = 272.1$, $r = 0.9915$, $p < 0.0001$) for OSSL male, ($F = 1367$, $r = 0.9983$, $p < 0.0001$) for OSSL females, ($F = 365.0$, $r = 0.9936$, $p < 0.0001$) for AESL male and ($F = 3215$, $r = 0.9993$, $p < 0.0001$) for AESL females.

Values of the correlation coefficients obtained for the male and female rat showed that females had more decrease erythrocyte counts than males. The percentage contributing effects of leachates in the observed erythrocyte counts was more prominent (92.73 %; OSRL and AERL, $p < 0.0001$ and 94.82 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachate on the sex (2.61 %; OSRL and AERL, $p = 0.0445$ and 1.50 %; OSSL and AESL, $p<0.0001$) and sex alone (2.67 %; OSRL and AERL, $p < 0.0001$ and 2.66 %; OSSL and AESL, $p<0.0001$) (figures 4.12 and 4.13).

Figures 4.14 and 4.15 show a concentration dependent, significant ($p < 0.05$) decrease in the platelet count of rats exposed to the leachate samples compared to negative control. Comparing the level of significance of the tested concentrations of the leachates with the negative control showed that for OSRL and AERL samples, all tested concentrations were significant ($p < 0.05$) with the correlation coefficient between leachate exposure and decrease platelet counts ($F = 4351$, $r = 0.9995$, $p < 0.0001$) for OSRL male, ($F = 4889$, $r = 0.9995$, $p < 0.0001$) for OSRL female, ($F = 3627$, $r = 0.9994$, $p < 0.0001$) for AERL male and ($F = 2470$, $r = 0.9991$, $p < 0.0001$) for AERL female. Also, OSSL and AESL samples at all tested

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concentrations were significant ($p < 0.05$) with correlation coefficients of ($F = 1356$, $r = 0.9983$, $p < 0.0001$) for OSSL male, ($F = 2290$, $r = 0.9990$, $p < 0.0001$) for OSSL female, ($F = 1381$, $r = 0.9983$, $p < 0.0001$) for AESL male and ($F = 2298$, $r = 0.9990$, $p < 0.0001$) for AESL female. The leachate samples caused lower platelet counts in female rats than males according to the values of their correlation coefficients. The effects of leachate in the observed decrease in platelet counts was prominent (95.80 %; OSRL and AERL, $p < 0.0001$ and 90.67 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachate on the sex (2.08 %; OSRL and AERL, $p < 0.0001$ and 6.06 %; OSSL and AESL, $p<0.0001$) and sex alone (2.00 %; OSRL and AERL, $p < 0.0001$ and 3.00 %; OSSL and AESL, $p<0.0001$).

Table 4.33 Alterations in leucocyte and differential counts in rats exposed to Olusosun landfill leachates.

	Conc (%)	WBC (x 10^3 µL)	LYM (%)	NEU (%)	MXD (%)
OSRL	MALE				
	DW	13.92 ± 0.47	72.37 ± 0.69	21.50 ± 1.13	6.13 ± 1.82
	1	13.41± 0.39	72.12 ± 0.35	22.92 ± 0.20	4.96 ± 0.48
	2.5	13.14 ± 0.10	72.23 ± 1.28	23.67 ± 1.13	4.10 ± 2.40^a
	5	11.43 ± 0.40^b	70.80 ± 0.52	25.67 ± 0.65^a	3.53 ± 0.18^b
	10	6.02 ± 0.32^c	63.47 ± 0.23^b	32.60 ± 0.21^b	3.93 ± 0.15^a
	25	4.72 ± 0.51^c	41.27 ± 0.24^c	55.63 ± 0.18^c	3.10 ± 0.42^b
	CYP	5.64 ± 0.35^c	55.23 ± 0.97^c	40.79 ± 2.29^b	3.98 ± 2.80^a
	FEMALE				
	DW	13.13 ± 0.62	69.77 ± 1.15	25.50 ± 1.19	4.73 ± 2.13
	1	13.02 ± 0.33	68.69 ± 0.65	26.74 ± 0.68	4.57 ± 0.41
	2.5	12.06 ± 0.09	67.63 ± 0.72	27.84 ± 0.69^a	4.53 ± 0.03
	5	11.09 ± 0.22^a	63.16 ± 0.52^a	32.73 ± 0.52^b	4.11 ± 1.04
	10	5.95 ± 0.16^c	46.42 ± 0.23^c	49.61 ± 0.14^c	3.97 ± 0.24^a
	25	4.69 ± 0.39^c	38.60 ± 1.13^c	58.19 ± 1.00^c	3.21 ± 0.26^a
	CYP	5.61 ± 0.21^c	54.33 ± 0.79^b	42.47 ± 1.04^c	3.20 ± 0.85^a
OSSL	MALE				
	DW	13.92 ± 0.47	72.37 ± 0.69	21.50 ± 1.13	6.13 ± 1.82
	1	13.43 ± 1.15	72.13 ± 3.36	22.60 ± 0.20	5.27 ± 1.05
	2.5	13.23 ± 0.40	72.02 ± 0.24	22.79 ± 0.15	5.19 ± 0.12
	5	12.85 ± 0.49	69.73 ± 0.46^a	25.47 ± 0.41^a	4.80 ± 0.12^a

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10	9.02 ± 0.21[c]	62.40 ± 0.51[b]	36.63 ± 0.71[c]	3.97 ± 1.18[b]
25	7.80 ± 0.03[c]	57.60 ± 0.15[c]	39.13 ± 0.19[c]	3.27 ± 0.18[c]
CYP	5.64 ± 0.35[c]	55.23 ± 0.97[c]	40.79 ± 2.29[c]	3.98 ± 2.80[b]
FEMALE				
DW	13.13 ± 0.62	69.77 ± 1.15	25.50 ± 1.19	4.73 ± 2.13
1	13.09 ± 0.65	69.47 ± 0.73	26.66 ± 0.15	3.87 ± 0.78
2.5	12.86 ± 0.75	68.95 ± 0.63	27.25 ± 0.66	3.80 ± 0.21
5	11.92 ± 0.67[a]	64.57 ± 0.98[a]	31.80 ± 1.22[a]	3.63 ± 2.20[a]
10	9.95 ± 0.02[b]	58.60 ± 0.78[c]	38.06 ± 1.06[b]	3.34 ± 0.37[a]
25	7.40 ± 0.02[c]	49.60 ± 0.51[c]	47.11 ± 0.46[c]	3.29 ± 0.97[b]
CYP	5.61 ± 0.21[c]	54.33 ± 0.79[c]	42.47 ± 1.04[c]	3.20 ± 0.85[b]

Values are in mean (± SE).

Superscripts differ significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) from corresponding DW using Dunnett's multiple post hoc test.

DW- Distilled water.

CYP- Cyclophosphamide (40 mg/kg/bw).

WBC – White blood cells
LYM – Lymphocytes
NEU – Neutrophils
MXD – (Monocytes, Eosinophils and Basophils).

Table 4.34 Alterations in leucocyte and differential counts in rats expose to Aba-Eku landfill leachates

Conc (%)	WBC (x 10^3µL)	LYM (%)	NEU (%)	MXD (%)
ABA-EKU RAW LEACHATE (AERL)				
MALE				
DW	13.92 ± 0.47	72.37 ± 0.69	21.50 ± 1.13	6.13 ± 1.82
1.0	13.81 ± 0.51	71.69 ± 3.36	22.41 ± 1.13	5.90 ± 2.26
2.5	13.74 ± 0.57	71.57 ± 1.79	22.65 ± 1.10	5.78 ± 2.89
5.0	10.17 ± 0.62[b]	69.43 ± 0.24	25.64 ± 0.67	4.93 ± 0.47[a]
10	7.54 ± 0.49[c]	61.67 ± 1.01[b]	33.60 ± 0.93[c]	4.73 ± 0.15[a]
25	5.12 ± 0.62[c]	46.80 ± 0.67[c]	49.67 ± 0.15[c]	3.53 ± 0.73[b]
CYP	5.64 ± 0.35[c]	55.23 ± 0.97[c]	40.79 ± 2.29[c]	3.98 ± 2.80[a]
FEMALE				
DW	13.13 ± 0.62	69.77 ± 1.15	25.50 ± 1.19	4.73 ± 2.13
1.0	13.05 ± 0.57	67.89 ± 1.76	27.54 ± 2.97	4.57 ± 1.21
2.5	12.95 ± 0.55	65.47 ± 0.69	30.10 ± 0.15[a]	4.43 ± 0.84
5	10.88 ± 0.60[a]	63.40 ± 1.69[a]	32.73 ± 0.12[b]	3.87 ± 1.81[a]
10	6.44 ± 0.53[c]	58.53 ± 0.67[c]	37.84 ± 0.18[c]	3.63 ± 0.81[a]
25	4.98 ± 0.09[c]	44.67 ± 1.21[c]	52.32 ± 1.07[c]	3.01 ± 2.28[a]
CYP	5.61 ± 0.21[c]	54.33 ± 0.79[b]	42.47 ± 1.04[c]	3.20 ± 0.85[a]
ABA-EKU SIMULATED LEACHATE (AESL)				
MALE				
DW	13.92 ± 0.47	72.37 ± 0.69	21.50 ± 1.13	6.13 ± 1.82

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1	13.44 ± 0.19	71.83 ± 1.27	23.17 ± 0.46	5.00 ± 1.73
2.5	12.72 ± 0.59	71.50 ± 0.55	23.53 ± 0.61	4.97 ± 0.06
5	10.85 ± 0.48[a]	70.37 ± 0.65	25.42 ± 0.67[a]	4.21 ± 1.30[a]
10	10.31 ± 0.24[a]	68.40 ± 1.33[a]	27.53 ± 0.98[a]	4.07 ± 0.35[a]
25	8.83 ± 0.34[c]	59.37 ± 1.28[c]	37.20 ± 0.70[c]	3.43 ± 0.58[c]
CYP	5.64 ± 0.35[c]	55.23 ± 0.97[c]	40.79 ± 2.29[c]	3.98 ± 2.80[c]
FEMALE				
DW	13.13 ± 0.62	69.77 ± 1.15	25.50 ± 1.19	4.73 ± 2.13
1	12.74 ± 0.67	69.60 ± 0.21	25.88 ± 0.78	4.52 ± 0.64
2.5	12.49 ± 0.66	69.57 ± 0.78	26.30 ± 0.21	4.13 ± 0.94
5	10.08 ±0.48[a]	63.53 ± 0.35[a]	33.46 ± 0.49[b]	3.01 ± 0.15[a]
10	9.85 ± 0.59[a]	60.57 ± 0.24[a]	36.49 ± 0.09[c]	2.94 ± 0.26[b]
25	8.44 ± 0.31[b]	56.57 ± 0.69[c]	40.80 ± 0.65[c]	2.63 ± 0.06[b]
CYP	5.61 ± 0.21[c]	54.33 ± 0.79[c]	42.47 ± 1.04[c]	3.20 ± 0.85[a]

Values are in mean (± SE).

Superscripts differ significantly (a = p<0.05; b = p<0.01; c = p<0.001) from corresponding DW using Dunnett's multiple post hoc test.

DW- Distilled water.

CYP- Cyclophosphamide (40 mg/kg/bw).

WBC – White blood cells
LYM – Lymphocytes
NEU – Neutrophils
MXD – (Monocytes, Eosinophils and Basophils).

Figures 4.16 and 4.17 show significant ($p < 0.05$) decrease in the haematocrit count of rats exposed to the leachate samples compared to negative control. Comparing the level of significance of the concentrations of the leachates with the negative control showed that the different concentrations of OSRL and AERL samples were significant ($p < 0.05$) except for 1.0 % concentrations that was insignificant ($p > 0.05$). The correlation coefficient between leachate exposure and decreased haematocrit count are ($F = 50.51$, $r = 0.9558$, $p < 0.0001$) for OSRL male, ($F = 36.85$, $r = 0.9405$, $p < 0.0001$) for OSRL female, ($F = 61.56$, $r = 0.9635$, $p < 0.0001$) for AERL male and ($F = 80.23$, $r = 0.9717$, $p < 0.0001$) for AERL female. Also, percentage haematocrit counts of rats exposed to different concentrations of OSSL and AESL samples were significant ($p < 0.05$) different from the negative control except for 1.0 and 2.5 % concentrations.

The correlation coefficients between leachate exposure and decrease in percentage haematocrit count are ($F = 31.08$, $r = 0.9302$, $p < 0.0001$) for OSSL male, ($F = 49.15$, $r = 0.9547$, $p < 0.0001$) for OSSL female, ($F = 60.64$, $r = 0.9629$, $p < 0.0001$) for AESL male and ($F = 57.63$, $r = 0.9611$, $p < 0.0001$) for AESL female. The values of the correlation coefficients showed that female rats had lower percentage haematocrit counts than males. The effects of leachate in the observed percentage haematocrit counts was prominent (89.07 %; OSRL and AERL, $p < 0.0001$ and 86.44 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachate on the sex

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(1.77 %; OSRL and AERL, p = 0.1412 and 0.87 %; OSSL and AESL, p = 0.1330) and sex alone (1.33 %; OSRL and AERL, p = 0.0005 and 1.01 %; OSSL and AESL, p = 0.0247).

Figures 4.18 and 4.19 show significant ($p < 0.05$) decrease in the haemoglobin content in leachate treated rats compared to negative control. Comparing the level of significance of the various concentrations of the tested leachates with the negative control using Dunnett's multiple posthoc test showed that except at the 1.0 % concentration, other tested concentrations of the leachate samples were significant ($p < 0.05$).

The correlation coefficient between leachate exposure and haemoglobin concentrations in treated rats are ($F = 817.1$, $r = 0.9972$, $p < 0.0001$) for OSRL male, ($F = 1430$, $r = 0.9984$, $p < 0.0001$) for OSRL female, ($F = 824.4$, $r = 0.9972$, $p < 0.0001$) for AERL male, ($F = 1244$, $r = 0.9981$, $p < 0.0001$) for AERL female. ($F = 619$, $r = 0.9962$, $p < 0.0001$) for OSSL male, ($F = 760.7$, $r = 0.9969$, $p < 0.0001$) for OSSL female, ($F = 861.6$, $r = 0.9973$, $p < 0.0001$) for AESL male and ($F = 1181$, $r = 0.9980$, $p < 0.0001$) for AESL female.

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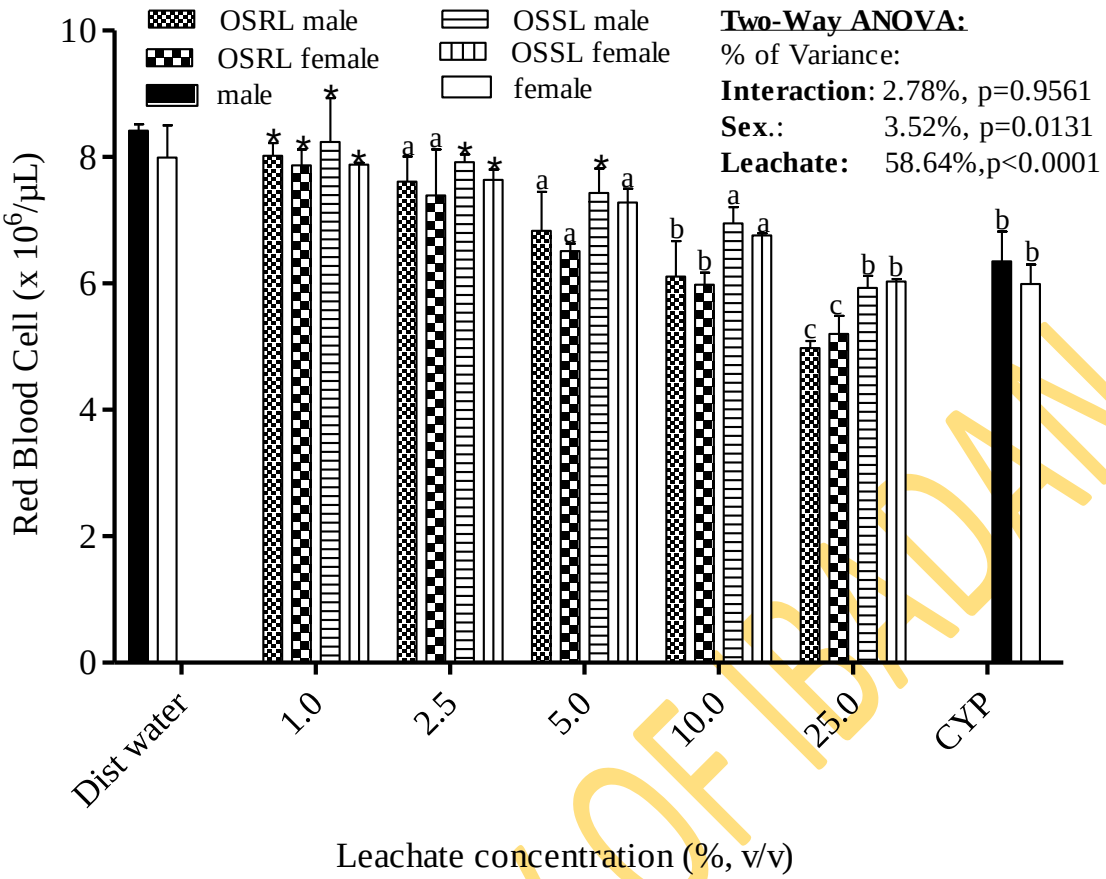

Fig. 4.12. Effects of Olusosun landfill raw and simulated leachates on erythrocyte count in rat. End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist. Water – Distilled water (negative control)

CYP – Cyclophosphamide (40 mg/Kg bwt).

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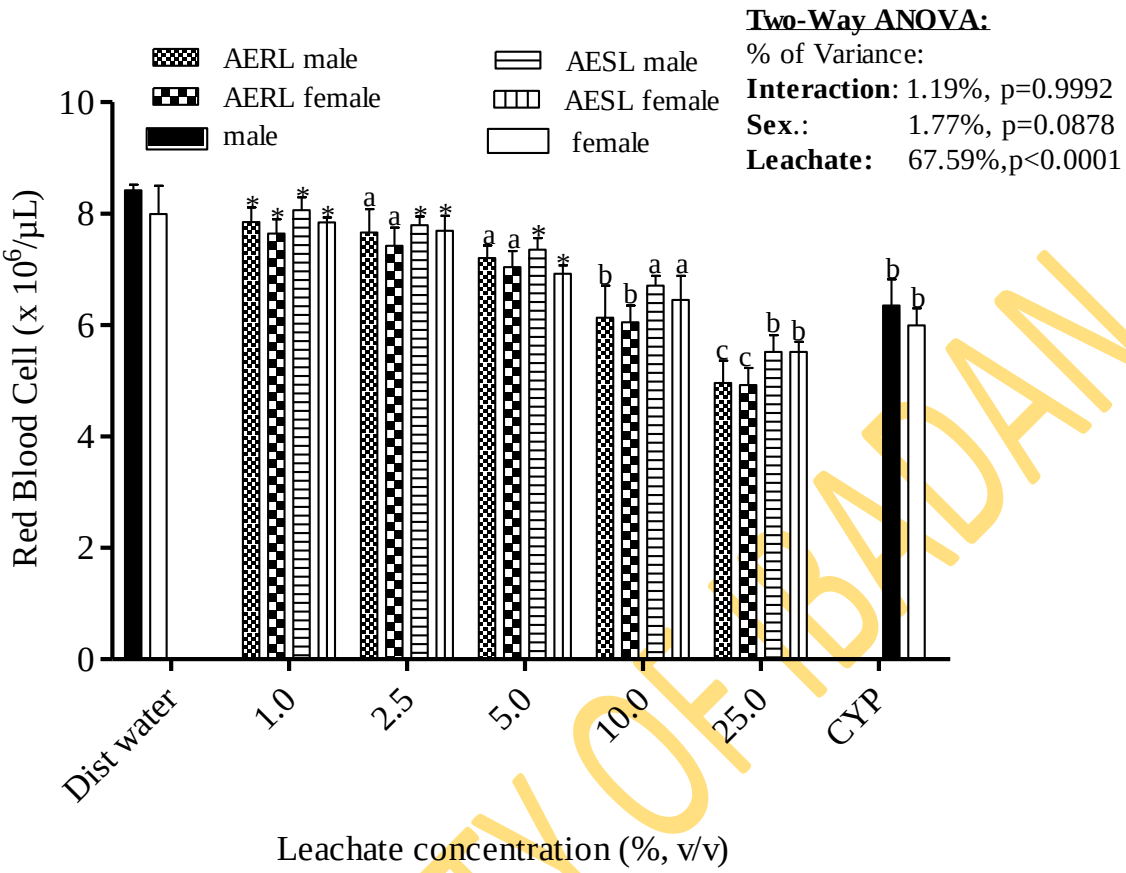

Fig. 4.13. Effects of Aba-Eku landfill raw and simulated leachates on Erythrocyte count in rat. End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist. Water – Distilled water (negative control)

CYP – Cyclophosphamide (40 mg/Kg bwt).

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Haemoglobin concentrations of leachate exposed rats were higher in males than females. The effects of leachate in the observed decrease in haemoglobin content was prominent (91.51 %; OSRL and AERL, $p < 0.0001$ and 91.02 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachate on the sex (1.72 %; OSRL and AERL, $p < 0.0001$ and 0.93 %;

OSSL and AESL, $p < 0.0001$) and sex alone (5.13 %; OSRL and AERL, $p < 0.0001$ and 7.51 %; OSSL and AESL, $p < 0.0001$).

Figures 4.20 and 4.21 show concentration independent, significant ($p < 0.05$) increase in the Mean Cell Haemoglobin (MCH) of rats exposed to the leachate samples compared to negative control. Comparing the MCH values of the negative control rats and leachate treated rats at the different concentrations using Dunnett's multiple posthoc test showed that only 10 and 25 % concentrations of the leachate samples were significant ($p < 0.05$).

The correlation coefficients between leachate exposure and increase MCH in rats are ($F = 801.4$, $r = 0.9971$, $p < 0.0001$) for OSRL male, ($F = 465.7$, $r = 0.9950$, $p < 0.0001$) for OSRL female, ($F = 206.8$, $r = 0.9888$, $p < 0.0001$) for AERL male and ($F = 1781$, $r = 0.9987$, $p < 0.0001$) for AERL female, ($F = 696$, $r = 0.9967$, $p < 0.0001$) for OSSL male, ($F = 434.8$, $r = 0.9947$, $p < 0.0001$) for OSSL female, ($F = 3044$, $r = 0.9992$, $p < 0.0001$) for AESL male and ($F = 4382$, $r = 0.9995$, $p < 0.0001$) for AESL female.

Though, MCH was higher in the female than male leachate exposed rats, but the effects of leachate samples in the MCH values of exposed rats was prominent (79.60 %; OSRL and AERL, $p < 0.0001$ and 82.76 %; OSSL and AESL, $p < 0.0001$) than interactive effects of the leachate on sex (16.88 %; OSRL and AERL, $p < 0.0001$ and 12.01 %; OSSL and AESL, $p < 0.0001$) and sex alone (3.19 %; OSRL and AERL, $p < 0.0001$ and 3.51 %; OSSL and AESL, $p < 0.0001$).

Figures 4.22 and 4.23 show concentration independent but significant ($p < 0.05$) decrease in the Mean Cell Haemoglobin Concentration (MCHC) of rats exposed to the leachate samples compared to negative control. The MCHC values of the various concentrations of the leachate and CYP treated rats compared with the negative control was significantly ($p<0.05$) different (Dunnett's multiple posthoc test). The correlation coefficient of leachate exposure and MCHC value in exposed rats are ($F = 5363$, $r = 0.9996$, $p < 0.0001$) for OSRL male, ($F = 1920$, $r = 0.9988$, $p < 0.0001$) for OSRL female, ($F = 3812$, $r = 0.9994$, $p < 0.0001$) for AERL male, ($F = 1833$, $r = 0.9987$, $p < 0.0001$) for AERL female, ($F = 3439$, $r = 0.9993$, $p < 0.0001$) for OSSL male, ($F = 4446$, $r = 0.9995$, $p < 0.0001$) for OSSL female, ($F = 4444$, $r = 0.9993$, $p < 0.0001$) for AESL male and ($F = 685$, $r = 0.9966$, $p < 0.0001$) for AESL female. MCHC was lower in the leachate exposed female rats than males.

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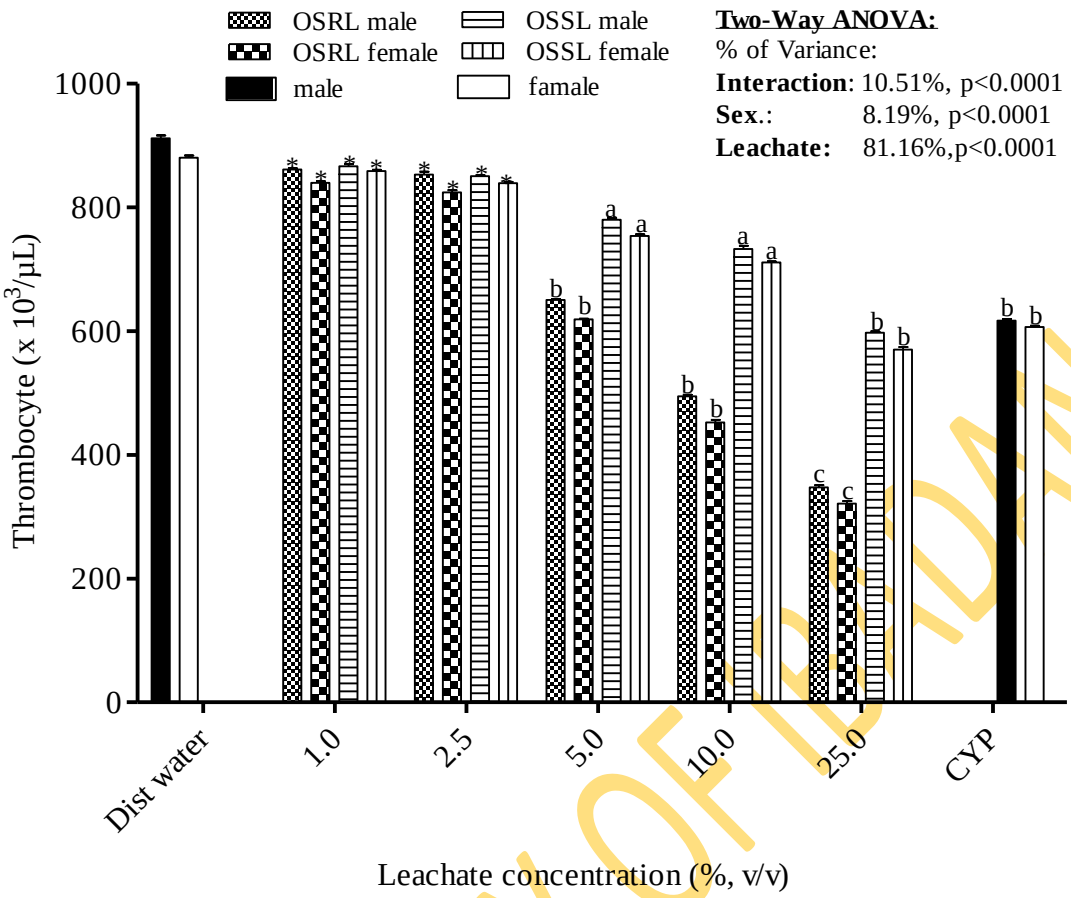

Fig. 4.14. Effects of Olusosun landfill raw and simulated leachates on platelet counts in rat.

End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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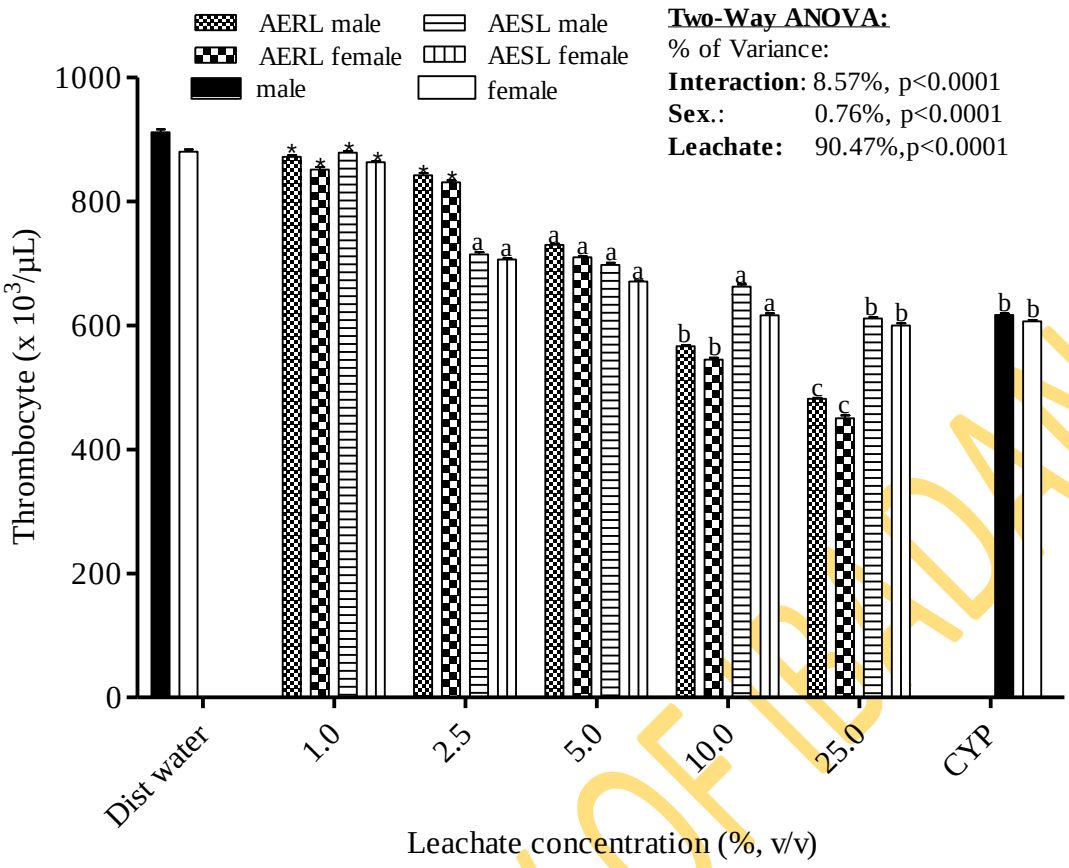

Fig. 4.15. Effects of Aba-Eku landfill raw and simulated leachates on platelet count in rat.

End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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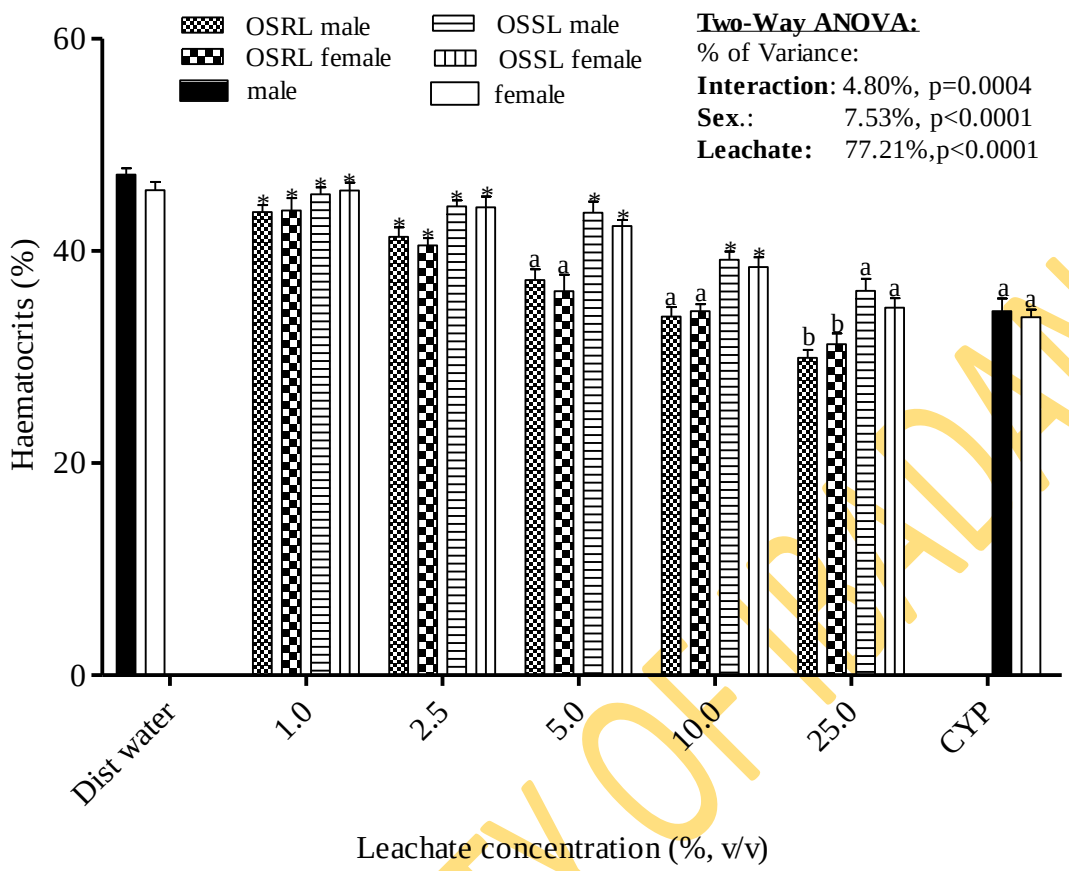

Fig. 4.16 Effects of Olusosun landfill raw and simulated leachates on percentage haematocrits in rat.

End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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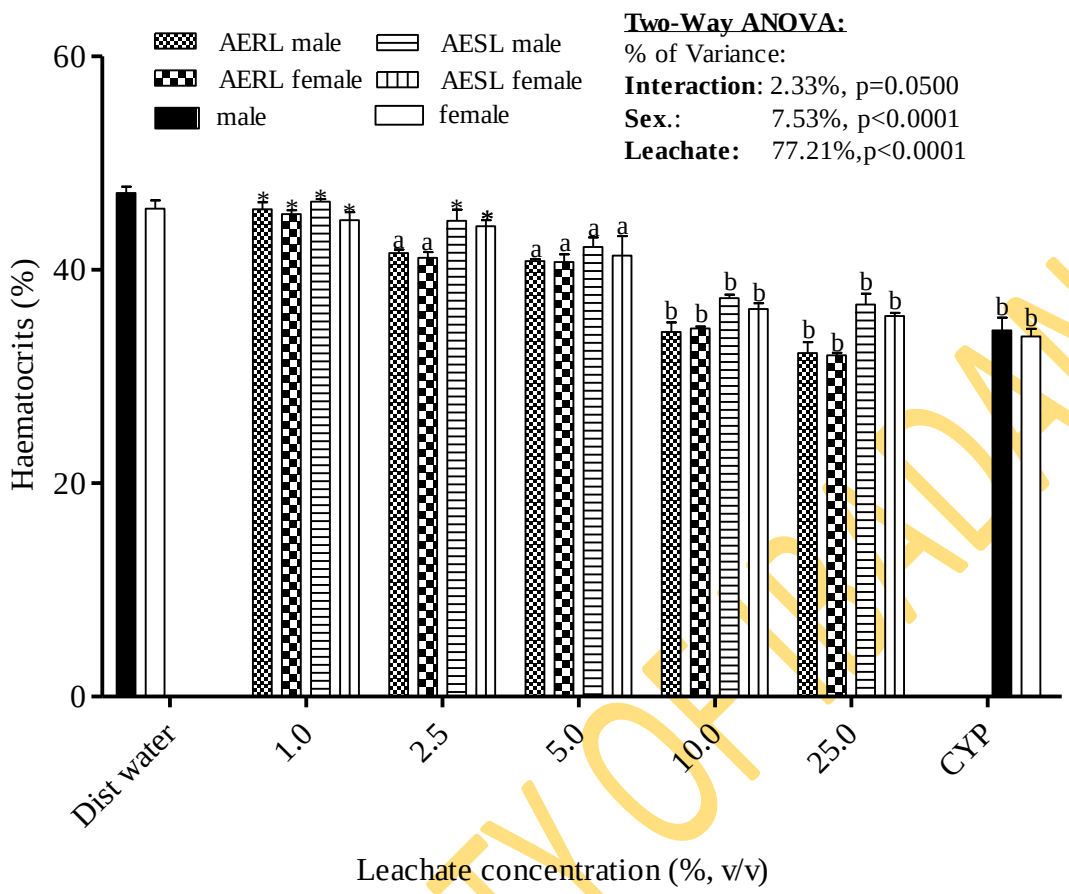

Fig. 4.17 Effects of Aba-Eku landfill raw and simulated leachates on percentage haematocrits in rat.

End point represents mean (± SE) for 5 rats/sex.

Values are significantly different (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$; * = $p>0.05$, insignificant) compared to negative control.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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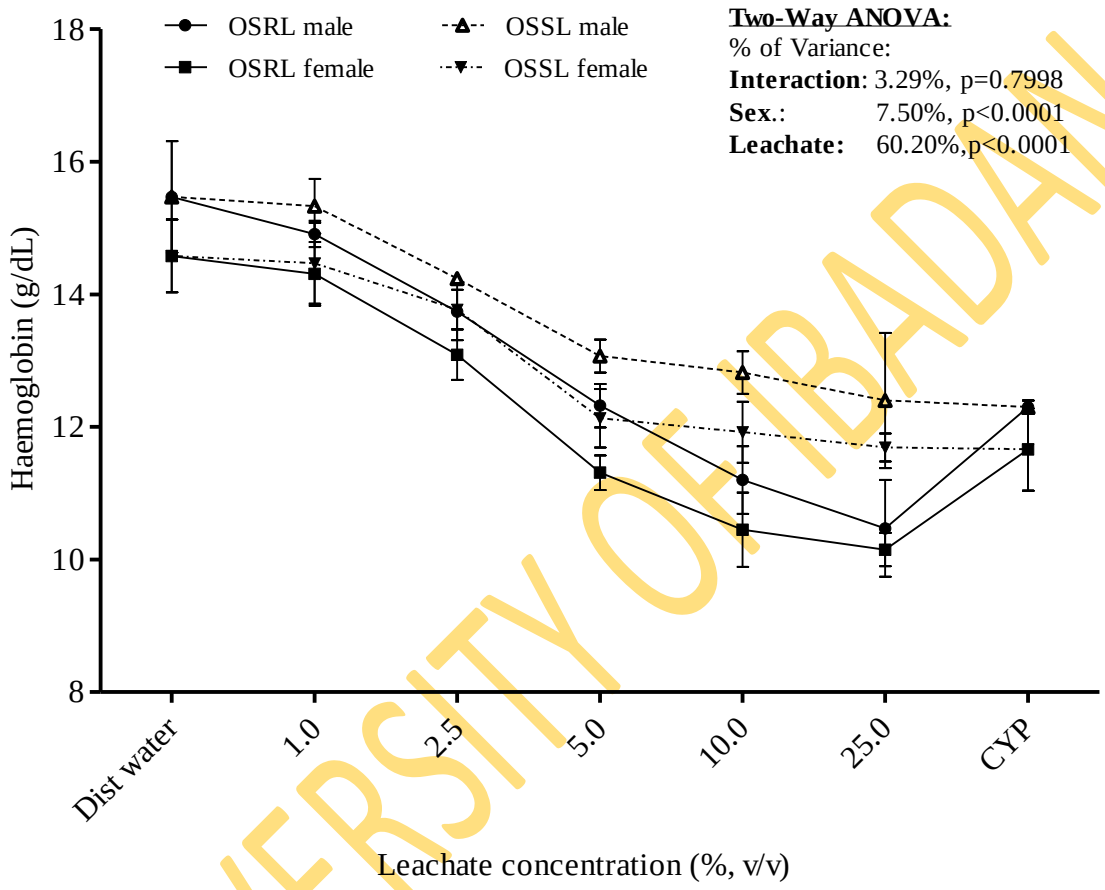

Fig. 4.18 Effects of Olusosun landfill raw and simulated leachates on haemoglobin concentrations in rat.

End point represents mean (± SE) for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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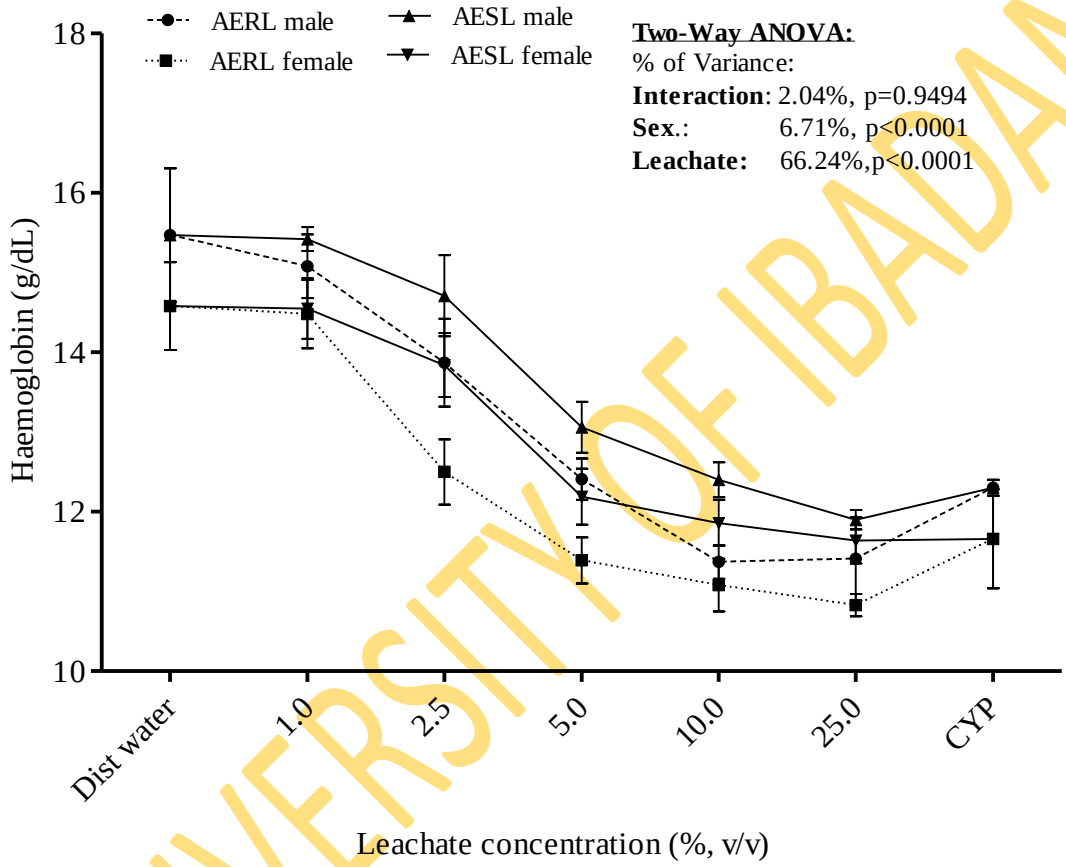

Fig. 4.19 Effects of Aba-Eku landfill raw and simulated leachates on haemoglobin concentrations in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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The percentage contribution of leachates in the observed MCHC value was prominent (91.91 %; OSRL and AERL, $p < 0.0001$ and 89.11 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachate on the sex (6.99 %; OSRL and AERL, $p < 0.0001$ and 8.76 %; OSSL and AESL, $p < 0.0001$) and sex alone (0.93 %; OSL and AEL, $p < 0.0001$ and 1.90 %; OSSL and AESL, $p < 0.0001$).

Figures 4.24 and 4.25 show concentration independent but significant ($p < 0.05$) increase in the Mean Cell Volume (MCV) of rats exposed to the leachate samples compared to negative control. Comparing MCV values of rats treated with various concentrations of the leachates with the negative control using Dunnett's multiple posthoc test was significant ($p < 0.05$) except for 1.0 % OSSL treated male rats. The correlation coefficients between leachate exposure and increase in MCV are ($F = 17860$, $r = 0.9999$, $p < 0.0001$) for OSRL male, ($F = 16440$, $r = 0.9999$, $p < 0.0001$) for OSRL female, ($F = 37720$, $r = 0.9999$, $p < 0.0001$) for AERL male and ($F = 22460$, $r = 0.9999$, $p < 0.0001$) for AERL female, ($F = 17290$, $r = 0.9999$, $p < 0.0001$) for OSSL male, ($F = 5340$, $r = 0.9996$, $p < 0.0001$) for OSSL female, ($F = 6865$, $r = 0.9997$, $p < 0.0001$) for AESL male and ($F = 31920$, $r = 0.9999$, $p < 0.0001$) for AESL female. MCV was higher in the leachate treated female rats than male. The percentage contribution of leachates in the observed MCV value was prominent (82.43 %; OSRL and AERL, $p < 0.0001$ and 83.59 %; OSSL and AESL, $p < 0.0001$) than the interactive effects of leachates on sex (14.44 %; OSRL and AERL, $p < 0.0001$ and 11.98 %; OSSL and AESL, $p < 0.0001$) and sex alone (2.87 %; OSRL and AERL, $p < 0.0001$ and 4.39 %; OSSL and AESL, $p < 0.0001$).

4.4.7 Morphological changes in sizes and shapes of red blood cell of exposed rats

The leachate samples adversely affected the shapes and sizes of red blood corpuscles. There was concentration dependent, significant ($p < 0.05$) increase in the abnormal shapes and sizes of erythrocyte of leachate treated rats compared to the negative control (Tables 4.35 and 4.36). The observed erythrocyte morphological abnormalities include acanthocytosis, schizocytosis, target cells, echinocytosis, tear drop poikilocytes (figure 4.26). Acanthocytes and echinocytes were predominantly observed than target cells, schizocytes and tear drop cells in the treated rats than negative control group. The raw leachates treated rats showed higher erythrocyte morphological abnormalities than the simulated leachate treated rats.

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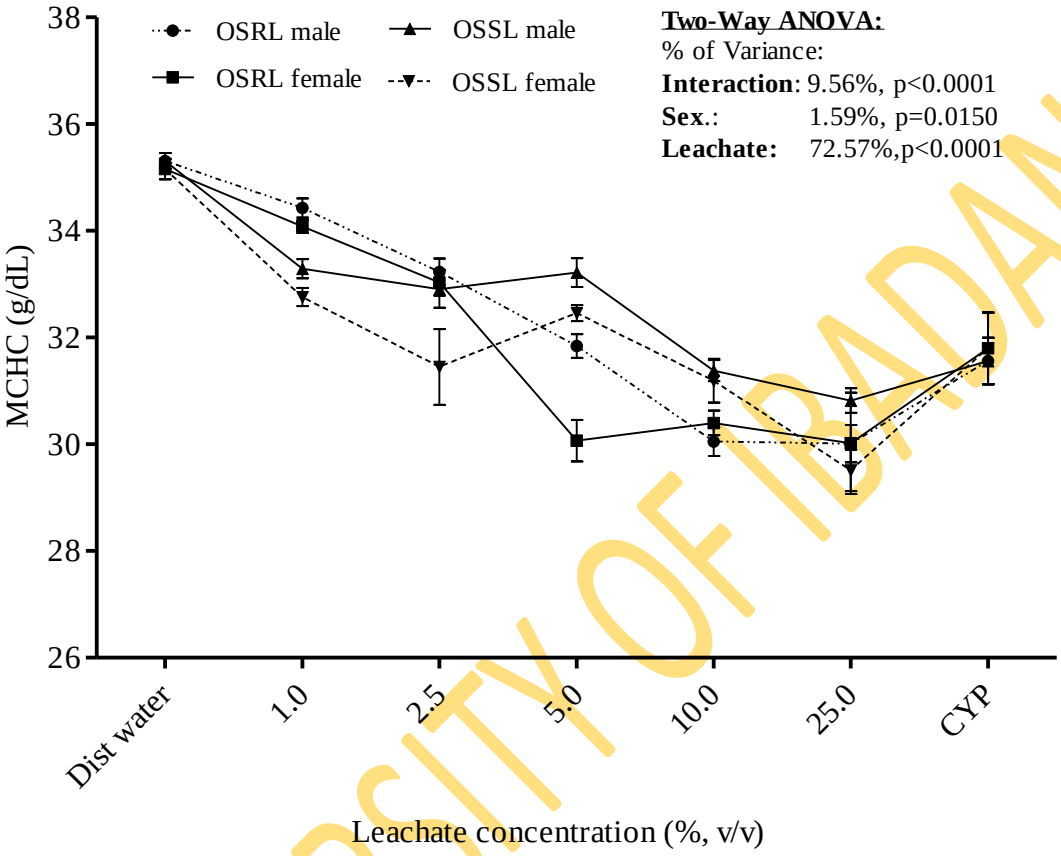

Fig. 4.20 Effects of Olusosun landfill raw and simulated leachates on Mean Corpuscular Haemoglobin Concentration in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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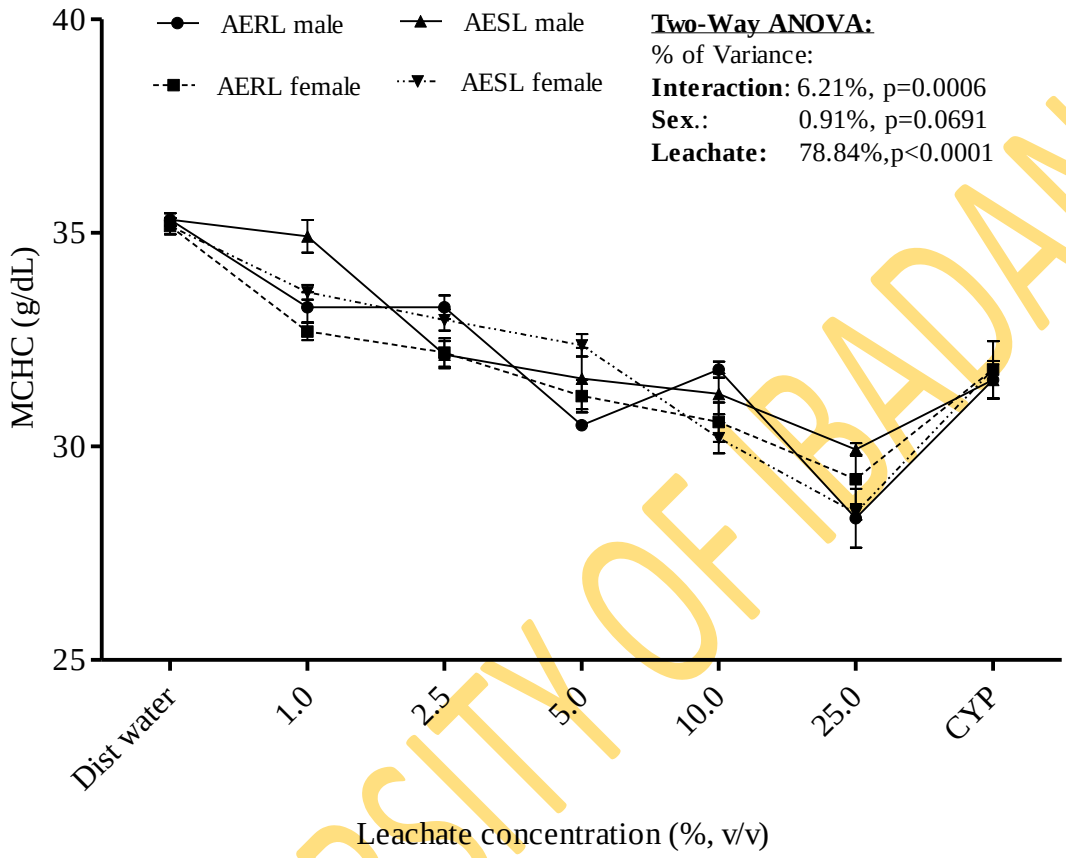

Figure 4.21Effects of Aba-Eku landfill raw and simulated leachates on Mean Corpuscle Haemoglobin Concentration in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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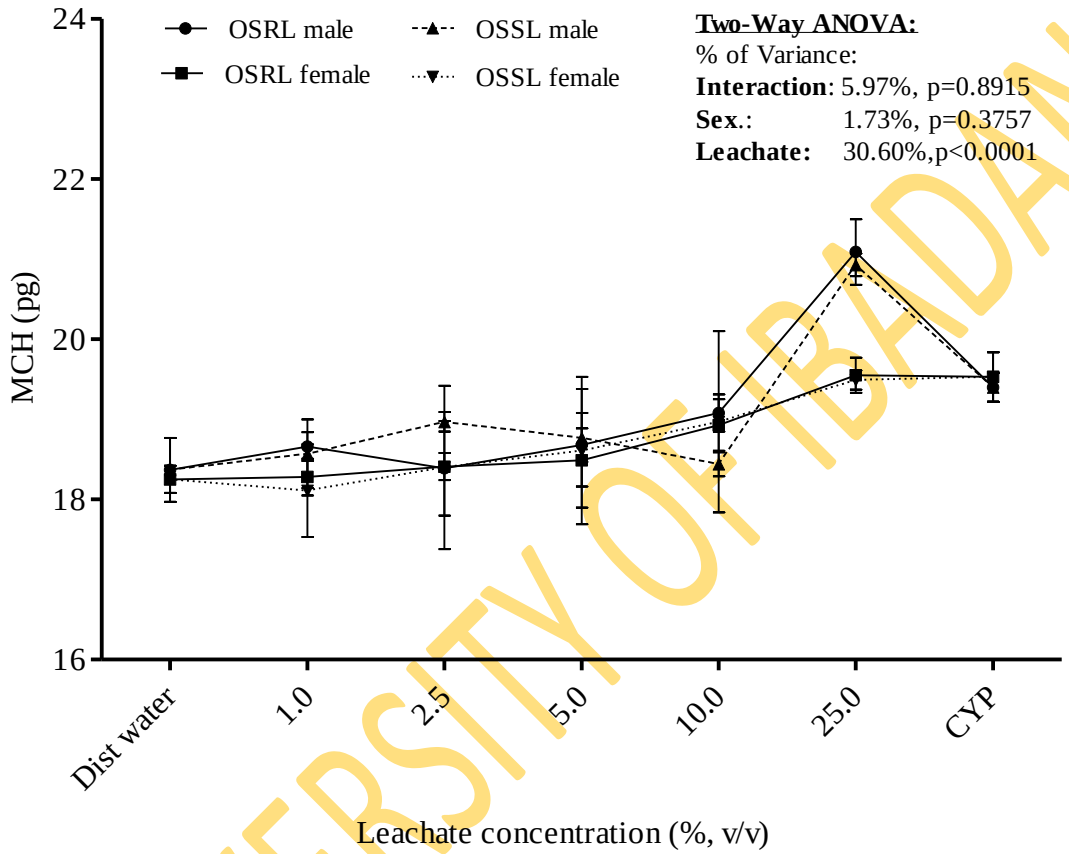

Fig. 4.22 Effects of Olusosun landfill raw and simulated leachates on Mean Corpuscle Haemoglobin in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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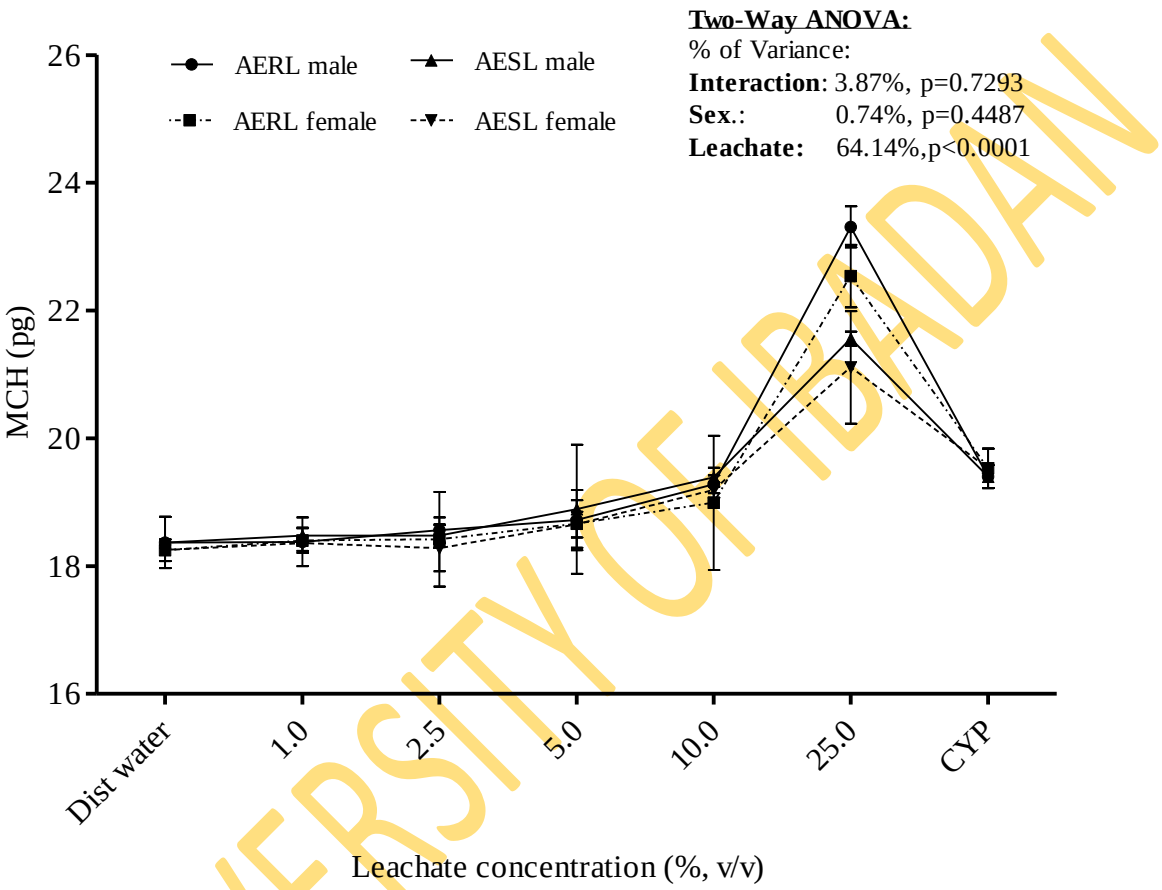

Fig. 4.23 Effects of Aba-Eku landfill raw and simulated leachates on Mean Corpuscle Haemoglobin in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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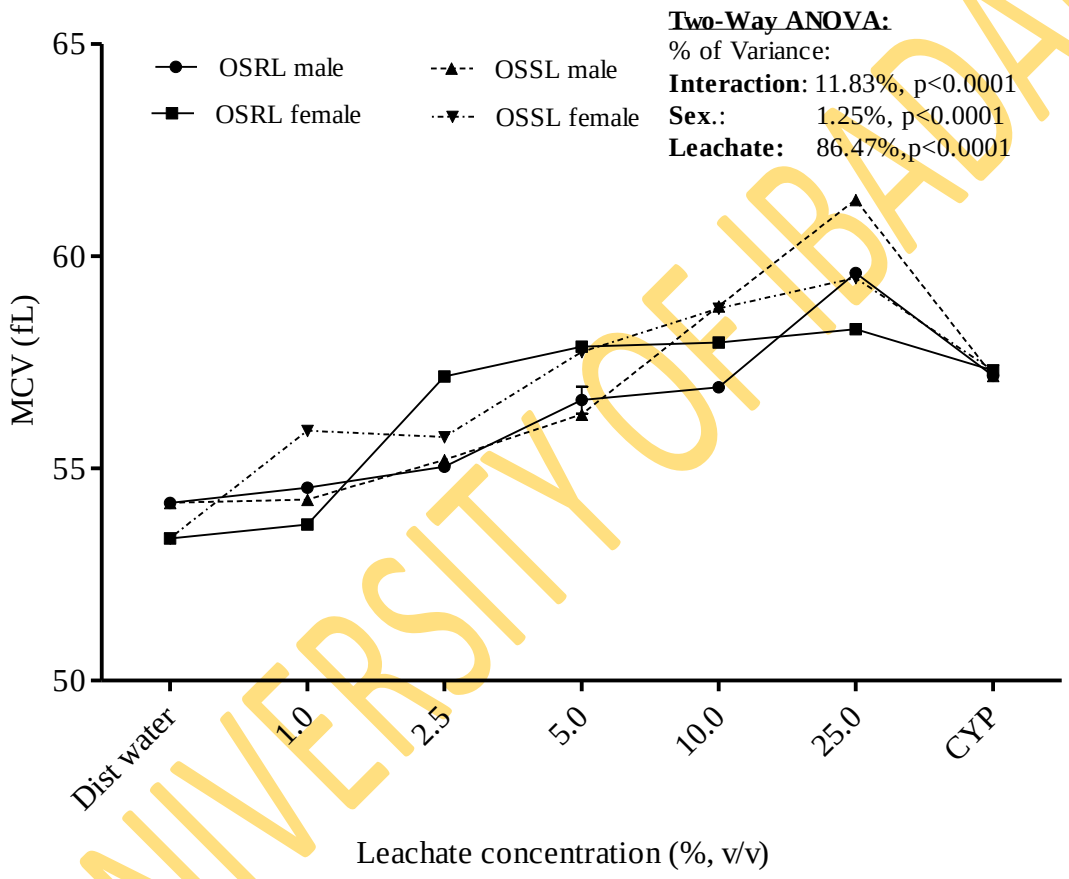

Fig. 4.24 Effects of Olusosun landfill raw and simulated leachates on Mean Corpuscle Volume in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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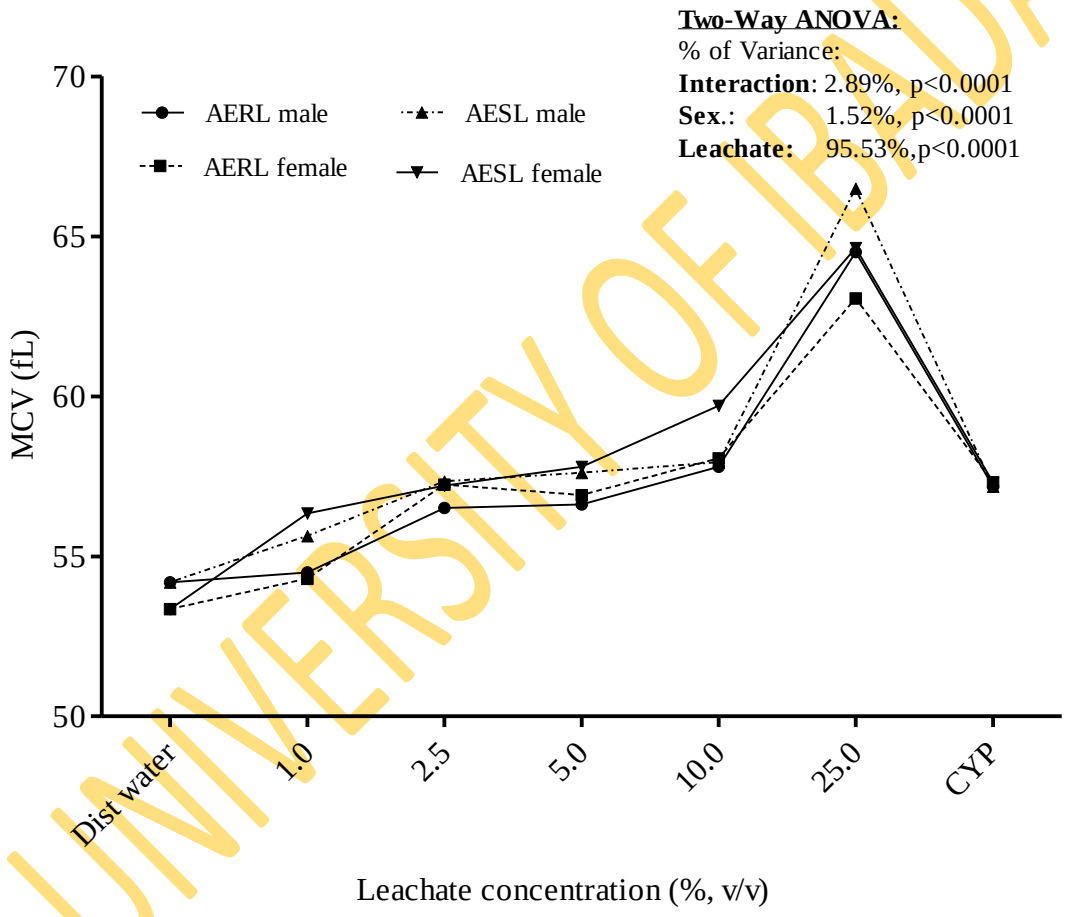

Fig. 4.25 Effects of Aba-Eku landfill raw and simulated leachates on Mean Corpuscle Volume in rat.

End point represents mean ± SE for 5 rats/sex.

Dist water – distilled water.

CYP – Cyclophosphamide (40 mg/Kg bwt).

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Table 4.35 Red blood cell morphological abnormalities in rats exposed to Olusosun landfill leachates

Conc (%)	Schizocyte	Tear drops	Target cell	Acanthocytes	Echinocyte
			OLUSOSUN RAW LEACHATE (OSRL)		
MALE					
DW	0.74±0.12	0.39±0.18	1.30±0.11	2.14±0.43	1.98±0.13
1.0	1.62± 0.33	0.64±0.16	4.22±0.41^{a}	14.72±0.92^{b}	10.12±1.23^{b}
2.5	4.21±0.40^{a}	0.94±0.25	10.68±1.42^{c}	23.50±2.10^{c}	16.94±0.92^{c}
5.0	10.43±0.40^{c}	3.73±0.13^{a}	15.19±0.61^{c}	24.72±1.22^{c}	21.76±1.41^{c}
10	27.14±0.12^{c}	7.46±1.01^{b}	26.73±1.23^{c}	29.51±0.25^{c}	25.19±0.63^{c}
25	34.16±0.31^{c}	11.14±0.44^{c}	31.16±0.24^{c}	35.18±1.12^{c}	32.24±1.09^{c}
CYP	19.63 ± 0.41^{c}	6.32±0.28^{b}	28.24±1.53^{c}	27.18±3.10^{c}	30.13±2.17^{c}
FEMALE					
DW	0.81±0.67	0.28±0.27	1.7±0.13	3.32±1.03	1.62±0.32
1.0	3.32 ± 0.53	0.73±0.15	3.39±0.18	15.12±0.51^{b}	10.23±1.32^{b}
2.5	4.51 ± 0.29	0.81±0.11	11.12±1.03^{b}	24.32±0.07^{c}	15.51±0.73^{c}
5.0	14.23±0.12^{c}	2.34±0.21^{a}	21.31±0.13^{c}	27.54±2.11^{c}	19.27±1.12^{c}
10	26.17±0.76^{c}	5.22±0.33^{c}	29.45±1.23^{c}	27.57±1.32^{c}	26.42±0.41^{c}
25	37.81±0.13^{c}	7.14±0.92^{c}	32.43±0.92^{c}	37.53±0.16^{c}	34.79±2.11^{c}
CYP	21.61 ± 0.27^{c}	6.24±1.23^{c}	29.24±1.04^{c}	33.40±1.15^{c}	32.18±1.09^{c}
			OLUSOSUN SIMULATED LEACHATE (OSSL)		
MALE					
1.0	1.10± 0.32	0.34±0.23	3.89±0.23	11.64±0.87^{a}	9.31±0.16^{b}
2.5	3.92±0.23^{a}	0.79±0.13	8.19±0.52^{b}	17.11±0.92^{b}	12.32±0.12^{b}
5.0	8.23±0.30^{b}	3.27±0.46^{a}	13.87±0.24^{b}	20.34±0.91^{c}	17.24±0.46^{c}
10	24.19±1.42^{c}	6.97±0.91^{b}	19.29±1.23^{c}	24.42±1.74^{c}	19.85±0.17^{c}
25	29.24±0.91^{c}	10.21±1.21^{c}	23.28±1.95^{c}	30.74±2.15^{c}	26.26±1.75^{c}
FEMALE					
1.0	2.91±0.21	0.51±0.12	2.11±0.12	12.31±0.71^{b}	8.82±0.28^{b}
2.5	4.07±0.19^{a}	0.76±0.37	9.35±1.01^{b}	18.48±0.49^{c}	12.12±0.35^{b}
5.0	13.72±1.24^{b}	2.12±0.72^{a}	15.31±0.28^{c}	24.46±1.32^{c}	17.23±0.98^{b}
10	24.29±0.98^{c}	4.76±0.98^{c}	21.24±1.41^{c}	26.29±1.78^{c}	18.94±1.72^{b}
25	32.21±1.02^{c}	6.55±0.19^{c}	29.52±0.86^{c}	31.29±1.82^{c}	24.26±1.06^{c}

Values are in mean (± SE).

Superscripts differ significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) from corresponding DW using Dunnett's multiple post hoc test.

DW- Distilled water.

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CYP- Cyclophosphamide (40 mg/kg/bw).

Table 4.36 Red blood cell morphological abnormalities in rats exposed to Aba-Eku landfill leachates.

Conc (%)	Schizocyte	Tear drops	Target cell	Acanthocytes	Echinocyte
			ABA-EKU RAW LEACHATE (AERL)		
MALE					
DW	0.74±0.12	0.39±0.18	1.30±0.11	2.14±0.43	1.98±0.13
1.0	1.19± 0.38	0.61±0.21	4.34±0.23^{a}	12.13±0.12^{a}	11.64±0.98^{b}
2.5	4.01±0.26^{a}	0.89±0.32	10.67±0.87^{b}	22.95±1.23^{b}	14.56±0.17^{b}
5.0	10.32±0.71^{b}	3.58±0.67^{a}	14.98±0.36^{b}	24.72±1.22^{c}	23.78±0.67^{c}
10	24.87±1.31^{c}	7.09±1.93^{c}	26.28±1.05^{c}	28.17±1.73^{c}	26.34±0.24^{c}
25	32.23±1.72^{c}	10.25±0.35^{c}	29.97±1.32^{c}	37.27±2.45^{c}	30.35±2.26^{c}
CYP	19.63 ± 0.41^{c}	6.32±0.28^{c}	28.24±1.53^{c}	27.18±3.10^{c}	30.13±2.17^{c}
FEMALE					
DW	0.81±0.67	0.28±0.27	1.7±0.13	3.32±1.03	1.62±0.32
1.0	3.02±0.83^{a}	0.63±0.15	3.21±0.76	14.19±0.16^{b}	10.15±0.29^{b}
2.5	4.49±0.59^{a}	0.79±0.14	10.73±0.73^{b}	25.12±1.19^{c}	15.01±1.91^{b}
5.0	12.29±0.98^{b}	2.14±0.76^{a}	19.21±0.93^{b}	26.09±1.16^{c}	18.16±0.32^{c}
10	24.24±0.72^{c}	5.19±0.72^{c}	26.37±1.76^{c}	27.15±0.44^{c}	24.26±0.23^{c}
25	36.12±2.43^{c}	6.98±0.19^{c}	30.28±2.32^{c}	35.14±0.27^{c}	32.27±1.37^{c}
CYP	21.61 ± 0.27^{c}	6.24±1.23^{c}	29.24±1.04^{c}	33.40±1.15^{c}	32.18±1.09^{c}
			ABA-EKU SIMULATED LEACHATE (AESL)		
MALE					
1.0	1.21± 0.01	0.41±0.54	3.34±0.91^{a}	9.72±0.27^{b}	9.01±0.35^{b}
2.5	3.17±0.38^{a}	0.69±0.74	8.27±0.28^{b}	16.12±0.14^{b}	10.45±0.71^{b}
5.0	9.36±0.25^{b}	3.19±0.34^{a}	11.12±0.92^{b}	19.45±0.88^{c}	15.32±0.53^{c}
10	23.73±1.54^{c}	6.74±0.17^{b}	17.29±0.53^{c}	22.67±1.25^{c}	17.95±0.64^{c}
25	28.64±1.83^{c}	9.97±0.84^{c}	21.28±1.32^{c}	29.18±1.24^{c}	24.84±1.19^{c}
FEMALE					
1.0	1.95±0.56	0.51±0.12	2.19±0.87	11.45±0.96^{b}	7.23±0.45^{b}
2.5	4.19±0.37^{a}	0.66±0.96	9.58±0.71^{b}	17.63±0.78^{b}	11.56±0.73^{c}
5.0	12.58±0.12^{b}	2.46±0.35^{a}	16.72±0.34^{b}	22.35±1.75^{c}	16.43±0.23^{c}
10	23.27±0.29^{c}	4.86±0.93^{c}	22.19±1.36^{c}	27.35±1.19^{c}	19.11±0.56^{c}
25	29.39±1.86^{c}	5.98±0.47^{c}	27.47±2.74^{c}	32.47±2.65^{c}	22.93±1.36^{c}
CYP	21.61 ± 0.27^{c}	6.24±1.23^{c}	29.24±1.04^{c}	33.40±1.15^{c}	32.18±1.09^{c}

Values are in mean (± SE).

Superscripts differ significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) from corresponding DW using Dunnett's multiple post hoc test.

DW- Distilled water.

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CYP- Cyclophosphamide (40 mg/kg/bw).

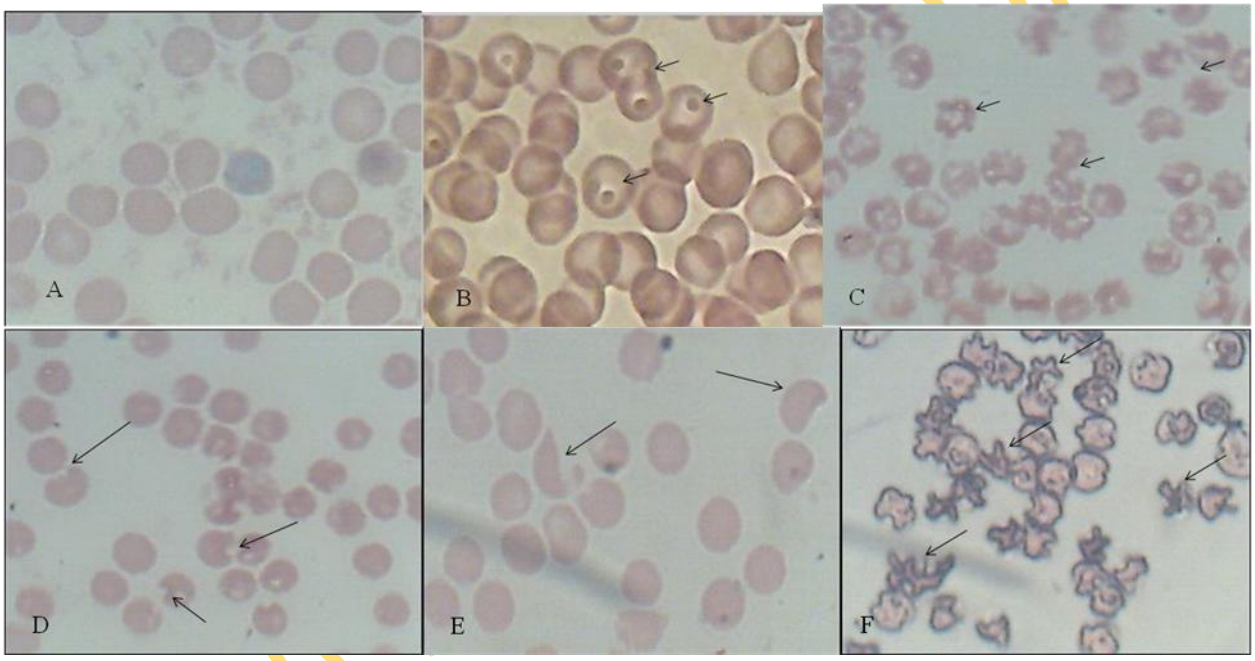

Figure 4.26. Red blood cell abnormal morphologies (arrowed) observed in rats exposed to Olusosun and Aba-Eku landfill leachates:

(a) normal red blood cells in the control groups

(b) target cells or codocytes (Heinz bodies resembling Bull eyes)

(c) acanthocytes (crenated): cells with shrink cell membranes

(d) schizocytes (fragmented red blood cells)

(e) tear drops (RBCs with spindle or sickle shape)

(f) echinocytes (burr cells): RBCs with star-like shapes

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4.5 *In vitro* cytogenotoxicity induced by Pb, Cr, Cu, Mn singly and in combination in cytokinesis – block WIL2-NS cells for the evaluation of potential interactive effects of leachate constituents

Pb, Cr, Cu, Mn and the combinations of these metals at different concentrations and folate status of the medium induced significant ($p < 0.05$) cytotoxic and genotoxic effects in WIL2–NS cells during the 72 hours culture. Pb, Cr, Cu, Mn and the combinations of the various tested concentrations of the metals, PMCC (lead; P, manganese; M, chromium; C and copper; C) induced different frequencies of micronucleated binucleated (MNed BN), nuclear bud binucleated (Nbud BN) and nucleoplasmic bridge binucleated (NPB BN) formation in WIL2–NS cells. Figures 4.27 – 4.31 showed in the frequencies of micronucleated binucleated WIL2–NS cells induced by the individual metals and their combinations in Low Folate (LF) and High Folate (HF) media. The induction of MNed BN WIL-2 NS cells was concentration dependent and significant ($p < 0.05$). The frequency of BNed MN cells was higher in LF than in HF medium for all the metals. Comparing the frequencies of MNed BN cell induction by the different concentrations of the metals with the corresponding controls (0.00µM) at the different folate status was significant ($p<0.05$) at higher concentrations of the metals. No viable WIL-2 NS cells were scored for MNed BN at 1000 µM concentrations of Mn and Cu, 100 and 1000 µM concentrations of Cr and 10, 100 and 1000 µM concentrations of PMCC since they all were totally necrotic. The percentage contributions of these metals in the induction of MNed BN WIL2–NS cells (82 – 93 % of the total variance) was higher than the effects of folic acid (FA; 2 – 8 %) and the interactive effects of metals and FA (2 – 4 %) as determined by two–way ANOVA. The genotoxic potentials of these metals from their percentage contributions is in the trend PMCC > Cr > Mn > Cu > Pb.

Individual and combination of the different concentrations of the tested metals induced significant ($p < 0.05$) increase in the frequencies of NPB in WIL-2 NS cells. The frequencies of NPB induction were higher in LF cells than in HF cells. Comparing the frequencies of induction of NPB in BN cells at the different concentrations of the tested metals with the corresponding control (0.00µM) using Dunnett's posthoc test was significant ($p < 0.05$) mostly at the higher concentrations of the metals (Figures 4.32 – 4.36). No viable WIL-2 NS cells were scored for NPB at 1000 µM concentrations of Mn and Cu, 100 and 1000 µM concentrations of Cr and 10, 100 and 1000 µM concentrations of PMCC since all the WIL-2 cells were totally necrotic. The percentage contributions of these metals in the induction of NPB WIL2–NS cells (75 – 83 % of the total variance) was higher than the effects of FA (5 – 23 %) and the interactive effects of

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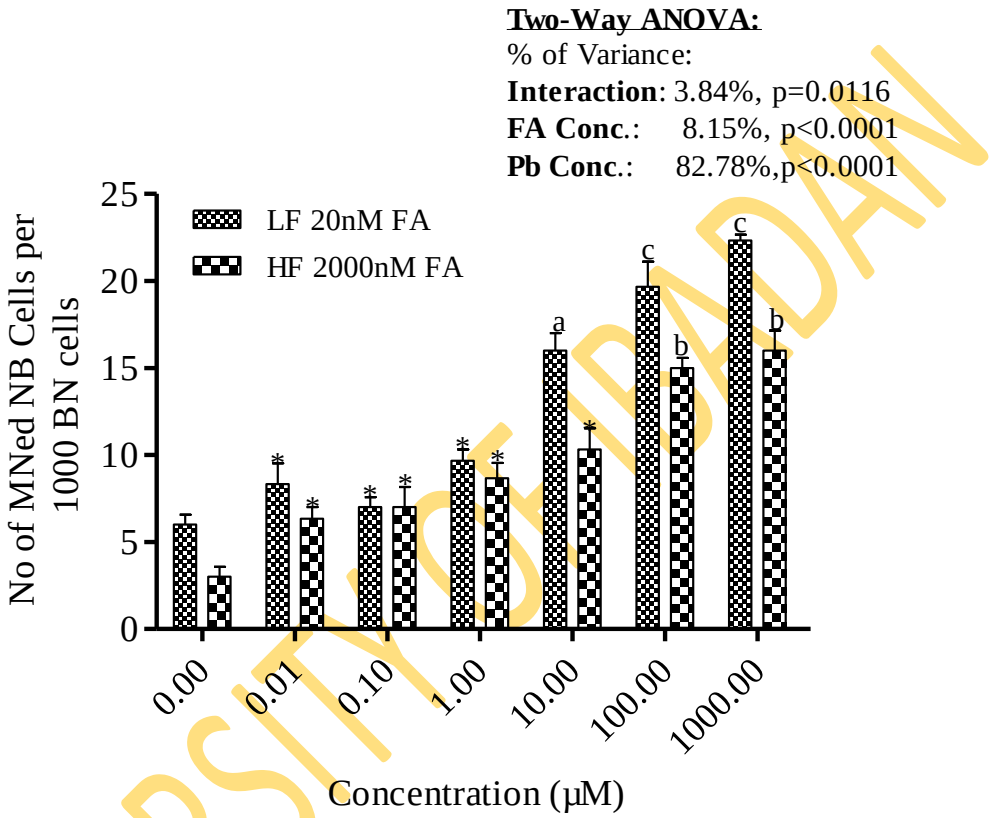

Figure 4.27 Frequency of MNed BN WIL2–NS cells exposed to different concentrations of lead (Pb) salt at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

MNed = Micronucleated

BN = Binucleated

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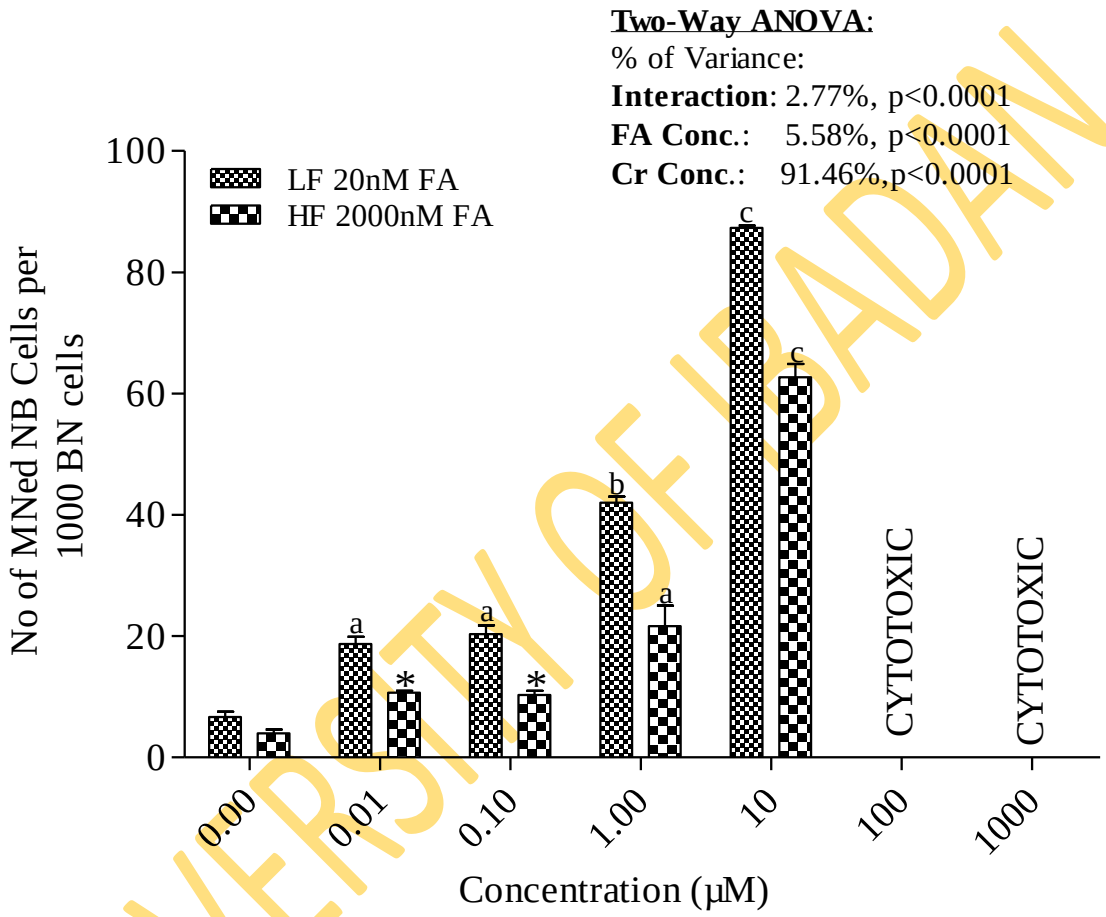

Figure 4.28 Frequency of MNed BN WIL2–NS cells exposed to different concentrations of chromium (Cr) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

MNed = Micronucleated

BN = Binucleated

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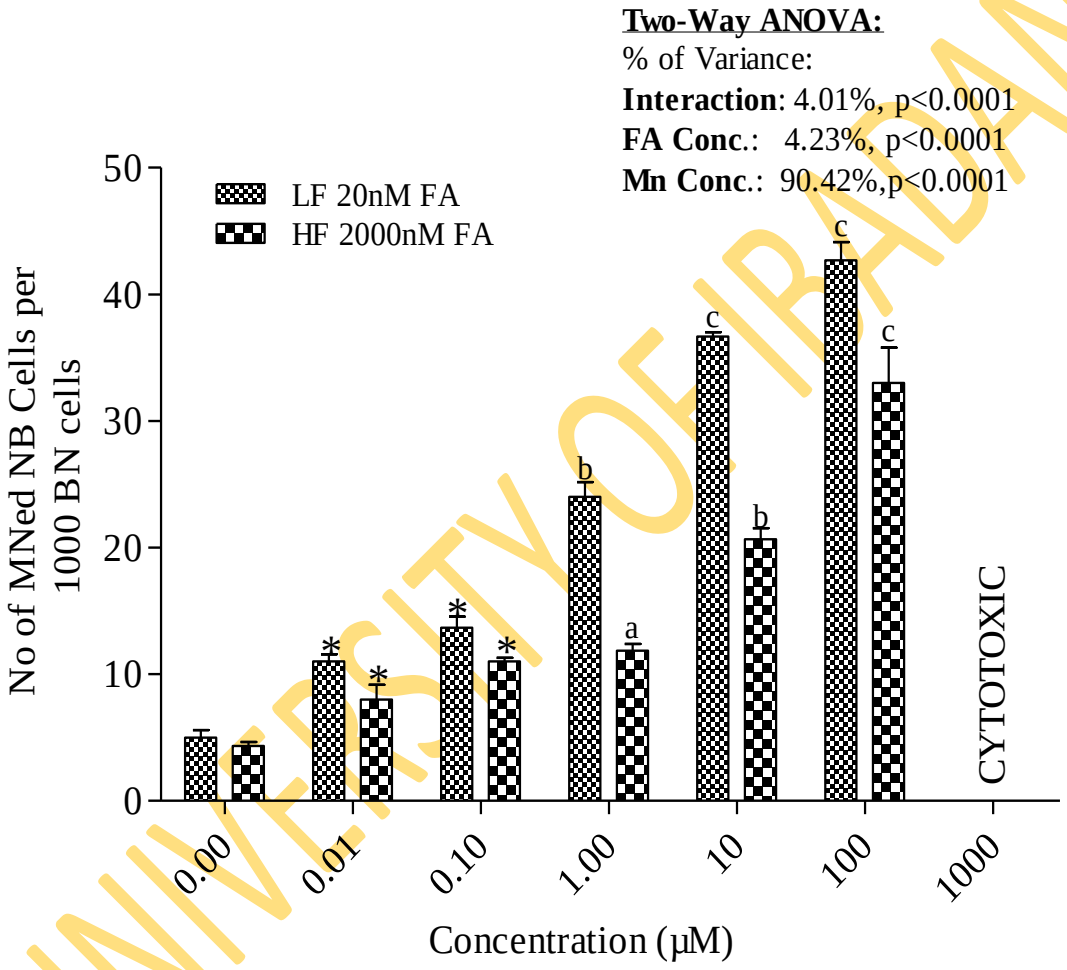

Figure 4.29 Frequency of MNed BN WIL2–NS cells exposed to different concentrations of manganese (Mn) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

MNed = Micronucleated

BN = Binucleated

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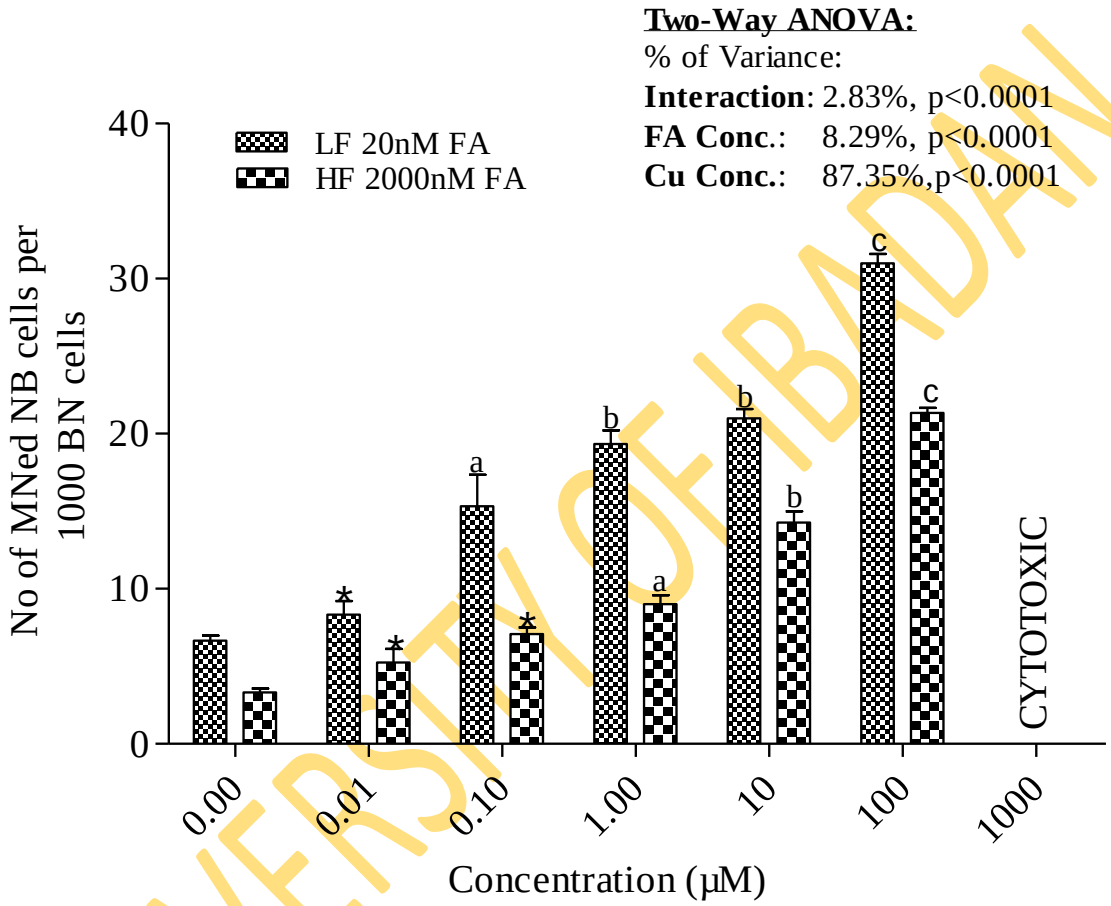

Figure 4.30 Frequency of MNed BN WIL2–NS cells exposed to different concentrations of copper (Cu) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

MNed = Micronucleated

BN = Binucleated

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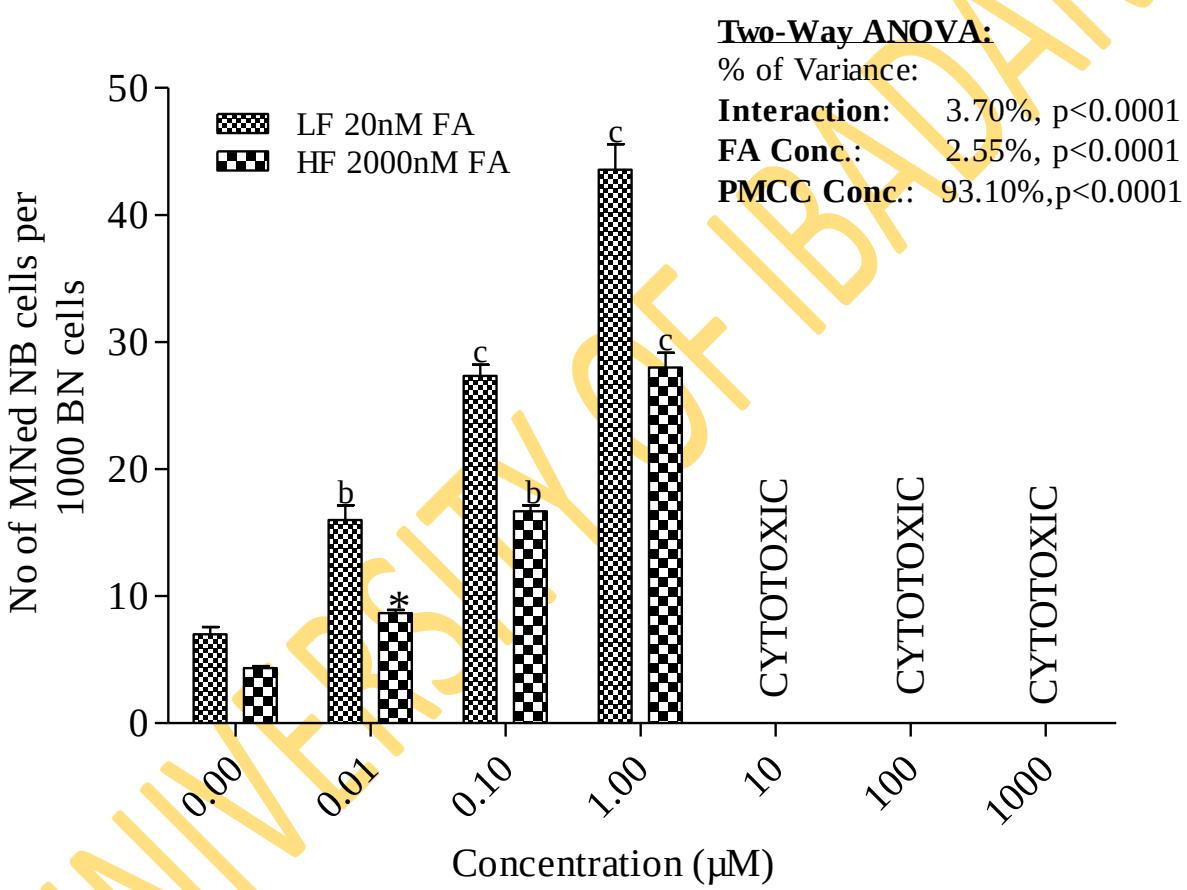

Figure 4.31 Frequency of MNed BN WIL2–NS cells exposed to different concentrations of PMCC at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

MNed = Micronucleated

BN = Binucleated

PMCC = Lead, Manganese, Copper and Chromium.

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metals and FA (5 – 15 %) as determined by two–way ANOVA. The genotoxic potentials of these metals from their percentage contributions is in the trend PMCC > Cr > Mn > Cu > Pb.

The individual and combined metals induced concentration dependent significant ($p < 0.05$) increase in the frequency of NBud in treated WIL2–N cells. The frequencies of NBud WIL2–NS cells induced by the tested metals in LF were higher than HF supplemented medium. Comparing the frequencies of NBud inductions at the different concentrations of the metals with the corresponding controls (0.00µM) was significant ($p < 0.05$) at higher concentrations of the metals. No viable WIL-2 NS cells were scored for NBud BN at 1000 µM concentrations of Mn and Cu, 100 and 1000 µM concentrations of Cr and 10, 100 and 1000 µM concentrations of PMCC since they all were totally necrotic. The percentage contributions of these metals in the induction of NBud BN WIL2–NS cells (68 – 88 % of the total variance) was higher than the effects of FA (3 – 8 %) and the interactive effects of metals and FA (3 – 8 %) as determined by two–way ANOVA. The genotoxic potentials of these metals from the percentage contributions values is in the order PMCC > Cr > Mn > Cu > Pb (figure 4.37 – 4.41).

Percentage frequencies of necrosis induction by the tested metals individually and their combinations in exposed WIL2–NS cells, showed concentration dependent and significant ($p < 0.05$) increase. Comparing the frequencies of necrosis at the different concentrations of the metals with the corresponding controls (0.00µM) was significant ($p < 0.05$) at higher concentrations of the metals. The frequencies of necrosis were higher in LF cells than in HF cells. At 1000 µM concentrations of Mn and Cu, 100 and 1000 µM concentrations of Cr and 10, 100 and 1000 µM concentrations of PMCC there was 100 percent necrosis. The percentage variance by metals and folic acids concentrations and their interactions on the frequencies of necrotic WIL-2 NS cell formation showed that metals (with percentage variances between 88 – 99 %) had stronger impacts than FA (percentage variance range of 0.10 – 1.44 %) and their interactions (percentage variance range of 0.22 – 1.56 %) in the causation of necrosis in WIL2–NS cells (Figures 4.42 – 4.46). The

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percentage contributions of the metals in the induction of necrosis in WIL2–NS cells as determined by two way ANOVA showed that the cytotoxic potentials of tested metals is in the order PMCC > Mn > Cr > Cu > Pb.

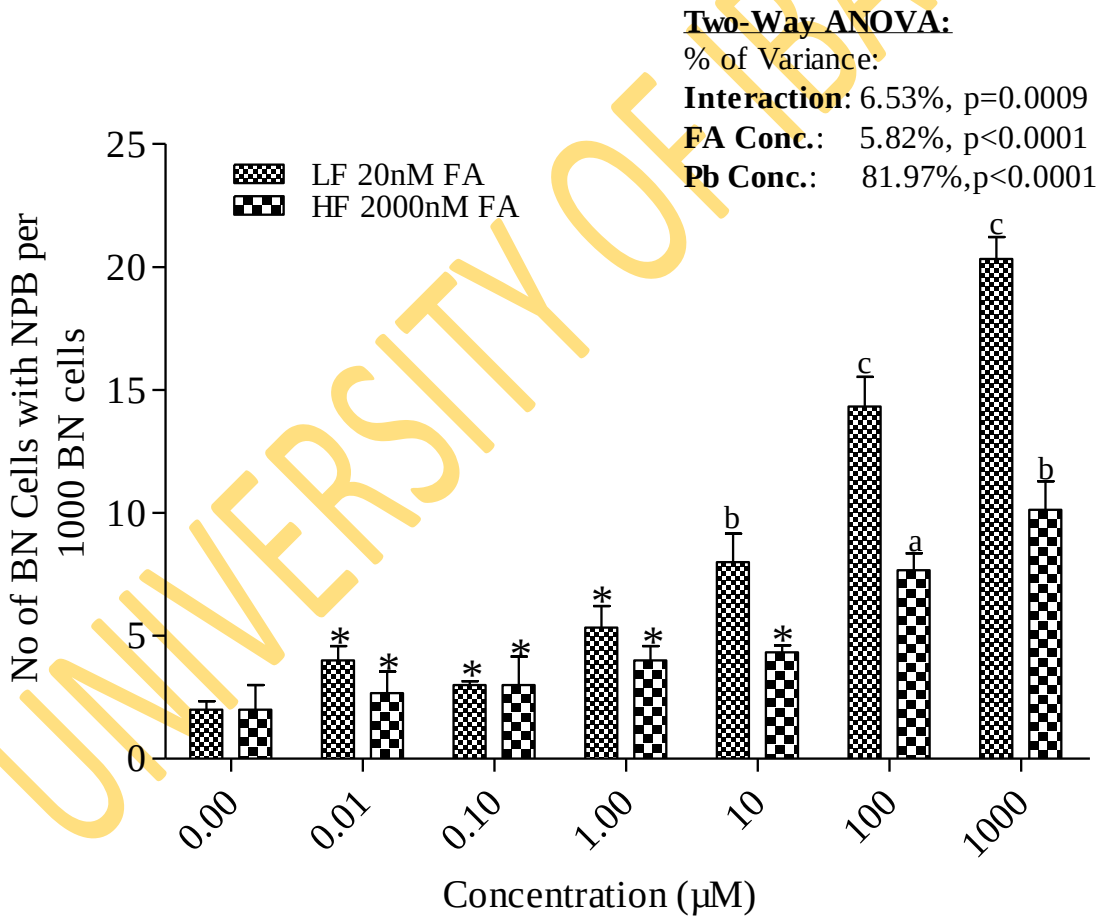

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Figure 4.32 Frequency of NPB BN WIL2–NS cells exposed to different concentrations of lead (Pb) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

BN = Binucleated

NPB = Nucleoplasmic bridge

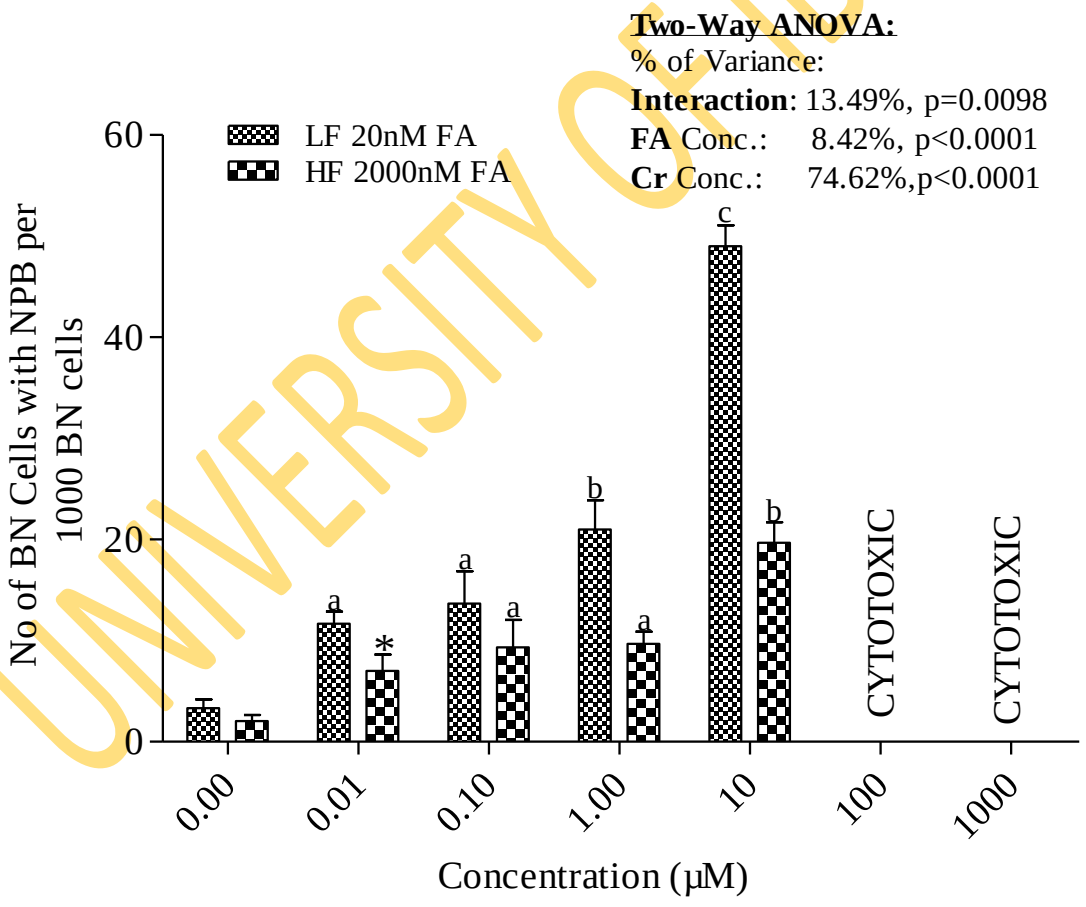

Figure 4.33 Frequency of NPB BN WIL2–NS cells exposed to different concentrations of chromium (Cr) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

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* Not insignificantly ($p < 0.05$) different from corresponding controls

BN = Binucleated

NPB = Nucleoplasmic bridge

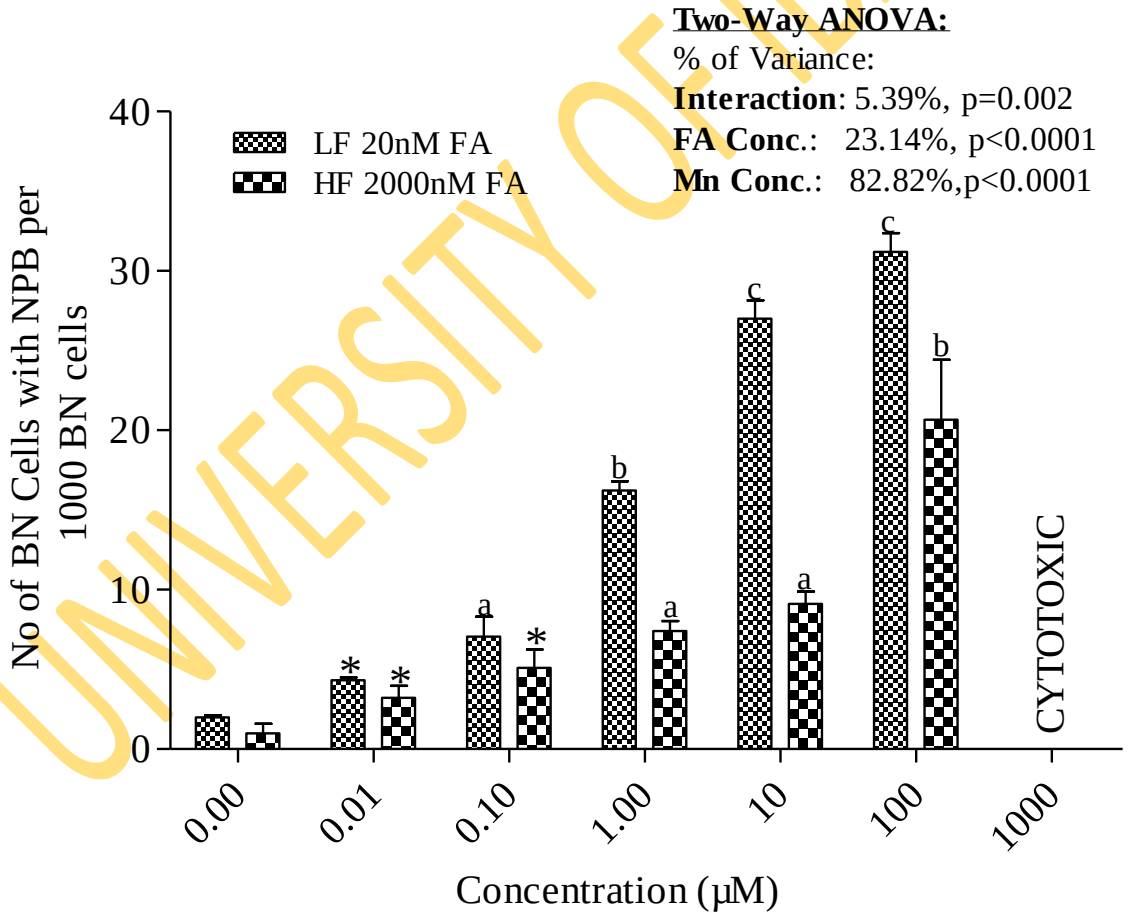

Figure 4.34 Frequency of NPB BN WIL2–NS cells exposed to different concentrations of manganese (Mn) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

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* Not insignificantly ($p < 0.05$) different from corresponding controls

BN = Binucleated

NPB = Nucleoplasmic bridge

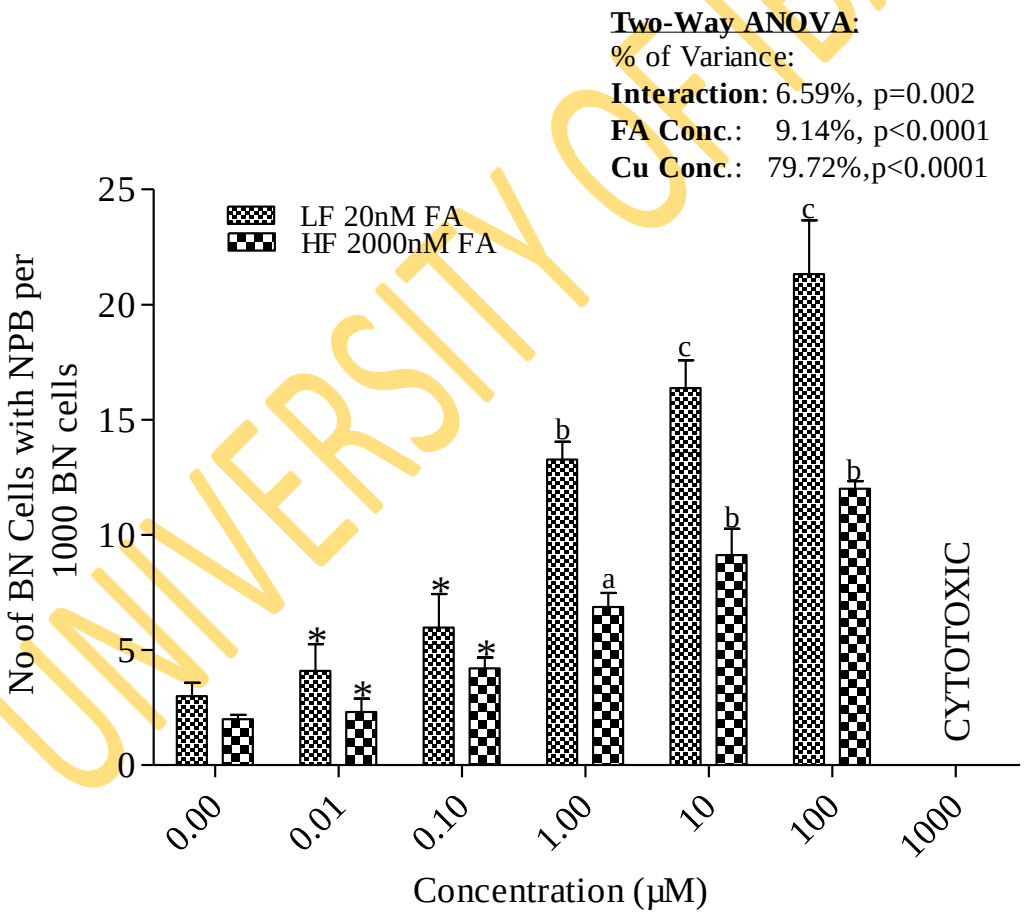

Figure 4.35 Frequency of NPB BN WIL2–NS cells exposed to different concentrations of copper (Cu) at different folate status.

[a, b, c]Superscripts significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from controls

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* Not insignificantly ($p < 0.05$) different from corresponding controls

BN = Binucleated

NPB = Nucleoplasmic bridge

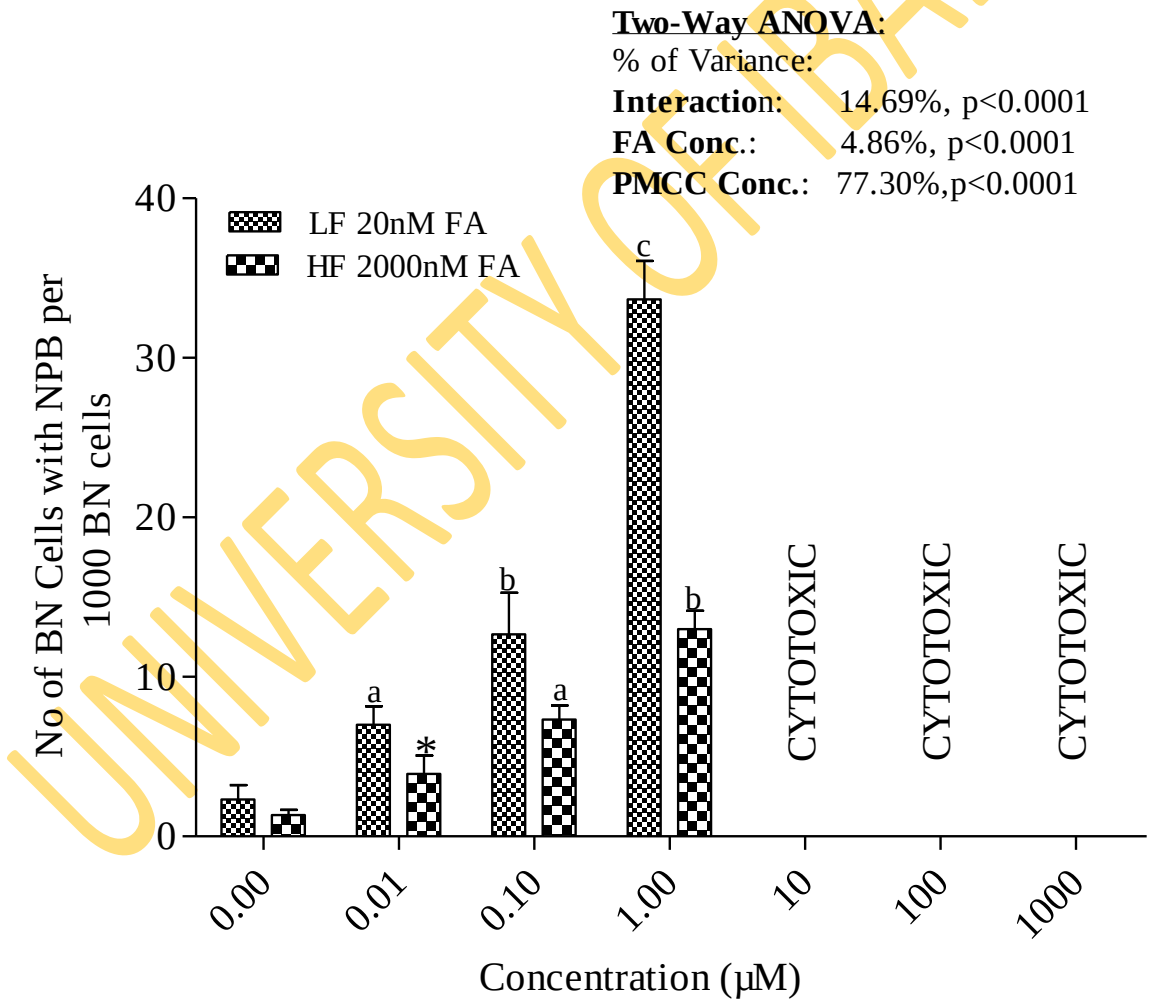

Figure 4.36 Frequency of NPB BN WIL2–NS cells exposed to different concentrations of PMCC at different folate status.

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[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

BN = Binucleated

NPB = Nucleoplasmic bridge

PMCC = Lead, Manganese, Chromium and Copper

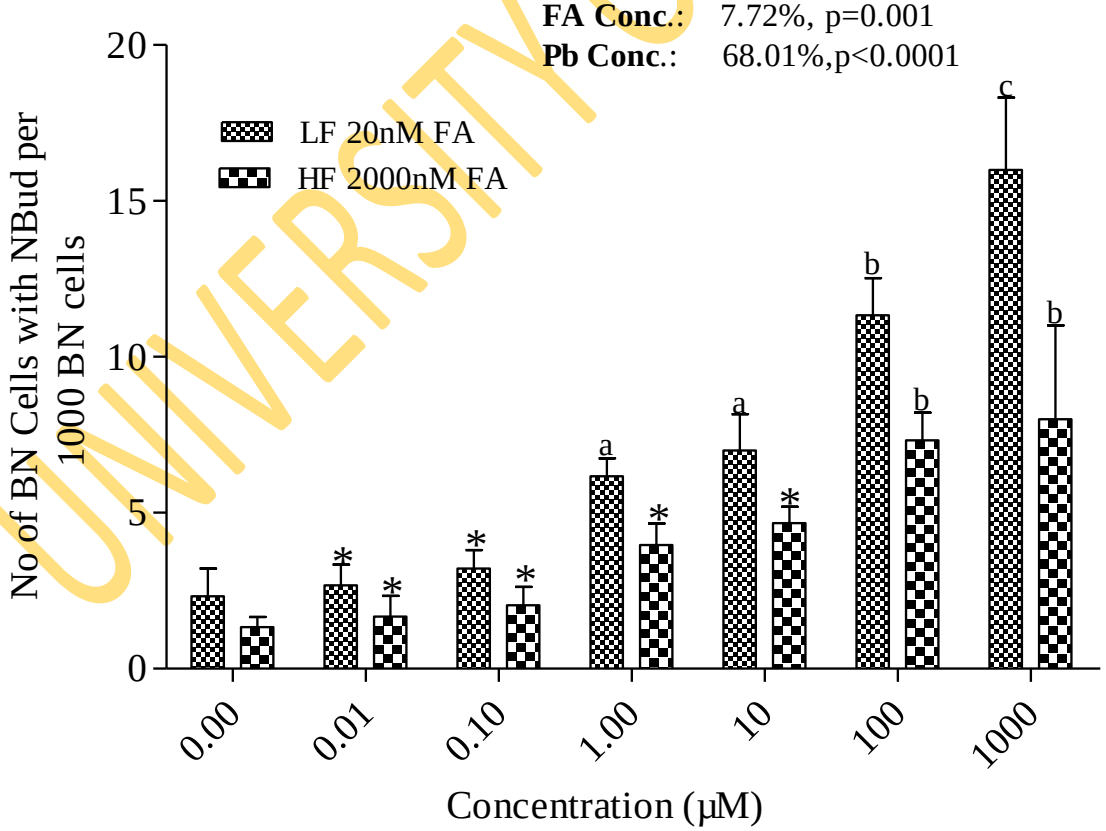

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Figure 4.37 Frequency of NBud BN WIL2–NS cells exposed to different concentrations of lead (Pb) at different folate status.

[a, b, c]Superscripts significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from controls

* Not insignificantly ($p < 0.05$) different from corresponding controls

BN = Binucleated

NBud = Nuclear bud

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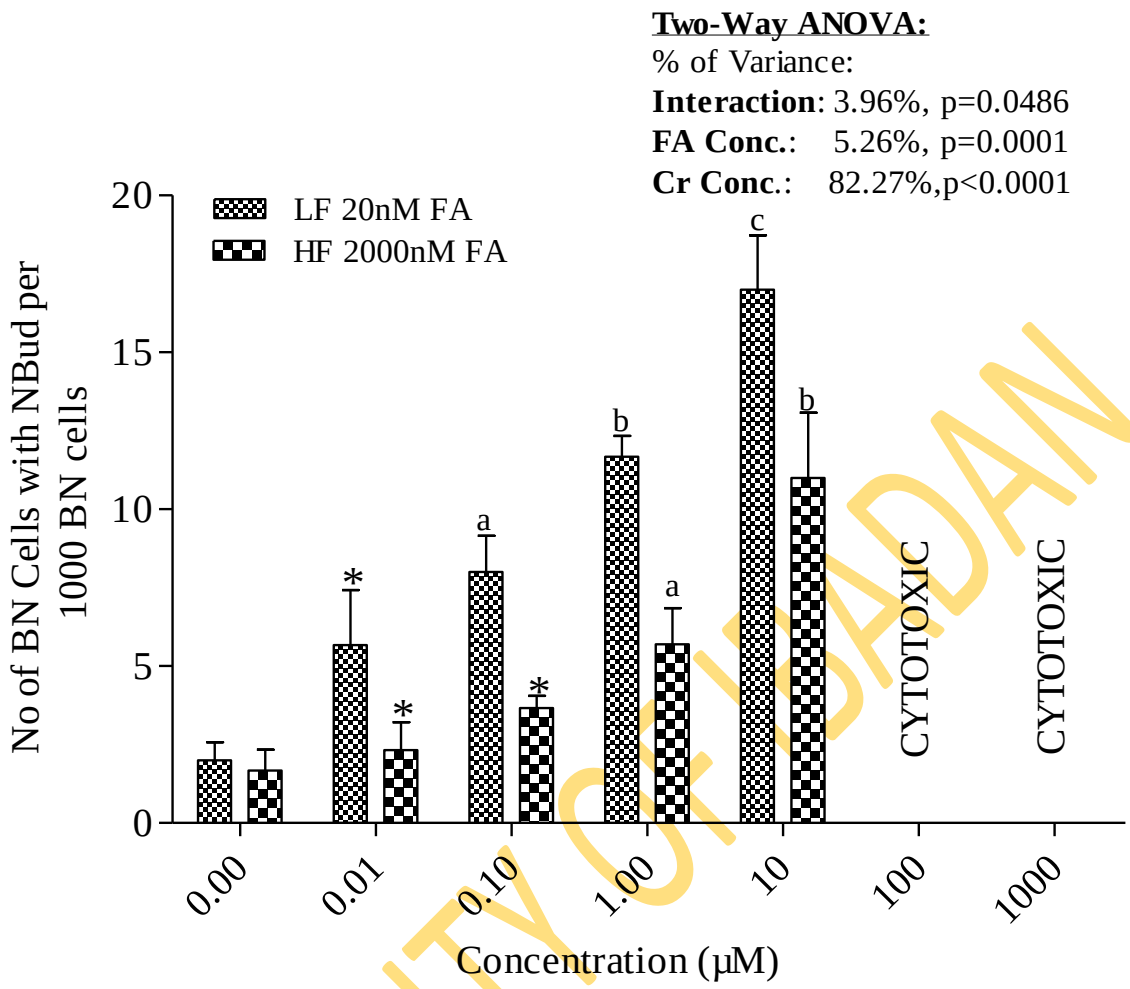

Figure 4.38 Frenquency of NBud BN WIL2–NS cells exposed to different concentrations of chromium (Cr) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

BN = Binucleated

NBud = Nuclear bud

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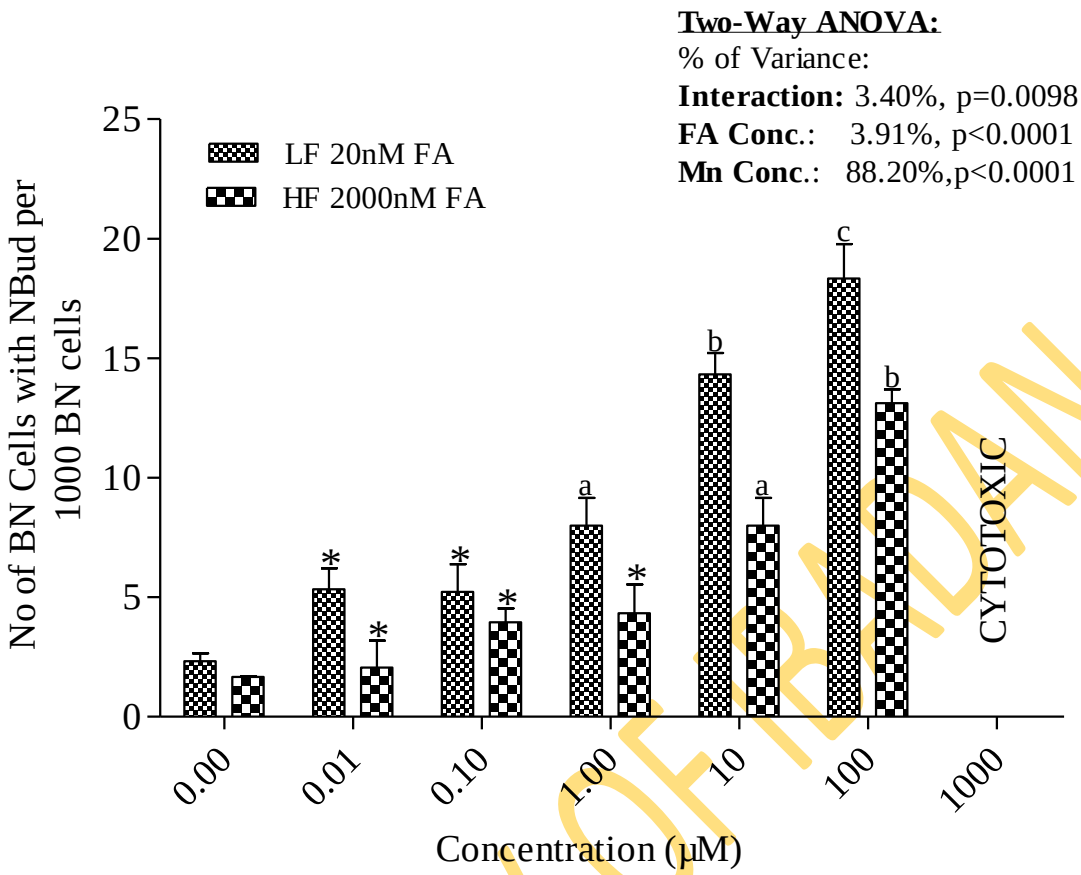

Figure 4.39 Frequency of NBud BN WIL2–NS cells exposed to different concentrations of manganese (Mn) at different folate status.

[a, b, c]Superscripts significantly (a = $p<0.05$; b = $p<0.01$; c = $p<0.001$) different from controls

* Not insignificantly ($p < 0.05$) different from corresponding controls

BN = Binucleated

NBud = Nuclear bud

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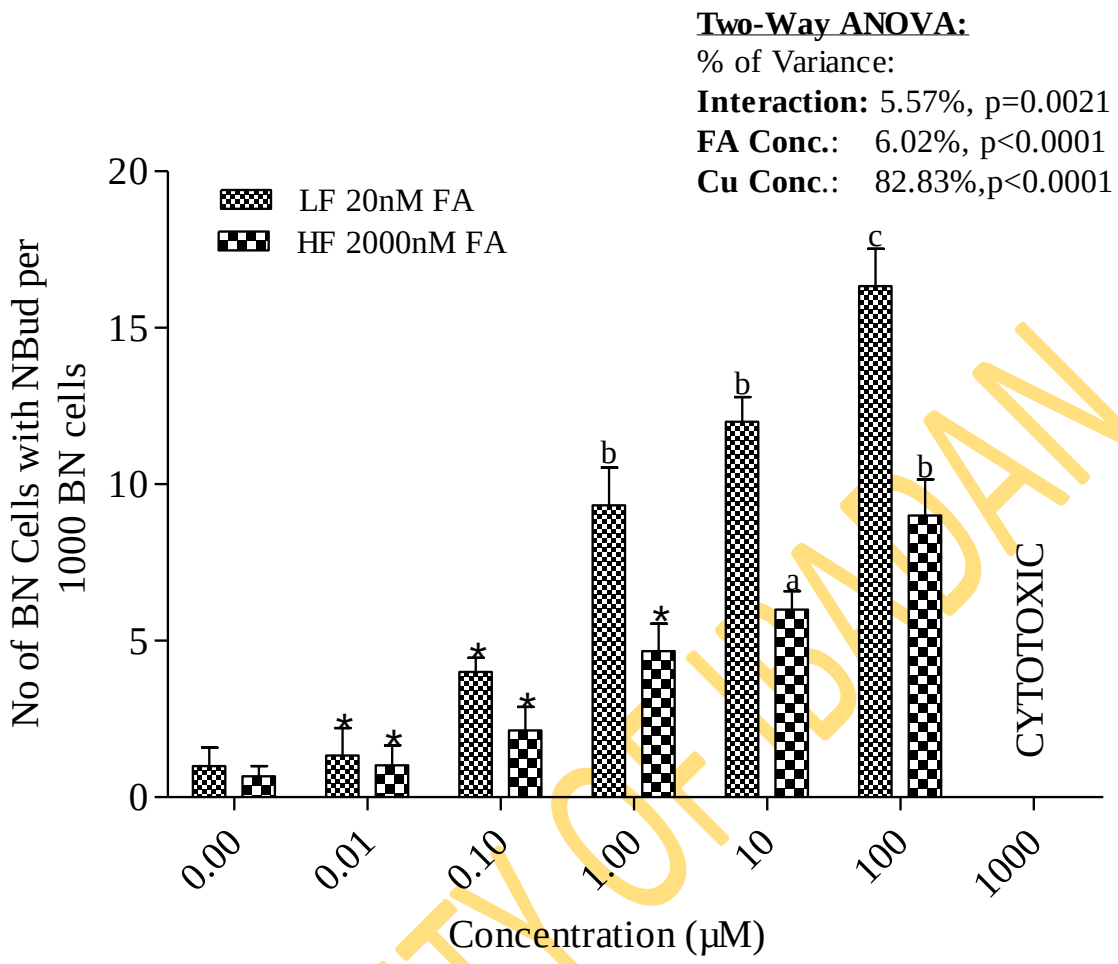

Figure 4.40 Frequency of NBud BN WIL2–NS cells exposed to different concentrations of copper (Cu) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

BN = Binucleated

NBud = Nuclear bud

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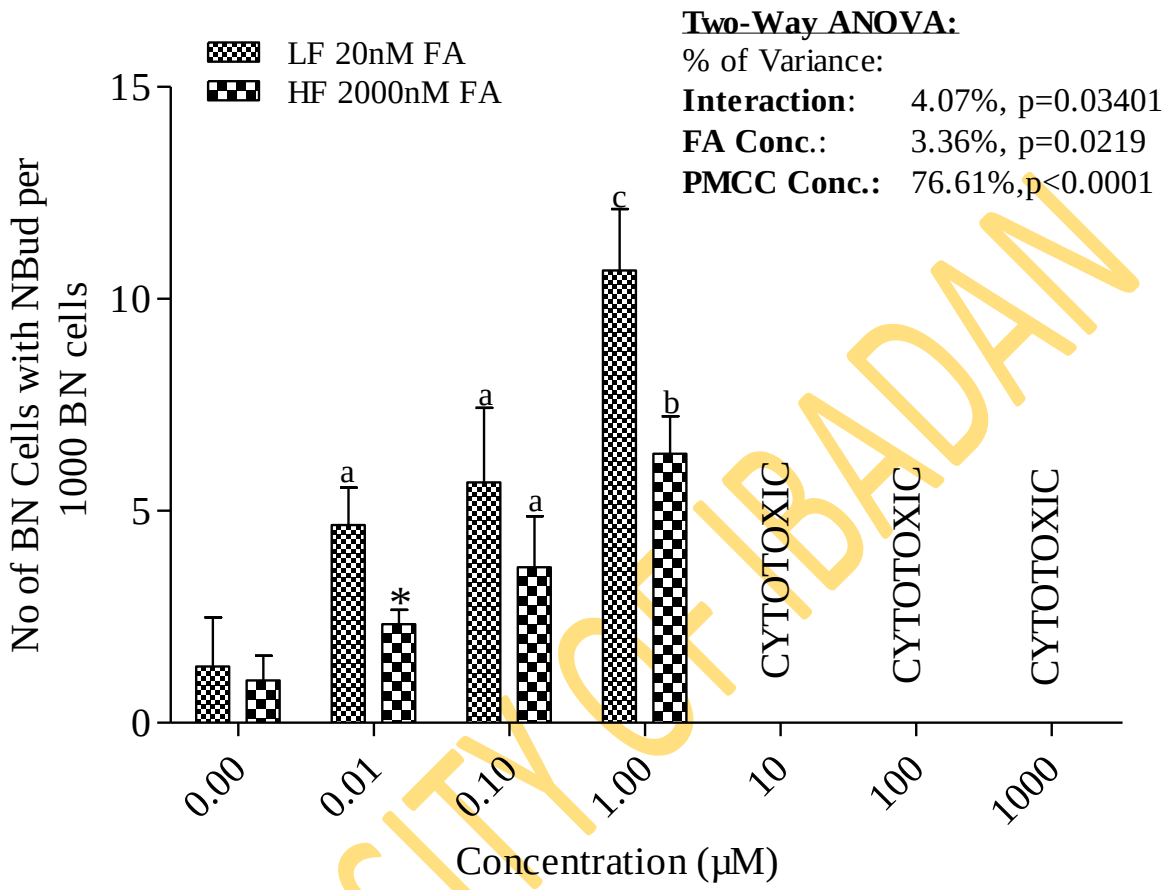

Figure 4.41 Frequency of NBud BN WIL2–NS cells exposed to different concentrations of PMCC at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

BN = Binucleated

NBud = Nuclear bud

PMCC = Lead, Manganese, Chromium and Copper

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Table 4.37 shows the nuclear division index (NDI) of the various concentrations of the metals and their combinations. The results showed a dose related and significant ($p < 0.05$) decrease in NDI for the tested metals. At 100 and 1000 µM, Cr was totally cytotoxic, while Mn and Cu showed total cytotoxicity at 1000 µM. Pb was not totally cytotoxic at these concentrations. Meanwhile PMCC, metal combination, showed total cytotoxicity even at 10 µM. Metal induced lower NDI in LF cells than in HF cells. The NDI for the various concentrations of the metals showed significance from control ($p < 0.05$) and metals exhibited different cytotoxicity in WIL2-NS cells with different folate status in the following trend PMCC > Cr > Mn > Cu > Pb.

Figure 4.48a – f showed some cells scored as micronucleated binucleated, nuclear bud, nucleoplasmid bridge and necrotic cells.

4.6 Health impacts associated with residents around Olusosun and Aba Eku landfills

A total number of two hundred and sixty one (261; exposed group) and two hundred and thirty six (236; unexposed group) questionnaires from Olusosun sampling locality and one hundred and ninety two (192; exposed group) and one hundred and forty six (146; unexposed group) questionnaires from Aba-Eku sampling area were voluntarily completed and returned by the human populations who participated in the study.

Table 4.39 shows the demographic and socio-economic characteristics of the exposed (within 2 km) and unexposed (6 km away) populations from Olusosun and Aba Eku landfills. There were more males in the exposed group for both studied sites than unexposed groups. Majority of the subjects were mainly in the 20 – 39 age brackets in both study sites. Subjects in the 10 – 19 age brackets were the least in Aba-Eku study sites while subjects in their 50s of age and above were the least recorded in Olusosun study site. Majority of the exposed group in Aba-Eku community are mainly farmers and professionals (crafty and traders), while scavengers and civil servants were more in Olusosun study site. A higher proportion of the subjects had secondary and tertiary education. A higher proportion of the subjects in both study sites reported a monthly income of the range #10000 – 20000 (ten – twenty thousand naira).

Table 4.40 shows the possible sources of human exposure to chemicals from Olusosun and Aba Eku landfills. The main sources of exposure to chemicals and vermin from the study sites as reported by the subjects can be classified into inhalation, dermal contact, vector proliferations and ingestion of contaminated food and water. It was observed that exposure to chemicals from the landfills through inhalation of odours, dusts and smokes were common to

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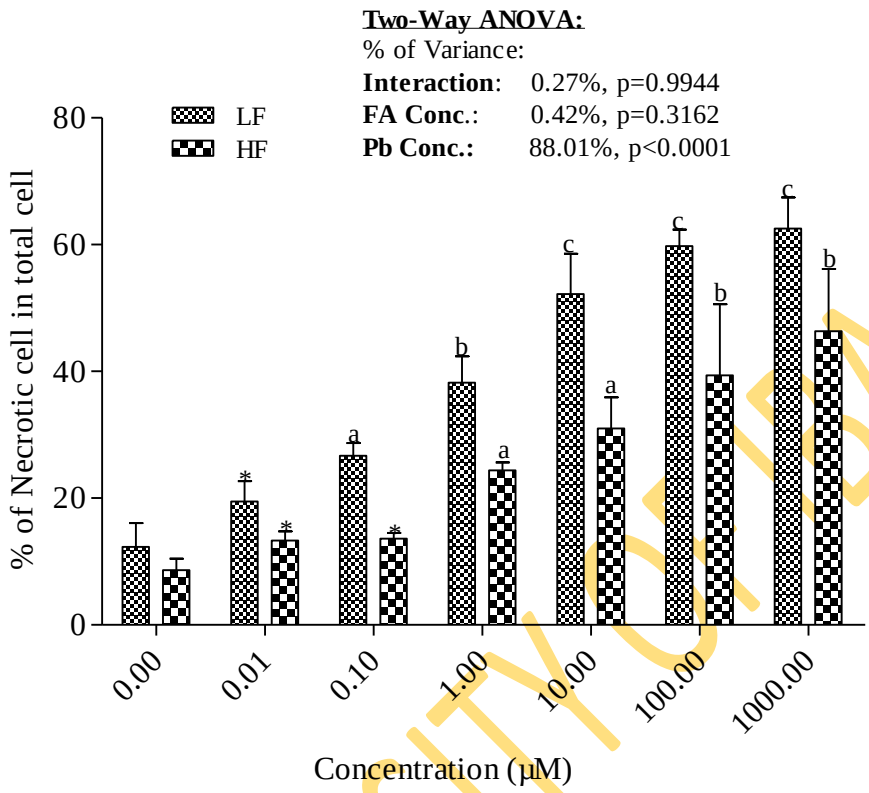

Figure 4.42 Frequency of necrotic WIL2–NS cells exposed to different concentrations of lead (Pb) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

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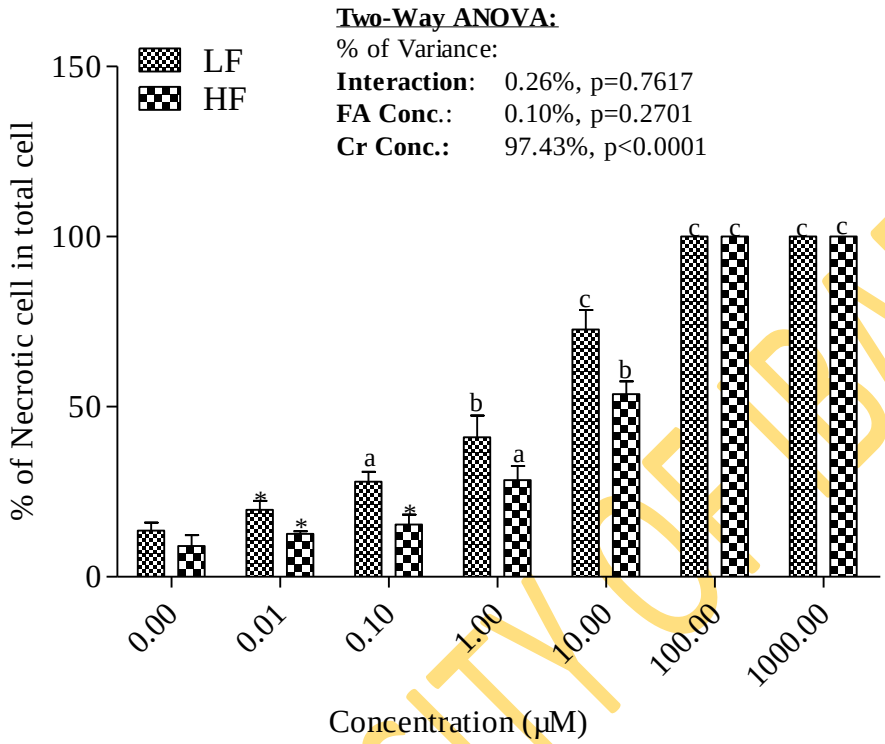

Figure 4.43 Frequency of necrotic WIL2–NS cells exposed to different concentrations of chromium (Cr) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

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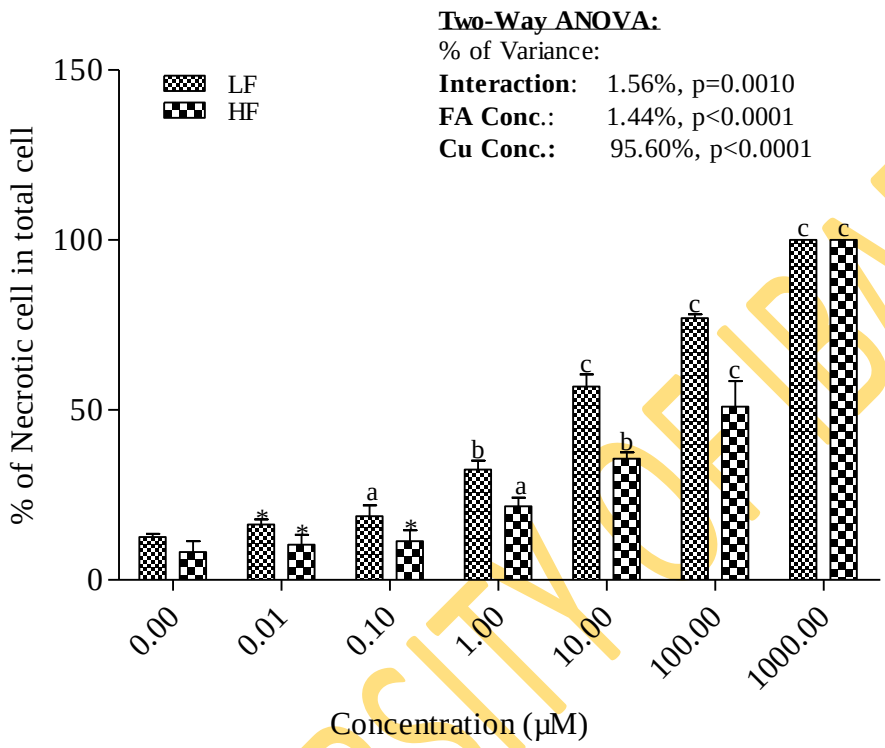

Figure 4.44 Frequency of necrotic WIL2–NS cells exposed to different concentrations of copper (Cu) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

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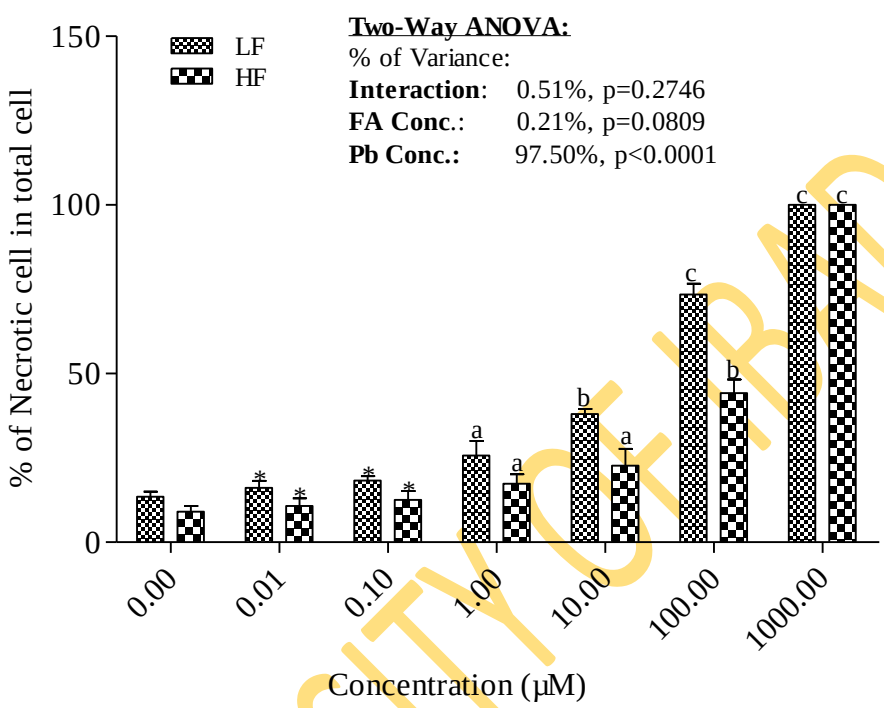

Figure 4.45 Frequency of necrotic WIL2–NS cells exposed to different concentrations of manganese (Mn) at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

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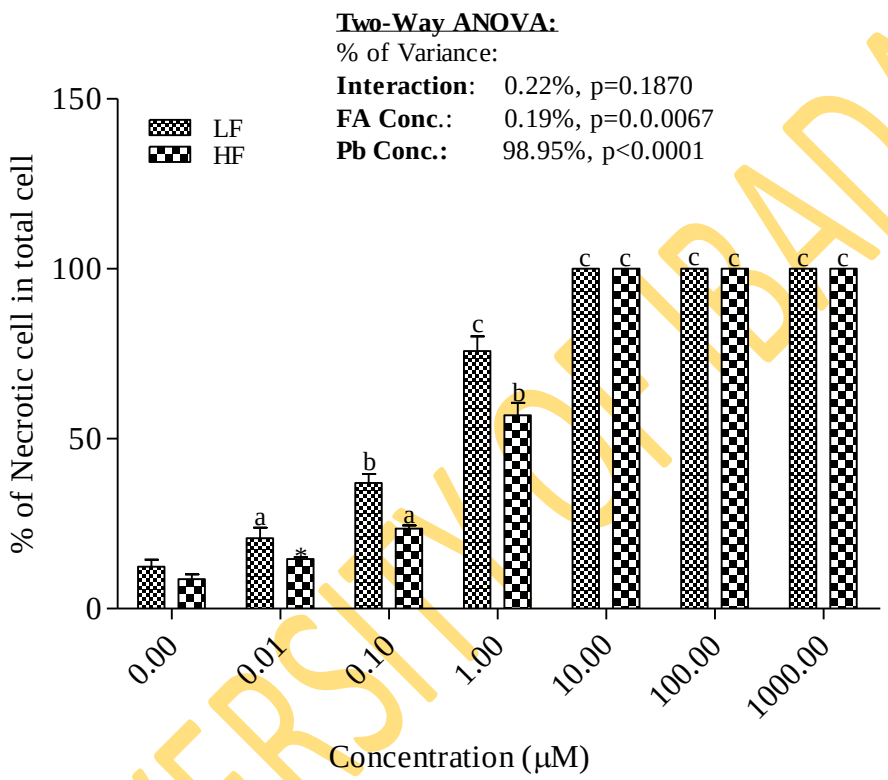

Figure 4.46 Frequency of necrotic WIL2–NS cells exposed to different concentrations of PMCC at different folate status.

[a, b, c]Superscripts significantly (a = p<0.05; b = p<0.01; c = p<0.001) different from controls

* Not insignificantly (p < 0.05) different from corresponding controls

PMCC = Lead, Manganese, Chromium and Copper

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Table 4.37 shows the nuclear division index (NDI) of WIL2–NS cells exposed to the different metallic solutions.

Conc. (µM)	Pb	Cr	Cu	Mn	PMCC
LF (20 nM)					
0.00	1.94 ± 0.41	1.87 ± 0.15	1.96 ± 0.13	1.88 ± 0.04	1.85 ± 0.21
0.01	1.88 ± 0.07	1.67 ± 2.03[a]	1.83 ± 0.01	1.75 ± 0.06	1.53 ± 0.24[b]
0.10	1.89 ± 0.10	1.63 ± 0.01[a]	1.86 ± 1.20	1.70 ± 0.70[a]	1.33 ± 0.45[c]
1.00	1.68 ± 0.90[b]	1.51 ± 0.12[c]	1.62 ± 0.01[b]	1.56 ± 0.12[c]	1.11 ± 0.17[c]
10.00	1.62 ± 0.11[b]	1.24 ± 0.04[c]	1.59 ± 0.06[c]	1.44 ± 0.17[c]	Cytotoxic
100.00	1.55 ± 0.04[c]	Cytotoxic	1.29 ± 1.80[c]	1. 22 ± 0.81[c]	Cytotoxic
1000.00	1.32 ± 0.16[c]	Cytotoxic	Cytotoxic	Cytotoxic	Cytotoxic
HF (2000 nM)					
0.00	2.21 ± 0.12	2.17 ± 0.22	2.15 ± 0.11	2.15 ± 0.32	2.08 ± 0.02
0.01	2.12 ± 0.36*	2.06 ± 0.01*	2.10 ± 0.06*	2.10 ± 0.01*	1.67 ± 0.90[b]
0.10	2.12 ± 0.01*	1.89 ± 0.46[a]	2.02 ± 0.04*	2.05 ± 0.21*	1.52 ± 0.23[b]
1.00	1.96 ± 0.80[a]	1.73 ± 0.17[a]	1.87 ± 0.07[a]	1.75 ± 0.14[b]	1.23 ± 0.02[c]
10.00	1.83 ± 0.21[b]	1.53 ± 0.12[b]	1.65 ± 0.42[b]	1.58 ± 0.25[b]	Cytotoxic
100.00	1.65 ± 0.06[b]	Cytotoxic	1.42 ± 0.09[c]	1.32 ± 0.18[c]	Cytotoxic
1000.00	1.43 ± 0.33[c]	Cytotoxic	Cytotoxic	Cytotoxic	Cytotoxic

Date presented as mean ± SE. Values significant ($p < 0.05$) and insignificantly (*$p < 0.05$) different from corresponding controls using Dunnett's multiple post hoc comparison test.

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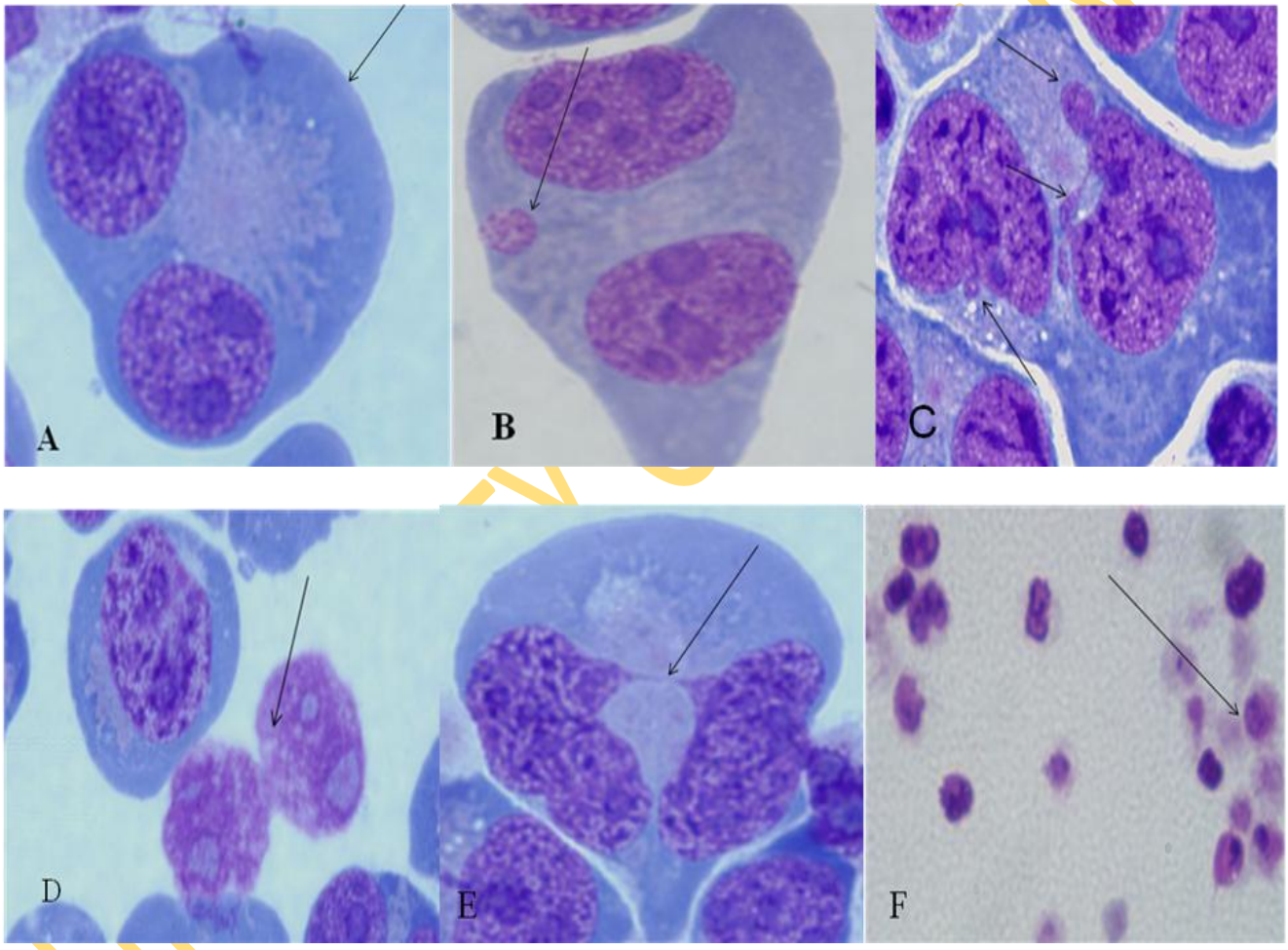

Fig. 4.47. Cytogenotoxic abnormalities (arrowed) observed in WIL2-NS cells treated with different concentrations of metals:

a . shows normal binucleated WIL2–NS lymphoblastoid cells.

b. shows binucleated WIL2–NS lymphoblastoid cell with a micronucleus.

c . shows a binucleated WIL2–NS cell with nuclear buds.

d. shows a necrotic binucleated WIL2–NS cell.

e. shows binucleated WIL2-NS cells with a nucleoplasmic bridge.

f . shows WIL2-NS cells at different stages of necrosis observed at higher concentrations of the metals.

both study sites. Also common to both landfills are exposure to water sources within the landfills either through ingestion (drinking) or by dermal contacts during bathing.

Exposure to vectors mainly houseflies and mosquitoes constitute a major potential source of infections to diseases among the exposed populations, as these flies and other insects were commonly found in the residential quarters mostly when the heat of the sun was scourging. Exposure to noise from heavy duty machines during disposal of wastes into the landfill was very common in the study sites. Setting of wastes on fire so as to reduce the volume and create spaces for the disposal of more wastes was common in the landfills (figures 4.49a and b, and 4.50d and f). This causes increase in smoke productions constituting a major source of exposure to chemicals from the wastes. At the Aba Eku study sites, majority of the communities are farmers, they cultivate their crops using decomposed solid wastes (organic humus) from the dumpsites and utilised Omi stream as source of water supply for irrigating these crops during the dry planting session. Also their domesticated animals forage on the landfill site and they also hunt free ranging animals around the site (figure 4.49). These features were not reported by respondents from Olusosun study site rather due to high population density in Lagos State, majority of Olusosun study site respondents reside in temporarily constructed residential structures within the landfill site. Commonly observed during the time of the study was flow of leachates from the dumpsites into the residential environment, buying and selling on the site, bore hole and well constructions (water sources) on the site, high prevalence of rodents, cats, flies and sometimes pigs, bathing, cooking and giving birth to and rearing of children on the site (figures 4.50).

Self reported health impacts obtained from the administered questionnaires in the two study sites that may be associated with solid waste management are combined into five composite variables as follows: respiratory problems, dermal infections, gastrointestinal problems, neuromuscular and psychological problems, reproductory and other problems.

Figure 4.51 showed the prevalence of respiratory symptoms among respondents around the landfills (exposed population) and control population (unexposed population). The health effects classified into respiratory problems are breathing difficulty, coughing, lung diseases, bronchitis,

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chest pain, asthma, tuberculosis and nose/throat irritations. Chi square analyses showed significant differences between exposed and unexposed populations in the frequencies of all the individually reported respiratory symptoms except for tuberculosis ($p = 0.1155$) reports from Aba Eku respondents. Odd ratios (ORs) calculated for each specific symptoms showed that these symptoms were more prevalent among people living (exposed) within ≤ 2 km of Olusosun and

Table 4.38. Demographic and socio-economic features of the exposed and unexposed populations around Aba Eku (AEL) and Olusosun (OSL) landfill sites.

Characteristics	Exposed (AEL) (n=192) (%)	Unexposed (AEL) (n=146) (%)	Exposed (OSL) (n=261) (%)	Unexposed (OSL) (n=236) (%)
Sex				
Male	106 (52.21)	55 (37.67)	173 (66.28)	132 (55.93)
Female	86 (44.79)	91 (62.33)	88 (33.71)	104 (44.07)
Age (years)				
10 – 19	19 (9.89)	9 (6.16)	50 (19.16)	40 (16.95)
20 – 39	96 (50.00)	79 (54.11)	112 (42.91)	113 (47.88)
40 – 49	48 (25.00)	42 (28.77)	81 (31.03)	69 (29.24)
≥50	29 (15.10)	16 (10.96)	18 (6.90)	14 (5.93)
Marital status				
Single	42 (21.88)	39 (26.71)	162 (62.07)	90 (38.14)
Married	121 (63.02)	98 (67.12)	84 (32.18)	135 (57.20)
Divorced	17 (8.85)	3(2.05)	5 (1.92)	7 (2.97)
Widower/widow	12 (6.25)	6 (4.11)	10 (3.83)	4 (1.69)
Highest Education				
None	19 (9.89)	6 (4.11)	31 (11.88)	9 (3.81)
Primary	42 (21.88)	31 (21.23)	42 (16.09)	42 (17.80)
Secondary	102 (53.13)	83 (56.85)	177 (67.82)	81 (34.32)
Tertiary	29 (15.10)	26 (17.81)	11 (4.21)	104 (44.07)
Occupation				
Farming	42 (21.88)	26 (17.81)	0 (0.00)	3 (1.27)
Professional	92 (47.92)	77 (52.74)	47 (18.01)	115 (48.73)
Civil Servant	23 (11.98)	34 (23.29)	73 (27.97)	104 (44.07)
Others	34 (17.71)	9 (6.16)	141 (54.02)	14 (5.93)
Monthly Income (Naira)				
< 5,000	25 (13.02)	9 (6.16)	29 (11.11)	14 (5.93)
< 10,000	50 (26.04)	19 (13.01)	73 (27.97)	33 (13.98)
< 20,000	102 (53.13)	63 (43.15)	115 (44.06)	80 (33.90)
> 20,000	15 (7.81)	55 (37.67)	44 (16.86)	109 (46.19)

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Table 4.39. Potential sources of human exposure to Olusosun (OSL) and Aba Eku (AEL) landfills.

Characteristics	Exposed (AEL) (n=192) (%)	Unexposed (AEL) (n=146) (%)	Exposed (OSL) (n=261) (%)	Unexposed (OSL) (n=236) (%)
1 Inhalation of odour	173 (90.10)	86 (58.90)	249 (95.40)	97 (41.10)
2. Exposure to dust	167 (86.98)	67 (45.89)	201 (77.01)	86 (36.44)
3. Exposure to smoke	141 (73.44)	59 (40.41)	211 (80.84)	76 (32.20)
4. Exposure through water sources				
i. Well water	106 (55.21)	76 (52.05)	124 (47.51)	119 (50.42)
ii. Borehole water	121 (63.02)	116 (79.45)	196 (75.10)	183 (77.54)
iii. Tap water	89 (46.35)	91 (63.33)	83 (31.80)	101 (42.80)
iv. Stream/River	112 (58.33)	66 (45.21)	48 (18.39)	32 (13.56)
v. Rain water	108 (56.25)	98 (62.12)	162 (62.07)	108 (45.76)
5. Consumption of plant materials				
i. Okra	52 (27.08)	6 (4.11)	0 (0)	0 (0)
ii. Tomatoes	47 (24.48)	4 (2.74)	0 (0)	0 (0)
iii. Maize	98 (51.04)	9 (10.27)	0 (0)	0 (0)
iv. Vegetables	103 (53.65)	13 (8.90)	0 (0)	0 (0)
v. Pepper	31 (16.15)	4 (2.74)	0 (0)	0 (0)
6. Consumption of animal materials				
i. Grasscutters	3 (1.56)	0 (0)	0 (0)	0 (0)
ii. Giant rats	4 (2.08)	0 (0)	0 (0)	0 (0)
iii. Snail	8 (4.17)	0 (0)	0 (0)	0 (0)
iv. Common rats	0 (0)	0 (0)	0 (0)	0 (0)
7. Exposure through organisms (vectors)				
i. Mosquitoes	178 (92.71)	96 (65.75)	258 (98.85)	171 (72.45)
ii. Flies	184 (95.83)	77 (52.74)	246 (94.25)	189 (80.08)
iii. Rat	98 (51.04)	54 (36.99)	173 (66.28)	104 (40.07)
iv. Cats	54 (28.13)	39 (26.71)	141 (54.02)	98 (41.53)
v. Snake	11 (5.73)	3 (2.05)	0 (0)	0 (0)
vi. Toads	26 (13.54)	5 (3.42)	2 (0.77)	0 (0)
8. Noise from machines	125 (65.10)	76 (52.05)	214 (81.99)	98 (41.53)
9. Exposure to fire	101 (52.60)	11 (7.53)	203 (77.78)	14 (5.93)
10. Dermal contacts	104 (54.17)	0 (0)	209 (80.08)	0 (0)

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11. Exposure through domestic animals				
i. Yes	22 (11.46)	0 (0)	0 (0)	0 (0)
ii. No	167 (86.98)	0 (0)	0 (0)	0 (0)

Aba Eku dumpsites than those living within ≥ 6 km (unexposed) in the two study sites. Olusosun respondents had higher the respiratory problems than Aba Eku respondents.

The Odd Ratio (OR) values showed significant relationship between living close to Olusosun landfill and breathing problems (OR = 9.65; 95% CI = 6.39 – 14.56; χ^2 = 129.9), coughing (OR = 8.23; 95% CI = 5.46 – 12.39; χ^2 = 112.5), lung diseases (OR = 7.03; 95% CI = 4.74 – 10.43; χ^2 = 101.5), bronchitis (OR = 6.11; 95% CI = 4.13 – 9.03; χ^2 = 88.0), chest pains (OR = 5.98; 95% CI = 4.02 – 8.90; χ^2 = 84.0), nose/throat pains (OR = 3.37; 95% CI = 2.31 – 4.93; χ^2 = 40.8), asthma (OR = 2.57; 95% CI = 1.56 – 4.22; χ^2 = 14.5) and tuberculosis (OR = 2.43; 95% CI = 1.41 – 4.18; χ^2 = 10.7). The responses obtained from Aba Eku also showed significant relationship for the specific type of respiratory problems except tuberculosis. They are chest pains (OR = 11.65; 95% CI = 6.91 – 19.64; χ^2 = 97.7), breathing problems (OR = 10.15; 95% CI = 6.13 – 16.80; χ^2 = 91.2), coughing (OR = 8.02; 95% CI = 4.91 – 13.09; χ^2 = 76.0), lung diseases (OR = 6.61; 95% CI = 4.03 – 10.84; χ^2 = 61.3), bronchitis (OR = 5.61; 95% CI = 3.46 – 9.10; χ^2 = 52.7), nose/throat pains (OR = 3.31; 95% CI = 2.06 – 5.31; χ^2 = 52.5), asthma (OR = 2.61; 95% CI = 1.34 – 5.09; χ^2 = 8.3), tuberculosis (OR = 1.85; 95% CI = 0.85 – 4.02; χ^2 = 2.5).

Figure 4.52 showed the results of dermal infection prevalence among exposed and unexposed populations from Olusosun and Aba Eku landfill study sites. Chi square analyses showed significant differences between exposed and unexposed populations in the frequencies of the individually reported dermal infections. ORs calculated for each specific symptoms showed that dermal infections were more prevalent among people living (exposed) within ≤ 2 km of Olusosun and Aba Eku dumpsites than those living within ≥ 6 km (unexposed) in the two study sites. Olusosun respondents had higher frequencies of dermal infections than Aba Eku respondents. The health effects classified into dermal infections includes eczema, skin rashes, skin itching and boils and warts. The results showed that there was significant relationship between living close to Olusosun dumpsites and skin rashes (OR = 7.18; 95% CI = 4.76 – 10.82; χ^2 = 97.4), skin itching (OR = 6.34; 95% CI = 4.29 – 9.37; χ^2 = 92.3), boils and warts (OR =

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4.79; 95% CI = 3.14 – 7.32; χ^2 =56.8) and eczema (OR = 2.98; 95% CI = 2.04 – 4.36; χ^2 = 32.8). The results obtained from Aba Eku also showed significant relationship for the dermal infections: skin itching (OR = 4.68; 95% CI = 2.92 – 7.47; χ^2 = 43.86), boils and warts (OR = 3.69; 95% CI = 2.02 – 6.75; χ^2 = 19.6), eczema (OR = 3.49; 95% CI = 2.14 – 5.67; χ^2 = 26.4) and skin rashes (OR = 3.03; 95% CI = 1.91 – 4.79; χ^2 = 23.0).

Figure 4.53 showed gastrointestinal problem prevalence among exposed and unexposed from the Olusosun and Aba Eku landfills. Chi square analyses showed significant differences

Figure 4.48 Activities observed on Aba Eku landfill site:

(a) burning of solid wastes with the release of smoke into residential areas

(b) domesticated goats source for food from wastes on the landfill.

(c) residential structures are within 500 m radius of the landfill. Human are usually exposed to flies, rodents, smoke, odour, leachates and noise from heavy trucks from the landfill.
(d) organic wastes (humus) from the decomposed wastes are use for cultivating plants.
(e) Omi stream usually receiving leachate from the leachate well (F) is used to irrigate plants.
(f) leachate well receiving leachate from a part of the landfill during leachate production.

Figure 4.49 Human activities (arrowed) capable of increasing exposure to Olusosun landfill chemicals:
(a) residential structures within or less than 500m and scavengers on the landfill

(b) leachate discharge into the surrounding of the temporary residential areas on the landfill.

(c) child exposure to flies, rodents, odour and leachates from the landfill.

(d) burning of wastes and human exposure to smoke on the landfill.

(e) Olusosun landfill is actually "home away from home" as residents take their bath, cook and eat, buy and sell on the site.

(f) bore hole was cited on the landfill to supply water for both domestic and commercial processes.

between exposed and unexposed populations in the frequencies of the individually reported gastrointestinal problems. ORs calculated for each specific symptoms showed that gastrointestinal infections were more prevalent among people living (exposed) within ≤ 2 km of Olusosun and Aba Eku dumpsites than those living within ≥ 6 km (unexposed) in the two study sites. Olusosun respondents had higher frequencies of the gastrointestinal problems than Aba Eku respondents. The health effects classified into gastrointestinal problems are nausea and diarrhaea. The results showed that there was significant relationship between living close to Olusosun dumpsites and diarrhaea (OR = 7.93; 95% CI = 6.22 – 12.06; χ^2 = 105.2) and nausea (OR = 3.53; 95% CI = 2.20 – 5.65; χ^2 = 29.3). The results obtained from Aba Eku also showed significant relationship for the gastrointestinal problems: diarrhaea (OR = 5.90; 95% CI = 3.57 – 9.75; χ^2 = 52.4) and Nausea (OR = 2.30; 95% CI = 1.21 – 4.35; χ^2 = 6.8).

Figures 4.54 showed the frequencies of neuromuscular and psychological symptom prevalence among the exposed and unexposed populations around Olusosun and Aba Eku landfill sites. The health impacts classified into neuromuscular and psychological problems include; headache, joint pains, upsetting of sleep, body pains, hearing problems, dizziness, depression, anxiety and anger. Chi square analyses showed significant differences between exposed and unexposed populations in the frequencies of the individually reported neuromuscular and psychological symptoms. ORs calculated for each specific symptoms showed that neuromuscular and psychological symptoms were more prevalent among people living (exposed) within ≤ 2 km of Olusosun and Aba Eku dumpsites than those living within ≥ 6 km (unexposed) in the two study sites. Olusosun respondents had higher frequencies of the neuromuscular and psychological symptoms than Aba Eku respondents. The results obtained from Olusosun study site showed significant relationship for the neuromuscular and psychological symptoms. They are dizziness (OR = 6.22; 95% CI = 4.09 – 9.46; χ^2 = 80.5), joint pains (OR = 5.96; 95% CI = 4.05 – 8.81; χ^2 = 87.0), body pains (OR = 5.81; 95% CI = 3.92 –

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8.61; χ^2 = 82.2), depressions (OR = 5.80; 95% CI = 3.89 – 8.64; χ^2 = 80.3), hearing problems (OR = 4.73; 95% CI = 2.57 – 8.73; χ^2 = 28.5), upsetting of sleep (OR = 4.71; 95% CI = 3.22 – 6.87; χ^2 = 67.4), anxiety (OR = 3.94; 95% CI = 2.69 – 5.77; χ^2 = 51.9), headache (OR = 3.60; 95% CI = 2.48 – 5.21; χ^2 = 47.5), anger (OR = 3.24; 95% CI = 2.20 – 4.79; χ^2 = 36.6). Also, there was significant relationship between living close to Aba Eku dumpsites and upsetting of sleep (OR = 5.63; 95% CI = 3.52 – 9.02; χ^2 = 55.2), joint pains (OR = 5.49; 95% CI = 3.28 – 9.19; χ^2 = 45.8), depressions (OR = 5.02; 95% CI = 1.68 – 10.29; χ^2 = 41.7), headache (OR = 4.63; 95% CI = 2.89 – 7.42; χ^2 = 43.1), anxiety (OR = 4.21; 95% CI = 2.56 – 6.90; χ^2 = 34.4), hearing problems (OR = 4.15; 95% CI = 3.01 – 8.35; χ^2 = 10.8), dizziness (OR = 4.07; 95% CI = 2.40 – 6.91; χ^2 = 29.1), anger (OR = 3.88; 95% CI = 2.35 – 6.39; χ^2 = 29.9), body pains (OR = 3.17; 95% CI = 1.92 – 5.26; χ^2 = 20.9).

Figures 4.55 showed the prevalence of reproductive problems and other diseases among expose and unexposed populations around Olusosun and Aba Eku landfill study sites. The health effects classified under reproductive problems and other diseases are stroke, diabetes, cancer, stillbirth, low birthweight and birth defects. Chi square analyses showed non significant differences between exposed and unexposed populations in the frequencies of the individually reported reproductive problems and other diseases except for low birthweight (p = 0.049) at the Olusosun study site. ORs calculated for each specific symptoms showed that reproductive problems and other diseases were more prevalent among people living (exposed) within ≤ 2 km of Olusosun and Aba Eku dumpsites than those living within ≥ 6 km (unexposed) in the two study sites. The results showed that there was significant relationship between living close to Olusosun dumpsites and stroke (OR = 4.18; 95% CI = 0.89 – 19.55; χ^2 = 3.9), low birth weight (OR = 4.18; 95% CI = 0.79 – 18.55; χ^2 = 3.9), still birth (OR = 3.70; 95% CI = 0.78 – 17.61; χ^2 = 3.1), diabetes (OR = 2.80; 95% CI = 0.89 – 8.79; χ^2 = 3.4), cancer (OR = 1.82; 95% CI = 0.33 – 10.04; χ^2 = 0.5), birth defects (OR = 1.52; 95% CI = 0.56 – 6.42; χ^2 = 0.3). The OR values of reproductive problems and other diseases obtained from Aba Eku also showed higher risk for these problems and diseases in exposed populations: stroke (OR = 4.68; 95% CI = 0.57 – 39.31; χ^2 = 2.4), still birth (OR = 3.88; 95% CI = 0.45 – 33.57; χ^2 = 1.8), diabetes (OR = 3.54; 95% CI = 0.75 – 16.65; χ^2 = 2.9), low birth weight (OR = 2.34; 95% CI = 0.62 – 8.82; χ^2 = 1.7), cancer (OR = 1.53; 95% CI = 0.14 – 17.01; χ^2 = 0.1), birth defects (OR = 1.53; 95% CI = 0.28 – 8.48; χ^2 = 0.2).

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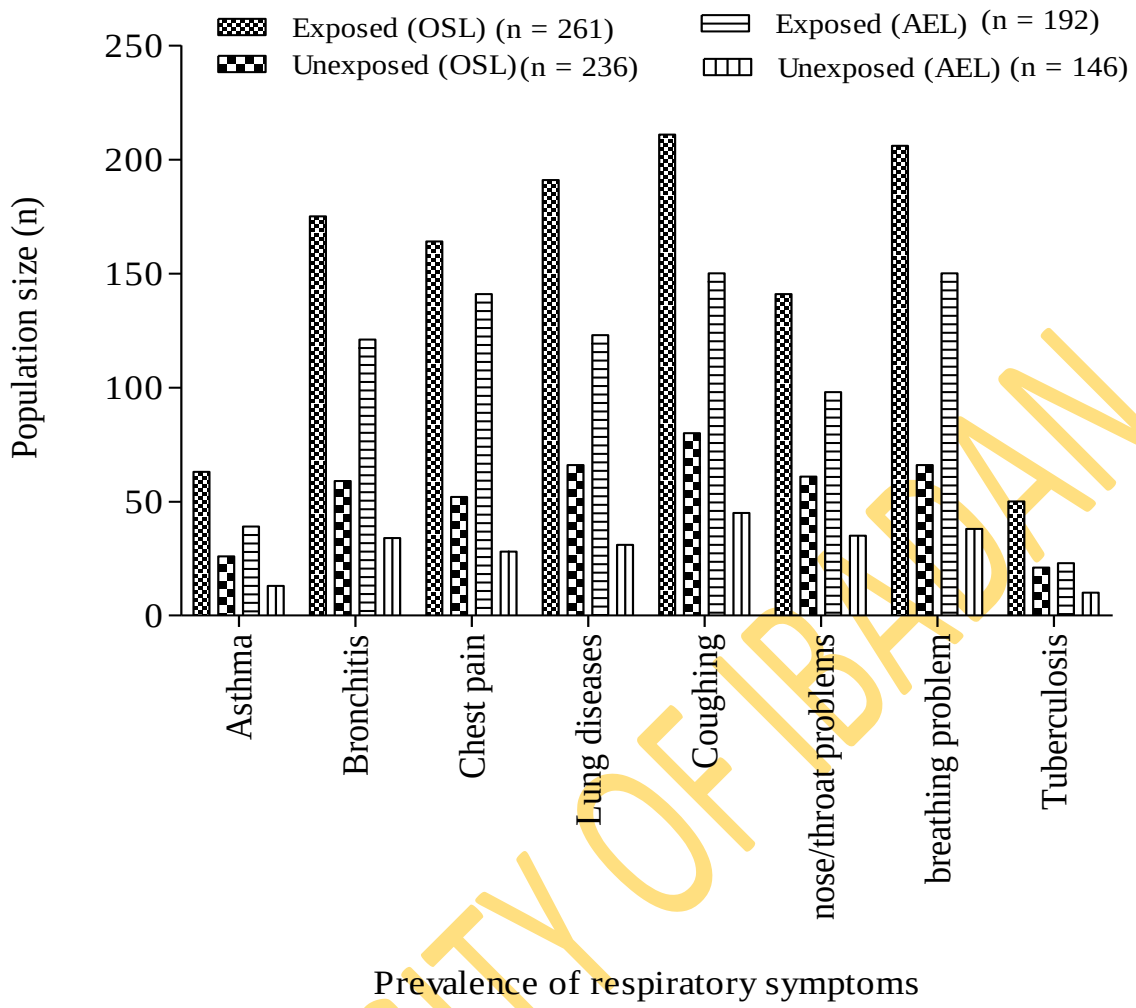

Figure 4.50. Prevalence of respiratory symptoms among exposed and unexposed populations around Olusosun and Aba Eku landfill sites.

OSL = Olusosun landfill.

AEL = Aba Eku landfill.

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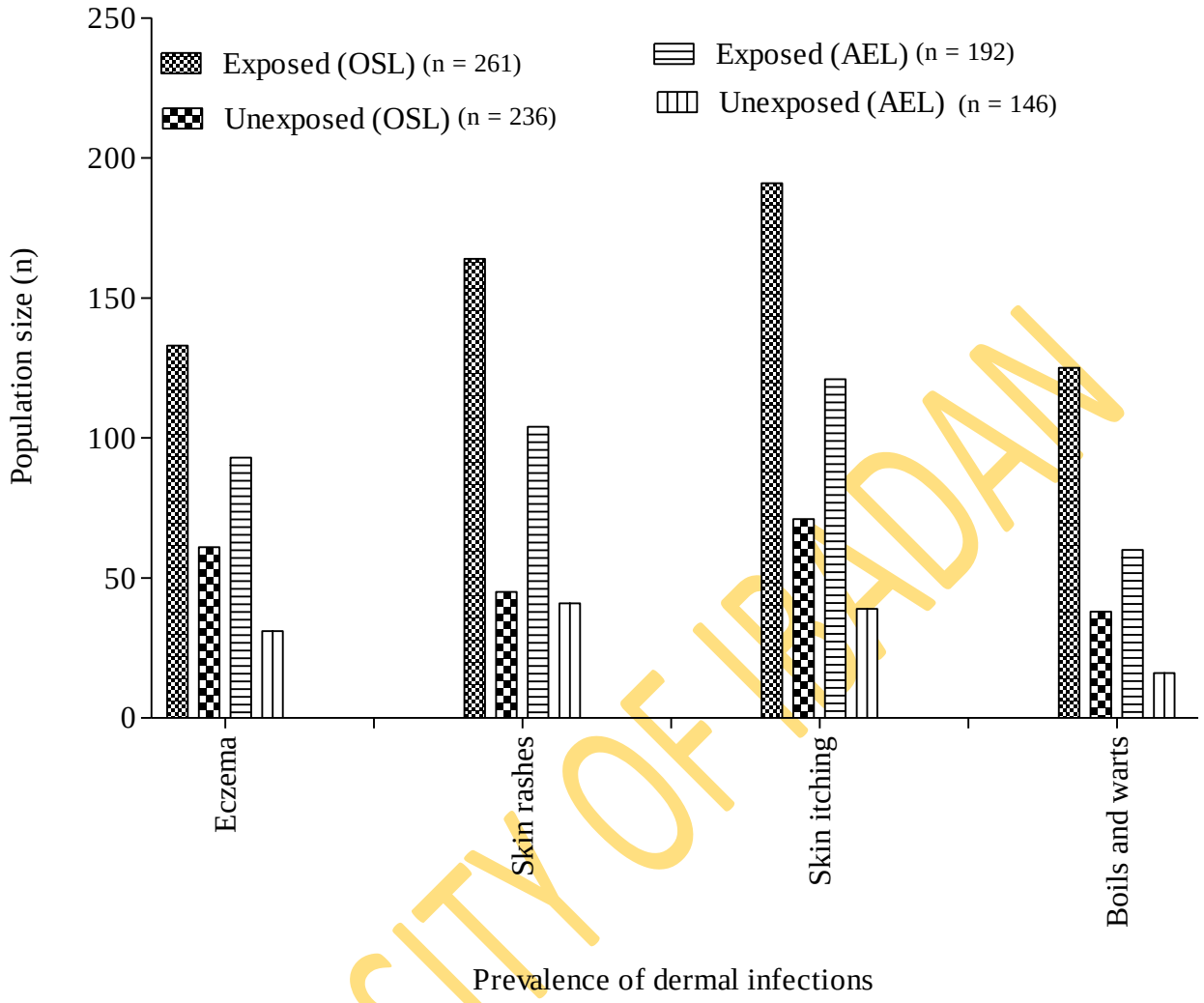

Figure 4.51 Prevalence of dermal infections among exposed and unexposed populations around Olusosun and Aba Eku landfill sites.

OSL = Olusosun landfill.

AEL = Aba Eku landfill.

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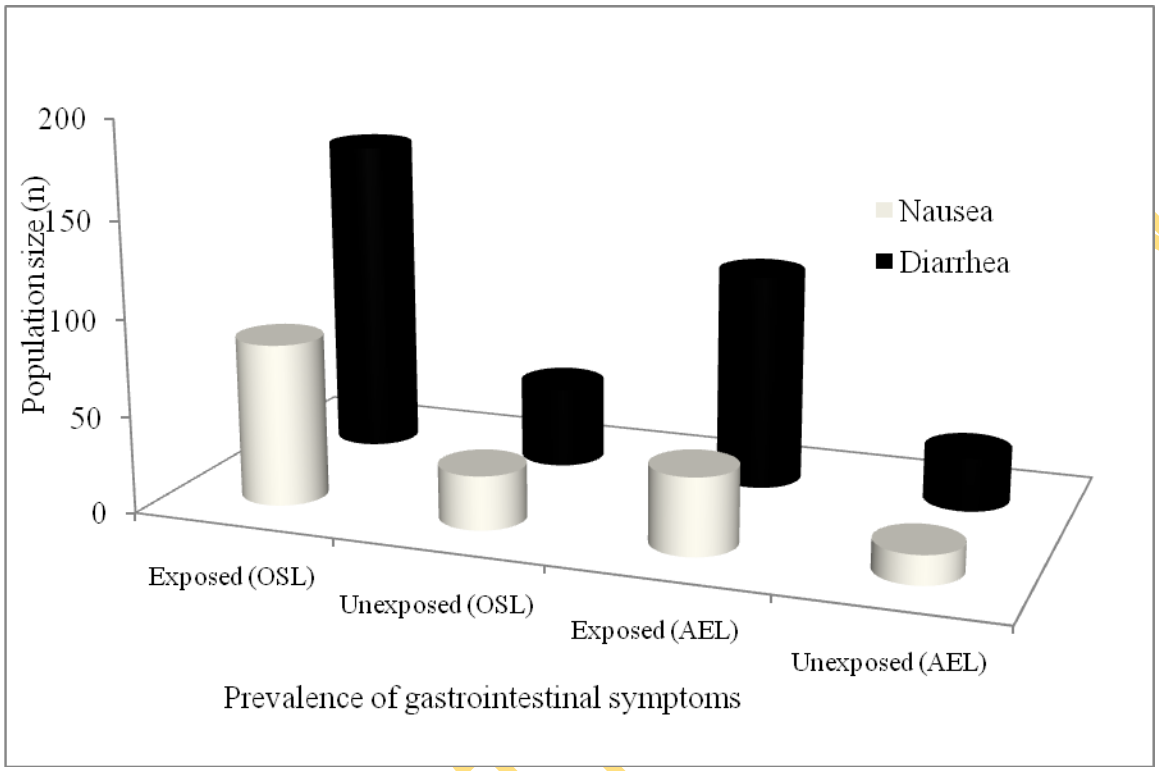

Figure 4.52 Prevalence of gastrointestinal symptoms among exposed and unexposed populations around Olusosun and Aba Eku landfill sites.

OSL = Olusosun landfill.

AEL = Aba Eku landfill.

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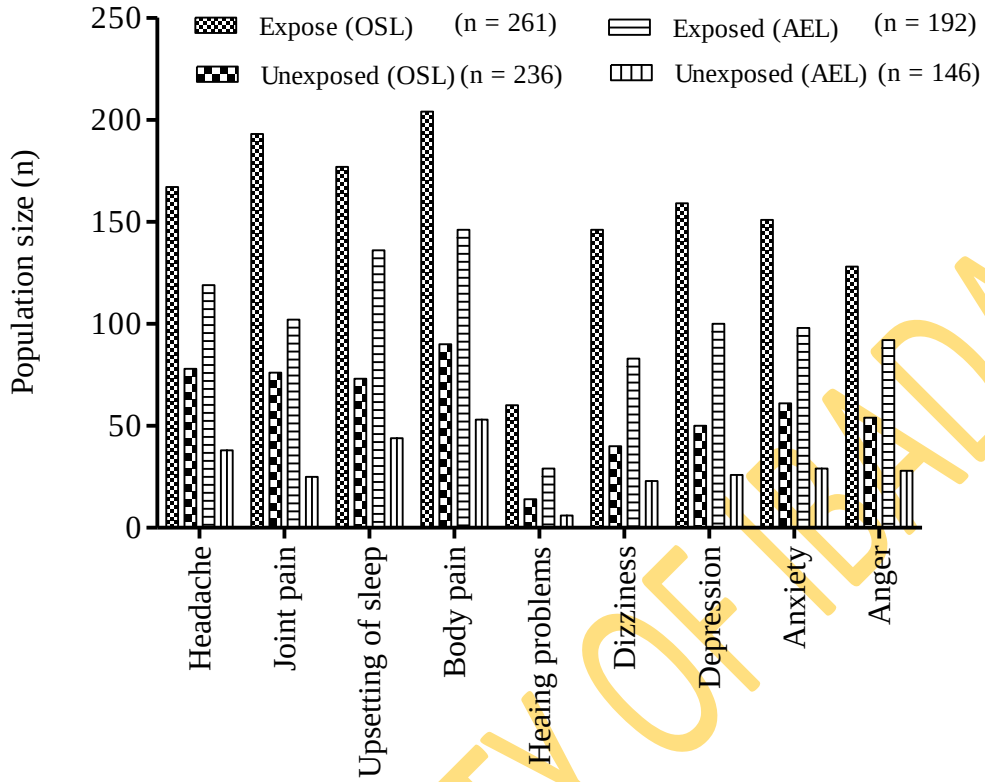

Figure 4.53 Prevalence of neuromuscular and psychological disorders among exposed and unexposed populations around Olusosun and Aba Eku landfill sites.

OSL = Olusosun landfill.

AEL = Aba Eku landfill.

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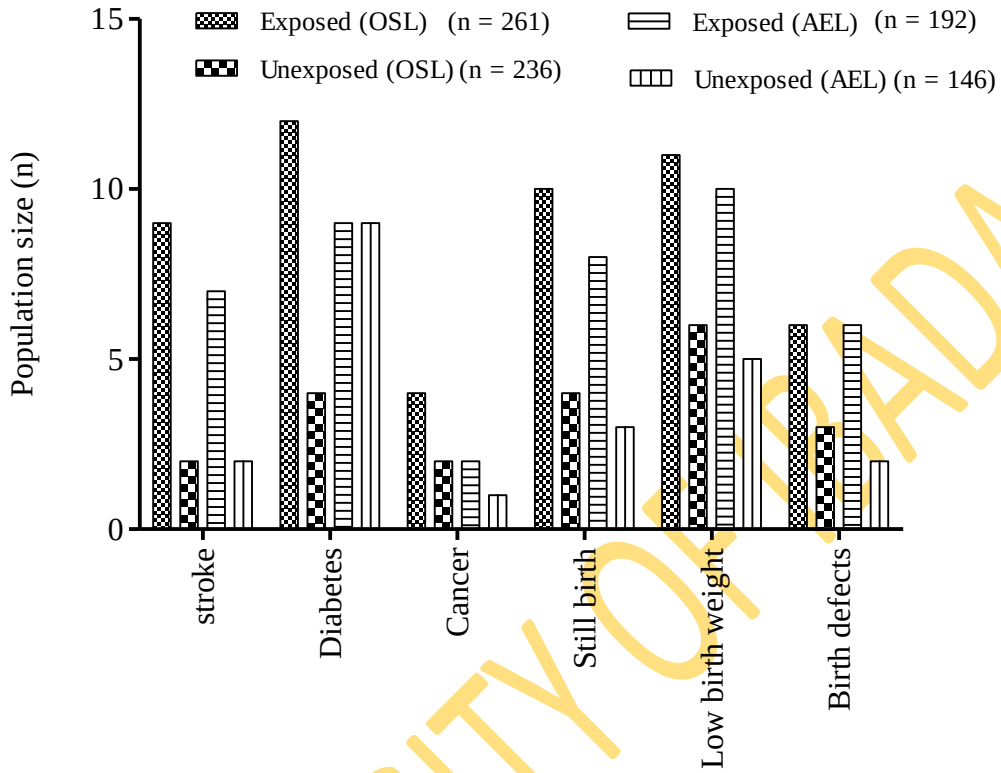

Figure 4.54 Prevalence of reproductive and other disorders among exposed and unexposed populations around Olusosun and Aba Eku landfill sites.

OSL = Olusosun landfill.

AEL = Aba Eku landfill.

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CHAPTER FIVE

DISCUSSION

Inappropriate disposal of municipal solid wastes by landfilling is still an issue of public concern due to the contamination of the environment (terrestrial, aquatic and arboreal) and biota by the constituents released from the landfills. The elevated heavy metals and anionic components of Olusosun and Aba Eku leachates than national and international permissible limits, is an indication of environmental contamination by these landfills. High concentrations of Cu, Pb, Cd, Mn, As, Ni and Cr in the leachates may be due to metal leachability during burning or decomposition processes of wastes (mostly industrial, medical and electronic) present in the landfills. It is also known that some toxic metals like Pb and Cd, existing as inorganic compounds, for instance chlorides, carbonates are highly volatile and can condense at low temperature. During ionisation process, metals can leach out easily into the environment (Valavanidis *et al*., 2008). Cr and Cu are slightly more difficult to leach out because their presence is mostly residual (White and Claxton, 2004), but their high concentrations in the leachates may be due to their ability to accumulate in sediments and landfills. Heavy metals readily accumulates in soil, sediments, water and biological materials hence are readily available in the ecosystem and can be toxic to living systems at higher concentrations (Pascual and Gold-Bouchot, 2004). High concentrations of ammonia, sulphates, phosphates, BOD and COD in the leachate samples may indicate the richness of organic compounds in the leachates. Many of these compounds are toxic to living organisms and usually lead to eutrophication and dissolved oxygen depletion of aquatic ecosystems. The inorganic, heavy metals and organic compounds in landfills may individually or in combination be responsible for landfill leachates systemic toxicity and cytogenotoxicity (Alimba *et al*., 2006; Alkassasbeh *et al*., 2009; Bakare *et al*., 2012; Li *et al*., 2010).

5.1 Cytogenotoxicity induction in catfish, Japanese quail and mice treated with leachates

Cytogenotoxic evaluation using MN assay is one of the primary tests among standard batteries of genotoxicity tests recommended by regulatory agencies and it has been widely used both in *in vivo* and *in vitro* studies as suitable end-points for genotoxic risk assessment in regulatory set-ups (Schmid, 1975; Vikram *et al*., 2007). Piscine and avian MN assays are considered useful cytogenetic methods for evaluating genotoxicity in aquatic (Al-Sabti and Metcalfe, 1995) and arboreal environments (Bhunya and Jane, 1992) respectively. They are

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considered biomarkers of clastogenicity and aneugenicity for assessing xenobiotics in the air and aquatic environments (Cavas and Ergene-Gazukara, 2005; Kursa and Bezrukov, 2007).

In this study, treatments of *C. gariepinus* (mud catfish) and *C. japonica* (Japanese quail) with Olusosun and Aba Eku landfill raw leachates induced significant increase in the frequencies of micronucleated cells in the peripheral erythrocytes, gill epithelial cells and kidney cells of *C. gariepinus* and peripheral erythrocytes and bone marrow cells of *C. japonica*. The increase in MN induction in the tissues of these animals indicates that OSRL and AERL constituents have clastogenic and or aneugenic properties and can induce chromosomal damage in the treated animals. Chromosomal damage can lead to genome instability, which can result in decreased cell survival or transformation, cancer formation and reproductive problems (Shugart, 2000).

The genotoxic effects of OSRL and AERL induced in *C. gariepinus* and *C. japonica* may be due to individual components of the leachate or combination of the toxicants acting through different mechanisms. It is possible that heavy metals present in the leachates crossed cell membranes of the treated animals, bind to DNA and cause the observed MN formation. This assertion is supported by the report that Cr^{3+} easily cross cell membranes by passive or facilitated diffusion (Ashby *et al*., 1995), bioccumulates to reach toxic levels (Stearns *et al*., 1995), binds to DNA (EPA, 1998) and produces genetic damage by its direct interactions with DNA (Blasiak and Kowalik, 2000). Cd induces genotoxicity mainly through single strand DNA breaks generated by direct Cd-DNA interactions as well as by action of incision nucleases and or DNA-glycosylases during DNA repairs (Privezentsev *et al*., 1996). It is also possible that DNA-damaging reactive oxygen species and inhibition of DNA repair mechanisms by metals are responsible for the observed MN formation in the leachate treated *C. gariepinus* and *C. japonica*. Bioaccumulation of Cu in tissues has been linked to increase free radicals generation and genotoxicity in cells (Bogdanova *et al*., 2002; Gwozdzinski, 1995). Ni compounds generate specific structural chromosomal damage, micronucleus, DNA-protein crosslinks and oxidative DNA base damage in exposed cells (Arrouijal *et al*., 1990; Sen *et al*., 1987; Kasprzak, 1991). Pb salts induced micronucleus was by disturbance of microtubule functions (Bonacker *et al*., 2005) indicating that Pb genotoxicity may involve indirect damage to DNA affecting the stabilization of chromatin (Johansson and Pellicciari, 1998).

The observation in this study on significant increase MN of peripheral erythrocytes of *C. gariepinus*, is similar to some previous studies on wastewaters. Das and Nanda (1986) observed significant increase in the induction of MN in peripheral erythrocytes of Indian catfish, *Heteropneustes fossilis*, exposed to mitomycin and paper mill effluent. Odeigah and Osanyipeju

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(1995) similarly observed concentration dependent and significant increase in the induction of MN in peripheral erythrocytes of *C. lazera* exposed to different concentrations of ethyl methane sulphate, textile mill and brewery effluents. Al-Sabti and Hardig (1990) also reported increase MN frequencies in erythrocytes of *Carassius auratus gibelio* treated with Cr^{3+}. Dose related and significant increase in the MN induction in peripheral erythrocytes of *Orechromis niloticus, O. aureus, Tilapia zilli* and *C. gariepinus* exposed to a textile mill effluent contaminated water and cyclophosphamide had been reported (Ali *et al*., 2008; Cavas and Ergene-Gozukara, 2003).

OSRL and AERL also induced dose related and significant increase MN in the gill epithelia cells and kidney cells of *C. gariepinus*. Significant increase in MN in the tissues of leachate treated mudfish may be due to the deleterious effects of the heavy metals and organic compounds (though not analysed in OSRL and AERL) present in the leachate samples. During exposure to the leachates, gill epithelial cells were directly exposed to the heavy metals and other constituents present in the leachates (aqueous medium) and chemicals probably absorbed from the leachates into the circulatory system of the leachate exposed fish. The gill epithelial cells are continuously dividing with higher mitotic index than the peripheral erythrocytes (Al-Sabti and Metcalfe, 1995; Hayashi *et al*., 1998), hence is suitable for the assay.

The kidney of mudfish is the main organ responsible for erythropoiesis (blood production process) as well as filtration. Therefore, significant increase in the micronucleated kidney cells observed in the leachate treated mudfish compared to the negative control may have been induced during erythropoiesis. Erythropoietic functions of the kidney may be responsible for the higher production of micronucleated cells in the kidney than the gill epithelial cells observed in OSRL and AERL treated *C. gariepinus*. Also, during exposure to xenobiotics, defective erythrocytes formed in the kidney are moved to the peripheral blood where they are removed by the hemocatheresis organs (Barsiene *et al*., 2006). This may account for the lower frequencies of MN in the peripheral blood than kidney cells observed in this study. The observation that the frequencies of MN in the examined organs and tissue was higher on the 3rd day than on the 6th day during exposure days may be due to the periods of the erythroblast cell cycle which occurs between 1–5 days in most species of fish (Al–Sabti and MetCalfe, 1995; Udroiu, 2006). While the higher values obtained during 9th and 14th days than the 6th day may be due to longer exposure duration to chemicals from the leachates (Udroiu *et al*., 2006).

Several studies have similarly reported micronucleus induction in different organs of fish species during *in vivo* and *in situ* exposure to individual or combination of chemicals. Jiraungkoorkul *et al*. (2007) reported high MN frequencies in the peripheral erythrocytes, gill,

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liver cells and fin cells of red-tailed tinfoil barb (*Puntius altus*) exposed to different concentrations of Cd salt at 24, 48, 72 and 96 hours. They also concluded from their findings that MN frequencies observed in the peripheral erythrocytes was most sensitive to Cd treatments than the gill, liver and fin cells. Cavas *et al*. (2005) reported higher frequencies of MN in the gill and liver cells than peripheral blood of Tilapia species (*Orechromis niloticus*) exposed to sublethal concentrations of cadmium chloride and copper sulphate in a subchronic study. In a study, the kidney and peripheral erythrocytes of two marine fishes, turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morua*) exposed to crude oil were examined for micronucleated cells. It was concluded that the cephalic kidney cells had two fold MN higher than peripheral blood cells (Barsiene *et al*., 2006). Talapatra *et al*. (2007) assessed MN induction in the kidney and gill cells of two fishes, *Labeo bata* and *Orechromis mossanbica*, exposed *in situ* to thermal power plants in Kolkata, India, they concluded that the frequencies of MN induction were higher in kidney than gill erythrocytes in both fish species examined.

The frequencies of MN induction in the three organs of the leachate treated *C. gariepinus* were higher in OSRL treated catfish than in AERL treated catfish. This observation can be supported by the leachate physicochemical composition which was higher in OSRL than AERL (table 4.1). Also, the toxicity factor for OSRL (1.25), which was higher than AERL (1.0) from the acute toxicity in *C. gariepinus* further supported the higher genotoxic effects of OSRL in *C. gariepinus* than AERL.

The induced micronucleated erythrocytes in OSRL and AERL treated *C. japonica* were higher in peripheral blood erythrocytes than in bone marrow cells. This may be attributed to the inability of the avian peripheral erythrocytes to undergo terminal differentiation and DNA or protein synthesis. Therefore, the MN induced in bone marrow erythroblasts were released into the peripheral blood as matured erythrocytes and may have accumulated in the peripheral blood (Guy *et al*., 1989).

The concentration dependent and significant increase in MN induced in both bone marrow and peripheral erythrocytes of *C. japonica* exposed to OSRL and AERL, may indicate that avian micronucleus assay is a sensitive tool for assessing the genotoxic impacts of xenobiotics. Although, no information exist on studies that assessed DNA damage in birds exposed to landfill leachates, but other studies have reported increase MN induction in peripheral blood of broiler chicks as an index of genotoxic exposure to cypermethrin (Sharaf *et al*., 2010), in pigeon and parrots (*Aratinga canicularis*) exposed to mitomycin-C (Gomez-Meda *et al*., 2006), in pigeons (*Columba livia* Gm) living around radioactive elements contaminated area

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(Ilyinskikh *et al*., 1997), in chickens in some farms exposed to contaminated food and water (Saleh and Sarhan, 2007) and in the bone marrow and peripheral erythrocytes of chicks exposed to monocrotophos (organophosphate insecticide) and lindane (organochlorine insecticide) (Bhunya and Jena, 1992;1993).

The simultaneous expressions of nuclear abnormalities (NAs) and MN in the peripheral erythrocytes of *C. gariepinus* and *C. japonica* exposed to OSRL and AERL further supports the DNA damaging effects of the constituents of the leachate samples. These chemicals induced problems like segregation tangled and attached chromosomes (abnormal chromosomal segregation) and gene amplifications (breakage-fusion-bridge) during the cell cycle of the erythropoietic organs of the leachate exposed catfish and Japanese quail. An attempt to eliminate the amplified DNA from the nucleus of the affected cells caused the formation of lobed and blebbed nuclei observed in the cytoplasm of the exposed organisms (Shimizu *et al*., 1998; 2000; Tolbert *et al*., 1982). In the instance, when the amplified DNA is selectively localised to specific sites on the periphery of the nucleus, nuclear bud is formed and when the bud is eliminated from the periphery of the nucleus it forms MN. This occurs mostly during the S phase of the cell cycle (Shimizu *et al*., 2000). Hence, the simultaneous expressions of nuclear bud and MN, with positive correction factor (between 0.73 and 0.84.) in human cells during exposure to xenobiotics (Serano-Garcia and Montero-Montaya, 2001). Binucleated erythrocytes observed in this study suggest that some of the characterised (and or uncharacterised chemicals) in OSRL and AERL exhibited cytochalasin B like properties of inhibiting microtubule assembly leading to cytokinesis blockage during M phase of the cell cycle (Umegaki and Fenech, 2000).

Studies on landfill leachates induced significant NAs in the erythrocytes of exposed catfish and Japanese quail are scarce, but NAs had been observed in parrots (*Aratinga canicularis*) exposed to different concentrations of mitomycin-C (Gomez-Meda *et al*., 2006) and in south polar skua (*Catharacta maccormicki*) exposed to environmental chemicals *in situ* (Kursa and Bezrukov, 2007). Also, in the erythrocytes of *Puntius altus* exposed to cadmium salt and *O. oreochromis* exposed to textile mill effluent in the order NT > LB > BN > BL (Jiraungkoorskul *et al*., 2007; Cavas and Ergene-Gozukara, 2003). Although, this order differs from BN > BL > LB > NT observed herein. Irrespective of the various order of occurrence of NAs in cytogenotoxicity studies, similar mechanisms led to their formations and their presence in cells can cause genetic imbalance, the basis of carcinogenesis (Mallick and Khuda-Bukhsa, 2003; Rodilla, 1993).

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Cytogenotoxic evaluation of OSRL and AERL exposure in mice using MN assay showed significant increase in the frequency of MN polychromatic erythrocytes (PCE) in both peripheral blood and bone marrow cells, and decrease in polychromatic and normochromatic erythrocyte ratio (PCE/NCE) in bone marrow cells of treated mice compared to the negative control. These findings further support previous observations in mud catfish and Japanese quail. It is possible that the constituents of the leachate samples distorted haemopoietic process in mice. The analysed metals, unidentified toxic constituents and their interactions are capable of inducing mutagenic effects through different mechanisms in the biologic systems. Copper can bind directly to the nitrogenous bases (has high affinity for G:C base pairs) and the phosphate group in DNA, disrupting the stacking structure of the DNA (Buttke and Sandstrom, 1994). Some leachate constituents can permeate cells and alter the cytosolic pH of the tissues. This alteration can affect both enzyme activities and DNA structure (Meng *et al*., 2002). Ni has been shown to produce deleterious effects by catalyzing the genotoxic effects of other heavy metals even when present at very low concentrations (Costa *et al*., 1994). It is also possible that leachate constituents (inorganic and organic components) induced free radical species (ROS) formation either by autoxidation or enzyme catalysed oxidation (Li *et al*., 2006a; 2010; Talorete *et al*., 2008), which reacted with lipids to produce lipid peroxidation of the cell membrane in exposed tissues, leading to oxidation of the nitrogenous bases and breakage in the DNA double helix (Li *et al*., 2006a). Mice orally administered copper sulphate showed several types of DNA damage, such as base alteration and DNA strand breaks, in their leucocytes due to ROS production (Saleha Banu *et al*., 2001). This result is in accordance with previous studies in which mice were gavaged with different concentrations of leachate and micronucleus induction was reported (Li *et al*., 2004). Municipal sludge leachate induced concentration dependent increase in MN PCE in exposed mice (Tewari *et al*., 2005). Cr and Ni induced genotoxic effects in peripheral lymphocytes of occupationally exposed welders as determined by comet and micronucleus assays (Danadevi *et al*., 2004).

The mouse-erythrocyte micronucleus (Mus-EMN) assay is a modified clastogenicity testing system (MacGregor *et al*., 1980; Salamone *et al*., 1980) from the mouse bone marrow erythrocyte micronucleus test (Heddle, 1973; Schmid, 1975) and it has been used to test for the clastogenicity of drinking water from a shallow well of a rural community in a monthly interval of 6 months study (Ma *et al*., 1987). Increased MN frequencies observed in erythrocytes of treated mice compared to the negative control using Mus-EMN test concluded that Mus-EMN assay is efficient in detecting clastogenicity of water pollutants even at very low concentrations

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(Ma *et al*., 1995). The concentration dependent and significant increase in the value of MN PCE observed in peripheral blood of OSRL and AERL treated mice using Mus-EMN test, further support the clastogenic or and aneugenic potentials of these leachate compositions in mice. Increased MN PCE frequencies in the peripheral blood cells than in bone marrow cells of leachate treated mice may indicate that micronucleated cells induced in the bone marrow accumulated in the peripheral blood of the mice. This observation is possible since in mice, spleen does not selectively destroy circulating micronucleated erythrocytes in the peripheral blood, hence they accumulate with time (Schlegel and MacGregor, 1982; Schlegel *et al*., 1986). This also showed that damage to bone marrow cells may be presented in the peripheral erythrocytes (MacGregor *et al*., 1980).

The use of micronucleus test in determining cytotoxic effects of chemicals and other xenobiotics by measuring PCE/NCE relationship was suggested (Krishna and Hayashi, 2000), and it was well adapted in assessing the cytotoxicity of an antimutagenic drug in exposed mice (Vilar *et al*., 2008). In this study, the concentration dependent and significant decrease in PCE/NCE ratio observed in OSRL and AERL treated mice compared to the negative control further supports the cytotoxic effects of landfill leachates in mice. It is possible that the leachate constituents caused damage or alterations to the normal proliferation of the bone marrow cells, hence the numbers of immature erythrocytes (PCE) is prejudiced in relation to mature erythrocytes (NCE) causing the PCE/NCE ratio to decrease (Vilar *et al*., 2008).

Sex specificity in the induction of genotoxicity and cytotoxicity observed in OSRL and AERL treated mice as assessed by MN assay, may be due to differences in the quantity of oestrogen production according to the sex of the mice. It has been previously reported that oestrogen may increase the induction of MN formation by mutagens (Nagae *et al*., 1991).

5.2 Systemic toxicity evaluation in rats treated with landfill leachates

Olusosun and Aba Eku landfill leachates induced different systemic toxicity in rats via changes observed in the clinical signs of toxicity, body and organ weight gain, haematology, biochemistry and histopathology of treated rats compared to the negative control. Some of these toxicological observations suggest the precise mechanisms of leachates induced genotoxic and cytotoxic effects in biologic systems.

5.2.1 Clinical signs of toxicity and mortality

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Clinical signs of toxicity observed in rats exposed to Olusosun and Aba Eku landfill leachate had been previously reported as symptoms of toxicity during exposure to chemicals some of which are found in landfill leachates. Anorexia (loss of appetite) observed in the leachate treated rats compared to the negative control is one of the common symptoms of liver injury attributed to the exposure of test chemicals and it is consistent with reduced body weight gain in exposed rats (Weingand *et al*., 1996). Labored breathing and sneezing observed at the 10 and 25 % concentrations of the tested leachates during the third and fourth weeks suggest respiratory disorder as a result of toxicosis of the chemicals in leachate (Kudyakov *et al*., 2004). Hair loss, exophthalmia, diarrhea, abscess, muscular disorder (muscular stiffness and decreased motor activities), reduced activities, blue discolouration and ungroomed hair are common symptoms of central nervous and immune systems dysfunction (Benlahcen *et al*., 2009).

There is no available information on clinical signs of landfill leachates induced toxicity in rats. Available information on mice intraperitoneally exposed to different concentrations of landfill leachate showed hair loss, anorexia, diarrhea and sluggishness (Bakare *et al*., 2003). Some clinical signs of toxicity observed in this study have been reported in rats exposed to chemical substances commonly found in landfill leachates. Rats exposed to 75 and 150 mg/kg herbicide showed reduced activities, increased weakness, slight diarrhea, molting in the abdominal region, hair loss and nasal hemorrhage (Tayeb *et al*., 2010). Also, rats exposed to 250 and 500 mg/L of lead acetate solution for 12 weeks, similarly displayed some of these signs (Benlahcen *et al*., 2009). Clinical signs of leachate toxicosis observed herein may be due to the deleterious actions of individual constituents or combined interactions of chemicals present in the tested leachates. These chemicals probably disrupted the functional integrities of the viscera responsible for maintaining the normal body metabolism in this group of mammals (Goldstein and Schnellmann, 1999; Hodgson and Goldstein, 2001). The observed clinical signs probably culminated and resulted into mortality in some rats during exposure.

5.2.2 Body and organ weight gain in landfill leachate treated rats

Body and organ weight changes had long been known as sensitive indicator of chemical induced changes to the organs and are widely accepted in the evaluation of test article associated toxicities (Michael *et al*., 2007; Seelers *et al*., 2007). In this study, the concentration dependent and significant decrease in the weekly and terminal weight gain in rats exposed to OSRL and AERL and significant decrease in rats body weight gain following exposure to OSSL and AESL may be due to the leachates induced anorexia. Concomitant increased in both absolute and

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relative liver, kidney, lung, heart and spleen, and decrease in thymus weight gain in leachate treated rats, may be due to obstructions of these organs by the leachate constituents.

Bakare *et al*. (2003) had previously reported that mice exposed to landfill leachates showed reduced terminal body weight gain. Li *et al*. (2010) similarly observed increase in relative kidney, liver and heart weight gain but decrease in relative spleen weight gain in mice exposed to different concentrations of leachate for 7 days. Changes in body and organ weight gain of leachate treated rats may have occurred due to bioaccumulation of heavy metals from the leachates during exposure. This assumption is supported by the finding of Sanchez-Chardi and Nadal (2007) and Sanchez-Chardi *et al*. (2007) that bioaccumulation of heavy metals from landfills in wood mouse is associated with increase relative liver and kidney weight gain. Simmons *et al*. (1988) exposed male F344 rats to 10 samples of complex mixtures of organic and inorganic components from solid wastes by oral exposure and at 24 hours post exposure, 9 of the 10 samples caused increase in relative liver weight gain. The authors concluded that these samples contained hepatotoxicants. Increase in liver weight gain in a less than 7 days duration of chemical exposure was associated with hepatic enzyme productions. Hence chemical treatment related liver weight gain has been associated with hepatocellular hypertrophy (like enzyme induction or peroxisome proliferation) (Amacher *et al*., 2006; Greaves, 2000; Juberg *et al*., 2006). Landfill leachates induced changes in kidney weight gain of exposed rats may reflect renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). The kidney is sensitive in predicting toxicity, physiologic perturbations and acute injury. Thymic and splenic weight changes are considered to be sensitive indicators of immunotoxicities (immune stimulation or depletion), stress and physiological perturbations (Seelers *et al*., 2007). Moreso histopathological changes in these organs correlates well with organ weight change (Michael *et al*., 2007). Rao *et al*. (1984) reported increase in splenic weight and decrease in thymic weight gain in albino rats exposed to 0.5 ml/kg body weight of kerosene for 6 days a week for a period of 35 days and they suggested chemical interference with the immune system in rats. Landfill leachate induced toxicity on the spleen and thymus as characterized by increase and decrease splenic and thymic weight respectively observed in this study may indicate the immunotoxic potentials of landfill leachates.

The involvement of mammalian viscera in the modulation of different processes like assimilation, metabolism, immunity, detoxification and elimination, may predispose these organs to direct pathological lesions and oxidative stress from the leachate constituents. Also, chemicals in mammalian body are readily absorbed into the blood and during transportation to the lungs for

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blood cell oxygenation, the alveoli of the lungs may become injured by these chemicals. This is an indication that landfill leachate constituents induced injuries in these organs. The type of lesions induced in these organs is identified from the histopathological evaluation of the organs, since change in organ weight gain correlates well with histopathology (Michael *et al*., 2007).

5.2.3 Histological changes in organs of landfill leachate treated rat

Histopathological evaluation of tissues provides information about acute and or chronic effects of toxic substances that may not be detected by other biomarkers and correlates well with biochemical analysis and organ weight gain (Amacher *et al*., 2006; Jadhav *et al*., 2007). Hepatic necrosis and cellular infiltrations with inflammatory cells observed in the liver; degeneration of epithelia of the renal tubules and renal necrotic changes could be induced by direct interaction of chemical components of the leachates or by exogenous and endogenous generated reactive oxygen species (ROS) in the liver and kidney of leachate exposed rats. The histological lesions observed herein have been similarly reported in the biopsy from liver of workers exposed to chemicals from industrial wastes (Cheong *et al*., 2007). Also, *Apodemus sylvaticus* and *Crocidura russula* (rodents) inhabiting Garraf landfill sites in Spain showed significant increase in quantity and severity of foci of cell necrosis, apoptosis and cytoplasmic vacuolization in the liver and kidney (Sanchez-Chardi *et al*., 2008). These lesions were induced by bioaccumulated cadmium, lead, mercury, zinc, copper, manganese, molybdenum and chromium in these organs (Sanchez-Chardi and Nadal, 2007; Sanchez-Chardi *et al*., 2007). Simmons *et al*. (1988) exposed rats to 0.5 – 5.0 ml/kg of 10 different complex waste mixtures for 24 hour. Eight among these samples induced centrilobular degenerations and necrosis. These may indicates that chemicals in landfill wastes are capable of inducing lesions in the liver.

The susceptibility of the kidney cells to xenobiotics is raleted to their functions in filtering toxic chemicals during ultrafiltrations. This may accounts for the lesions observed in the kidneys of leachate treated rats in this study. The findings herein had been similarly reported in yellow-necked mice inhabiting steelworks and zinc smelters in Poland and exposed to lead, cadmium and zinc *in situ* (Damek-Poprawa and Sawicka-Kapusta, 2003).

Thymus is a sensitive target organ for most immunotoxicants during exposure. Decrease in size or weight of this organ is often one of the first noted measures of toxicity (Schuurman *et al*., 1992). Necrosis and other multiple histopathological changes in the thymus and spleen of leachate exposed rats could be attributed to adverse immunotoxic effects of xenobiotics to the

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organs (Kuper *et al*., 2000). Ikechukwu *et al*. (2010) exposed rats to different concentrations of crude oil for 28 days and observed degenerative, necrotic and inflammatory changes in the section of spleen of exposed rats compared to control. Histopathological lesions observed in the thymus and spleen of leachate treated rats along with concomitant change in the weight gain of these organs indicates the immunotoxic properties of chemicals present in the tested leachates.

Histopathological changes in the lungs of leachate treated rats indicates that the lungs are susceptible to damage from leachate constituents irrespective of method of exposure, either by inhalation or oral gavaging of the leachates. Lungs have not been well assessed for histopathological lesions in landfill toxicity despite its frequent exposure to landfill gases. It is possible that the observed lesions in leachate treated rats were by oxidative stress. That the histopathology of the heart in most of the leachate treated rats did not differ significantly from the negative control, may indicate that the heart is not readily affected by the landfill leachates compared to other examined viscera.

5.2.4 Changes in serum biochemistry of landfill leachate exposed rat

A good number of serum biochemistry has been used in *in vivo* studies to evaluate the toxic potentials of chemical compounds. Some of these biochemical analyses have been validated as organ-specific biomarkers and may be useful in determining possible mechanisms of toxicity (Travlos, 1996). Biochemical analyses validated as liver and kidney biomarkers were considered in this study. Liver and kidney were selected among all viscera due to their importance in metabolism, detoxification, storage and excretion of xenobiotics and their metabolites and are especially vulnerable to damage (WHO, 1992). Moreso biochemical analysis correlates well with histological lesions to confirm the induction of organ-specific effects of xenobiotics.

In several organs, cell damage is followed by the release of a number of cytoplasmic enzymes into the circulatory system, this provides the basis for clinical diagnosis (Sundberg *et al*., 1994). Alanine aminotransferase (ALT) is found in greatest concentration (highly specific) in the liver of rats and dogs while aspartate aminotransferase (AST) can be found in various concentrations in muscles, heart, liver, kidney and intestine, but concomitant increase in the activities of both enzymes in the serum signifies acute hepatocellular injuries and xenobiotic induced necrosis (Clermont *et al*., 1967; Davis, 1992). Serum concentration of AST and ALT are the most commonly used biochemical markers of hepatocellular necrosis (Friedman *et al*., 1996; Henderson *et al*., 1983) and have long been considered sensitive indicators of hepatic injury

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(Molander *et al*., 1955). These enzymes are localized in the periportal hepatocytes, reflecting their role in oxidative phosphorylation and gluconeogenesis (Sturgill and Lambert, 1997).

Changes in the serum levels of ALT and AST have been used as a tool to study cell viability, cell death (apoptosis) and changes in cell membrane permeability (Dasgupta *et al*., 1996; Davies, 1992; Vonen and Morland, 1984). The marked treatment related increase in the activities of serum ALT and AST and hepatocellular necrosis observed in this study indicates that leachate constituents (mostly heavy metal) induced damage to the hepatocytes. This damage probably affected the liver cell membrane causing cell death (apoptosis), and increased the cell membrane permeability (Boyd, 1983). Similar increase in ALT and AST was observed in workers exposed to chemicals from industrial waste vapours (Cheong *et al*., 2007) and in residents of Hardeman county USA, who ingested water contaminated by numerous chemicals from a landfill (Meyer, 1983). Silva *et al*. (1999) observed increased serum levels of ALT in rats after 7 days of river-water intake and they concluded that their observation reflected the toxic effects of water contaminants on the cell membrane, leading to increase permeability and increase leakage of this hepatic intracellular enzyme. Wistar rats exposed to individual and combination of cadmium and ethanol showed increase in serum ALT and AST levels with concomitant deformities in the histology of the liver. These observations showed that Cd and ethanol administered separately and especially in combination caused injuries to liver cells of exposed rats (Brzoska *et al*., 2003). Male Fischer-344 rats were exposed to 10 different complex waste mixtures and 8 among these waste mixtures caused increase serum ALT and AST with concomitant induction of necrosis and degeneration in the centrolobular region of the liver. It was concluded that interaction among the many chemicals in these mixtures, which may include a combination of synergies and antagonisms as well as additives, caused the observed effects (Simmons *et al*., 1988).

Serum creatinine and urea concentrations are biomarkers of renal injury (Induski and Lutz, 2005; Travlos *et al*., 1996) and the elevation of these biomarkers is usually associated with impairment of renal function (Kumer *et al*., 2005). If the glomerular filtration decreases due to restriction of the renal blood supply, or due to functional impairment of nephrons by physical or chemical agents, the retention of the waste products of metabolism occurs in blood. Increase in serum urea and creatinine concentrations observed in leachate treated rats may be due to depression of glomerular filtration rate and renal tubular cell injury (Ulutas *et al*., 2006). These biochemical changes in leachate treated rats along with the observed treatment related kidney lesions agrees with Finco (1989) that approximately 75% of the nephrons must be non functional

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for the observed changes in the concentrations of urea and creatinine to occur. Similar observations were made in rats exposed to Cr^{3+} compound (Laborda *et al*., 1986) and in rabbits exposed to cyanide (Okolie and Osagie, 1999), and renal damage was suggested. Silva *et al*. (1999) observed significant increase in serum creatinine of rats after 7 and 30 days exposure to river-water intake and they concluded that river-water contaminants induced kidney injury in exposed rats. Wistar rats exposed to individual and combination of cadmium (Cd) and ethanol showed increase in Cd accumulation in the kidneys after 12 weeks of exposure to different concentrations of Cd salt with increase in serum creatinine and urea and concomitant deformities in the morphology of the kidney. These observations showed that Cd and ethanol administered separately and especially in combination caused injuries to kidney cells of exposed rats (Brzoska *et al*., 2003). Male Wistar rats intraperitoneally injected with 0.25 to 2 mg/kg of $CdCl_2$ for 40 days showed that Cd led to the elevation of serum urea by 194 to 316 % and serum creatinine by 14 to 90 %. This confirmed renal damage by cadmium intoxication (Moshtaghie *et al*., 1991).

Changes in serum total protein and serum albumins during toxicity studies have been suggested as supplementary indicators of hepatic synthetic functions (Meyer and Harvey, 2004) and nephrotoxicity (Induski and Lutz, 2005) and are included in the minimal recommendations for clinical pathology testing in preclinical studies (Weingand *et al*., 1996). Albumin is a protein synthesized in the liver and the most abundant plasma protein accounting for about 60 % of the total serum protein. Its functional role makes it a reliable marker for the diagnosis of liver diseases (Benoit *et al*., 2000). The concentration dependent decrease in serum total proteins and albumin observed in leachate treated rats are indicative of disorders in protein synthesis, metabolism and necrosis (Goldwasser and Feldman, 1997). Meyer (1983) observed reduced albumin in humans who consumed water contaminated with leachate from a waste dump site.

Decreased serum albumin observed in this study accompanied with concomitant decrease in serum total protein has been associated with hepatocellular damage (Woodman, 1996). Male rats exposed to different concentrations of Cd showed reduced serum protein concentration with concomitant increase in serum urea and creatinine indicating functional damage to kidney (Morowati, 2001; Moshtaghie *et al*., 1991). The association between decrease serum albumin and total protein concentrations and treatment related histopathological lesions in kidneys and liver of leachate treated rats suggest that leachate constituents induced liver and kidney dysfunctions.

5.2.5 Changes in haematological indices of leachate exposed rat

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Haematological characteristics of animals have been widely used in diagnosing diseases and pathologies of humans and domestic animals. Blood cells are exposed to any agent absorbed or injected into the bloodstream, even those that are rapidly metabolized and excreted. This is because blood is an important liquid connective tissue participating in all vital functions in the animals' body. Haematological data from *in vivo* toxicological studies is one of the most predictive measures for human risk (Evans, 2008) since changes in blood parameters are mainly due to injuries and or infections of some tissues and organs. Changes in blood parameters may be an adaptive response of bone marrow or peripheral blood cells to physiological and immunological changes due to exposure to xenobiotics, stress, diseases and hypoxia (Blaxhall, 1972; Duthie and Tort, 1985).

Olusosun and Aba Eku landfill leachates induced concentration dependent decrease in the total erythrocyte counts, haemoglobin concentrations, percentage haematocrit and platelet count observed in the leachate treated rats compared to the negative control. The observed decrease in red blood cells may be due to haemolysis of the red blood cells due to xenobiotic in the leachates induced haemorrhages and reduced erythropoiesis (Mandal *et al*., 1986). Decrease in the percentage haematocrit may be attributed to failure in erythropoiesis, destruction of mature red blood cells and increase plasma volume (Malhotra and Srivastava, 1978), and it is an indicator of stress probably induced by the constituents of leachates on the animal health and O_2 carrying capacity of blood (Larsson *et al*., 1985). Decrease in haemoglobin content may be attributed to decrease in the number of red blood cells and this will impact on the oxygen carrying capacity of the rats (Malhotra and Srivastava, 1978). These changes in haematological parameters have been previously reported in rats exposed to sub-lethal concentrations of cypermethrin for 21 days and rats exposed to mercury chlorides for 7, 14 and 21 days (Mahour and Saxena, 2009), in mice (*Mus musculus*) exposed to different concentrations of distillery soil leachate for 30 days.

Red blood cell indicators: MCV, MCH and MCHC, are dependent on red blood corpuscle count, haemoglobin concentration and haematocrit. Increase in MCV may suggest an intensified compensating activity of the haemopoietic system, which may be in response to haemolytic action of toxicants in the tested leachates (Sharma *et al*., 2007). MCHC is an expression of the average concentration of haemoglobin in red blood cells and give the ratio of the weight of haemoglobin to the volume of red blood cells. Decrease in MCHC 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 seen in iron deficiency (Fischbach, 1984). Reduction in MCHC further confirmed poor haemoglobin carrying capacity of the erythrocytes

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(Lynch *et al*., 1969; Eaton and Klaassen, 1998). The reduction in the erythrocytic parameters of leachates exposed rats with concomitant increase in MCV and decrease in MCHC (erythrocyte indices) suggests that leachate treated rats suffered macrocytic and hypochromic anaemia (Barger, 2003). Increase in MCH had been previously reported in Algerian mice in habiting an area contaminated with heavy metals and it was linked to macrocytic anaemia that was probably caused by hepatic or pulmonary diseases (Nunes *et al*., 2001). Swiss albino mice exposed to distillery soil leachate similarly present increased MCV, MCH and decreased MCHC (Sharma *et al*., 2007b). Also were mice exposed to textile dye wastewater (Mathur *et al*., 2003).

Quantitative investigations of total white blood cells and differentials (non specific immune cells) formed a basic examination mostly included in immunotoxic studies (Tryphonas, 2001). These components of blood cells are useful in signaling clinically relevant haematologic changes that may result in clinical identifiable autoimmune disorders and various forms of leukemia (Lee *et al*., 1999). Significant decrease in the total white blood cell counts observed in the leachate treated rats compared to the negative control, is due to decrease in lymphocyte count (lymphocytopenia). Decrease in lymphocyte counts may be due to toxic effects of the leachate constituents (particularly heavy metals) on the lymphoid tissues, hence reduction in the quantity of lymphocytes released into the circulation. This assertion lends credence to histopathological lesions and changes in the organ weight gain observed in the thymus and spleen (lymphoid organs). Also, since it is known that inorganic and organic components of landfill leachates are capable of inducing oxidative stress (Li *et al*., 2006a; 2010; Talorete *et al*., 2008), it is possible that ROS induced the secretion of cortisol in the treated rats during stress reaction, which shortened the life span of the lymphocytes and promotes their apoptosis (Verburg van-Kemanade *et al*., 1999; Wyets *et al*., 1998) and reduced their proliferation leading to compromised immune response (Witeska, 2005). This was similarly observed in *Heteropneustes fossilis* (fish) exposed to10 mg/L of cadmium chloride for 96 hours (Radhakrishnan, 2010), in Perch dwelling in natural bodies polluted with mixture of heavy metals containing Zn, Cu, Pb, Cd and Hg (Larsson *et al*., 1985) and in humans occupationally exposed to 10 ppm concentration of Benzene (Chang, 1972).

Concentration dependent increase in neutrophils count (neutrophilia), along with concomitant decrease in MXD suggest that the constituents of the tested leachate samples, likely the heavy metals, compromised the phagocytotic activities of these cells in the treated rats. Previous studies (Efuntoye *et al*., 2011; Oshode *et al*., 2008) showed the presence of faecal coliforms which have been implicated in gastroenteritis, in leachates from Aba Eku sampling

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site. The production of enterotoxin by these diarrhea-causing bacteria may be responsible for the elevated neutrophils due to their phagocytic activities against the bacterial infections in tissue.

The observed neutrophils count has been previously reported in mice exposed to whole-body gamma irradiation (Nunia *et al*., 2006), carp and rainbow trouts exposed to Cu at lethal concentrations (Svobodova *et al*., 1994) and in Bonnet monkeys exposed to 60 mg/ kg of cyclophosphamide once and blood sampled at 96th hour post-exposure (Mythili *et al*., 2004).

Landfill leachates adversely affected the shape of red blood corpuscles in treated rats. Significant increase in altered red cell morphologies (like acanthocytes, schizocytes, target cells, echinocytes, tear drop poikilocytes, anisocytes and sickle cells) compared to the negative control rats, is consistent with findings in the erythrocytic parameters. The presence of abnormal RBC may be related to leachate induced disturbances in osmoregulatory system of the blood cells (Shaw *et al*., 1991) and/or oxidative injury to the cell membrane (Christopher, 2004). Leachates induced oxidative damage to RBC membrane might have enhanced leakage of free denatured haemoglobin (Everse and Hsia, 1997), thus accounting for the findings of the erythrocytic indices. Abnormal erythrocyte morphology has been similarly reported in mice exposed to distillery soil leachates (Sharma *et al*., 2007). Erythrocytes serve as the principal vehicle for effective transportation of oxygen and carbon dioxide between the lungs and tissues/organs. During this process, oxygen and carbon dioxide molecules coupled to the haem group of the erythrocytes may be exposed to heavy metals (particularly Pb) and other leachates constituents in the plasma. It is plausible that free radicals are generated during this process and may account for the different shapes of the erythrocytes. Moreso, erythrocytes are more vulnerable to lipid peroxidation (LPO) (Eritsland, 2000; Jadhav *et al*., 2007). LPO is capable of inducing profound alterations in the structure and functions of the cell membrane, via decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes, and loss of essential fatty acids (van Ginkel and Sevanian, 1994). Heavy metals analysed in the tested samples have been linked to oxidative damage in erythrocyte membrane lipid bilayer (Tkeshelashvili *et al*., 1989), and peroxidation of erythrocyte membrane lipids (Misiewicz *et al*., 1999). These findings on haematological changes associated with haematopathology may be due to the consequences of bone marrow impairment or direct damage to blood cells (Bloom, 1993).

The consequences of damage to erythrocytes are life-threatening and may clinically manifest as anaemia, hypoxia, and several health disorders.

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5.3 *In vitro* cytogenetic study of Pb, Cu, Cr, Mn and their combinations using CBMN Cytome with WIL2-NS cell line

In this study, Cr, Cu, Mn and Pb in their individual and joint forms induced different levels of genotoxic, cytotoxic and cytostatic effects on WIL2-NS human B lymphoblastoid cells as assessed using CBMN Cyt assay. The possible mechanisms of MN, NPB and NBud formation (genotoxic effect) may be through direct interactions of the metals with the cells and/ or indirect through the production of reactive Oxygen species (free radical species).

Chromate salts induced cytogenetic effects both *in vivo* and *in vitro* had been previously reported. Hexavalent form of chromate (Cr^{6+}) produce varieties of genotoxic related effects through the induction of DNA-strand breaks, sister chromatid exchange, DNA-protein complexes, MN formation and structural chromosome aberrations (Godet *et al*., 1996; Biederman and Landolph, 1990). Li *et al*. (1992) reported microtubule bundling and depolymerization (cytoskeletal disturbances) in 3T3 cell line exposed to 3.13 and 25µM doses of the hexavalent chromate salt *in vitro*. Seoane and Dulout (2001) utilized kinetochore staining in cytokinesis-block micronucleus assay and confirmed that Cr^{6+} salts are able to induce kinetochore positive and negative micronucleus in human diploid fibroblast (MRC-5) and they concluded that the aneugenic and clastogenic ability of potassium dichromate in chromate genotoxicity was linked to malsegregation induction by disrupting microtubules.

It is also possible that chromate salts may have undergone transformation into a more genotoxic component, which induced the observed genotoxic effects. This assertion is supported by the findings of Tsapakos and Wetterhahn (1983) and Kadiiska *et al*. (1994) that hexavalent chromate salts permeated cells via sulphur-anion channel on cell membrane and undergo a reductive process that produces trivalent and pentavalent species, Cr^{3+} and Cr^{5+} intermediates, which induced variety of damage to DNA. Trivalent chromium is a stable form of chromate which binds directly to DNA forming DNA monoadducts, as well as, DNA-DNA and DNA-protein crosslinks (Snow and Xu, 1991). Blasiak and Kowalik (2000) concluded from their findings that DNA strand breaks induction by Cr^{3+} and Cr^{6+} in isolated human blood lymphocytes, was by reactive oxygen species formation.

Lead (Pb) is one of the most ubiquitous heavy metal that has been detected in virtually all media of the environment and biological systems and its genotoxic effects evaluated. Pinto *et al*. (2000) observed significant higher frequencies of CA and sister chromatid exchange (SCE) in cultured blood lymphocytes and increase frequency of MN in buccal cells from occupational exposed human population to lead compounds. Duydu *et al*. (2001) reported a significant

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increase in DNA breaks in workers exposed to Pb, as determined by the comet assay (single cell gel electrophoresis). Palus *et al*. (2003) reported a significant increase in the frequency of MN of clastogenic and aneugenic origin among workers occupationally exposed to Pb. Dose-response induction of MN in V79 cells from peripheral blood lymphocytes of workers occupationally exposed to Pb has been reported (Vaglenov *et al*., 2001). Vaglenov *et al*. (1998) reported a significant increase in the frequency of binucleated cells with MN in occupational exposure to Pb and they concluded that exposure to Pb at concentrations higher than 1.20 µM may pose an increase in genetic risk. But an occupationally exposed population examined for blood lead levels had 3 µM of lead compared to 0.20–0.27 µM of control group (Palus *et al*., 2003). This may indicate a higher genetic risk among populations with higher blood lead levels.

Valverde *et al*. (2001) exposed CD-1 male mice by inhalation to low non cytotoxic concentrations (0.01, 0.1 and 1.0 µM) of $Pb(CH_3COO)_2$ for 1 hour and observed increase lipid peroxidation and free radicals in different organs. Minozzo *et al*. (2004) observed in their study that inorganic Pb interfares with cellular signal transduction pathways, in particular with members of the calcium – dependent proteins kinase C (PKC) family, which play important role in cell proliferation. These findings led to the conclusion that Pb ions are weak mutagens in mammalian cell systems, but are mitogens capable of inducing genotoxicity or carcinogenicity by indirect interactions through oxidative stress. Hartwig and Schwerdtle (2002) reported that besides direct interactions with repair enzymes, Pb salts are also capable of interfering with Ca regulatory processes involved in the regulation of DNA replication, maintenance and or repair systems.

The results of this study that Pb induced increase frequency of MN and cytostatic (NDI) effects in WIL-2 NS cells, had being reported in workers occupationally exposed to 18-fold increase in blood Pb content compared to a control group (Minozzo *et al*., 2004). It can be deduced that lead induced genotoxic, cytotoxic and cytostatic effects was mainly by indirect mechanisms, through free radical formation (Minozzo *et al*., 2004; Valverde *et al*., 2001).

The findings herein that manganese salt induced genotoxic, cytotoxic and cytostatic effects in WIL-2 NS cells have been reported. $MnCl_2$ and $KMnO_4$ induced breaks, sister chromatid exchange and fragments in exposed cultured mammalian cells Umeda and Nishimura (1979), mutagenic effects in lambda prophage in *E. coli* WP2s (lambda) (Rossman *et al*., 1984). Joardar and Sharma (1990) exposed mice to different concentrations of $KMnO_4$ and $MnCl_2$ and observed significant increase in the frequencies of chromosomal aberrations and micronuclei in bone marrow cells and sperm-head abnormalities in the epididymis of exposed mice compared to

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control. El Deiry *et al*. (1984) reported that Mn^{2+} altered the accuracy of base selection and the specificity of hydrolysis of DNA polymerase I in *E. coli*. In a recent study, Flora *et al*. (2008) investigated the genotoxic, clastogenic and cytotoxic effects of $MnCl_2.4H_2O$ in different phases of cell cycle using short-term human lymphocyte cultures *in vitro*. They examined mitotic index (MI), chromosomal aberrations and DNA damage index detected by cytogenetic and comet assays of the lymphocyte exposed to 15, 20 and 25 µM. They observed from their study that Mn had high cytotoxic potentials since it caused decrease in mitotic index (MI) in all the phases of the cell cycle. Chromosomal aberrations and DNA damage were only observed at the highest dose of Mn (25 µM) exposure and at the G2 phase of the cell cycle after 3 hours of the treatment.

They concluded from their findings that Mn readily induce cytotoxicity since at all tested doses and stages of the cell cycle (G1, S, G2 and M) they observed decrease MI and its DNA damaging property may be through interference with cell replication or blockage of DNA repair mechanisms. Significant decrease in NDI (cytostatic effects) and concomitant percentage increase in necrotic cells (cytotoxic effects) may support that manganese induce reactive oxygen species formation.

Copper salts have been shown to significantly increase MN formation in the root tips of *Vicia faba* and *Pisum sativum* in a concentration dependent pattern when these plants were exposed to different concentrations of Cu^{2+} in hydroponic solution (Souguir *et al*., 2008). Saleha Banu *et al*. (2004) exposed mice to various concentrations (0, 1.25, 2.50, 5.00, 7.50, 10.00 and 12.50 mg/kg body weight) of copper sulphate by oral gavaging for 1–2 weeks and observed dose dependent significant DNA damage in the peripheral blood leukocytes using comet assay. Also accumulated copper in the hepatocytes of Long-Evans Cinnamon rats induced DNA strand breaks as detected by the comet assay (Hayashi *et al*., 2000). Increase lipid peroxidation in cell membranes and DNA damage by copper has been related to its role in the generation of free radicals (Bremner, 1998). Study on copper induced significant DNA strand break and increase frequency of MN erythrocytes and the role of curcumin which exerted genoprotective effects in exposed mice (Corona-Rivera *et al*., 2007), further supported free radical formation of copper.

Copper salt significantly decrease NDI and concomitantly increased percentage necrotic cell formation in WIL2-NS cells may indicate that the observed genotoxic effects of Cu^{2+} was by the generation of reactive oxygen species (ROS).

The combinations of Cr, Mn, Pb and Cu induced concentration dependent and significant genotoxic, cytotoxic and cytostatic effects in WIL2-NS cells was mainly at low concentrations (0.01, 0.01 and 1.0 µM) of the combined metals, which was more than as observed in individual

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metals. At 10 µM and above, there was total cytotoxic effects (total necrotic cell induction) compared to other metals. It may be difficult to understand the actions of the individual metals in the mixture, since they have different solubility, absorbability, chemical reactivity and complexes formed within cells (Stohs and Bagchi, 1995). It may be suggested that in their combined state, these metals elicited more reactive oxygen species which induced higher cytotoxic, genotoxic and cytostatic effects in WIL2-NS cells than the individual effects of the metals. This assertion is supported by the findings that oxidative stress caused by the mixture of eight metals was mediated by the conversion of accumulated hydrogen peroxide (H_2O_2) to hydroxyl group (OH^-), increased oxidative injury in peripheral erythrocytes of exposed rats (Gregus and Klaassen, 2001).

It is evident from the combined actions of these metals that the genotoxic induction in WIL2-NS cells is related to the induction of necrosis as no viable cell was seen in the culture even at 10 µM compared to that of the individual metals. Fenech *et al*. (1999) reported a positive correlation in the induction of MN and necrosis in WIL2-NS cells after exposure to different concentrations of H_2O_2 indicating indirect DNA damage induction. Necrosis is an alternative form of cell death caused by damage to cellular membranes, organelles and/or critical metabolic pathways required for cell survival such as energy metabolism (Fenech, 2007). Necrosis was shown to be the major route through which damaged DNA were eliminated from cultured lymphocytes (Fenech *et al*., 1999). Similarly, landfill leachates induced cytotoxic effects and stress in MCF-7 mammalian cells after 6 days of incubation using DNA fragmentation assay was by necrosis (Talorete *et al*., 2008). Moreso, significant reduction in the number of PCE/NCE cells in leachate treated mice compared to negative control (cytotoxicity) and concomitant increase in micronuclei formation observed in this study further supports the *in vitro* cytogenetic results of heavy metals exposure on WIL2-NS cells.

5.4 Mechanisms of cytogenetic and systemic toxicity of landfill leachates

It is now well known from *in vivo* and *in vitro* studies that leachates from landfills are capable of inducing toxicity and DNA damage in both eukaryotic and prokaryotic systems. Li *et al*. (2006a,b) showed that leachate increased the level of TBARS (endogenous lipid peroxidation) and induced changes in the activities of Cu, Zn-SOD, Se-dependent GPx and CAT in hearts, kidneys, livers, brains and spleens of leachate treated mice. Li *et al*. (2010) reported that landfill leachates induced protein oxidation (as assessed by protein carbonyl content) and DNA-protein crosslinks (DPC) in viscera of treated mice. Their findings led to the conclusion

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that landfill leachates, a mixture of organics, inorganics, heavy metals and some unidentified xenobiotics (Pivato and Gaspari, 2006; Schrab *et al*., 1993; Yasuhara *et al*., 1997), induced toxic effects and DNA damage by autoxidation or by enzyme catalyzed oxidation through free radical formation.

The findings from the systemic toxicity and heavy metal induced cytogenetic effects using CBMN-Cytome in WIL2-NS cells observed in this study supported the free radical mechanisms. Two possible processes may be involved during the free radical oxidative mechanism:

1. It is well known that metals; Cu, Fe, Cd, Pb, Ni, Cr, Mn and As exhibit their toxic effects in tissues and organs through reactive oxygen species (ROS) formation (Stohs and Bagchi, 1995; Romeo *et al*., 2000; Jadhav *et al*., 2007). Weinberger *et al*. (2002) reported that increase in urinary levels of thiobarbituric-acid-reacting substances (TBARS), which are proportional to lipid peroxidation and oxidation stress, correlated with elevated serum transaminases levels in preterm infants. Sanchez-Chardi *et al*. (2007) reported that the bioaccumulation of Pb, Hg, Cd, Fe, Mg, Zn, Cu, Mn, Mo and Cr in the tissues of wood mice (*Apodemus sylvaticus*) inhabiting Garraf landfill, correlated with elevation of serum aminotransferases in these mice. While Li *et al*. (2006a,b) reported that mice exposed to different concentrations of Xingou landfill leachate showed significant increase in thiobarbituric acid reactive substances (TBARS) level of the organs. It has long been known that marked release of AST and ALT into the circulation is an indication of severe damage to tissue membranes (majorly hepatic tissues) (Molander *et al*., 1955; Zimmerman, 1999).

Considering the preceding information, it can be deduced that increase in serum transaminases along with histopathological lesions on the tissues (mostly on liver hepatocytes) and damage to red blood cell membrane observed in this study suggests that leachate constituents (mostly the heavy metals) provoked loss of functional integrity of the cellular membranes of the leachate exposed rats through free radical formation. This deduction is well supported by *in vitro* study of Talorete *et al*. (2008), wherein they exposed human breast cancer MCF-7 cells to different concentrations of leachates from two landfills in Tunisia using modified E-screen assay and they concluded that leachate induced cytotoxicity was through damage to cell membranes.

2. The histopathological findings in virtually all the examined viscera showed that there was increase in the number of apoptotic lymphocytes and macrophages (Kupffer cells), cellular infiltrations (by neutrophils), congestions, degenerations and necrosis in the thymus, liver, kidney and spleen of leachate treated rats compared to the negative control. The inflammatory

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cells observed in landfill leachate treated rats were in response to tissue damage and their presence indicate the generation of reactive oxygen species by endogenous means.

The propensity for cells to undergo necrosis and or apoptosis (programmed cell death) depends on the intracellular oxidant status, the level of ATP in the cell and the extent of induced membrane damage and this may determine the extent of observed DNA damage in the cell (Fenech *et al*., 1999). WIL2-NS cells exposed to heavy metals in this studies induced genotoxic and cytotoxic effects and it was suggested that the cytotoxic effects (increase percentage of necrosis) caused the observed genotoxic effect. This claim may be supported by the histological observations of inflammatory cells (macrophages, neutrophils and lymphocytes) production in leachate treated rats. These cells were elicited in response to the presence of invading microorganisms (mostly feacal coliforms) (Efuntoye *et al*., 2011; Oshode *et al*., 2008), heavy metals and other unanalysable chemicals present in Olusosun and Aba Eku landfill leachates.

Inflammatory cells function by destroying and removing produced apoptotic and necrotic cells (cellular debris) through phagocytosis (Jaeschke, 2000; Ramaiah and Jaeschke, 2007). These phagocytic cells do not attack healthy cells but respond to distressed or dying cells (Jaeschke, 2006). These may be supported by the report that apoptotic and necrotic cells trigger neutrophilic inflammatory process (Faouzi *et al*., 2001; Lawson *et al*., 1998). During phagocytosis, mediators of neutrophilic inflammation generated by Kupffer cells including tumour necrosis factor α (TNF- α), interleukins (IL-1 and IL-6), chemokines and reactive oxygen species (ROS) (Sweet and Hume, 1996) activate neutrophil induced tissue damage. The presence of neutrophil in cells triggers a long-lasting oxidative stress through Nicotinamide adenosine dinucleotide hydrogen (NADH) oxidase. NADH oxidase generates superoxides from oxygen and hydrogen peroxides (ROS), which is highly diffusible (Gujral *et al*., 2004; Jaeschke, 2003).

These intracellular reactive oxygen species formed through autoxidation (endogenous source) (Adamson and Billings 1992; Jaeschke 2000, 2003; Gujral *et al*., 2004), have been associated with genotoxic damage (Chellman *et al*., 1986) and cytotoxicity (Schacter *et al*., 1988). Also, ROS, whether exogenously or endogenously generated have been associated with lipid peroxidation, protein and DNA damage, aging and carcinogenicity (Frenkel *et al*., 1986; Nag, 2009). Decrease in serum albumin and total proteins observed in this study may be due to free radical formation which caused protein damage or protein degradation through oxidative stress induction in rats (Frenkel *et al*., 1986; Li *et al*., 2010; Nag, 2009). If the affected cellular proteins are those responsible for ion balance, it can lead to disruption of actin filament assembly and ATP production (Cullen, 2005; Watanabe and Phillips, 1986).

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ROS can cause damage to any base or sugar moiety in DNA leading to strand breaks. It is plausible that leachate constituents generated ROS through multiple exogenous and endogenous sources or acted directly in fish, bird and mammals to cause alterations in the DNA structures leading to the observed MN formation in these organisms. This may also account for clinical signs, body and organ weight changes, haematological and histopathological alterations observed in rat exposed to the Olusosun and Aba Eku landfill leachates.

5.5 Health impacts associated with human exposure to landfill emissions among residents near Olusosun and Aba Eku landfills

Association of ill health with landfill emissions is an issue of concern to those living within and closer to municipal landfills. The findings herein showed that there was an association between emissions from Olusosun and Aba Eku landfill sites and health status of population residents within and nearer to the landfills than those farther away.

The finding that the monthly income of most exposed population was less than twenty thousand naira (#20,000) per month is an indication that they were economically disadvantaged. The high standards of living in the States may have informed their choices of living closer to landfills. For instance in Lagos State, the economy is more expensive, that majority of the economically disadvantage populations cannot afford accommodation rent, hence it is a common observation to build temporary structures on and within landfills and other available spaces like under pedestrian bridge. This assertion is supported by the finding that all residential property values decreased with closeness to landfill sites in Lagos state (Akinjare *et al*., 2011). Likewise in Oyo State, the drive to become 'landlords' and to avoid embarrassments from surveying agents due to escalation of house rents made many economically disadvantage population to acquire parcel of landed properties around landfills since they are cheaper to purchase and developed into residential structures. This population also constituted the scavengers, who sort and pick recyclable materials from the landfills for sales to cope with the increasing standards of living of the country and their survival.

According to the findings herein, the potential pathways of exposure among the exposed population in the Olusosun study sites are air transport of contaminants, ingestion of water and food (probably contaminated with landfill chemicals, microbes and other particulate matters), noise from heavy duty machines, dermal contact and transmissions of pathogens through vermin (vectors of diseases), while at Aba Eku landfill; air transport of contaminants, ingestion of water and food products (likely contaminated with landfill contaminants) and transmissions of

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pathogens through vermin (vectors of diseases) were possibilities. Although in this study, environmental media (air, water and soil) from the study sites were not examined for contaminants likely to predispose residents to ill health, available information from previous studies on the sites and other landfills, had reported higher concentrations of contaminants in these media. For instance, water samples obtained from 20 randomly selected wells and boreholes used as sources of water supply by the exposed population and leachate samples collected from 10 different locations on Olusosun landfill sites contained high cobalt (Co), cadmium (Cd), lead (Pb) and copper (Cu) (heavy metals) than permissible limits in Nigeria and United States (Oyeku and Eludoyin, 2010). Longe and Enekwechi (2006) and Ogundiran and Afolabi (2008) had earlier reported higher concentrations of heavy metals (Cr, Cd and Cu) and physicochemical characteristics from surface an underground water sources and leachates from Olusosun landfill. Likewise, Nubi *et al*. (2008) and Nubi and Ajuonu (2011) reported higher physicochemical parameters and heavy metal concentrations from Omi river and source of water supply within 2 km radius of Aba Eku landfill than permissible limits in Nigeria. Higher values for physicochemical parameters and heavy metals were found in soil from dumpsites in Port Harcourt municipality and the environments (Ogbonna *et al*., 2009). A common feature around landfills is blockage of drainages and canals by wastes, this leads to flooding. The flood water contaminates wells, other sources of drinking water and can disperse heavy metals on soils.

Information is relatively scarce on the evaluation of organic pollutant contents of leachates from Olusosun and Aba Eku landfill, but organics like phenanthrene, fluoranthrene and pyrene had been reported in soil elutriates from Aba Eku landfill site (Bakare *et al*., 2009). Also, over 40 organic compounds have been identified in leachate contaminated waters (Vasanthi *et al*., 2008) and 200 organic identified in landfill leachates (Paxeus, 2000). Radioactive substances were also detected in wastes around Ibadan (Odunaike *et al*., 2009), uranium was detected in landfill leachates in Norway (Oygard and Gjengedal 2009), microbes capable of transmitting infections of different types have been isolated and classified in Aba Eku landfill leachates (Oshode *et al*., 2008; Efuntoye *et al*., 2011), while water supply for domestic and commercial purpose around Ojota (Olusosun landfill site) had been reported to contain higher loads of microorganisms (Okonko *et al*., 2008).

Airborne emissions from the landfills, via odour, smoke, fire and dust, are a well established source of exposure pathway. Organic and inorganic compounds, microorganisms and particulates matters have been isolated from indoor air within 2 km radius of residential quarters closer to landfills in previous studies (Schrapp and Al-Mutairi, 2010; USEPA, 2008). These

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inorganic and organic compounds may be responsible for the strong rotten and pungent odours which saturated the air as complained by the exposed populations during this study. The odour emanating from the landfills in particular was very worrisome that many passers-bye complain bitterly calling for the intervention by government during the time of this study (2007–2009). The effect of the smell obviously constitutes serious health hazard to persons living near the dump site. Based on personal interview, some of the people living around Olusosun saw nothing wrong in the smell that oozes from the landfill. They even described it in clear terms as an outstanding feature that has consistently defined the area. A particular respondent who lived with his family in Oregun area said "If the smell disappears, then something must be really wrong. Most of us have lived in this area for such a long time that our noses no longer perceive any smell". Exposure to odours and loss of smell as described by many of the exposed population describes human health condition known as anosmia. It has been reported that exposure to odours from landfills caused loss of smell in humans may result in exposure to lethal quantities of chemicals in the odour. This condition results basically from exposure to sulphides and has been proven to cause anxiety, depression and other negative psychological reactions (Lee and Jones-Lee, 1991; 2003).

The study sites contain organic matter which can be biodegraded by living organisms. This makes the landfills suitable as breeding and germinating sites for spores, bacteria, viruses, houseflies, mosquitoes (insects) and rodents. Hence, populations living around landfills are exposed to all manners of sicknesses, including encephalitis (inflammation of the brain), dengue fever, gastrointestinal, dermal, parasitic diseases, plague as well as Hantaviruses which can easily be transmitted to humans by these organisms (Tauhid-Ur-Rahman, 2006). In Aba Eku study site, residents (exposed) cultivated crop plants on and around the landfill using decomposed soil from the sites and river Omi to irrigate the crops. Also their domesticated animals mainly goats and birds were always foraging on the dumpsites in search of food materials from the wastes, and leachate from the dumpsite seems to be the only available drinking water for these animals. These observations may cause poor yield in the crop plants and death and loss of economic plants and the domesticated animals. These corroborated the reports of Bakare and Wale-Adeyemo (2004). It is also possible that these plants and animals can bioaccumulate heavy metals and organic chemicals from the landfill soil and leachate contaminated Omi River over a period of time. The bioaccumulated metals and organic compounds may biomagnify to lethal concentrations in humans along the food chain when these animals and plants are consumed. This assumption is supported by the findings of Sanchez-

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Chardi and Nadal (2007) and Sanchez-Chardi *et al*. (2007) that heavy metals were bioaccumulated in rodents in habiting landfills in Barcelona, Spain. It was shown that vegetables grown on three urban waste dumpsites in Kumasi, Ghana, bioaccumulated heavy metals from the dumpsite soils with Cd and Pb levels higher than WHO/FAO permissible limits (Odai *et al*., 2008). Also, high quantity of PCB was obtained in the blood of Swedish children who consumed fish from leachate polluted river (Cuadra *et al*., 2006).

There were associations between residential proximity to Olusosun and Aba Eku landfills and adverse respiratory symptoms. The incidence of asthma, bronchitis, chest pain, lung diseases, coughing, nose/throat problems, breathing problems and tuberculosis among exposed populations around the two landfills increased significantly. The excess risk in respiratory problems could be associated with exposure to landfill chemicals present in the smoke, fire, odour and particulate matters in air that was often breathe in during gaseous exchange by the residents. The constituents of air around landfills like molds, dust and metal fumes can act as allergens for respiratory problems like asthma, coughing, nose/throat problems and breathing problems while microorganisms, organic and inorganic chemicals in the air and particulate matters can increase in the incidence of lung disease, breathing problems bronchitis and tuberculosis. During the course of this study, one of the respondents noted that many people have contracted different respiratory infections because of the fumes that pervade the atmosphere. She informed in a statement "some of my neighbours were forced to move out of here because of constant sickness. One particular man lost his three children to asthma attacks and had to pack out when his wife became pregnant." Besides, studies have shown that landfill air (gases, smoke, open fire and odour) contains deleterious chemicals and particulate matters which can cause the observed health effects directly or by compromising immune system. Pukkala and Ponka (2001) reported increase in the incidence of asthma among cohorts who built their residential structures on closed dumpsites in Helsinki, Finland. Different respiratory problems were observed in children who lie in close proximity to clinical wastes in Cameroon (Mochungong *et al*., 2011). Elevated asthma in young children was associated with occupational exposure to volatile organic compounds (Eggleston *et al*., 2005). Kudyakov *et al*. (2004) examined the rates of hospitalization for respiratory diseases in relation to residences in zip codes with hazardous wastes (persistent organic pollutions, POPs) sites in the New Yolk City. Elevated chronic bronchitis and chronic airway obstruction were reported among population living near POPs-contaminated site compared to the control. Increase in serum concentrations of organochlorine correlated with significant increase in asthma, atopic disorders and infections

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among children living in close proximity to a landfill. It was suggested that these chemicals cause immunosuppression in the exposed children which affected their health (Bunger *et al*., 2000).

Increased dermal infections and gastrointestinal problems were also associated with living close to Olusosun and Aba Eku landfills. Significant increase in the incidence of eczema, skin rashes, skin itching, boils and warts, nausea and diarrhea with high odd ratios in exposed than unexposed population may be associated with inorganic and organic chemicals and microorganisms present in the air, water and soil, the likely sources of exposure from the studied landfills. Insect and rodent proliferation and prevalence in and around the landfills may have contributed to increase in the incidence of dermal and gastrointestinal problems observed among the exposed populations. Oshode *et al*. (2008) and Efuntoye *et al*. (2011) had previously isolated faecal coliforms, implicated in gastroenteritis, from Aba Eku landfill leachate, while Okonko *et al*. (2008) isolated Enterobacter from water samples used for domestic purposes from Ojota area, the location of Olusosun landfill. These enterotoxin producing bacteria, along with heavy metals and organic components present in landfills may have caused irritation of the linings of the intestinal tracts in exposed populations leading to increase in the prevalence of diarrhea and nausea (gastrointestinal disorders) among exposed than unexposed populations. These same microorganisms and chemicals can cause alterations in the immune systems of exposed populations. This assumption along with the activities of mosquitoes, houseflies) and rodents can be implicated with increase in dermal and gastrointestinal disorders observed among exposed than unexposed populations. Studies have similarly reported elevated gastrointestinal disorders among Danish waste collectors compared to other Danish work force (Michelozzi *et al*., 1998) and dermal and gastrointestinal disorders among the residence closer to Al-Shuyoukh landfill in Kuwait (Schrapp and Al-Mutairi, 2010). The authors concluded that the populations were exposed to endotoxins, fungi and organic chemicals present in the air.

Significant elevation of dizziness, depression, anxiety, anger, headache, body pain, sleep disturbance and hearing problems (classified as neuromuscular and psychological disorders) were also observed among exposed population to Aba Eku and Olusosun landfill emissions than unexposed. Also, there was higher prevalence of stroke, diabetes, cancer, stillbirth, low birth weight and birth defects (reproductive and other disorders) among the exposed population compared to unexposed, though these symptoms were not significantly observed. The observed psychological disorders can be associated with exposure to noise from heavy machines that bring wastes to the sites, smoke produced during burning of wastes, irritating odour and chemicals

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emanating from the landfills. These reproductive, neuromuscular, psychological and other observed disorders among exposed populations nearer Olusosun and Aba Eku landfills had been previously reported among exposed populations to the emissions from landfills Jeleeb Al-Shuyoukh landfill in Kuwait. The prevalence of these disorders was associated with high airborne dusts around the residential houses containing many genera and species of molds and bacteria along with high concentrations of about 112 volatile organic compounds including acetone, 1,1-Dichloroethylene, ethylbenzene, methylene chloride, styrene, toluene, chlorethane, methane, pinene, nonanal and benzaldehyde (Schrapp and Al-Mutairi, 2010).

Studies have shown that adverse birth defects (congenital abnormalities), low birth weight, preterm birth and intrauterine growth retardation were associated with maternal residence's closeness to landfills (Berry and Bove, 1997; Dolk *et al*., 1998; Eggleston *et al*., 2005; Elliott *et al*., 2001; Fielder *et al*., 2000; Geschwind *et al*., 1992). Also reported were increase incidences of liver dysfunctions, neurotoxic effects, diabetes, immune system disorder, stroke, endocrine diseases, respiratory infections, gastrointestinal and dermal disorders, cancer formation and chromosomal aberrations among residence exposed to landfill emissions (Dalton, 2003; Kouznetsova *et al*., 2007; Kudyakov *et al*., 2004; Ma *et al*., 2007; Meyer, 1983; Palmer *et al*., 2005). These studies considered residence within 2 km radius of the landfills as surrogate for exposure, while relatively few among these studies determined the possible environmental contaminants likely to be responsible for health effects around these landfills (Choi *et al*., 2006; Schrapp and Al-Mutairi, 2010). The study herein showed that residential proximity to Olusosun and Aba Eku landfills is associated with different health impacts in residents within 2 km radius of the landfills.

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5.6 CONCLUSION AND RECOMMENDATIONS

This study showed that solid wastes disposal into Olusosun and Aba Eku landfills emit leachates and gases into the environment. The physical and chemical analysis of the leachates from these landfills contained higher concentrations of inorganic compounds, particularly; heavy metals, COD, BOD, ammonia and sulphates, than standard permissible limits. These constituents

are capable of polluting surface and underground water, soil and air and can induce deleterious effects and or endanger life.

Cytogenotoxicity evaluations of the leachates showed induction of DNA damage in *Clarias gariepinus* (mudfish), *Coturnix japonica* (Japanese quail) and *Mus musculus* (albino mice) through increasing the frequencies of micronucleus formation and nuclear abnormalities in these organisms. They also caused decrease in polychromatic and normochromatic erythrocyte ratio in bone marrow cells of *M. musculus*. The observed cytogenotoxicity suggest leachates-induced genetic instability in vertebrates. Genetic instability has been associated with decrease cell survival or transformation and cancer formation. These leachates also exhibited different signs of systemic toxicity in Wistar albino rats (*Rattus novergicus*). These changes include; clinical signs of toxicity, change in body and organ weight gain, alterations in some serum biochemical parameters, alterations in haematological indices and erythrocyte morphology and histopathological lesions.

Cytogenotoxic induction by Cu, Mn, Pb, Cr and their mixture in cytokinesis-block WIL2-NS cells in this study, along with the information from the systemic toxicity of Olusosun and Aba Eku landfill leachates, showed that loss of functional integrity of the cellular membranes and the induction of apoptosis and necrosis in biologic systems which triggers neutrophilic inflammatory processes may be involved in the mechanisms of landfill leachates-induced cytogenotoxicity through autoxidation and free radical formation.

Living in close proximity (within 2 km) to these unsanitary waste disposal facilities increased the expressions of respiratory, gastrointestinal, dermal, reproductive, psychological, neuromuscular disorders and other health impacts among human populations. This indicates that Olusosun and Aba Eku unsanitary landfills'emissions contained harmful xenobiotics capable of impacting on public health and contaminating the environmental media. These findings may suggest health risk to living things including human population exposed to chemical substances from Olusosun and Aba Eku solid waste landfills.

Lack or inadequate environmental regulation and enforcement of rules guiding solid waste disposal in Ibadan and Lagos gave rise to open dumping and unsanitary landfilling as the predominate solid waste management methods. These methods are associated with open burning of wastes. The preceeding factors along with high poverty rate among most inhabitants in Lagos and Oyo states and ignorance about the role of unsanitary landfill management in the causation of environmental pollution and ill health, increased the risk of exposure to landfill

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chemicals. Moreso, asides scavengers, many people who are not solid waste personnel live and work in the immediate proximity to these open dumps and landfills. Therefore, adequate measures need be taken to minimise, if not totally stoped, environmental pollution and health effects from landfill management.

Specific recommendations arising from the study are:

- Airborne contamination from particulates matter, bioaerosols and gases from biodegradation of organic wastes, is one among the greatest threats to solid waste workers, waste pickers and residents in close proximity to landfills. There should be regular air monitoring, particularly during loading and off-loading, transfer and disposal of wastes in the landfills. This should be done by direct-reading instruments that measure methane and oxygen deficiency; these should include combustible gas indicators, flame ionization detectors, and oxygen meters.
- Researchers, medical practitioneers and occupational health institutions should be involved in studying the health status of solid waste workers and residents around landfill facilities and compared with appropriate baseline control populations, so as to establish epidemiological data.
- Scavengers who make their livelihood from collecting, sorting, or otherwise handling of wastes, can reduce diseases and injury risk by wearing protective clothing, goggles, shoes/boots, gloves and respiratory equipment that provides proper air filtration and ventilation. Also, solid waste workers and residents around landfills should be vaccinated for hepatitis A and B, tetanus, diphtheria, polio, typhoid, encephalitis and rabies.
- Medical surveillance standards and protocols, including baseline and follow-up medical examinations should be routinely conducted for workers and probably exposed populations to landifill chemicals.
- Policy makers and environmental managers should promulgate rules against illegal waste disposal and training on effective health education on the hazardousness of emissions from landfills.
- Regulatory authorities in Nigeria should improve economic developments to enable the construction of engineered (sanitary) landfill for the disposal of wastes.
- It is well known that chemical induced immunosuppressions and immunostimualtions account for the health impacts observed during exposure. It is therefore recommended that residential location around the studied landfills and immune markers like

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immunoglobulin levels, mitogen stimulation assay, helper: suppressor (CD4:CD8) ratio, percentage of T cells (CD3) and B cells (CD19) be investigated to further understand landfill toxicity among Nigerian.

- Cytogenoxicity has been associated with increase cancer formation and congenital and other reproductive abnormalities. It is suggested that cytogenetic examination of residents in close proximity to these landfills be evaluated to ascertain the level of exposure.
- Research should be conducted to determine the level of serum bioaccumulation of heavy metals and some organic compounds like Polychlorinated Biphynyls and biochemical enzymes like antioxidising enzymes and endocrine disruptors.

UNIVERSITY OF IBADAN

REFERENCES

Abdou, M.H. 2007. Health impacts on workers in landfill in Jeddah City, Saudi Arabia. *Journal of Egyptian Public Health Association*, 82.3-4: 319-329.

Adamson, G.M. and Billings, R.E. 1992. Tumor necrosis factor induced oxidative strss in isolated mouse hepatocytes. *Archive of Biochemistry and Biophysics*, 294: 223-229.

Aderibigbe, Y. 2010. Ugly side of the Olushosun scavengers. *The Nation* http://www.thenationonlineng.net/web2/articles/49807/1/Ugly-side-of–the-olusosun-scavengers/page1.home.

Adeyeye, J. 2006. 52 percent of Nigerians drink dirty water. *The Punch* 17(19701) (www.punchontheweb.com).

Agency for Toxic Substances and Disease Registry (ATSDR). 2001. *Landfill Gas Primer: An overview for Environmental Health Professionals.* Washington: Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdcgov/HAC/landfill/html/toc.htm/.

Akinjare, O.A., Ayedun, C.A., Oluwatobi, A.O. and Iroham, O.C. 2011. Impact of Sanitary Landfills on Urban Residential Property Value in Lagos State, Nigeria. *Journal of Sustainable Development*, 4.2: 48-60.

Akinloye, M.K. and Olomo, J.B. 2005. The radioactivity in some grasses in the environment of nuclear research facilities located within the OAU, Ile-Ife, Nigeria. *Nigeria Journal of Physics*, 17S; 219-225.

Alabi, A.O. and Bakare, A.A. 2011. Genotoxicity and mutagenicity of electronic waste leachates using animal bioassays. *Toxicology and Environmental Chemistry*, 93.5: 1073–1088.

Albert, R.E. 1987. Health risks of concern. In: Andelman JB; Underhill DW. Health effects from hazardous waste sites. Chelsea, Michigan, USA: Lewis Publishers. Pp. 143-147.

Ali, F., El-Shehawi, A.M. and Seehy, M.A. 2008. Micronucleus test in fish genome: A sensitive monitor for aquatic pollution. *African Journal of Biotechnology*, 7: 606 – 612.

Alimba , C.G., Bakare, A.A. and Latunji, C.A. 2006. Municipal landfill leachates induced chromosome aberration in rat bone marrow cells. *African Journal of Biotechnology* 5.22: 2053-2057.

Alkassasbeh, J.Y.M., Heng, L.Y. and Surif, S. 2009. Toxicity testing and the effects of landfill leachate in Malaysia on behaviour of common Carp (Cyprinus carpio L. 1758; Pisces, Cyprinidae). *American Journal of Environmental Sciences*, 5.3: 209 – 217.

Al-Sabti, K. and Hardig, J. 1990. Micronucleus test in fish for monitoring the genotoxic effects of the industrial waste products in Baltic Sea, Sweden. *Comparative Biochemistry and Physiology*, 97C: 179-182.

Al-Sabti, K. and Metcalfe, C.D. 1995. Fish micronuclei for assessing genotoxicity in water. *Mutation Research*, 343:121-135.

UNIVERSITY OF IBADAN

Amacher, D.E., Schomaker, S.J., Boldt, S.E. and Mirsky, M. 2006. The relationship among microsomal enzyme induction, liver weight and histological change in Cynomolgus monkey toxicology studies. *Food and Chemical Toxicology*, 44.4: 528 – 537.

Amahdar, L., Anouar, A., Ababou, B., Verschaeve, L. and Hilali, A. 2009. *In vitro* genotoxicity of Settat town landfill leachate, Morocco. *Arh Hig Rada Tokiskologi*, 60: 179–184.

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. Washington, DC: American Public Health Association (APHA, AWWA, WPCF).

American Public Health Association, (APHA). 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association (APHA, AWWA, WPCF), Washington DC.

American Fisheries Society (AFS), (2004). Guidelines for the use of fishes in research. Copyright by the American Fisheries Society, Bethesda, MD.Pp 1 – 57. Available on the internet: www.fisheries.org/afs/publicpolicy/guidelines2004.pdf.

American Society for Testing and Materials. 1992. Standard Test Method for Shake Extraction of Solid Waste with Water, ASTM Designation: D3987-85. West Conshohocken, PA: ASTM. pp 4.

Arrouijal, F.Z., Hildebrand, G., Vophi, H. and Marzin, D. 1990. Genotoxic activity of nickel subsulphide α-Ni3S2. *Mutagenesis*, 5:583-589.

Ashby, J.A., Tinwell, H. Lefevre, P.A., Browne, M.A. 1995. The single cell gel electrophoresis assay for induced DNA damage (comet assay): Measurement of tail length and movement. *Mutagenesis*, 10:85-90.

Aspinwall. 2001. Environmental monitoring at Nant-y-Gwyddon landfill site, Cardiff: Environ Aspinwall Report E5169/June/1994/L8:26.

Atwater, J.W., Jasper, S., Mavinic, D.S. and Koch, F.A. 1983. Experiments using Daphnia to measure landfill leachate toxicity. *Water Research*, 17.12: 1855- 1861.

Ayininuola, G.M. and Muibi, M.A. 2008. An engineering approach to solid waste collection system: Ibadan North as case study. *Waste Management*, 28:1681-1687.

Aziz, H. A., Alias, S., Adlan, M. N., Faridah, Asaari, A. H. and Zahari, M. S. 2007. Colour removal from landfill leachate by coagulation and flocculation processes. *Bioresource Technology*, 98.1: 218-220.

Bacud, L., Sioco, F., and Majam, J. 1994. A descriptive study of the water quality of drinking wells around the Payatas dumpsite, Unpublished B.Sc. Thesis, University of the Philippines, College of Public Health.

Bakare, A.A. 2001. The potential mutagenic effect of the leachates from rural solid waste landfill on *Allium cepa* (L). SINET: *Ethiopia Journal of Science*, 24.2:283–291.

UNIVERSITY OF IBADAN

Bakare, A.A., Mosuro, A.A. and Osibanjo, O. 1999. Cytotoxic effects of landfill leachate on *Allium cepa* (L.). *Bioscience Research Communication*, 11.1:1–13.

Bakare, A.A., Mosuro, A.A., Osibanjo, O. 2000a. Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* (L.). *Journal of Environmental Biology*, 21.3:263–271.

Bakare, A.A., Mosuro, A.A., Osibanjo, O. and Sobowale, M.A. 2000b. Comparative toxicity of raw and simulated leachate using root elongation in *Allium cepa* (L.). *Journal of Applied Science and Environmental Management*, 4:71-81.

Bakare, A.A., Mosuro, A.A. and Osibanjo, O. 2003. Landfill leachate induced toxicity in mice. *Journal of Environmental Biology*, 24.4:429-435.

Bakare, A.A. and Wale-Adeyemo, A.R. 2004. The mutagenic and cytotoxic effects of leachates from domestic solid wastes and Aba-Eku landfill, Nigeria on *Alliums cepa. Nature Environment and Pollution Technology* 3.4:455-462.

Bakare, A.A., Mosuro, A.A. and Osibanjo, O. 2005. An *in vivo* evaluation of induction of abnormal sperm morphology in mice by landfill leachates. *Mutation Research* 582:23-34.

Bakare, A.A., Pandey, A.K., Bajpayee, M., Bhargav, D., Chowdhuri, D.K., Singh, K.P., Murthy, R.C., and Dhawan, A. 2007. DNA damage induced in human peripheral blood lymphocytes by industrial solid waste and municipal sludge leachate. *Environmental and Molecular Mutagenesis*, 48: 30-37.

Bakare, A.A. 2009. Wastes: Toxicity and potential health hazards of leachate, In: W*aste management: Research, Technology and Developments*. Ed. James R. Lavelle. NOVA Science publishers, Inc. USA (ISBN: 978-1-60456-256-9):1- 40.

Bakare, A.A., Patel, S., Pandey, A.K., Bajpayee, M. and Dhawan, A. 2012. DNA and oxidative damage induced in somatic organs and tissues of mouse by municipal sludge leachate. *Toxicology and Industrial Health*, 28.7:614 – 623.

Barger, A.M. 2003. The complete blood cell count: a powerful diagnostic tool. *Veterinary Clinical: Small Animal Practice*, 33.6: 1207-1222.

Barlaz, M. A., Ham, R. K. and Schaefer, D. M. 1992. Microbial, chemical and methane production characteristics of anaerobically decomposed refuse with and without leachate recycling. *Waste Management and Research*, 10.3:257-267.

Barsiene, J., Dedonyte, V., Rybakovas, A., Andreikenaite, L. and Andersen, O.K. 2006. Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil. *Aquatic Toxicology*, 78; 99–104.

Bassey, B.E., Benka-Coker, M.O. and Aluyi, H.S.A. 2006. Characterization and management of solid medical wastes in the federal capital territory, Abuja Nigeria. *African Health Sciences*, 6.1: 58-63.

UNIVERSITY OF IBADAN

Benlahcen, K., Sansar, W., Belhabri, L. and Slimani, M. 2009. Lead in water: neurotoxicity and stressful effects on wistar rat. *Global Journal of Environmental Research*, 3.1: 52-60.

Benoit, R., Breville, D., Rambourdin, F., Bayle, G., Capitan, P. and Obled, C. 2000. Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. *American Journal of Physiology and Endocrinology Metabolism*, 279.2: 244–255

Bernard, C., Guido, P., Colin, J. and Du-Delepierre-A-le. 1996. Estimation of hazard of landfills through toxicity testing of leachates. I. Determination of leachate toxicity with a battery of acute tests. *Chemosphere*, 33.11: 2303-2320.

Bernier, J., Brousseau, P., Krzystyniak, K., Tryphonas, H. and Fournier, M. 1995. Immunotoxicity of heavy metals in relation to Great lakes. *Environmental Health Perspective*, 103 (*suppl* 9):23-34.

Berry, M. and Bove, F. 1997. Birth weight reduction associated with residence near a hazardous waste landfill. *Environmental Health Perspectives*,105:856–61.

Bhunya, S.P. and Jena, G.B. 1992. Genotoxic potential of the organochlorine insecticide lindane (γ- BHC): an *in vivo* study in chicks. *Mutation Research*, 272: 175-181.

Bhunya, S.P. and Jena, G.B. 1993. Studies on the genotoxicity of monocrotophos, an organophosphosphate insecticide, in the chick *in vivo* test system. *Mutation Research*, 292:231-239.

Biedermann, K.A. and Landolph, J.R. 1990. Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis and anchorage independence in diploid human fibroblasts by carcinogenic metal salts. *Cancer Research*, 50:7835–7842.

Blankers, L.C. 1996. Illegal transports of waste: Tricks of the trade. Fourth international conference on environmental compliance and enforcement. Vol. 2. Pp 1-7 www.inece.org/4thvol2/blankers.pdf.

Blasiak, J. and Kowalik, J. 2000. A comparison of the *in vitro* genotoxicity of tri- and hexavalent chromium. *Mutation Research*, 469:135-145.

Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. *Journal of Fish Biology*, 4:593–605.

Bloom, J.C. 1993. Principle of haematotoxicology: laboratory assessment and interpretation of data. *Toxicological pathology.* 21:130 – 134.

Bogdanova, A.Y., Gassmann, M. and Nikinmaa, M. 2002. Copper ion redox state is critical for its effects on ion transport pathways and methaemoglobin formation in trout erythrocytes. *Chemical and Biological Interactions*, 139:43-59.

Boller, K. and Schmid, W. 1970. Chemical mutagenesis in mammals. The Chinese hamster bone marrow as an *in vivo* test system. Hematological findings after treatment with trenimon. *Humangenetik*, 11.1: 35–54.

UNIVERSITY OF IBADAN

Bonacker, D. Stoiber, T., Bohm, K.J., Prots, I., Wang, M, Unger, E., Their, R., Bolt, H.M. and Degen, G.H. 2005. Genotoxicity of inorganic lead salts and disturbance of microtubule function. *Environmental and Molecular Mutagenesis*, 45:346-353.

Bortolotto, T., Bertoldo, J.B., da Silveira, F.Z., Defaveri, T.M., Silvano, J. and Pich, C.T. 2009. Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays. *Environmental Toxicology and Pharmacology*, 28: 288 – 293.

Boyd, J.W. 1983. The mechanisms relating to increase in plasma enzymes and isoenzymes in diseases of animals. *Veterinary and Clinical Pathology*, 12: 9–24.

Bremner, I. 1998. Manifestations of copper excess. *American Journal of Clinical Nutrition*, 67(suppl): 1069S-1073S.

Brenneke, H.P.U.H. 1937. Die Ursache des herabgesetzten Wurfgröße bei Mäusen nach Röntgenbestrahlung des Samens, Z.A.V. In: Kirsch-Volders, M., Sofuni, T., Aardemac, M., Silvio Albertini, S., Eastmond, D., Fenech, M., Motoi Ishidate, M. Jr., Kirchner, S., Lorge, E., Morita, T., Norppa, H., Surrallés, J., Vanhauwaert, A. and Wakata, A. 2003. Report from the in vitro micronucleus assay working group, *Mutation Research*, 540:153–163.

Bridges, O., Bridges, J., Potter, J. 2000. A generic comparison of the airborne risks to human health from landfill and incinerator disposal of municipal solid waste. *Environmentalist*, 20.4: 325-334.

Brown, G., 1992. Haematology tests in toxicology: Time for a re-think? *Comparative Haematology International*. 2, 231 – 235.

Brzoska, M.M., Moniuszko-Jakoniuk, J., Pilat-Marcinkiewicz, B. and Sawicki, B. 2003. Liver and kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol and Alcoholism*, 38, 2–10.

Buczynska, A., Cyprowski, M., Szadkowska-Stanczyk, I. 2011. Indoor disposal of household waste as a source of environmental biohazard exposure. *Polish Journal of Environmental Studies*, 20.4: 851–856.

Bunger, J., Antlauf-Lammers, M., Schulz, T.G., Westphal, G.A., Müller, M.M., Ruhnau, P., Hallier, E. 2000. Health complaints and immunological markers of exposure to bioaerosols among biowaste collectors and compost workers. *Occupational and Environmental Medicine*, 57:458–464.

Buttke, T.M. and Sandstrom, P.A. 1994. Oxidative stress as a mediator of apoptosis. *Immunology Today*, 15: 7-10.

Cabrera, G.L. and Rodriguez, D.M.G. 1999. Genotoxicity of leachates from a landfill using three bioassays. *Mutation Research*, 426:207–210.

Cameron, R.D. and Koch, F.A. 1980. Toxicity of landfill leachate. *Journal of Water Pollution and Control Federation*, 52.4: 761-769.

UNIVERSITY OF IBADAN

Carrasco, K.R., Tilbury, K.L. and Myers, M.S. 1990. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. *Canadian Journal of Fishery and Aquatic Science*, 47: 2123-2136.

Cavas, T., and Ergene-Gozukara, S. 2003. Evaluation of the genotoxic potential of lambda – cyhalothrin using nuclear and nucleolar biomarkers on fish cells. *Mutation Research*, 534: 93–99.

Cavas, T. and Ergene-Gozukara, S. 2005. Micronucleus test in fish cells: A bioassay for *in situ* monitoring of genotoxic pollution in the marine environment. *Environmental and Molecular Mutagenesis*, 46:64-70.

Chandra, S., Chauhan, L.K.S., Pande, P.N. and Gupta, S.K. 2004. Cytogenetic effects of leachates from tannery solid waste on the somatic cells of *Vicia faba*. *Environmental Toxicology*, 19:129–133.

Chandra, S., Chauhan, L.K.S., Murthy, R.C., Saxena, P.N., Pande, P.N. and Gupta, S.K. 2005. Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Science of the Total Environment*, 347:46–52.

Chandra, S., Chauhan, L.K.S., Dhawan, A., Murthy, R.C. and Gupta, S.K. 2006. *In vivo* genotoxic effects of industrial waste leachates in mice following oral exposure. *Environment and Molecular Mutagenesis*, 47:325-333.

Chang, I. 1972. Study on the threshold limit value of benzene and early diagnosis of benzene poisoning. *Journal of Catholic Medical College*, 23: 429-434.

Cheesbrough, M., 2005. District Laboratory practice in tropical countries: Haematological test. Part 2, 2nd edition. Cambridge Low Price Editions. Pp 263–347.

Chellman, G.J., Bus, J.S. and Working, P.K. 1986. Role of epididymal inflammation in the induction of dominant lethal mutations in Fischer 344 rat sperm by methylchloride. *Proceedings of National Academic of Science*, USA 83: 8087-8092.

Cheong, H., Kim, E.A., Choi, J., Choi, S., Suh, J., Choi, D.S. and Kim, J.R. 2007. Grand rounds: an outbreak of toxic hepatitis among industrial waste disposal workers. *Environmental Health Perspective*, 115: 107-112.

Cheung, K.C., Chu, L.M. and Wong, M.H. 1993. Toxic effects of landfill leachate on microalgae. *Water Air Soil Pollution*, 69.3-4:337-349.

Christensen, T.H., Kjeldsen, P., Albrechtsen, H.J., Heron, G., Nielsen P.H., Bjerg, P.L. and Holm, P.E. 1994. Attenuation of Landfill Leachate pollutants in Aquifers. *Critical Reviews in Environmental Science and Technology*, 24: 119–202.

Christensen, J.B., Jensen, D.L., Filip Z., Gron C. and Christensen T.H. 1998. Characterization of the dissolved organic carbon in landfill polluted groundwater, *Water Reserch*, 32:125.

UNIVERSITY OF IBADAN

Christensen, T.H., Kjeldsen, P., Bjerg, P.L., Jensen, D.L., Christensen, J.B., Baun, A., Albrechtsen, H.J., Heron, C. 2001. Biogeochemistry of landfill leachate plumes. *Applied Geochemistry*, 16: 659-718.

Christopher, M.M. 2004. Interpretation of red blood cell morphology (advanced level). 29th World congress of the world small animal Veterinary Association. 6-9th october. Rhodes Greece (http://www.vin.com/proceedings.plx?CID).

Choi, A. L; Levy, J. I.; Dockery, D.W; Ryan L. M; Tolbert, P.E; Altshul, L. M; and Korrick, S. A; 2006. Does living Near a Superfund Site Contribute to Higher Polychlorinated Biphenyl (PCB) Exposure?. *Environmental Health Perspective*, 114.7: 1092-1097.

Clement, B., Delolme, C., Winiarski, T and Bouvet, Y.1993. The risk of contamination by leachates of freshwater ecosystems. Proceedings of the 4th International Symposium on Landfill, Margherita di Pula, pp: 1155-1166.

Clermont, R.J. and Chalmers, T.C. 1967. The transaminases tests in liver disease. *Medicine*, 46: 197-207.

Coker, A. and Sridhar, M.C.K. 2010. Increase in healthcare facilities and rapid environmental degradation: A technological paradox in Nigeria's urban centres. *African Journal of Environmental Science and Technology*, 4.9: 577 – 585.

Coleman, S.P.E. and Schultes, J.P.E. 1996. Cradle-to-grave compliance tracking of U.S./Mexico transboundary hazardous waste: the haztraks tracking system. Fourth international conference on environmental compliance and enforcement. Vol. 2. Pp 609-629. www.inece.org/4thvol2/coleman.pdf.

Corona-Ravera, A., Urbina-Cano, P., Bobadilla-Morales, L., Vargas-Lares, J., Ramirez-Herrera, M.A., Mendoza-Magana, M.L., Troyo-Sanroman, R., Diaz-Esquivel, P. and Corona-Rivera, J.R. 2007. Protective in vivo effects of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. *Journal of Applied Genetics*, 48.4: 389-396.

Costal, M., Salnikov, K., Cosentino, S., Klein, C.B., Huang, X, Zhuang, Z. 1994. Molecular mechanisms of nickel carcinogenesis. *Environmental Health Perspective*, 102:127-130.

Countryman, P.I. and Heddle, J.A. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutation Research*, 41:321–332.

Cuadra, S.N., Linderholm, L., Athanasiadou, M., Jakobson, K. 2006. Persistent Organochlorine Pollutants in children working at a waste disposal site and in young females with high fish consumption in Managua, Nicaragua. *Ambio*, 35.3:109-116.

Cullen, J.M. 2005. Mechanistic classification of liver injury. *Toxicologic Pathology*, 33: 6-8

UNIVERSITY OF IBADAN

Cunningham, V.L., Perino, C., D'Aco, V.J., Hartmann A. and Bechter, R. 2010. Human health risk assessment of carbamazepine in surface waters of North America and Europe. *Regulatory Toxicology and Pharmacology*, 56: 343–351.

Dacie, J.V. and Lewis, S. M. 1984. *Practical Haematology*, 4th Ed. Churchill livingstone, Edinburgh, London; pp 420.

Dalton, P. 2003. Upper airway irritation, odor perception and health risk due to airborne chemicals. *Toxicology Letters*, 140-141: 239-248.

Damek-Poprawa, M. and Sawicka-Kapusta, K. 2003. Damage to liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, 186: 1-10.

Danadevi, K., Rozati, K., Banu, B.S. and Grover, P. 2004. Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the comet and micronucleus assays. *Mutagenesis*, 19.1: 35–41.

Das, R.K. and Nanda, N.K. 1986. Induction of micronuclei in peripheral erythrocyte of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent. *Mutation Research*, 175: 67-71.

Dasgupta, S., Ghosh, S. and Das, K.K. 1996. Transaminase activities in some metabolically active tissues in nickel-treated rats under protein restriction. *Indian Journal of Physiology and Allied Sciences*, 50: 27-33.

Daskalopoulos, E., Badr, O. and Probert, S.D. 1998. An integraded approach to municipal solid waste management. *Resources, Conservation and Recycling*, 24.1: 33-50.

Daughton, C.G. and Ternes, T.A. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environmental Health Perspective*, 107 (*suppl* 6): 907-938.

Davies, D.T. 1992. Enzymology in preclinical safety evaluation. *Toxicologic Pathology*, 20: 501-505.

De Flora, S., Begnasco, M., Serra, D., Zanacchi, P. 1990. Genotoxicity of chromium compounds: a review. *Mutation Research*, 238:99-172.

Deguchi, Y., Toyoizumi, T., Masuda, S., Yasuhara, A., Mohri, S., Yamada, M., Inoue, Y. and Kinae, N. 2007. Evaluation of mutagenic activities of leachates in landfill sites by micronucleus test and comet assay using goldfish. *Mutation Research*, 627:178–185.

Denison, R.A. and Silbergeld, E.K. 1988. Risks of municipal solid waste incineration: an environmental perspective. *Risk Analysis*, 8.3: 343–355.

Department for Environment, Food and Rural Affairs (DEFRA). 2004. Review of Environmental and Health Effects of Waste Management: Municipal Solid Waste and Similar Wastes. http://www.hmso.gov.uk/.

UNIVERSITY OF IBADAN

Dolk, H., Vrijheid, M., Armstrong, B., Abramsky, L., Bianchi, F., Garne, E., Nelen, V., Robert, E., Scott, J.E.S., Stone, D. and Tenconi, R. 1998. Risk of congenital anomalies near hazardous waste landfill sites in Europe: the Eurohazion study. *Lancet*, 352:423–427.

Donnelly, K.C., Brown, K.W. and Thomas, J.C. 1988. Bacterial mutagenicity of leachate from municipal sewage sludge-amended soils. Environmental Toxicology and Chemistry, 9.4: 443-451.

Doumas, B.T., Watson, W.A. and Brigo, H.G. 1982. Bilirubin total and conjugated: Modified methods in selected methods of clinical chemistry vol. 9 (WR Faulker and S Mats, eds). Washington D.C. pp 113.

Dunnett, C. W. 1964. New tables for multiple comparisons with a control. *Biometrics*, 20:482-491.

Duthie, G.G., and Tort, L. 1985. Effect of dorsal aortic cannulation on the respiration and haematology of the Mediterranean dog-fish *Scyliorhinus canicula*. *Comparative Biochemistry and Physiology*, 81:879-883.

Duydu, Y., Suzen, H.S., Aydin, A., Cander, O., Uysal, H., Isimer, A., Vural, N. 2001. Correlation between lead exposure indicators and sister chromatid exchange (SCE) frequencies in lymphocytes from inorganic lead exposed workers. *Archives of Environmental Contamination and Toxicology*, 41: 241–246.

Eaton, D.L. and Klaassen, C.D. 1998. Principals of toxicology. In: Klaassen C.D. (Ed) Toxicology. The Basic Science of Poison. 5th edn, pp 335–354.

Eduljee, G. 1992. Assessing the risks of landfill activities, Harwell Waste Management Symposium. new developments in landfill proceedings; Harwell, Didcot, Oxfordshire, UK. Oxfordshire, UK: AEA Environment and Energy;

Efuntoye, M.O., Bakare, A.A. and Sowunmi, A.A. 2011. Virulence factors and antibiotic resistance in Staphylococcus aureus and Clostridium perfrigens from landfill leachate. *African Journal of Microbiological Research*, 5.23: 3994 – 3997.

Eggleston, P.A., Diette, G., Lipsett, M., Lewis, T., Tager, T., McConnell, R., Chrischilles, E., Lanphear, B., Miller, R. and Krishnan, J. 2005. Lessons Learned for the Study of Childhood Asthma from the Centers for Children's Environmental Health and Disease Prevention Research. *Environmental Health Perspective*, 113:1430–1436.

El-Fadel, M., Findikakis, A.N. and Leckie J.O. 1997. Environmental impacts of solid waste landfilling. *Journal of Environmental Management*, 50: 1-25.

Elliott, P., Briggs, D., Morris, S., de Hoogh, C., Hurt, C., Jensen, T.K., Maitland, I., Richardson, S., Wakefield, J. and Jarup, L. 2001. Risk of adverse birth outcomes in populations living near landfill sites. *British Medical Journal*, 323:363–368.

EPA. 1998. Toxicological review of hexavalent chromium, Washington, DC: National center for environmental assessment, office of research and development. 64 pp.

UNIVERSITY OF IBADAN

Eritsland, J. 2000. Safety considerations of polyunsaturated fatty acids. *American Journal of Clinical Nutrition* 71:197S–201S.

Ernst, W.R., Hennigar, P., Doe, K., Wade, S. and Julien, G. 1994. Characterization of the chemical constituents and toxicity to aquatic organisms of a municipal landfill leachate. *Water Pollution Research Journal of Canada*, 29.1:89-101.

Esakku, S., Palanivelu, K., Joseph, K., 2003. Assessment of heavy metals in a municipal solid waste dumpsite. Proceedings of a workshop on sustainable landfill management 3 – 5 December Chennai, India, pp. 139 – 145.

European Council. 2002. Council Decision of 19 December 2002 establishing criteria and procedures for the acceptance of waste at landfills pursuant to Article 16 of and Annex II to Directive 1999/31/EC. *Official Journal of the European Communities*, L 11:27– 49.

Evans, G.O. 2008. Animal haematotoxicology: A practical guide for toxicologists and biomedical researchers. CRC press. (ISBN 142 0080091, 97814400), pp 14–78.

Evans, H.J. Neary, G.J. and Williamson, F.S. 1959. The relative biological efficiency of single doses of fast neutrons and gamma rays in Vicia faba roots and the effect of oxygen. Part II. Chromosome damage; the production of micronuclei. *International Journal of Radiation Biology*, 1:230–240.

Everse, J. and Hsia, N. 1997. The toxicities of native and modified haemoglobins. *Free Radical and Biological Medicine*, 22:1075–1099.

Fan, H.J., Shu, H.Y., Yang, H.S. and Chen, W.C. 2006, Characteristics of landfill leachates in central Taiwan. *Science of The Total Environment*, 361.1-3: 25-37.

Faouzi, S., Burckhardt, B.E., Hanson, J.C., Campe, C.B., Schrum, L.W., Rippe, R.A. and Maher, J.J. 2001. Anti-Fas induces hepatic chemokines and promotes inflammation by an NF-kappa B-independent, caspase-3- dependent pathway. *Journal of Biology and Chemistry*, 276: 49077-49082.

Farmer, A. and Hjerp, P. 2001. Municipal solid waste incineration: health effects and public communication. A report to the National Society for Clean Air and Environmental Protection. Institute for European Environmental Policy (IEEP).

Federal Environmental Protection Agency (FEPA). 1991. Guidelines and standard for industrial Effluents, Gaseous emission and hazardous waste management in Nigeria.

Fenech, M. 2007. Cytokinesis-block micronucleus cytome assay. *Nature Protocols*, 2.5:1084-1104.

Fenech, M. and Crott, J.W. 2002. Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. *Mutation Research*, 504: 131-136.

Fenech, M., Crott, J.W., Turner, J. and Brown, S. 1999. Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block

UNIVERSITY OF IBADAN

micronucleus assay: description of the method and results for hydrogen peroxide. *Mutagenesis*, 14.6: 605 – 612.

Fenech, M. and Morley, A. A. 1986. Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation. *Mutation Research*, 161: 193-198.

Feng, S., Wang, X., Wie, G., Peng, P., Yang, Y. and Cao, Z. 2007. Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity. *Chemosphere*, 67: 1133–1137.

Ferrari, B., Radetski, C.M., Veber, A.M. and Ferard, J.F. 1999. Ecotoxicological assessment of solid wastes: A combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers. *Environmental Toxicology and Chemistry*, 18:1195–1202.

Fielder, H. M. P., Poon-King, C. M., Palmer, S. R., Moss, N. and Coleman, G. 2000. Assessment of impact on health of residents living near the Nant-y-Gwyddon landfill site: retrospective analysis. *British Medical Journal*, 320:19-23.

Finco, D.R. 1989. Kidney function In: J.J. Kaneko (ed), Clinical Biochemistry of Domestic Animals, Academic Press, san Diego, CA, Pp 496–542.

Fischbach, F. 1984. A manual of laboratory diagnostic tests. JB Lippincott Company, New York, pp 1 – 898.

Flora, S.J.S., Mittal, M. and Mehta, A. 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian Journal of Medical Research*, 128:501-523.

Forester, W.S. and Skinner, J.H. 1987. International perspectives on hazardous waste management: report from 12 ISWA countries. Report from the International Solid Wastes and Public Cleansing Association (ISWA) Working Group on Hazardous Wastes. London, Academic Press. ISBN 0122621654.

Frenkel, K., Chrzan, K., Troll, W., Teebor, G.W. and Steinberg, J.J. 1986. Radiation-like modification of bases in DNA exposed to tumor promoter–activated polymorphonuclear leukocytes. *Cancer Research*, 46: 5533-5540.

Friedman, L.S., Martin, P. and Munoz, S.J. 1996. Liver function tests and the objective evaluation of the patient with liver disease. In: Zakin D, Boyer TD, eds. Hepatology: A textbook of liver disease. 3rd ed. Philadelphia: WB Saunders 971-833.

Gad, S.C. 2007. Laws and regulations governing animal care and use in research. In animal model in toxicology. 2nd ed. Taylor and Francis group. New York pp 901-921.

Gaidajis, G., Angelakoglou, K. and Aktsoglou, D. 2010. E-waste: environmental problems and current management**.** *Journal of Engineering Science and Technology Review* 3.1: 193-199.

Galaris, D. and Evangelou, A. 2002. The role of oxidative stress in mechanisms of metal induced carcinogenesis. *Critical Review of Oncology and Haematology*, 42;93-103.

UNIVERSITY OF IBADAN

Geschwind, S.A., Stowijk, F.A.J., Bracken, M., Fitzgerald, E., Stark, A., Olsen, C. and Melius, J. 1992. Risk of congenital Malformations Associated with Proximity to Hazardous waste Sites. *American Journal of Epidemiology*,135: 1197-1207.

Godet, F., Babut, M., Burnal, D., Verler, A.M. and Vasscur, P. 1996. The genotoxicity of iron and chromium in electroplating effluents. *Mutation Research*, 370:19-28.

Goldstein, R.S. and Schnellmann, R.G. 1999. Toxic responses of the kidney: in Casarett and Doull's Toxicology McGraw – Hill Co 5th ed. 417–422.

Goldwasser, P. and Feldman, J. 1997. Association of serum albumin and mortality risk. *Journal of Clinical Epidemiology*, 50: 693-703.

Gomez-Meda, B.C., Zamora-Perez, A.L., Luna-Aguirre, J., Gonzalez-Rodrıguez, A., Ramos-Ibarra, M.L., Torres-Bugarın, O., Batista-Gonzalez, C.M. and Zuniga-Gonzalez, G.M.. 2006. Nuclear abnormalities in erythrocytes of parrots (*Aratinga canicularis*) related to genotoxic damage. *Avian Pathology*, 35.3:206-210.

Greaves, P. 2000. Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation, 2nd edition. Elsevier Science, Amsterdam.

Greer, L.E. 1984. Definition of hazardous waste. *Hazardous waste*. 1: 309-322.

Gregus, Z. and Klaassen, C.D. 2001. Mechanism of toxicity. In: Klaassen CD, (ed) Casarett and Doull_s toxicology: the basic science of poisons. 6th ed. McGraw-Hill, New York, pp 35–81.

Gujral, J.S., Liu, J., Farhood, A., Hinson, J.A. and Jaeschke, H. 2004. Functional importance of ICAM-1 in the mechanism of neutrophil-induced liver injury in bile duct-ligated mice. *American Journal of Physiological Gastrointestine and Liver Physiology*, 286: G499-507.

Guy, S.P., Bradshaw, T.K. and Itzhaki, P.F. 1989. Chromatin characteristics of carcinogen-treated avain erythrocyte nuclei and effects of reactivation. *Cell Biology and International Reproduction*, 13: 759-771.

Gwozdzinski, K. 1995. Structural changes in erythrocytes components induced by copper and mercury. *Radiation, Physics and Chemistry*, 45:877-882.

Hall, H.I., Kay, W.E., Gensburg, L.S. and Marshall, E.G. 1996. Residential proximity to hazardous waste sites and risk of end-stage renal disease. *Journal Environmental Health*, 59:17–21.

Harmon, M.P. and Coe, K. 1993. Cancer Mortality in U.S. Counties with Hazardous Waste Sites. *Population and Environment*, 14.5: 463-480.

Hartwig, A. and Schwerdtle,T. 2002. Interaction by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicological Letter*, 127:47–54.

UNIVERSITY OF IBADAN

Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Soatome, K., Tanaka, N., Takai, A., Sasaki, Y.F. Asano, N. Sofuni, T. and Ojima, Y. 1998. Development of genotoxicity assay systems that use aquatic organisms. *Mutation and Research*, 399:125–133.

Hayashi, M., Kuge, T., Endoh, D., Nakayama, K., Arihawa, J., Tahazawa, A. and Okuit, T. 2000. Hepatic Cu accumulation induces DNA strand breaks in the liver cells of Long-Evans Cinnamon strain rats. *Biochemistry and Biophysics Research Communication*, 276: 174–178.

Heddle, J.A. 1973. A rapid *in vivo* test for chromosome damage. *Mutation Research*, 18:187–190.

Helma, C., Mersch-Sunderman, V., Houk, V.S., Glass-Brenner, U., Klein, C., Wenquing, L., Kassie, F., Schulte-Hermann, R. and Knasmuller, S. 1996. Comparative evaluation of four bacterial assays for the detection of genotoxic effects in the dissolved water phases of Aqueous Matrices. *Environmental Science and Technology*, 30:897-907.

Helma, C., Sommer, R., Schulte-Hermann, R. and Knasmuller, S. 1994. Enhanced clastogenicity of contaminated groundwater following UV irradiation detected by the *Tradescantia* micronucleus assay. *Mutation Research*, 323:93-98.

Henderson, B.E., Preston-Martin, S., Edmondson, H.A., Peters, R.L. and Pike, M.C. 1983. Hepatocellular carcinoma and oral contraceptives. *Britain Journal of Cancer*, 48: 437-440.

Henry, A.S., Marian, M. and Alexis, P.N. 1974. Life term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *Journal of Nutrition*, 104: 239 – 243.

Hertzman, C., Hayes, M., Singer, J. and Highland, J. 1987. Upper Ottawa street landfill site health study. *Environmental Health Perspectives*, 75: 173–195.

Hodgson, E. and Goldstein, J.A. 2001. Metabolism of toxicants: Phase 1 reactions and pharmacogentics. In Introduction to Biochemical Toxicology. 3rd ed. New York. Wiley.

Ikem, A., Osibanjo O., Sridhar M. K. C. and Sobande A. 2002. Evaluation of groundwater quality characteristics near two waste sites in Ibadan and Lagos, Nigeria. *Water, Air and Soil Pollution* 140:307-333.

Ilyinskikh, N.N., Ilyinskikh, E.N., Ksenz, A.S. and Yurkin, A.Y. 1997. An assessment of frequencies of micronucleated erythrocytes in peripheral blood of Pigeons (Columba livia Gm) living in the polluted radioactive area around the Siberian chemical plant. *Environmental pollution*, 98.1:119-122.

Indulski, J.A.and Lutz, W. 1995. Biomarkers used for the assessment of health hazards in populations living in the vicinity of communal and industrial waste dump sites: Review. *International Journal of Occupational Medicine and Environmental Health*, 8: 11-16.

UNIVERSITY OF IBADAN

Iwegbue, C.M.A., Arimoro, F.O. and Nwajei, G.E. 2007. The genotoxicity screening of simulated leachate from semi-urban waste dumps in Niger-Delta, Nigeria analysed by the Allium test. *Research Journal of Environmental Sciences*, 1.6:310-316.

Jadhav, S.N., Sarkar, S.N., Aggarwal, M., Tripathi, H.C. 2007. Induction of oxidative stress in erythrocytes of male rats subchronically exposed to a mixture of eight metals found as groundwater contaminants in different parts of India. *Archives of Environmental Contamination and Toxicology*, 52: 145-151.

Jaeschke, H. 2000. Reactive oxygen and mechanisms of inflammatory liver injury. *Journal of Gastroenterology and hepatology*, 15:718-724.

Jaeschke, H. 2003. Molecular mechanisms of heatic ischemia-repurfusion injury and preconditioning. *American Journal of Physiological Gastrointestine and Liver Physiology*, 284: G15-26.

Jaeschke, H. 2006. Mechanisms of neutrophils-mediated liver cell injury during ischemia-reperfusion and other acute inflammatory conditions. *American Journal of Physiological Gastrointestine and Liver Physiology*, 290: 1083-1088.

Janerich, D.T., Burnett, W.S., Feck, G., Hoff, M., Nasca, P., Polednak, A.P., Greenwald, P.and Vianna, N. 1981. Cancer incidence in the Love canal area. *Science.* 212: 1404–1407.

Jibiri, N.N., Farai, I. P. and Alausa, S.K. 2007. Activities concentration of Ra-226, Th-228 and K-40 in different food crops from a high background radiation area in Bisichi Jos Plateau State, Nigeria. *Radiation and Environmental Biophysics*, 46: 53-59.

Jiraungkoorskul, W., Sahaphong, S., Kosai, P. and Kim, M. 2007. Micronucleus test: the effects of Ascorbic acid on cadmium exposure in fish (*Puntius altus*). *Research Journal of Environmental Toxicology*, 1.1:27-36.

Joardar, M. and Sharma, M. 1990. Comparison of clastogenicity of inorganic Mn administered in cationic and anionic forms in vivo. *Mutation Research*, 240: 159-163.

Johansson, L and Pellicciari, C.E. 1988. Lead induced changes in the stabilization of the mouse sperm chromatin. *Toxicology*, 51:11-24.

Johnson, B.L. 1997. Hazardous waste: human health effects. *Toxicology and Industrial Health*, 13:121-143.

Johnson, B.L. 1999. *Impact of hazardous waste on human health*. CRC press Inc., New York.

Jones, D. L., Williamson, K. L. and Owen, A. G. 2006. Phytoremediation of landfill leachate. *Waste Management*, 26.8: 825-837.

Jonsson, S., Ejlertsson, J., Ledin, A., Mersiowsky, I. and Svensson, B.H. 2003. Mono- and diesters from o-phthalic acid in leachates from different European landfills. *Water Research*, 37:609–17.

UNIVERSITY OF IBADAN

Juberg, D.R., Mudra, D.R., Hazelton, G.A. and Parkinson, A. 2006. The effect of fenbuconazole on cell proliferation and enzyme induction in the liver of female CD1 mice. *Toxicology and Applied Pharmacology*, 214.2: 178–187.

Kadiiska, M.B., Xiang, Q.H. and Mason, R.P. 1994. *In vivo* free radical generation by chromium (VI): an electron spin resonance spin-trapping investigation. *Chemical Research and Toxicology*, 7: 800-805.

Kamiya, A., Ose, Y. and Sakagami, Y. 1989. The mutagenicity of refuse leachate from a municipal incinerator. *Science of the Total Environment*, 78:131-145.

Kasprzak, K.S. 1991. The role of oxidative damage in metal carcinogenicity. *Chemical Research and Toxicology*, 4:604-615.

Kasperczyk, S., Birkner, E., Kasperczyk, A. and Kasperczyk, J. 2005. Lipids, lipid peroxidation and 7-ketocholesterol in workers exposed to lead. *Human Experiment and Toxicology*, 24:287–295.

Kharrazi, M., Von Behren, J., Smith, M., Lomas, T., Armstrong, M., Broadwin, R., Blake, E., McLaughlin, B., Worstell, G. and Goldman, L. 1997. A community based study of adverse pregnancy outcomes near a large hazardous waste landfill in California, *Toxicology and Industrial Health*, 13:229-310.

Kilburn, K.H. 1999. Neurotoxicity from Airborne Chemicals Around a Superfund Site. *Environmental Research Section A*, 81.2: 92-99.

Kimani, N.G. 2007. Environmental pollution and impacts on public health: implications of Dandora municipal dumping sites in Nairobi, Kenya. Report summary.

Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A. and Christensen, J.B. 2002. Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Critical Reviews in Environmental Science and Technology*, 32. 4: 297-336.

Kouznetsova, M., Huang, X., Ma, J., Lessner, L. and Carpenter, D.O. 2007. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. *Environmental Health Perspectives*, 115: 75 – 79.

Krishna, G. and Hayashi, M. 2000. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research*, 455: 155-166.

Kudyakov, R., Baibergenov, A.A., Zdeb, M. and Carpenter, D.O. 2004. Respiratory disease in relation to patient residence near hazardous waste sites. *Environmental Toxicology and Pharmacology*, 18:249-57.

Kumar, G., Banu, S.G., Kannan, V. and Pandian, R.M. 2005. Antihepatotoxic effects of β – carotene on paracetamol induced hepatic damage in rats. *Indian Journal of Experimental Biology*, 43: 351 – 355.

UNIVERSITY OF IBADAN

Kuper, C. F., Harleman, J. H., Richter-Reichelm, H. B., and Vos, J. G. 2000. Histopathologic approaches to detect changes indicative of immunotoxicity. *Toxicologic Pathology*, 28: 454–466.

Kurniawan, T.A., Lo, W.H. and Chan G.Y.S. 2006. Physico-chemical treatments for removal of recalcitrants contaminants from landfill leachates. *Journal of Hazardous Materials*, 129.1-3: 80-100.

Kursa, M. and Bezrukov, V. 2007. Health status in an Antartic top predator: Micronucleus frequency and white blood cell differentials in the south polar skua (*Catharacta maccormicki*). *Polarforschung*, 77.1:1-5.

Laborda, R., Diaz-Mayans, J. and Nunez, A. 1986. Nephrotoxic and hepatotoxic effects of chromium compounds in rats. *Bulletin of Environmental Contamination and Toxicology*, 36: 332-336.

Laniyan, T.A., Kehinde-Phillips, O.O., and Elesha, L. 2011. Hazards of heavy metal contamination on the groundwater around a municipal dumpsites in Lagos, Southwestern, Nigeria. *International Journal of Engineering and technology*, 11.5: 61 – 69.

Lanning, L.L., Creasy, D.M., Chapin, R.E., Mann, P.C., Barlow, N.J., Regan, K.S. and Goodman, D.G. 2002. Recommended approaches for the evaluation of testicular and epididymal toxicity. *Toxicologic Pathology*, 30.4: 507-520.

Larsson, A., Haux, C., and Sjobeck, M. 1985. Fish physiology and metal pollution, results and experiences from laboratory and field studies. *Ecotoxicology and Environment*, 9:250–281.

Lasisi, K.S.A. 2002. Composition of Landfill leachate from existing Landfills in Lagos State. A paper presented at an in- house seminar for officers of Lagos State Environmental Protection Agency. Pp 1–12.

Lawson, J.A., Fisher, M.A., Simmons, C.A. Farhood, A. and Jaeschke, H. 1998. Parenchymal cell apoptosis as a signal for sinusoidal sequestration and transendothelial migration of neutrophils in murine models of endotoxin and Fas-antibody induced liver injury. *Hepatology*, 28: 761-767.

Lee, G.F and Jones, R.A. 1991. Municipal solid waste management: long-term public health and environmental protection. A paper prepared for workshop on landfills and groundwater quality. NWWA national outdoor action conference, Las Vegas, Neveda, May, 15.

Lee, G.F. and Jones-Lee, A. 1994. Impact of municipal and industrial non-hazardous waste landfills on public health and the Environment: An over view prepared for California environmental protection Agency's Comparative risk project.

Lee, G.F. and Jones-Lee, A. 2003. Association between hazardous chemical sites and illness. Report of G. Fred Lee & associates, El Macero, CA. www.gfredlee.com.

UNIVERSITY OF IBADAN

Lee, G.R., Forster, J., Lukens, J., Paraskevas, F., Greer, J.P. and Rodgers, G.M. 1999. Wintrobe's Clinical Haematology, 10th ed. Vol. 1 part VII, Haematologic Malignancies. Baltimore MD: Williams and Wilkins.

Leenheer, J.A., Nanny, M.A. and McIntyre, C. 2003. Terpenoids as major precursors of dissolved organic matter in landfill leachates, surface water and groundwater. *Environmental Science and Technology*, 37:2323– 2331.

Lewis, E.L., Kallenbach, L.R., Geary, N.S., Melius, J.M., Ju, C.L., Orr, M.F. and Forand, S.P. 1998. Investigation of cancer incidence and residence near 38 landfills with soil gas migration conditions: New York State, 1980–1989. ASTDR/HS-98-93 Agency for Toxic Substances and Disease Registry.

Li, G., Sang, N. and Zhao, Y. 2004. Micronuclei induced by municipal landfill leachate in mouse bone marrow cells *in vivo*. *Environmental Research*, 95: 77–81.

Li, G., Sang, N. and Wang, Q. 2006a. Oxidative damage induced in brains and livers of mice by landfill leachate. *Ecotoxicology and Environmental Safety*, 65:134-139.

Li, G., Sang, N. and Guo, D. 2006b. Oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachate. *Chemosphere* 65: 1058-1063.

Li, G., Yun, Y., Li, H. and Sang, N. 2008. Effect of landfill leachate on cell cycle, micronucleus, and sister chromatid exchange in *Triticum aestivum*. *Journal of Hazardous Materials*, 155: 10–16.

Li, H., Han, M., Hou, L., Li, G. and Sang, N. 2010. Landfill leachate ingestions induced protein oxidation and DNA–protein crosslinks in mouse viscera. *Journal of Hazardous Materials*, 174: 54-8.

Li, W., Zhao, Y. and Chou, I. 1992. Cytoskeletal injury induced by by hexavalent chromate. *Toxicology In Vitro*, 5:433-444.

Longe, E.O., Enekwechi, L.O. 2007. Investigation on potential groundwater impacts and influence of local hydrogeology on natural attenuation of leachate at a municipal landfill. *International Journal of Environmental Science and Technology*, 4, 1: 133-140.

Lynch, M.J., Rakhael, S.S., Miller, L.D., Space, D.D., and Inwood, M.J.H. 1969. Medical laboratory technology and clinical pathology, 2nd edn. WB Saunders Co, Phaladelphia, pp 1- 299.

Ma, J., Kouznetsova, M., Lessner, L. and Carpenter, D.O. 2007. Asthma and infectious respiratory disease in children-correlation to residence near hazardous waste sites. *Paediatric Respiratory Reviews*, 8; 292-298.

Ma, T.H., Neas, R.E., Harris, M.M., Xu, Z., Cook, C. and Swofford, D. 1987. *In vivo* tests (Tradescantia-and Mouse-Micronucleus) and chemical analysis on drinking water of rural communities. In: Short-term bioassays in the analysis of complex mixtures V. (S.S. Sandhu, D. DeMarini. M. J. Mass, M.M. Moore and J.L. Mumford, Eds.) Plenum Press. New York, pp. 189-205.

UNIVERSITY OF IBADAN

Ma, T., Zhou, X., Loarca, G.F., Arreola, G.G., and Lecona, S. 1995. Mouse-erythrocyte micronucleus (Mus-EMN) assay on the clastogenicity of industrial wastewater. *Revista Internacional de Contaminacion Ambiental*, 11.2: 95-98.

MacGregor, J.T., Wehr, C.M. and Gould, D.H. 1980. Clastogen-induced micronuclei in peripheral erythrocytes: the basis of an improved micronucleus test. *Environmental Mutagenesis*, 2: 509-514.

Mahour, K., and Saxena, P.N. 2009. Assessment of haematotoxic potential of mercuric chloride in rat. *Journal of Environmental Biology*, 30.5:927–928.

Malhotra, O. P. and Srivastava, D. K. 1978. Cadmium induced changes in lipid peroxidation, blood hematology and biochemical parameters of male rats. *Biochemistry and Biophysics Acta*, 526:1–12.

Mallick, P. and Khuda-Bukhsh, A.R. 2003 Nuclear anomalies and blood protein variations in fish of the Hooghly-Matlah River System, India, as an indicator of genotoxicity in water, *Bulletin of Environmental Contamination and Toxicology*, 70:1071–1082.

Mandal, A., Chakraborty, S., and Lahiri, P. 1986. Haematological changes produced by lindane (-HCH) in six species of birds. *Toxicology*, 40:103–111.

Marcomini, A., Capel, P.D., Lichtensteiger, T.H., Brunner, P.H. and Giger, W. 1989. Behaviour of aromatic surfactants and PCBs in sludge-treated soil and landfills. *Journal of Environmental Quality*, 18:523–528.

Marsh, G. and Caplan, R. 1987. Evaluating health effects of exposure at hazardous waste sites: a review of the state-of-the-art, with recommendations for future research. In Andelman J.B.; Underhill D.W. Health effects from Hazardous Waste Sites. USA; Lewis Publishers.

Marshall, E.G., Gensburg, L.J., Deres, D.A., Geary, N.S., Cayo, M.R. 1997. Maternal residential exposure to hazardous wastes and risk of central nervous system and musculoskeletal birth defects. *Archives of Environmental Health*, 52.6., 416-425.

Mathur, N., Krishnatrey, R., Sharma, S., Pathak, S. and Sharma, K.P. 2003. Certain haematological responses in Swiss albino mice following exposure to textile dye wastewater. *Journal of Environmental Biology*, 24.2: 161–164.

Matos, R.C., Vieira, C., Morais, S., Pereira, M.L. and Pedrosa, J. 2010. Toxicity of chromate copper arsenate: A study in mice. *Environmental Research*, 110: 424-427.

McDaniels, A.E., Reyes, A.L., Wymer, L.J., Rankin, C.C. and Stelma, Jr. G.N. 1993. Genotoxic activity detected in soils from a hazardous waste site by the Ames test and an SOS colorimetric test. *Environment and Molecular Mutagenesis*, 22:115-122.

Meng, Z.Q. Sang, N. and Zhang, B. 2002. Effects of derivatives of sulphur dioxide on micronuclei formation in mouse bone marrow cells *in vivo*. *Bulletin Environment Contamination Toxicology*, 69: 257–264.

UNIVERSITY OF IBADAN

Meyer, C.R. 1983. Liver dysfunction in residents exposed to leachate from a toxic waste dump. *Environmental Health Perspective*, 48: 9-13.

Meyer, D.J. and Harvey, J.W. 2004. Hepatobiliary and skeletal muscle enzymes and liver function tests. In: Meyer DJ and Harvey J Weds. Veterinary Laboratory Medicine: Interpretation and Diagnosis. 3rd ed. St. Louis, MO: Saunders: 169 – 192.

Michael, B., Yano, B., Sellers, R.S., Perry, R., Morton, D., Roome, N., Johnson, J.K. and Schafer, K. 2007. Evaluation of organ weights for rodents and non-rodents toxicity studies: A review of regulatory guidelines and a survey of current practices. *Toxicological Pathology*, 35: 742-750.

Michelozzi, P., Fusco, D., Forastiere, F., Ancona, C., Dell'Orco, V., Perucci, C.A. 1998. Small area study of mortality among people living near multiple sources of air pollution *Occupational and Environmental Medicine*,55:611–615.

Minozzo, R., Luiz Irineu Deimling, L.I., Gigante, L.P. and Santos-Mello, R. 2004. Micronuclei in peripheral blood lymphocytes of workers exposed to lead. *Mutation Research*, 565:53–60.

Misiewicz, A., Radwan, K., Misiewicz, A. and Dziewit, T. 1999. Malonyldialdehyde concentration in red blood cells of workers engaged in the production of iron-manganese alloys. *Medical Practice*, 50:277–281.

Mochungong, P.I.K., Gulis, G. and Sodemann, M. 2011. Morbidity among children living around clinical waste treatment and disposal site in the Northwest region of Cameroon. *Journal of Public Health in Africa*, 2.13: 54–57.

Molander, D.W., Wroblewsk, F. and La Due, J.S. 1955. Transaminase compared with cholinesterase and alkaline phosphatase: an index of hepatocellular integrity. *Clinical and Research Proceedings*, 3:20-24.

Morowati, M. 2001. Biochemical and histopathological changes in serum creatinine and kidney induced by inhalation of Thimet (phorate) in male Swiss Albino mouse, *Mus musculus*. *Environmental Research*, 87: 31–36.

Moshtaghie, A.A., Raisi, A. and Goodarzi, H. 1991. A study of the effects of cadmium toxicity to proteinuria in male rats. *Journal of Islamic Academic and Science*, 4.3: 192–195.

Muhlich, M., Scherrer, M. and Daschner, F.D. 2003. Comparison of infectious waste management in European hospitals. *The Journal of Hospital Infection*, 55.4: 260-268.

Mythili, M.D., Nair, S.C. and Gunasekaran, S. 2004. Effect of cyclophosphamide pretreatment on hematological indices of Indian Bonnet monkeys. *Indian Journal of Pharmacology*, 36.3:175-180.

Nag, A. 2009. Role of antioxidants in oxidative stress management. *Research in Environment and Life Sciences*, 2.2: 53-60.

UNIVERSITY OF IBADAN

Nagae, Y., Miyamoto, H., Suzuki, Y. and Shimizu, H. 1991. Effect of estrogen on induction of micronuclei by mutagens in male mice. *Mutatation Research*, 263:21-26.

National Environmental Standards and Regulation Enforcement Agency (Federal Republic of Nigeria Official Gazette), 2009. National Environmental (sanitation and waste control). Published by Federal Government of Nigeria printer, Abuja FGP 112/102009/L000 (OL54). No.60 (96); Pp. 1057 – 1102

National Research Council. 1991. Use of biological markers in assessing human exposure to airborne contaminants. In: National Research Council, ed. Human Exposure Assessment for Airborne Pollutants. Washington, D.C.: National Academy of Science.

Nubi, O.A., Osibanjo, O. and Nubi, A.T. 2008. Impact assessment of dumpsite leachate on the qualities of surface water and sediment of river Eku, Ona-Ara Local Government, Oyo State, Nigeria. *Science World Journal*, 3.3: 17-20.

Nubi, O.A. and Ajuonu, N. 2011. Impacts of industrial effluent and dumpsite leachate discharges on the quality of groundwater in Oyo state, Nigeria. *Journal of Biodiversity and Environmental Sciences*, 1.3: 13-18.

Nunes, A.C., Mathias, M.D.L., and Crespo, A.M., 2001. Morphological and haematological parameters in the Algerian mouse (*Mus spretus*) inhabiting an area contaminated with heavy metals. *Environmental Pollution*. 113: 87–93.

Nunia, V., Sancheti, G. and Goyal, P.K. 2006. Protection of Swiss albino mice against whole – body and gamma irradiation by diltiazem. *The British Journal of Radiology*, 6:1-8.

Nwogu, S. 2010. Complaints mount over olusosun waste dump. *The Punch*. http://www.punchng.com/articl.aspx?theartic=Art20101108135921 37.

Obidoska, G. Jasińska, D. 2008. Phytotoxicity and potential genotoxicity of Radiowo municipal landfill leachate. *Annals of Warsaw University of Life Sciences – SGGW Land Reclamation*, 40: 39–44.

Odai, S. N., Mensah, E., Sipitey, D., Ryo, S. and Awuah, E. 2008. Heavy metals uptake by vegetables cultivated on urban waste dumpsites: case study of Kumasi, Ghana. *Research Journal of Environmental Toxicology*, 2.2: 92-99.

Odeigah, P.G.C. and Osanyipeju, O.A. 1995. Genotoxic effects of two industrial effluents and ethyl methane sulfonate in *Clarias lazera*. *Food and Chemical and Toxicology*, 33.6: 501-505.

Odunaike, R. K., Laoye, J.A., Alausa, S.K., Ijeoma, G.C. and Adelaja, A.O. 2008. Radiation emission characterization of waste dumpsites in the city of Ibadan in Oyo State of Nigeria. *Research Journal of Environmental Toxicology*, 2.2: 100 – 103.

Ogbonna, D.N., Kii, B.L. and Youdeowei, P.O. 2009. Some physico-chemical and heavy metal levels in soils of waste dumpsites in Port Harcourt Municipality and Environs. *Journal of Applied Science and Environmental Management*, 13.4: 65-70.

UNIVERSITY OF IBADAN

Ogundiran, O. O.; Afolabi, T. A. 2008. Assessment of the physico-chemical parameters and heavy metals' toxicity of leachates from municipal solid waste open dumpsite. *International Journal of Environmental Science and Technology*, 5.2: 243-250.

Ogungbuyi, O., Nnorom, I. C., Osibanjo, O. and Schluep, M. 2012. E-waste country assessment Nigeria. E-waste African project of the secretariat of the Basel convention. Pp. 1 - 94 http://www.ewasteguide.info/files/ogungbuyi-2012_BCCC- Empa.pdf .

Okonko, I.O., Adejoye, O.D., Ogunnusi, T.A., Fajobi, E. A. and Shittu, O. 2008. Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *African Journal of Biotechnology*, 7.5: 617-621.

Okolie, N.P. and Osagie, A.U. 1999. Liver and Kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. *Food and Chemical Toxicology*, 37: 745-750.

Omura, M., Inamasu, T. and Ishinishi, N. 1991. Mutagenicity assays of leachate from domestic waste landfills in Japan. The establishment of a protocol for measuring mutagenicity levels of leachate. *Bulletin of Environmental Contamination and Toxicology,* 46:561-568.

Omura, M., Inamasu, T. and Ishinishi, N. 1992. Mutagenic activity of the leachate of municipal solid waste landfill. *Mutation Research*, 298:125-129.

Oni, A.A., Hassan, A.T., and Li, P. 2011. Toxicity of leachates from Aba-Eku Landfill Leachate Lagoon, Ibadan, South-Western, Nigeria. *Advanced and Applied Scientific Research*, 2.2: 450-460.

Osaki, K., Shosaku, K., Norihisa, T. and Yoshiro, O. 2006. Toxicity testing of leachate from waste landfills using Medaka (*oryzias latipes*) for monitoring environmental safety. *Environmental Monitoring Assessment*, 117: 73-84.

Oshode, O.A., Bakare, A.A., Adeogun, A.O., Efuntoye, M.O. and Sowunmi, A.A. 2008. Ecotoxicological assessment using *Clarias gariepinus* and microbial characterization of leachate from municipal solid waste landfill. *International Journal of Environmental Research*, 2: 391 – 400.

Oyeku, O.T. and Eludoyin, A.O. 2010. Heavy metal contamination of groundwater resources in a Nigerian urban settlement. *African Journal of Environmental Science and Technology*, 4.4: 201-214.

Oygard, J.K. and Gjengedal, E. 2009. Uranium in municipal solid waste landfill leachate. *International Journal of Environmental Research*, 3.1: 61 – 68.

Palmer, S.R., Dunstan, F.D.J., Fielder, H., Fone, D.L., Higgs, G. and Senior, M.L. 2005. Risk of congenital Anomalies after t6he Opening of Landfill sites. *Environmental Health Perspectives*, 113.10:1362-1365.

UNIVERSITY OF IBADAN

Palus, J., Rydzynski, K., Dziubaltowska, E., Wyszynska, K., Natarajam, A.T. and Nilsson, R. 2003. Genotoxic effects of occupational exposure to lead and cadmium. *Mutation Research*, 540:19–28.

Patnaik, B.B., Howrelia, J.H., Mathews, T. and Selvanayagam, M. 2011. Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sublethal concentrations of lead and cadmium. *African Journal of Biotechnology*, 10.57: 12218-12223.

Parker, T., Dottridge, J. and Kelly, S. 2002. Investigation of the composition and emissions of trace components in landfill gas. R and D Technical Report P1-438/TR. Environment Agency, Bristol. Pp. 145.

Pascual, A., Gold-Bouchot, G. 2004. Heavy metals and hydrocarbons in sediments from three Lakes from San Miguel, Chiapas, México. *Bulletin of Environmental Toxicology*,73:762-769.

Paxeus, N. 2000. Organic components in municipal landfill leachates. *Water Research and Technology*, 42: 323 – 333.

Perez, H.R., Frank, A.L. and Zimmerman, N.J. 2006. Health effects associated with organic dust exposure during the handling of municipal solid waste. *Indoor and Built Environment*, 15.3: 207 –212.

Pheby, D., Grey, M., Giusti, L., and Saffron, L. 2002. *Waste management and public health: the state of the evidence. Epidemiological research on the impact of waste management activities on health.* South West Public Health Observatory (www.swpho.org.uk). Pp 1-72.

Pinto, D., Ceballo, J.M., Garcia, G., Guzman, P., Del Razo, L.M., Vera, E., Gomez, H., Garcia, A. and Gonsebatt, M.E. 2000. Increased cytogenetic damage in outdoor painters. *Mutation Research*, 467:105–111.

Pivato, A. and Gaspari, L. 2006. Acute toxicity test of leachates from traditional and sustainable landfills using luminescent bacteria, *Waste Management*, 26:1148–1155.

Polednak, A.P. and Janerich, D.T. 1989. Lung cancer in relation to residence in census tracts with toxic-waste disposal sites: A case-control study in Niagara County, New York. *Environmental Research*, 48.1.: 29-41.

Porta, D., Milani, S., Lazzarino, A.I., Perucci, C.A. and Forastiere, A. 2009. Systematic review of epidemiological studies on health effects associated with management of solid waste. *Environmental Health*, 8,60: 1–14.

Powers, D.A. 1989. Fish as model systems. *Science*, 246: 352-358.

Preston, R.J. 1996. Aneuploidy in germ cells: Disruption of chromosome mover components. *Environment and molecular mutagenesis*, 28:176-181.

UNIVERSITY OF IBADAN

Preston, R.J., Bender, M.A., Brewen, J.G., Carrano, A.V., Heddle, J.A., Macfee, A.F., Wolff, S. and Wasson, J.S.1981. Mammalian in vivo and in vitro cytogenetic assays. A report of the U.S. EPA's Gene Tox Program. *Mutation Research*,87. 2:143-188.

Pukkala, E. and Ponka, A. 2001. Increased incidence of cancer and asthma in houses built on a former dump Area. *Environmental Health Perspectives*, 109.11: 1121-1125.

Radetski, C.M., Ferrari, B., Cotelle, S., Masfaraud, J.F. and Ferard, J.F. 2004. Evaluation of the genotoxic, mutagenic and oxidant stress potentials of municipal solid waste incinerator bottom ash leachates. *Science of the Total Environment*, 333:209–216.

Radhakrishnan, M.V. 2010. Immunological Effect of cadmium on *Heteropneustes fossilis*. *Global Veterinaria*, 4. 6: 544-547.

Ramaiah, S. and Jaeschke, H. 2007. Role of neutophils in the pathologenesis of acute inflammatory liver injury. *Toxicologic Pathology*, 35:757-766.

Ray, M.R., Roychondhury, S., Mukherjee, G., Roy, S. and Lahiri, T. 2005. Respiratory and general health impairments of workers employed in a municipal solid waste disposal at an open landfill site in delhi. *International Journal of Hygiene and Environmental Health*. 208.4:255-262.

Reinhard, M., Goodman, N.L. and Barker, J.F. 1984. Occurrence and distribution of organic chemicals in two landfill leachate plumes. *Environmental Science and Technology*,18:953–961.

Reinhart, D.R. 1993. A review of recent studies on the sources of hazardous compounds emitted from solid waste landfills: a US experience. *Waste Management and Research*, 11:257–268.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glucose oxaloacetate and glutamic pyruvate transaminases. *American Journal of Clinical Pathology*, 28: 53-56.

Remigios, M.V. 2010. An overview of the management practices at solid waste disposal sites in African cities and towns. *Journal of Sustainable Development in Africa*, 12.7: 233-239.

Rodriguez, J., Castrillon, L., Maranon, E., Sastre, H. and Fernandez, E. 2004. Removal of non-biodegradable organic matter from landfill leachates by adsorption. *Water Research*, 38.14-15: 3297-3303.

Rodilla, V.1993. Origin and evolution of binucleated cells and binucleated cell with micronuclei in cisplatin–treated CHO cultures. *Mutation Research*, 300: 281-291.

Romeo, M., Bennani, N., Gnassia-Barelli, M., Lafaurie, M. and Girard, J.P. 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquatic Toxicology*, 48: 185-194.

UNIVERSITY OF IBADAN

Rossman, T.G., Molina, M. and Meyer, L.W. 1984. The genetic toxicology of metal compounds: Induction of lambda prophage in *E. coli* WP2s (Lambda). *Environmental Mutagenesis*, 6: 59-69.

Saha, R., Chattopadhyay, A. and Naidu, B.R. 2003. Pollution potential of leachate from East Kolkata solid waste dumpsite. Workshop on sustainable landfill management 3–5 December Chennai, India, 303–307.

Salamone, M., Heddle, J., Stuart, E. and Katz, M. 1980. Towards an improved micronucleus test: studies on 3 model agents, mitomycin C, cyclophosphamide and dimethyl benzanthracene. *Mutation Research*, 74: 347-356.

Saleh, K. and Sarhan, M.A.A. 2007. Clastogenic analysis of chicken farms using micronucleus test in peripheral blood. *Journal of Applied Sciences Research*, 3.12: 1646-1649.

Saleha Banu, B., Danadevi, K., Mahboob, M. and Kaiser, J. 2001. In vivo genotoxic effect of zinc sulphate in mouse peripheral blood leukocytes using comet assay. *Drug and Chemical Toxicolology*, 24: 63–73.

Sanchez-Chardi A, Nadal J. Bioaccumulation of metals and effects of landfill pollution in small mammals. Part I. The great white-toothed shrew, *Crocidura russula*. *Chemosphere*. 2007; 68: 703-711.

Sanchez-Chardi, A., Penarroja-Matutano, C., Ribeiro, C.A.O. and Nadal, J. 2007. Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The wood mouse, *Apodemus sylvaticus*. *Chemosphere*, 70:101-109.

Sanchez-Chardi, A., Marques, C.C., Gabriel, S.I., Capela-Silva, F., Cabrita, A.S., López-Fuster, M.J., Nadal, J. and Mathias, M.L. 2008. Haematology, genotoxicity, enzymatic activity and histopathology as biomarkers of metal pollution in the shrew (*Crocidura russula*). *Environmental Pollution*, 156:1332–1339.

Sang, N. and Li, G. 2004. Genotoxcity of municipal landfill leachate on root tips of *Vicia faba*. *Mutation Research*, 560: 159-165.

Sang, N. and Li, G. 2005.Chromosomal aberrations induced in mouse bone marrow cells by municipal landfill leachate. *Environmental Toxicology and Pharmacology*, 20: 219-224.

Sang, N., Li, G. and Xin, X. 2006. Municipal landfill leachate induced cytogenetic damage in root tips of *Hordeum vulgare*. *Ecotoxicology and Environmental Safety*, 63:469–473.

Saskatchewan Ministry of Environment (SME). 2006. Health and environmental effects of burning municipal solid waste. EPB 185 Jan 10. www.environment.gov.sk.ca

Sawaittayothin, V. and Polprasert, C. 2007. Nitrogen mass balance and microbial analysis of constructed wetlands treating municipal landfill leachate. *Bioresource Technology*, 98.3: 565-570.

UNIVERSITY OF IBADAN

Schacter, E., Beecham, E.J., Covey, J.M., Kohn, K.W. and Potter, M. 1988. Activated neutrophils induced prolonged DNA damage in neighboring cells. *Carcinogenesis*, 9: 2297–2304.

Schlegel, R., MacGregor, J.T., Everson, R.B. 1986. Assessment of cytogenetic damage by quantitation of micronuclei in human peripheral blood erythrocytes. *Cancer Research*, 46:3717-3721.

Schlegel, R. and MacGregor, J. T. 1982. The persistence of micronuclei in peripheral-blood erythrocytes: detection of chronic chromosome breakage in mice. *Mutation Research*, 104, 367–369.

Schmid, W. 1975. The micronucleus test. *Mutation Research*, 31: 9-15.

Schrab, G.E., Brown, K.W. and Donnelly, K.C. 1993. Acute and genetic toxicity of municipal landfill leachate. *Water, Air and Soil Pollution*, 69: 99–112.

Schrapp, K. and Al-Mutairi, N. 2010. Associated health effects among residences near Jeleeb Al-Shuyoukh landfill. *American Journal of Environmental Sciences*, 6.2:184–190.

Schuurman, H.J., Van Loveren, H., Rozing, J. and Vos, J. G. 1992. Chemicals trophic for the thymus: Risk for immunodeficiency and autoimmunity. *International Journal of Immunopharmacology*, 14: 369–75.

Schwartz, G.G., Skinner, H.G. and Duncan, R. 1998. Solid waste and pancreatic cancer: an ecologic study in Florida, USA. *International Journal of Epidemiology*, 27: 781-787.

Schwarzbauer, J., Heim, S., Brinker, S., Littke, R. 2002. Occurrence and alteration of organic contaminants in seepage and leakage water from a waste deposit landfill. *Water Research*, 36: 2275– 87.

Sellers, R.S., Morton, D., Michael, B., Roome, N., Johnson, J.K., Yano, B.L., Perry, R.and Schafer, K. 2007. Society of toxicologic pathology Position Paper: Organ weight recommendations for toxicology studies. *Toxicologic Pathology*, 35: 751-755.

Sen, P., Conway, K. and Costa, M. 1987. Comparison of the localization of chromosome damage induced by cadmium and nickel compounds. *Cancer Research*, 47:2142-2147.

Seoane, A.I. and Dulout, F.N. 2001. Genotoxic ability of Cadmium, Chromium and Nickel salts studied by kinetochore staining in the Cytokinesis-Blocked Micronucleus Assay. *Mutation Research*, 490:99-106.

Serrano-Garcia, L. and Montero-Montoya, R. 2001. Micronuclei and chromotid buds are the result of related genotoxic events. *Environmental and Molecular Mutagenesis*, 38:38-45.

Sha'Ato, R., Aboho, S.Y., Oketunde, F.O., Eneji, I.S., Unazi, G., Agwa, S. 2007. Survey of solid waste generation and composition in a rapidly growing urban area in Central Nigeria. *Waste Management*, 27: 352 – 358.

UNIVERSITY OF IBADAN

Sharaf, S., Khan, A., Khan, M.Z., Aslam, F., Saleemi, M.K., and Mahmood, F. 2010. Clinico-haematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. *Experimental and Toxicological Pathology*, 62: 333-341.

Sharma, S., Sharma, A., Singh, P.K., Soni, P., Sharma, S., Sharma, P., and Sharma, K.P., 2007. Impact of distillery soil leachate on haematology of swiss albino mice (*Mus musculus*). *Bulletin of Environmental Contamination and Toxicology*, 79: 273–277.

Shaw, B.P., Dash, S. and Panigrahi, A.K. 1991. Effect of methyl mercuric chloride treatment on haematological characteristics and erythrocyte morphology of Swiss mice. *Environmental Pollution*. 73:43–52.

Shcherbatykh, I., Huang X., Lessner, L. and Carpenter, D.O. 2005. Hazardous waste sites and stroke in New York State. *Environmental Health: A Global Access Science Source*, 4.18: 1 – 8.

Shenker, B.J., Rooney, C., Vitale, L. and Shapiro, I.M. 1992. Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. I: Suppression of T-cell activation. *Immunopharmacology and Immunotoxicology*, 14.3:539-553.

Shimizu, N., Itoh, N., Utiyama, H. and Wahl, G.M. 1998. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. *Journal of Cell Biology*, 140: 1307-1320.

Shimizu, N., Shimura, T. and Tanaka, T. 2000. Selective elimination of acentric double minutes from cancer cells through the extrusion of micronuclei. *Mutation Research*, 448: 81-90.

Shugart, L.R. 2000. DNA damage as a biomarker of exposure. *Ecotoxicology*, 9:329-340.

Shusterman, D., Lipscomb, J., Neutra, R. and Satin, K. 1991. Symptom prevalence and odor-worry interaction near hazardous waste sites. *Environmental Health Perspectives*, 94:25-30.

Sia Su, G.L. 2007. Impact on drinking water sources in close proximity to the Payatas dumpsite, Philippines. *Journal of Public Health*, 15:51–55.

Siddique, H.R., Gupta, S.C., Dhawan, A., Murthy, R.C., Saxena, D.K. and Chowdhuri, D.K. 2005. Genotoxicity of industrial solid waste leachates in *Drosophila melanogaster*. *Environment and Molecular Mutagenesis*, 46:189–197.

Siddique, H.R., Gupta, S.C., Mitra, K., Murthy, R.C., Daya K. Saxena, D.K. and Chowdhuri, D.K. 2008. Induction of biochemical stress markers and apoptosis in transgenic *Drosophila melanogaster* against complex chemical mixtures: Role of reactive oxygen species. *Chemico-Biological Interactions*, 169:171–188

Silkworth, J.B., Cutler, D.S. and Sack, G. 1989. Immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in a complex environmental mixture from the Love Canal. *Fundamental and Applied Toxicology*, 12.2: 303-312.

UNIVERSITY OF IBADAN

Simmons, J.E., DeMarini, E. and Berman, E. 1988. Lethality and hepatotoxicity of complex waste mixtures. *Environmental Research*, 46:74-85.

Simmons, J.E., Yang, R.S.H and Berman, E. 1985. Evaluation of the nephrotoxicity of complex mixtures containing organics and metals: advantages and disadvantages of the use of real-world complex mixtures. *Environmental Health Perspectives*, 103 (Suppl 1):67-71.

Singh, I. 1984. Induction of gene conversion and reverse mutation by manganese sulphate and nickel sulphate in *Saccharomyces cerevisiae*. *Mutation Research*, 137: 47 49.

Sisinno, C.L.S., E.C. Oliveira-Filho, E.C., Dufrayer, E.M.C., Moreira, J.C. and Paumgartten, F.J.R. 2006. Toxicity evaluation of a municipal dump leachate using zebrafish acute tests. *Bulletin of Environmental Contamination and Toxicology*, 64: 107-113.

Slack, R.J. Gronow, J.R. Voulvoulis, N. 2005. Household hazardous waste in municipal landfills: contaminants in leachate. *Science of the Total Environment*, 337: 119–137.

Slack, R. J., Gronow, J. R., Hall, D. H. and Voulvoulis, N. 2007. Household hazardous waste disposal to landfill: Using LandSim to model leachate migration. *Environmental Pollution*, 146.2: 501-509.

Snow, E.T. and Xu, L. 1991. Chromium (III) bound to DNA templates promotes increase polymerase progressivity and decreased fidelity during replication *in vitro*. *Biochemistry*, 30:11238-11245.

Solbe, J.F. 1995. Fresh water in: *Handbook of Ecotoxicology* (Edited by Peter Calins) Black Well Science Ltd. Pp 1-683.

Sosniak, W.A., Kaye, W.E. and Gomez, T.M. 1994. Data Linkage to explore the risk of low birth weight associated with maternal proximity to hazardous waste sites from the National Priorities List. *Archives of Environmental Health*, 49: 251–255.

Souguir, D., Ferjani, E., Ledoigt, G. and Goupil, P. 2008. Exposure of *Vicia faba* and *Pisum sativum* to copper-induced genotoxicity. *Protoplasma*, 233:203–207.

Stearns, D.M., Belbruno, J.J., Wetterhahn, K.E. 1995. A prediction of chromium (III) accumulation in human from chromium dietary supplements. FASEB Journal, 9: 1650-1657.

Stegeman, J.J. and Lech, J.J. 1991. Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environmental Health Perspective*, 90:101-109.

Stohs, S.J. and D. Bagchi. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biological Medicine*, 18: 321–36.

Sturgill, M.G. and Lambart, G.H. 1997. Xenobiotic–induced hepatotoxicity: Mechanisms of liver injury and methods of monitoring hepatic functions. *Clinical Chemistry*, 43.8: 1512-1526.

UNIVERSITY OF IBADAN

Sundberg, A., Appelkvist, E.L., Dallner, G. and Nilsson, R. 1994. Glutathione transferase in the urine–Sensitive methods for detection of kidney damage induced by nephrotoxic agenst in humans. *Environmental Health Perspective*, 102: 293–296.

Svobodova, Z., B. Vykusova and J. Machova, 1994. Sublethal chronic effects of pollutants on freshwater induced in fish. Ed. R. Muller in R. Lloyd Lugano, pp: 39-52.

Sweet, M.J. and Hume, D.A. 1996. Endotoxin signal transduction in macrophages. *Journal of Leukocytic Biology*, 60:8-26.

Talapatra, S.N., Ganguly, P., Mukhopadhyay, A. and Banerjee, S.K. 2007. Assessment of genetic biomarkers with special reference to micronucleated and binucleated erythrocytes in two fish species grown at industrial vicinity of thermal power plants, Kolkata, India. *Asian Journal of Water, Environment and Pollution*, 4.1:139-144.

Talorete, T., Limam, A., Kawano, M., Jenhani, A.B.R., Ghrabi, A. and Isoda, H. 2008. Stress response of mammalian cells incubated with Landfill leachate. *Environmental Toxicology and Chemistry*, 27.5: 1084 – 1092.

Tauhid-Ur-Rahman, M. 2006. Domestic waste disposal practice of Sylhet city. Journal od Applied Sciences, 6.7: 1506 – 1512.

Tayeb, W., Nakbi, A., Trabelsi, M., Attia, N., Miled, A. and Hammami, M. 2010. Hepatotoxicity induced by sub-acute exposure of rats to 2,4-Dichlorophenoxyacetic acid based herbicide "Desormone Lourd". *Journal of Hazardous Materials*, 180: 225-233.

Taylor, D.J., Green, N.P.O. and Stout, G.W.1998. *Biological Science*. Cambridege Low Edition Edition. Edited by Soper, R. Cambridge University Press. 167-191.

Teo, T. and Fenech, M. 2008. The interactive effect of alcohol and folic acid on genome stability in human WIL2-NS cells measured using the cytokinesis-block micronucleus cytome assay. *Mutation Research*, 657.1:32-38.

Tewari, A., Chauhan, L.K.S., Kumar, D. and Gupta, S.K. 2005. Municipal sludge leachate-induced genotoxicity in mice-A subacute study. *Mutation Research*, 587:9–15.

Thoday, J. M. 1951. The effect of ionizing radiation on the broad bean root. Part IX. Chromosome breakage and the lethality of ionizing radiations to the root meristem. *Brazilian Journal of Radiology*, 24: 276–572.

Thomas, P., Umegaki, K., Fenech, M. 2003. Nucleoplasmic bridges are a sensitive measure of chromosome reaarangement in the cytokinesis-block micronucleus assay. *Mutagenesis*, 18.2: 187–194.

Tkeshelashvili, L.K., Tsakadze, K.J. and Khulusauri, O.V. 1989. Effect of some nickel compounds on red blood cell characteristics. *Biological Trace Element Research*, 21:337–342.

Tolbert, P.E., Shy, C.M. and Allen, C.M.J.W. 1982. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutation Research*, 271:69-77.

UNIVERSITY OF IBADAN

Torres, E., Subida, R. and Rabuco, L. 1991. Health Profile of Child Scavengers in Smokey Mountain, Balut, Tondo, Manila, German Agency for Technical Cooperation/ International Labor Organization/College of Public Health, University of the Philippines, Manila.

Travlos, G.S., Morris, R.W., Elwell, M.R., Duke, A., Rosenblum,.S. and Thompson, M.B. 1996. Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13 – week toxicity studies in rats. *Toxicology*, 107: 17–29.

Treitz, W. 1970. Fundamentals of clinical chemistry with clinical correlation. W.B. saunders, Philadelphia vary B. detaeselier pp. 280–284.

Tryphonas, H. 2001. Approaches to detecting immunotoxic effects of environmental contaminants in human. *Environmental Health perspectives*, 109:877–884.

Tsapakos, M.J. and Wetterhahn, K.E. 1983. The interaction of Chromium with nucleic acids. *Chemical and Bioloical Interaction*, 46:265-277.

Tubb, A., and Iwugo, K. 2000. Organisation of municipal solid waste management system. Bristol: University of the West of England.

Udroiu, I. 2006. The micronucleus test in piscine erythrocytes. *Aquatic Toxicology*, 79:201–204.

Udroiu, I., Ieradi, L.A., Cristaldi, M., Tanzarella, C., 2006. Detection of clastogenic and aneugenic damage in newborn rats. *Environmental and Molecular Mutagenesis*, 47: 320–324.

Umeda, M., and Nishimura, M.1979. Inducibility of chromosomal aberrations by metal compounds in cultured mammalian cells. *Mutation Research*, 67: 221-223.

Umegaki, K. and Fenech, M. 2000. Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils. *Mutagenesis*, 15.3:261-269.

United Nation Environmental Programme (UNEP). 1994. *Technical report*. No.17.

United States Environmental Protection Agency (USEPA). 1980. *Toxicity of leachates*, EPA office of research and development, municipal environmental research lab, EPA-600/2-80-057. pp. 1-93.

United States Environmental Protection Agency (USEPA). 1996a. Acid digestion of sediments sludges and soils, Method-3050B.USEPA: Washington, DC. www.epa.gov/safewater/mcl.html.

United States Environmental Protection Agency (USEPA). 1996b. Biochemical test guidelines. OPPTS. 880.3550 immunotoxicity (office of prevention, pesticides and toxic substances).

UNIVERSITY OF IBADAN

United States Environmental Protection Agency (USEPA). 1998. Office of Solid Waste and Emergency Response. Greenhouse gas emissions from the management of some selected materials in municipal solid wastes.

United States Environmental Protection Agency. 2006. National recommended water quality criteria. http://www.epa.gov./ost/criteria/wqctable/.

United States Environmental Protection Agency (USEPA) (Office of Air and Radiation Protection Agency). 2008. Frequently Asked Questions About Landfill Gas and How It Affects Public Health, Safety, and the Environment. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2006. U.S. EPA, Office of Atmospheric Programs. EPA/430/R-08-005. *http://www.epa.gov/climatechange/emissions/usinventoryreport*.

Ulutas, B., Kiral, F. and Birincioglu, S. 2006. Unable to protect gentamicin-induced nephrotoxicity with allopurinol in rats. *Ankara Universitesi Veteriner Fakultesi Derg*, 53: 65-68.

Vaglenov, A., Carbonell, E. and Marcos, R. 1998. Biomonitoring of workers exposed to lead: genotoxic effects, its modulation by polyvitamin treatment and evaluation of the induced radioresistence. *Mutation Research*, 418:79–92.

Vaglenov, A., Creus, A., Lalchev, S., Petkova, V., Pavlova, S. and Marcos, R. 2001. Occupational exposure to lead and induction of genetic damage. *Environmental Health Perspective*, 109: 295–298.

Valavanidis, A., Iliopoulos, N., Gotsis, G. and Fiotakis, K. 2008. Leachability, heavy metals, PAHs and PCBs in fly and bottom ash of a medical waste incineration facility. *Waste Management Research*, 26: 247–255.

Valverde, M., Trejo, C. and Rojas, E. 2001. Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA–metal interaction? Mutagenesis 16: 265–270.

van Ginkel, G. and Sevanian, A. 1994. Lipid peroxidation-induced membrane structural alterations. *Methods in Enzymology*, 233:273–288.

Vasanthi, P., Kaliappan, S. and Srinivasaraghavan, R. 2008. Impact of poor solid waste management on ground water. *Environmental Monitoring Assessment*, 143:227–238.

Venkatesan, N. and Chandrakasan, G. 1995. Modulation of cyclophosphamide induced early lung injury by curcumin, an anti-inflammatory antioxidant. *Molecular and Cellular Biochemistry* 142: 79-87.

Verburg van Kemenade, B.M., Nowak, B., Engelsma, M.Y. and Wyets, F.A. 1999. Differential effects of cortisol on apoptosis and proliferation of carp B lymphocytes from head, kidney, spleen and blood. *Fish Shellfish immunology*, 9:405–415.

Vianna, N.J. and Polan, A.K. 1984. Incidence of low birth weight among Love canal residents. *Science*. 226: 1217–1219.

UNIVERSITY OF IBADAN

Vilar, J.B., Ferreira, F.L., Ferri, P.H., Guillo, L.A. and Chen Chen, L. 2008. Assessment of the mutagenic, antimutagenic and cytotoxic activities of ethanolic extract of araticum (*Annona crassiflora* Mart. 1841) by micronucleus test in mice. *Brazilian Journal of Biology*, 68.1: 141-147.

Vine, M.F., Stein, L., Weigle, K., Schroeder, J., Degnan, D., Tse, C.J., Hanchette, C. and Backer, L. 2000. Effects on the immune system Associated with Living Near a Pesticide Dumpsite. *Environmental Health Perspective*, 108.12:1113 – 1124.

Vitram, A., Ramarao, P. and Jena, G. 2007. Prior bleeding enhances the sensitivity of peripheral blood and bone marrow micronucleus test in rats. *Mutagenesis*, 22.4:287-291.

Vonen, B. and Morland, J. 1984. Isolated rats hepatocytes in suspension: potential hepatotoxic effects of six different drugs. *Archives Toxicology*, 56: 33–35.

Vrijheid M. 2000. Health effects of residence near hazardous waste landfill sites: a review of epidemiologic literature. *Environmental Health Perspectives*, 108 (Suppl 1):101-112.

Vrijheid, M., Dolk, H., Armstrong, B., Abramshy, L., Bianchi, F., Fazarinc, I., Garne, E., Ide, R., Nelen,V., Robert, E., Scott, J.E.S., Stone, D. and Tenconi, R. 2002. Chromosomal congenital anomalies and residence near hazardous waste landfill sites. *Lancet*, 359: 320–322.

Walling, E., Walston, A., Warren, E., Warshay, B. and Wilhelm, E. 2004. Municipal solid waste management in developing countries: Nigeria a case study. http://www.2dnr.cornell.edu/saw44/NTRES33/produts/spring%202004/papers/solidwaste management. pdf. 1 - 17.

Ward, R.S., Williams, G.M. and Hills, C.C. 1996. Changes in major and trace components of landfill gas during subsurface migration. *Waste Management Research*,14:243-261.

Washburn, P.C. and Di Giulio, R.T. 1989. Stimulation of superoxide production by nitrofurantoin, p-nitrobenzenoic acid and m-dinitrobenzene in hepatic microsomes of three species of freshwater fish. *Environmental Toxicology and Chemistry*, 8:171-180.

Watanabe, S. and Phillips, M.J. 1986. Acute phalloidin toxicity in living hepatocytes. Evidence for a possible disturbance in membrane flow and for multiple functions for actin in the liver cell. *American Journal of Pathology*, 122:101-111.

Weatherburn, M.W. 1967. Phenol–hypochlorite reaction for determination of ammonia. *Analytical Chemistry*, 39.8: 971 – 974.

Weingand, K., Brown, G., Hall, R., Davies, D., Gossett, K., Neptun, D., Waner, T., *et al*. 1996. Harmonization of animal clinical pathology testing in toxicity and safety studies. *Toxicological Science*, 29: 198–201.

White, P.A., and Claxton, L.D. 2004. Mutagens in contaminated soil: A review. *Mutation Research*, 567, 227–345.

UNIVERSITY OF IBADAN

Witeska, M. 2005. Stress in fish: Haematological and immunological effects of heavy metals. *Electronic Journal Ichthyology*. 1:35–41.

Williams, A. and Jalaludin, B. 1998. Cancer incidence and mortality around a hazardous waste depot. *Australian and New Zealand Journal of Public Health*, 22.3: 342-346.

Williamson, D.M, White, C.M., Poole, C., Kleinbaum, D., Vogt, R. and North, K. 2006. Evaluation of serum immunoglobulins among individuals Living Near Six superfund sites. *Enviromental Health Perspective*, 114.7:1065-1071.

Woodman, D.D. 1996. Assessment of hepatic function and damage in animal species. *Journal of Applied Toxicology*, 8: 249-254

World Health Organisation (WHO). 1990. Environmental health criteria for methylmercury, *Environmental Health Criteria* 101, 144 Geneva.

World Health Organisation. 1992. Environmental Health Critaria, 134 Cadmium. IPCS, Geneva.

World Health Organisation. 2006, *Guidelines for drinking-water quality*, WHO press, Geneva.

World Health Organisation Working Group. 2000. Evaluation and Use of Epidemiological Evidence for Environmental Health Risk Assessment: WHO Guideline Document. *Environmental Health Perspectives*,108.10: 997-1002.

Wyets, F.A., Flikt, A.G. and Verburg van Kemenade, B.M.L. 1998. Cortisol inhibits apoptosis in carp neutrophils granulocytes. *Developmental and Comparative Immunology*, 22:563–572.

Yasuhara, A., Shiraishi, H., Nishikawa, M., Yamamoto, T., Uehiro, T., Nakasugi, O., Okumura, T., Kenmotsu, K., Fukui, H., Nagase, M., Ono, Y. Kawagoshi, Y., Baba, K. and Noma, Y. 1997. Determination of organic components in leachates from hazardous waste disposal sites in Japan by gas chromatography–mass spectrometry. *Journal of Chromatography*, A 774: 321– 332.

Zerbock, O. 2003. *Urban solid waste management: Waste reduction in developing nations* (written for the requirements of CE 5993 Field Engineering in the Developing World). Michigan Technological University, Houghton, MI.

Zimmerman, H.J. 1999. Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver. 2nd ed. Philadelphia, PA: Lippincott, Williams, and Wilkins.

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APPENDIX

Appndix I: Procedure for leachate simulation from solid waste soil samples

a. 250g solid waste soil sample was weighed into volumentary flask

b. 1000ml of distilled water was added to form soil solution.

c. Soil solution was mechanically stirred at 1 hr interval using glass rod for 24 hrs

d. Supernatant was filtered with 2.5µm filter to remove suspended particles.

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Appendix II: Procedure involved in the serum biochemical analysis

a. Leachate was administered to rat by oral gavaging for 30 days

b. Blood was collected into lithium heparinise bottle for biochemical analysis

c. Serum biochemistry was processed using kit methods (Radox, UK).

d. Absorbance of the biochemical parameters were read spectrophotometrically against blank.

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Appendix III. Procedure for the haematological analysis

a. Aliquot of blood was collected Into EDTA bottle for haematological analysis

b. Automated analyser machine for use to quantify haematological indices.

c. Results of the haematological parameters generated from the automated analyser machine

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Appendix IV: Self Reported Health Impact Assessment Questionnaire (SRHIAQ) used for the health impact assessment study.

CELL BIOLOGY AND GENETICS UNIT, DEPARTMENT OF ZOOLOGY, UNIVERSITY OF IBADAN, NIGERIA

Public Health Impact Assessment of Landfill/Dumpsite Management Questionnaire

Dear respondent,

This questionnaire is designed to assess the health impact of landfills/dumpsites management among Nigerians. The findings from this research will be useful to policy makers in instituting better waste management methods and planning effective health care programmes for your community. The information obtained would be treated with utmost confidentiality. Please feel free to answer all the questions as honestly as possible. Thank you.

SECTION A: SOCIO – DEMOGRAPHIC INFORMATION

1. Area of residence: (1) City ☐ (2) Town ☐ (3) Village ☐
2. How old are you? …….. …..
3. Sex: (a) Male ☐ (b) Female ☐
4. Marital Status: (1) Single ☐ (2) Married ☐ (3) Separated ☐
   (4) Divorced ☐ (5) Cohabiting ☐ (6) Widower/widow ☐
5. Religion: (1) Christianity ☐ (2) Islam ☐ (3) Traditional ☐ (4) Others specify ……
6. Highest educational level: (1) None ☐ (2) Primary Education ☐ (3) Secondary Education ☐
   (4) Tertiary ☐ (5) Others Specify……..

SECTION B: SOCIO – ECONOMIC INFORMATION

7. Occupation: (1) Farmer ☐ (2) Professional ☐ (3) Civil Servant ☐ (4) Others Specify……..
8. Monthly Income (Naira): (1) < 5,000 ☐ (2) <10,000 ☐ (3) < 20,000 ☐
   (4) above 20,000 ☐
9. Type of Residential Apartment: (1) Self contained ☐ (2) Flat ☐ (3) Bungalow ☐
   (4) Single Room ☐ (5) Others……..
11. Is your apartment (residence) close to a dumpsite? (1) Yes ☐ (2) No ☐
12. If yes to question 11, how close is your residence to the dumpsite? (1) Within 1m ☐
    (2) 1 – 3m ☐ (3) 3 – 6m ☐ (4) above 6m ☐
13. How long have you been living in the apartment…….

SECTION C: DUMPSITE/LANDFILL AND EXPOSURE PATHWAY CHARACTERISTICS

14. Does the landfill/dumpsite contain landfill liners, landfill caps and leachate collection units?
    (1) Yes ☐ (2) No ☐ (3) Don't Know ☐

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15. Which of the following type of wastes are disposed off in the dumpsite; you may tick more than one option: (1) Glass ☐ (2) Paper/ Board ☐ (3) Metals ☐ (4) Plastics ☐ (5) Wood ☐ (6) Organic wastes ☐ (7) Medical waste ☐ (8) Agricultural wastes ☐ (9) Industrial wastes ☐ (10) others specify……..
16. Are you exposed to odours from the dumpsite: (1) Yes ☐ (2) No ☐
17. If yes to question 16, how regular are the odours from the dumpsite? (1) Once a day ☐ (2) Once a week ☐ (3) Once a month ☐ (4) Always ☐ (5) Less often ☐ (6) Others specify……..
18. When are these odours common and very high? (1) Dry Season ☐ (2) Wet Season ☐
19. How long have you been experiencing these odours? .....
20. Where do you normally encounter the odour? (1) At home ☐ (2) In the garden ☐ (3) On the streets ☐ (4) others specify……..
21. How can you explain the strength of the odour? (1) Faint ☐ (2) Moderate ☐ (3) Strong ☐ (4) Very strong ☐
22. Which of the following describes the type of the smell from site? (1) Sweet ☐ (2) Gas like ☐ (3) Rotten/Decaying matter ☐ (4) Chemical – like ☐ (5) others specify……
23. Do you breathe in dust particles from the sites? (1) Yes ☐ (2) No ☐
24. If yes to question 23, how regular? (1) Daily ☐ (2) weekly ☐ (3) monthly ☐ (4) Always ☐ (5) Less often ☐ (6) Others specify……
25. When are you exposed to the dust mostly? (1) Dry Season ☐ (2) Wet Season ☐
26. For how long have you been inhaling these dust particles?.....
27. Are the wastes in the site burnt? (1) Yes ☐ (2) No ☐
28. If yes to question 27, do you inhale smoke from the burning? (1) Yes ☐ (2) No ☐
29. If yes to question 28, how regular? (1) once a daily ☐ (2)once a week ☐ (3) once a month ☐ (4) Always ☐ (5) Less Often ☐ (6) Others Specify……
30. What are the sources of your water supply? (1) Well ☐ (2) Bore hole ☐ (3) Tap water ☐ (4) Stream/River ☐ (5) Rain water ☐ (6) Others specify…….
31. Does the water have (1) taste ☐ (2) odour ☐ (3) colour ☐ (4) others specify……
32. Are the sources of these water supplies close to the dumpsite? (1) Yes ☐ (2) No ☐ (3) Not sure ☐
33. If yes, how close? (1) <500 ☐ (2) 500 – 1km ☐ (3) 1 – 2km ☐ (4) 2km and above ☐
34. What do you use the water for? (1) Drinking ☐ (2) Cooking ☐ (3) Washing ☐ (5) Bathing ☐ (6) others specify……..
35. Is dirty water (leachates) produced from the dumpsites? (1) Yes ☐ (2) No ☐ (3) Not aware ☐
36. If yes, how regular do you see it? (1) once a day ☐ (2) once a week ☐ (3)once a month ☐ (4) Always ☐ (5) Less often ☐ (6) others specify……..

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37. Does it flow to the surrounding soils? (1) Yes ☐ (2) No ☐
38. Does it flow to your water sources? (1) Yes ☐ (2) No ☐ (3) Not sure ☐
39. Do you try to make your water clean before use? (1) Yes ☐ (2) No ☐
40. If yes, how? (1) Boiling ☐ (2) Filtering ☐ (3) Addition of Alum ☐
    (4) others specify……
41. Do you plant crops on the decomposed organic soil around the dumpsite? (1) Yes ☐ (2) No ☐
42. If yes, which of these crops do you plant? (1) Okra ☐ (2) Tomatoes ☐ (3) Maize ☐
    (4) Vegetables ☐ (5) Pepper ☐ (6) Others Specify……
43. For how long have you been planting these crops? ……
44. Do you use humus from the dumpsite for growing crops in your garden? (1) Yes ☐ (2) No ☐
45. If yes, for how long have you been practicing this method? ……..
46. Do you hunt and eat animals from the dumpsite? (1) Yes ☐ (2) No ☐
47. If yes, which of these animals do you hunt? (1) Grasscutters ☐ (2) Giant rat ☐ (3) Snail ☐
    (4) Common rats ☐ (5) others specify……
48. For how long have you been hunting these animals now? ……
49. Do you harvest naturally growing eatable plants from the dumpsite? (1) Yes ☐ (2) No ☐
50. If yes, which of these plants? (1) Pawpaw ☐ (2) Vegetables ☐ (3) Banana ☐
    (4) Plantain ☐ (5) Others specify……..
51. For how long have you been eating these plants now? …….
52. Do animals (organisms) from the dumpsite disturb you? (1) Yes ☐ (2) No ☐
53. If yes, which of these animals? (1) Mosquitoes ☐ (2) Flies ☐ (3) Rats ☐ (4) Cats ☐
    (5) Snakes ☐ (6) Toads ☐ (7) Others Specify
54. How regular do they disturb you? (1) Once a day ☐ (2) Once a week ☐ (3) Once a month ☐
    (4) Always ☐ (5) Less often ☐ (6) Others specify……..
55. Are you disturbed by the noise of heavy machines in the dump sites? (1) Yes ☐ (2) No ☐
56. If yes, how regular are you disturbed? (1) Once a day ☐ (2) Once a week ☐ (3) Once a
    month ☐ (4) Always ☐ (5) Less often ☐ (6) Others specify……
57. Do your domesticated animals like goats, sheep and poultry feed from dumpsites? (1) Yes ☐
    (2) No ☐

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SECTION D: PERCEPTION OF HEALTH RISK FROM LANDFILL MANAGEMENT IN NIGERIA

Some people reported the statements below concerning dumpsites/landfills. Please tick the most appropriate option that best fits your opinion about the following statements on this 5 – point scale: Strongly Agree (SA), Agree (A), Not Sure (NS), Disagree (D) and Strongly Disagree (SD)

S/N	STATEMENTS	SA	A	NS	D	SD
58	Living close to dumpsites increases psychological disturbance in humans					
59	Individuals around dumpsite may develop one or more of headache, vomiting, nausea, fever, rashes, diarrhea, itching of skin					
60	Individuals around dumpsite may develop one or more of joint pain, general body pain, eye irritation and ear problems.					
61	People around dumpsites may have increased rate of respiratory problems like asthma, catarrh, bronchitis, and others.					
62	Children whose mothers lived within 2km or less from the dumpsites have greater possibility of being born with a major birth defect like cleft palate.					
63	Chemicals present in leachates and gases produced from dumpsites are not harmful to human health.					
64	People are exposed to these chemicals through breathing, ingestion or skin contact via air, water, soil or food					
65	People are comfortable living around these dumpsites					
66	Pregnant women living around dumpsites may have miscarriages, still births or produce children with low birth weights or may be barren.					
67	People living around dumpsites may develop problems like cancer, memory loss and other forms of diseases due to immune suppression, than individuals not staying close to dumpsites.					
68	The dirty water (leachate) produced from the wastes can cause the quality of bore hole and well water, streams, soils, and air to be polluted, when it gets into them.					
69	Nigerians are more concerned or worried about their health conditions and quality of the environment, when they are around					

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	landfills/dumpsites					

SECTION E: HEALTH INFORMATION OF RESIDENTS/WORKERS AROUND DUMPSITES/ LANDFILLS.

Please tick the appropriate box and give details where necessary.

70. Which of the following respiration illnesses have you ever suffered? (1) Asthma ☐ (2) Bronchitis ☐ (3) Chest pain ☐ (4) Lung diseases ☐ (5) Coughing ☐ (6) Irritation of nose and throat ☐ 7.breathings difficulty ☐ 8.tuberculosis ☐ (9). others specify ……

71. For how long now have you been having these problems……

72. What do you think may cause these problems ……

73. Which of these other problems or diseases have you ever suffered? (1) Eczema ☐ (2) skin rashes ☐ (3) vomiting ☐ (4) upsetting of sleep ☐ (5) constant headache ☐ (6) eye problem ☐ (7) muscle weakness in arms and legs ☐ (8) hearing problem ☐ (9) fever ☐ (10) pains ☐ (11) diarrhea ☐ (12) others specify……

74. For how long now have you been experiencing these problems……

75. What do you think is the cause of these problems ……

76. Do you experience the problems (1) daily ☐ (2) weekly ☐ (3) monthly ☐ (4) yearly ☐ (5) others explain……

77. Have you experience any case of (1) miscarriage ☐ (2) stroke ☐ (3) diabetes ☐ (4) cancer ☐ (5) brain disorder ☐ (6) still birth ☐ (7) children with low birth weight ☐ (8) children with birth defects ☐ (9) others specify ……

78. For how long now have you been experiencing these problems……

79. Do you experience them (1) daily ☐ (2) weekly ☐ (3) monthly ☐ (4) yearly ☐ (5) others specify……

80. Have you been experiencing (1) dizziness ☐ (2) anger ☐ (3) Depression ☐ (4) annoyance ☐ (5) restlessness ☐ (6) anxiety ☐ (7) others specify ……..

81. For how long now have you been experiencing these problems …….

82. What do you think is the cause of the conditions in **80** above……

83. Do you visit clinics/hospitals when you experience diseases and problems? (1) Yes ☐ (2)No ☐

84. If YES to question 83, do you follow physician's prescriptions? (1) Yes ☐ (2) No ☐

85. Do you buy drugs yourself? (1) Yes ☐ (2) No ☐

86. If YES to question **85**, what are the common drugs you buy ?.....

87. Which other ways do you manage the problems and diseases ……

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SECTION F : OCCUPATIONAL HISTORY

88. How long have you been on your profession ……

89. Are you exposed to chemicals in your work place? (1) Yes ☐ (2) No ☐

90. If yes, please list these chemicals ……..

91. Do you inhale dust particles or smoke when at work place? (1) Yes ☐ (2) No ☐

92. What are the sources of these dust particles or smoke? ……

93. How long have you been exposed to these dust particles and / or smoke (1) daily ☐

(2) weekly ☐ (3) monthly ☐ (4) yearly ☐ (5) others describe……

94. Do you smoke any tobacco products? (1) Yes ☐ (2) No ☐

95. For how long now and what quantity do you take per smoking? ……

96. Do you drink alcohol? (1) Yes ☐ (2) No ☐

97. For how long now and how many units of alcohol do you take per day?.....

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